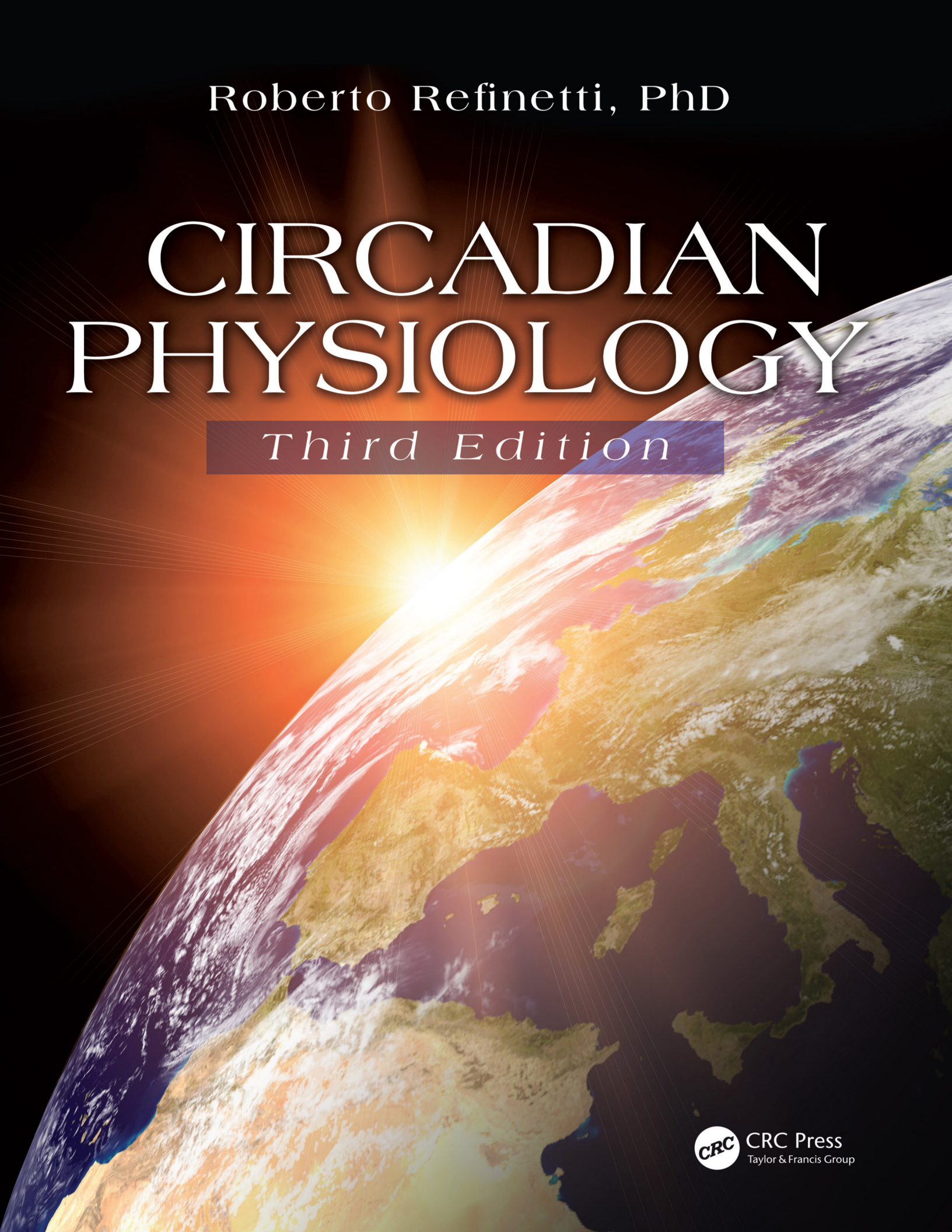
Roberto Refinetti, PhD

CIRCADIAN PHYSIOLOGY

Third Edition



CRC Press
Taylor & Francis Group

CIRCADIAN PHYSIOLOGY

Third Edition

This page intentionally left blank

CIRCADIAN PHYSIOLOGY

Third Edition

Roberto Refinetti, PhD
Boise State University
Idaho, USA

CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2016 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works
Version Date: 20160202

International Standard Book Number-13: 978-1-4665-1498-0 (eBook - PDF)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (http://www.copyright.com/) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978-750-8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com

Contents

Preface xiii
Acknowledgments xv
Author xvii
Software Installation xix

SECTION I History and Methods

Chapter 1 Early Research on Circadian Rhythms 3

1.1 Remote Past 3
1.1.1 Biological Rhythms 3
1.1.2 Endogenous Rhythmicity 5
1.2 Twentieth Century 9
1.2.1 1901–1950 9
1.2.2 1951–2000 10
1.3 Current Trends 13
1.3.1 Circadian Physiology and the World 14
1.3.2 Current Researchers 16
1.4 Ethics of Animal Research 22
1.4.1 Use of Animals 22
1.4.2 Ethical Issue 25
Summary 26
Exercises 26
Suggestions for Further Reading 27
Websites to Explore 28
References 28

Chapter 2 Research Methods in Circadian Physiology 33

2.1 Scientific Method 33
2.1.1 Philosophy and Science 33
2.1.2 Rules of the Method 38
2.1.3 Science and Religion 41
2.2 Research on Populations and Organisms 46
2.3 Research on Organs, Cells, and Molecules 53
2.3.1 Research on Organs 55
2.3.2 Research on Cells 56
2.3.3 Research on Molecules 57
2.4 Research on the Environment 60
2.4.1 Monitoring 60
2.4.2 Control 64
Summary 65
Exercises 66
Suggestions for Further Reading 68
Websites to Explore 69
References 69

Chapter 3 Analysis of Circadian Rhythmicity 79

3.1 Data Analysis 79
3.2 Parameters of Circadian Rhythms 82
3.2.1 Mean Level and Amplitude 83
3.2.2 Phase 86
3.2.3 Period 89
3.2.4 Waveform and Robustness 93
3.3 Statistical Significance 98
3.3.1 Regular Time Series 101
3.3.2 Educed Time Series 106
3.3.3 New Statistics 108
Summary 109
Exercises 109
Suggestions for Further Reading 111
Websites to Explore 112
References 112

SECTION II Phenomenology

Chapter 4 Ultradian and Infradian Rhythms 119

4.1 Environmental Rhythms 119
4.2 Ultradian Rhythms 123
4.2.1 Cardiac and Respiratory Rhythms 123
4.2.2 Neuroendocrine Rhythms 124
4.2.3 Tidal Rhythms 125
4.2.4 Other Ultradian Rhythms 126
4.3 Infradian Rhythms 128
4.3.1 Estrous Cycle 128
4.3.2 Weekly Rhythms 131
4.3.3 Lunar Rhythms 133
4.3.4 Other Infradian Rhythms 134
4.4 Annual Rhythms 136
4.4.1 Seasonal Rhythms 137
4.4.2 Circannual Rhythms 147
Summary 148
Exercises 149
Suggestions for Further Reading 151
Websites to Explore 152
References 152

Chapter 5 Daily and Circadian Rhythms 169

5.1 Environmental and Populational Rhythms 169
5.1.1 Environmental Rhythms 169
5.1.2 Populational Rhythms 171
5.2 Behavioral Rhythms 174
5.2.1 Locomotor Activity 175
5.2.2 Feeding and Excretion 181
5.2.3 Sensation and Perception 182
5.2.4 Learning and Other Processes 185

5.3 Autonomic Rhythms 187
5.3.1 Body Temperature 187
5.3.2 Cardiovascular Function 191
5.3.3 Melatonin and Cortisol Secretion 192
5.3.4 Metabolism and Sleep 195
5.3.5 Other Functions 197
Summary 198
Exercises 198
Suggestions for Further Reading 201
Websites to Explore 202
References 202

SECTION III Mechanisms

Chapter 6 Endogenous Mechanisms 237

6.1 Endogenous Rhythmicity 237
6.1.1 Concept of a Pacemaker 237
6.1.2 Free-Running Rhythms 239
6.1.3 Temperature Compensation 247
6.2 Inheritance Mechanisms 248
6.3 Single or Multiple Oscillators 255
6.3.1 Splitting 255
6.3.2 Spontaneous Internal Desynchronization 257
Summary 261
Exercises 261
Suggestions for Further Reading 262
Websites to Explore 262
References 263

Chapter 7 Photic Environmental Mechanisms 279

7.1 Nonparametric Theory of Entrainment 279
7.1.1 Phase Shifts 279
7.1.2 Entrainment 286
7.1.3 Range of Entrainment 293
7.1.4 Transients 296
7.1.5 Photoperiod 296
7.2 Photic Parameters 299
7.2.1 Masking 300
7.2.2 Aftereffects 302
7.2.3 Dark Adaptation 304
7.2.4 Some Bizarre Parametric Effects 307
7.3 Synthesis and Models 307
7.3.1 Mathematical Models 307
7.3.2 Functional Models 308
Summary 312
Exercises 313
Suggestions for Further Reading 315
Websites to Explore 315
References 315

Chapter 8 Nonphotic Environmental Mechanisms 325

8.1 Nonphotic Entrainment 325
8.1.1 Ambient Temperature 325
8.1.2 Food Availability 326
8.1.3 Physical Exercise 328
8.1.4 Social Interaction 330
8.1.5 Dark Pulses 333
8.1.6 Other Stimuli 334
8.2 Searching for a Common Mechanism 335
8.3 Separate Food-Entrainable Pacemaker 336
Summary 341
Exercises 341
Suggestions for Further Reading 342
Websites to Explore 343
References 343

Chapter 9 Integration of Mechanisms 351

9.1 Internal Temporal Order 351
9.1.1 Relationship between the Body Temperature Rhythm and the Activity Rhythm 352
9.1.2 Other Dependencies 358
9.2 Ecology and Evolution 360
9.2.1 Evolution 360
9.2.2 Diurnal and Nocturnal Niches 363
9.2.3 Natural Light Exposure 370
9.2.4 Environmental Conflicts 375
9.3 Lifetime Changes 376
9.3.1 Intra- and Interindividual Variability 376
9.3.2 Development 377
9.3.3 Rhythmic Interactions 379
9.3.4 Aging 384
9.3.5 Learning 387
9.3.6 Sexual Dimorphism 390
Summary 390
Exercises 391
Suggestions for Further Reading 392
Websites to Explore 393
References 393

Chapter 10 Homeostasis and Circadian Rhythmicity 413

10.1 Temperature Regulation 413
10.1.1 Homeostatic Control of Body Temperature 413
10.1.2 Circadian Control of Body Temperature 422
10.1.3 Integration of Homeostasis and Circadian Rhythmicity 426
10.2 Sleep, Feeding, and Energy Expenditure 431
10.2.1 Homeostatic and Circadian Control of Sleep 431
10.2.2 Homeostatic and Circadian Control of Feeding 434
10.2.3 Homeostatic and Circadian Control of Energy Expenditure 438
Summary 441
Exercises 442
Suggestions for Further Reading 443
Websites to Explore 443
References 444

SECTION IV Physical Substrates

Chapter 11 Receptors 471

11.1 Sensory Input 471
11.2 Photic Receptors 471
11.2.1 Eyes 471
11.2.2 Other Photic Receptors 479
11.3 Nonphotic Receptors 481
11.3.1 Skin Temperature 481
11.3.2 Core Temperature 483
11.3.3 Nutritional State 484
Summary 486
Exercises 486
Suggestions for Further Reading 487
Websites to Explore 488
References 488

Chapter 12 Pacemakers 499

12.1 Suprachiasmatic Nucleus 499
12.1.1 Lesion Studies 499
12.1.2 Monitoring of SCN Activity 502
12.1.3 Transplantation Studies 503
12.2 Cellular Processes 505
12.2.1 Anatomy of the SCN 505
12.2.2 Functional Properties of SCN Cells 506
12.2.3 Neurotransmitters in the SCN 509
12.3 Molecular Processes 511
12.3.1 Early Studies 512
12.3.2 Molecular Clock of the Fruit Fly 514
12.3.3 Molecular Clock of Other Simple Organisms 515
12.3.4 Mammalian Molecular Clock 516
12.3.5 Beyond the Loop 519
12.4 Other Pacemakers 522
12.4.1 Pineal Gland 522
12.4.2 Eyes 523
12.4.3 Liver 524
12.4.4 Other Peripheral Clocks 526
Summary 529
Exercises 529
Suggestions for Further Reading 530
Websites to Explore 531
References 531

Chapter 13 Afference and Efference 549

13.1 Afferent Pathways 549
13.1.1 Anatomy 549
13.1.2 Function 551
13.1.3 Pharmacology 555
13.1.4 Molecular Mechanisms 559
13.2 Efferent Pathways 561

13.2.1 Anatomy 561
13.2.2 Function 565
Summary 570
Exercises 571
Suggestions for Further Reading 573
Websites to Explore 573
References 573

SECTION V Applications

Chapter 14 Optimal Timing on Earth and in Space 589

14.1 Patterns of Human Mobility 589
14.2 Best Time for Sports and Intellectual Activities 592
14.2.1 Physical Activities 592
14.2.2 Intellectual Activities 593
14.2.3 Morningness–Eveningness Typology 597
14.3 Space Exploration 600
Summary 602
Exercises 604
Suggestions for Further Reading 604
Websites to Explore 605
References 605

Chapter 15 Jet Lag and Shift Work 611

15.1 Jet-Lag Syndrome 611
15.1.1 Diagnosis 611
15.1.2 Treatment 614
15.2 Shift-Work Malaise 620
15.2.1 Diagnosis 620
15.2.2 Treatment 623
15.3 Daylight-Saving Time 624
Summary 626
Exercises 626
Suggestions for Further Reading 627
Websites to Explore 627
References 627

Chapter 16 Human Medicine 633

16.1 Chronotherapeutics 633
16.1.1 Cardiovascular Disease 635
16.1.2 Cancer 636
16.1.3 Asthma 637
16.2 Sleep Disorders 638
16.3 Depression 643
16.3.1 Traditional Treatment of Affective Disorders 644
16.3.2 Circadian Rhythmicity and Affective Disorders 647
16.3.3 Seasonal Affective Disorder 650
Summary 652
Exercises 653
Suggestions for Further Reading 655
Websites to Explore 655
References 655

Chapter 17 Pet Selection and Veterinary Medicine 667

17.1 Pet Selection 667
17.2 Veterinary Medicine 669
Summary 676
Exercises 677
Suggestions for Further Reading 677
Websites to Explore 677
References 677

Appendix A: Assessment of Learning 683

Appendix B: Dictionary of Circadian Physiology 685

Appendix C: Organisms Used 701

This page intentionally left blank

Preface

It has been 10 years since the publication of the second edition of *Circadian Physiology*. Sales figures and comments from readers indicate that the book achieved its goal of serving as an accessible but comprehensive review of basic and applied research on circadian rhythms. A clear writing style and minimal requirement of background knowledge have allowed the book to serve both as a handbook for life scientists experienced in other fields but interested in expanding their research efforts into the study of circadian rhythms and as a textbook for undergraduate and graduate students. A Chinese translation was published in 2011. A new, updated English edition is needed now.

Readers who grew up after the universalization of the Google search engine often expect to find everything through a single online query. This is not a reasonable expectation when it comes to scientific knowledge. Scientific knowledge is very specific and requires vetting by specialists using restricted databases. Broad-minded scientists are needed to summarize the overwhelming amount of information by writing or editing a book. Several excellent books on circadian rhythms have been published in the past 10 years. Some are very readable but are targeted at general audiences that have no interest in physiological or molecular mechanisms. Others are very rigorous in content but lack comprehensive coverage of the field or adopt a writing style inaccessible to nonspecialists and students. *Circadian Physiology* remains the only book in press that successfully combines thorough and detailed coverage with an accessible writing style, providing a truly integrated view of the discipline that only a single-author book can achieve. Of course, no book can provide truly exhaustive coverage of a scientific discipline, and readers interested in more detailed information about the topics covered in this book will benefit from the detailed referencing of original sources by bibliographic footnotes in each chapter. This approach is in line with the reasoning that a good textbook is not only an effective summary of the scientific literature but also a guided gateway to this literature.

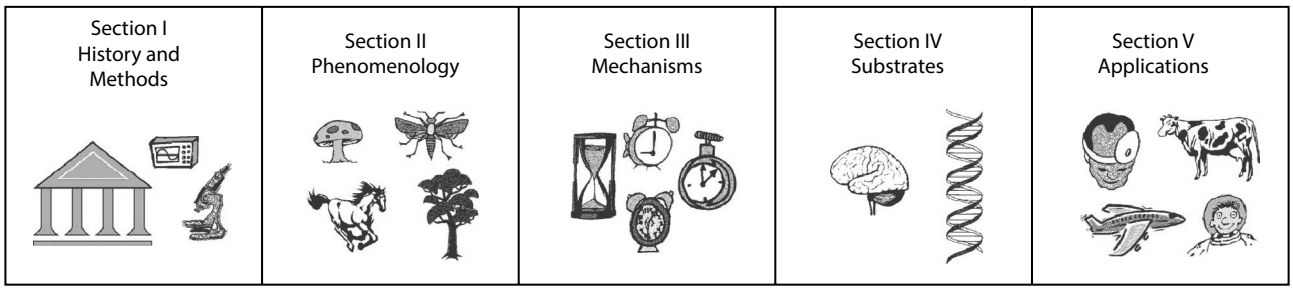
The organization of this edition is similar to that of the first and second editions because it remains the most logical and didactical. The book is divided into five sections, each with several chapters (see figure). Section I covers historical and methodological topics in the study of circadian rhythms. Section II deals with the phenomenology of biological rhythms, i.e., the description of the multiplicity of rhythmic phenomena in living organisms, including infradian, circadian, and ultradian rhythms. Section III addresses the physiological mechanisms, both endogenous and environmental, that control circadian rhythms. Section IV provides an insight into the physical substrates of circadian rhythms at the level of organs, cells, and molecules. Section V covers the multiple applications of circadian physiology in the planning of optimal times for physical and intellectual activity, the prevention of jet lag, the management of shift work, the treatment of sleep disorders, and many other endeavors.

If one thinks of biological rhythmicity as something to be learned, the five sections of the book will answer five natural questions:

Section I: How do we study it?
Section II: What is it?
Section III: How does it work?
Section IV: How is it built?
Section V: What can it be used for?

The fundamentals of circadian physiology have not changed, of course, in the 10 years since the publication of the second edition. Major advances have been made in the past decade, however, and each chapter has been updated with new material. More than a thousand references have been added, bringing the total to more than 6000 references.

This third edition of *Circadian Physiology*, like the previous ones, is intended to be accessible to a wide audience. Brief reviews of essential principles in physiology, biochemistry, molecular biology, neuroscience, statistics, computer science, and philosophy of science are provided in Chapters 2 and 3 as part of the discussion of research methods and data analysis procedures in circadian physiology. Beyond these essential principles, the required background knowledge generally does not exceed that expected of first-year university students or scientists from other fields of inquiry (and, when it does, additional background material is provided). As much as I tried to make all chapters equally readable, however, readers with different backgrounds may find some chapters to be "denser" than others. For readers who are medical or psychological practitioners, I must point out that this book was written from the perspective of circadian *physiology*, not circadian



pathology. Thus, the five sections of the book, and the chapters within them, are arranged according to basic biological processes, not according to disease types. Section V discusses important medical applications of circadian physiology, but I cannot, of course, claim to have covered circadian pathology exhaustively.

Professors adopting this edition of *Circadian Physiology* as a textbook will notice that 17 chapters are 2 chapters more than the 15 weeks of a typical university course. I felt that forcing the material into 15 chapters would disrupt the natural organization of the topics covered in the book without providing any real benefit, as many professors do not place equal emphasis on every chapter and often skip a few chapters or combine two chapters in one week. The choice of how to organize the course should rightfully remain the prerogative of the professor, not of the author of the textbook. Arrangement of the material into 17 thematically oriented chapters allows the book to present a well-organized view of the field that will be valuable not only to students but also to general readers, medical practitioners, and life scientists who are expanding their research programs into the study of circadian rhythms. Readers will notice that Chapters 8, 11, and 17 are considerably shorter than the others; they remain as separate chapters, however, for organizational reasons.

To facilitate its use as a textbook, this book contains summaries, suggestions for further readings, directions to pertinent websites, and exercises at the end of each chapter. A brief Assessment of Learning section, with 20 multiple-choice questions, appears after the last chapter. A companion CD provides computer programs designed to offer practical experience in a variety of topics. Instructions for software installation are given in a separate section before the first chapter, and programs for data analysis—and tutorials and simulation programs—are introduced at the appropriate points in the various chapters. A dictionary of circadian physiology—with information on meaning, etymology, and pronunciation—is included at the end of the book. For the benefit of international readers, the dictionary includes a table of equivalency of major circadian physiology terms in eight foreign languages. Also included are lists of standard international units of measurement and of conversion factors for various British units that are still in use in the United States. Readers—both researchers and students—are also encouraged to visit my laboratory's website (www.circadian.org) and to use the e-mail link to send me queries about specific issues.

Although it is unrealistic to expect that every reader will fall in love with this book, I hope that all readers will enjoy and benefit from reading it as much as I enjoyed and benefited from writing it. I believe that I have not only compiled a rigorous, scholarly selection of facts and theories in circadian physiology—with thorough documentation through figures and references—but have also clearly conveyed the importance and the fascination of past and current studies on the all-encompassing process of circadian rhythmicity.

Acknowledgments

Many people assisted me in the task of preparing this book. First and foremost, I thank my wife for her continued support of my academic endeavors. Early mentors—namely, Dora Ventura (University of São Paulo), Harry Carlisle and Steven Horvath (University of California, Santa Barbara), Evelyn Satinoff (University of Illinois), and Michael Menaker (University of Virginia)—were instrumental in the development of my research career. Frequent collaborations with other scientists—namely, Giuseppe Piccione and Giovanni Caola (University of Messina, Italy), Mutlu Kart Gür (Ahi Evran University, Turkey), Priyoneel Basu (Banaras Hindu University, India), Mamane Sani (University of Maradi, Niger), Lara Dugas and Amy Luke (Loyola University Chicago), and Khalid Abdoun (King Saud University, Saudi Arabia)—helped me maintain an active research program despite burgeoning administrative responsibilities. Intellectual exchanges with numerous students who worked in my laboratory over the years—particularly Aaron Osborne, Candice Brown, Adam Shoemaker, and Jonathan DeLonge—helped me avoid the stagnation of academic dogma. Several circadian researchers from around the world helped me compile the language equivalency table in the *Dictionary of Circadian Physiology*, and their names are listed in that section of the book. As the editor-in-chief of the *Journal of Circadian Rhythms*, I have also benefited greatly from the interaction with the numerous authors and members of the editorial board.

Exchanges of letters with Professor Jürgen Aschoff and Professor Franz Halberg, both pioneers in the field of circadian rhythms who are unfortunately no longer with us, helped me gain a broader historical perspective of the field.

For financial support of my research program, I thank the National Institute of Mental Health and the National Science Foundation. For comments on previous editions of *Circadian Physiology*, I thank Ralph Mistlberger (Simon Fraser University), William Timberlake (Indiana University), Colin Dawes (University of Manitoba), James Watrous (Saint Joseph's University), and Erik Maronde (Johann Wolfgang Goethe University). I also thank the various individuals and institutions that provided permission to reprint previously published diagrams and photographs, as well as individual scientists who provided original figures or their personal photographs.

Finally, this book would not have been published if it were not for the superb work of the staff at the Taylor & Francis Group. I am especially appreciative of the support and encouragement provided by Barbara Ellen Norwitz and the technical assistance provided by Jennifer Ahringer and Christine Selvan.

This page intentionally left blank

Author



Roberto Refinetti is a physiological psychology professor, circadian physiology researcher, and chair of the Department of Psychology at Boise State University, in Boise, Idaho. He is also a member of the graduate faculty of the University of Messina (Italy) and was formerly associated with the University of South Carolina, the University of Virginia, the University of Illinois, the University of São Paulo (Brazil), and the University of California at Santa Barbara (where he earned his doctorate in 1987). His research program in circadian physiology, which concentrates on the integration of circadian and homeostatic mechanisms, has been funded by the U.S. National Science Foundation and the National Institutes of Health and has yielded more than 200 publications in scientific journals. Dr. Refinetti is editor-in-chief of the *Journal of Circadian Rhythms* and of the interdisciplinary journal *Sexuality & Culture* and has served as an invited peer-reviewer for more than 70 biomedical journals. He is a member of the American Physiological Society, the Society for Neuroscience, the Philosophy of Science Association, the Association for Psychological Science, the Society for Research on Biological Rhythms, the American Statistical Association, and the World Association of Medical Editors. You may visit his website at www.circadian.org and contact him by e-mail at refinetti@circadian.org.

This page intentionally left blank

Software Installation

A CD containing the circadian physiology software package accompanies this book. Although the book can be read independently of installation and use of the software package, one's reading experience will be greatly enhanced by completion of the computer exercises that appear at the end of most chapters. Also, researchers interested in data analysis of circadian rhythms will benefit from the various data analysis programs included in the package. This section of the book explains how to install the software package and provides general information about its use.

HOW TO INSTALL THE SOFTWARE

Requirements

The programs will run under the Windows operating system. Despite recent gains in the mobile phone sector, the Mac OS continues to account for less than 7% of the desktop operating system market share, so that the development of separate versions of these programs for the Mac OS is not practical. The **Setup** program will automatically install the software package in personal computers running under Windows 10 or older versions back to Windows 95. For installation in network computers, users should consult their network administrator, who should read the **Readme** file in the distribution CD. Memory and disk space requirements are modest (no more than 40 Mb of RAM and 40 Mb of free disk space are required). A computer mouse (or equivalent) is required, but a printer is optional. Multimedia functionality (sound card and speakers) is required for only two of the programs.



Procedure

Insert the *Circadian Physiology* CD in your CD drive. If the drive is set to automatically read the CD, **Setup** will start automatically. Otherwise, navigate to the CD and run the **Setup** program. Follow the simple on-screen instructions. At the end of the installation, a Shortcut will be placed on the Desktop. If you cannot find the Shortcut, see the *Troubleshooting* section.

HOW TO USE THE SOFTWARE

All programs and data files will be located in the folder "\Program Files\Circadian" unless you designated a different folder during installation of the program (sample data files will be in the subfolder "\Data"). To simplify operation of the software package, you should use the banner program **Circadian** to access the other programs. You can start **Circadian** by double-clicking on its Shortcut icon on the Desktop.

When you start **Circadian**, a banner will appear at the top of your screen. The banner contains mini icons of the various programs (see figure). To run a program, just click on its mini icon. A single click is enough. To see a brief description of the program before activating it, rest the mouse pointer on the program's icon. For your convenience, the brief descriptions are listed in the following table. The table also indicates which chapters contain exercises involving each of the programs. Detailed descriptions of the data analysis programs (i.e., programs 1 through 10) are given in the main text of Chapter 3.

No.	Name	Description	Chapters
1	Plot	Plots data as Cartesian plots or actograms	2, 3, 7
2	Moving	Calculates moving averages	3
3	Onecycle	Detects temporal pattern of a single cycle	4
4	Rhythm	Detects rhythmicity in a data set	4
5	Fourier	Conducts spectral analysis	4
6	Rayleigh	Detects periodicity in a series of events	4
7	Acro	Calculates acrophase, mean level, and amplitude of a rhythm	5
8	Tau	Calculates circadian period by chi-square periodogram	5
9	LSP	Calculates circadian period by Lomb–Scargle periodogram	5
10	Cosinor	Calculates all parameters of a rhythm	5
11	Free-run	Demonstration of free-running rhythms	12
12	Wave	Tutorial on periodic processes	3
13	Entrain	Tutorial on entrainment of circadian rhythms	7
14	PRC	Compilation of phase response curves	7, 8
15	Model	Computer model of circadian pacemaker	6 through 8

(*Continued*)

No.	Name	Description	Chapters
16	Jet-lag	How to minimize jet lag	15
17	Health	How to control your own clock	14 through 17
18	Bioclock	Listen to music (*Bioclock Rhapsody*)	17
19	Organism	Convert species names	9
20		Close the banner program	

If you have just installed the software package and are impatient to test it out, you may want to try the program **Bioclock** (number 18). This program simply plays the musical composition *Bioclock Rhapsody* and does not require any background reading. All other programs are introduced at the appropriate point in the various chapters of the book.

The menu bar in each program (except **Bioclock**) contains a Help item. Clicking on the Help item will provide you with a general description of how the program operates. More detailed instructions are given in the end-of-chapter exercises (see table). If you plan to analyze your own data sets, you must be aware that the data analysis programs (i.e., programs 1 through 10) expect data files in a specific format. For equally spaced time series, standard ASCII files (text files with one value per line) are required. For unequally spaced time series (which include time series with missing values), files must contain two values per line *separated by a space* (ASCII 32): a time tag and the value to be plotted or analyzed. The time tag must be in 24-hour clock mode (e.g., 22.5 for 10:30 p.m.). If the file contains more than 1 day, the clock may be reset to 0 at midnight each day. Sample data files are provided with the software package, and you may inspect them with a word processor to verify the file format. The sample data files are described in the Exercises at the end of the various chapters and are also listed in the following table:

File	Time Tag?	Length	Description
A01.txt	No	7 days, 6-minute resolution	Body temperature (°C) of a Richardson's ground squirrel
A02.txt	No	8 days, 6-minute resolution	Body temperature (°C) of a degu (noisy record)
A03.txt	No	36 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a golden hamster
A04.txt	No	29 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a golden hamster
A05.txt	No	42 days, 6-minute resolution	Body temperature (°C) of a laboratory rat
A06.txt	No	19 days, 6-minute resolution	Locomotor activity (beam breaks per 6 minutes) of a pill bug
A07.txt	No	6 days, 6-minute resolution	Heat production (W) of a fat-tail gerbil
A08.txt	No	20 days, 6-minute resolution	Computer-generated cosine wave, no noise
A09.txt	No	20 days, 6-minute resolution	Computer-generated cosine wave, 60% noise
A10.txt	No	20 days, 6-minute resolution	Computer-generated cosine wave, 85% noise
A11.txt	Yes	10 days	Computer-generated cosine wave, no noise
A12.txt	Yes	10 days	Computer-generated cosine wave, 60% noise
A13.txt	Yes	10 days	Computer-generated cosine wave, 85% noise
A14.txt	Yes	7 days	Body temperature (°C) of a laboratory rat
A15.txt	Yes	7 days	Body temperature (°C) of a laboratory rat
A16.txt	No	7 days, 6-minute resolution	Body temperature (°C) of a fat-tail gerbil
A17.txt	No	7 days, 6-minute resolution	Body temperature (°C) of a tree shrew
A18.txt	Yes	1 day	Locomotor activity (counts per 6 minutes) of a 13-lined ground squirrel
A19.txt	Yes	1 day	Body temperature (°C) of a man
A20.txt	No	34 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a domestic mouse with a light-induced phase shift on day 23
A21.txt	No	29 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a domestic mouse with a light-induced phase shift on day 14
A22.txt	No	43 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a Nile grass rat transferred from DD to LD on day 26
A23.txt	No	30 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a golden hamster under LD 7:5 (LD included in the file)
A24.txt	No	33 days, 6-minute resolution	Running-wheel activity (revolutions per 6 minutes) of a domestic mouse transferred from LD to DD on day 17
A25.txt	No	10 days, 6-minute resolution	Computer-generated cosine wave with periodicities of 24 and 12 hours
A26.txt	No	10 days, 6-minute resolution	Computer-generated cosine wave with periodicities of 24, 12, 10, and 6 hours
A27.txt	No	10 days, 6-minute resolution	Computer-generated cosine wave with periodicities of 24.5 and 23.5 hours
A28.txt	Yes	2 days	Air relative humidity (%)
A29.txt	No	8 days, 3-hour resolution	Plasma urea concentration (mmol per liter) of a goat
A30.txt	No	4 years, 1-day resolution	Mean daily temperature in Chicago from January 1999 to December 2002

TROUBLESHOOTING

Although all programs in the package have been extensively debugged, the possibility of minor errors cannot be ruled out. Should you have difficulties in using the software, refer to the following table that lists some of the most common problems and their solutions.

Problem	Solution
Nothing happened when I placed the installation CD in the CD drive.	The **autorun** function of your CD drive is probably disabled. You must either enable it or access the CD directly by navigating to it using the tools in the Taskbar.
The software installation failed.	Most likely, you are trying to install the software in a network computer. Call your network administrator and ask him/her to read the **Readme** file in the CD. If the installation failed in a stand-alone computer, you may consult the **Readme** file yourself. If you have a minimal knowledge of the Windows operating system, you can install the software manually. If you have limited space on your hard drive, don't copy the two *wav* files (which will save you tens of megabytes but will also prevent you from using the program **Bioclock**).
The **Circadian** shortcut icon does not appear on the Desktop.	If **Setup** failed to create a shortcut for **Circadian**, you can access the program by navigating to the appropriate folder and double-clicking on the **Circadian** icon. You may also create a Shortcut yourself. First locate the **Circadian** program. Then right-click on its icon. Choose *Create Shortcut*. Follow the simple directions. When done, drag the Shortcut to your Desktop or to the Start Menu. If you wish to rename the shortcut, right-click on it and choose *Rename*.
The banner displayed by **Circadian** is not in a convenient location on my Desktop.	Close other programs, such as word processors and web browsers. None of the programs in the circadian physiology software package will conflict with the banner.
I don't like the background color of the banner.	The background color of the banner is the same as the background color of your Desktop (which may not be visible if you have added a wallpaper to the background). Check the Display settings in the Windows Control Panel.
The tool tips (brief program descriptions) are not being shown when I rest the mouse on the program icons.	Make sure that the banner is the active window on the Desktop. To cause it to be the active window, just click anywhere between the mini icons.
When I start a program, it flashes for a few seconds.	This is only a minor nuisance, but you can avoid it by *not* double-clicking on the icons. One click is enough to start any program from the banner.
One of the data analysis programs refuses to load a data set.	Make sure that the data file is in the correct format (see specifications mentioned earlier). In particular, a data set with time tags will not load if the program is expecting a data set without time tags, and vice versa. In rare cases, it may happen that the data set is too large to be loaded all at once. If this is the case, try breaking the file down into shorter files.
I believe I am obtaining spurious numerical results.	If you live outside the United States, it is likely that you are using commas instead of periods as decimal separators. Please check the international settings in your computer (Regional and Language Options). You should use some of the data sets in the end-of-chapter exercises to make sure that you obtain the expected results before you try to analyze your own data.
A program is not doing what it is supposed to do.	It is possible that *you* are doing something wrong. Check the Help item in the program's menu bar.
A program supposed to have audio functionality remains silent.	"You have the right to remain silent" should apply to people being arrested, not to computer programs! First of all, check the volume in your speakers. If this is not the problem, make sure that your computer has the necessary hardware (sound card, speakers, etc.).
Data analysis procedures take too long to execute.	No procedure should take more than a few seconds. If you have a large data set, you may be able to speed up processing by closing other programs that are simultaneously open. If your computer runs at less than 1 GHz, you may want to upgrade it.
When I print something, the page comes out blank.	Check your printer settings. All programs in this software package utilize the Windows printing routines for the default printer. If the Windows printer settings are not correct, the information will be lost on its way to the printer.
Some text appears in fonts that are too big or too small for the program window.	The programs use standard fonts in computers sold in the United States. In other countries, it is possible that the closest font set available in the computer will not be adequate. You should obtain and install font sets for MS Sans Serif (8 point and 10 point sizes) and Courier New (8 point size). Check Microsoft's website (www.microsoft.com).
In some programs, some words have spurious characters.	Encoding of characters does not have a universal standard. The programs in this package use Western European Windows encoding. If your computer is set for a different encoding, some characters will not print correctly. Check the Fonts settings in the Windows Control Panel.
The program window is too big and extends outside the borders of the screen.	Your video settings are archaic. Use the Windows Control Panel to set the resolution of your monitor to 1024 × 768 pixels or greater. Color settings should not be a problem (a 16-bit color scheme is sufficient).
I tried everything in this Troubleshooting list and am still having problems.	Ask for help from the author of the program. Send an e-mail message to Dr. Refinetti at refinetti@circadian.org. Please include information about your computer and a detailed description of the problem.

This page intentionally left blank

Section I

History and Methods

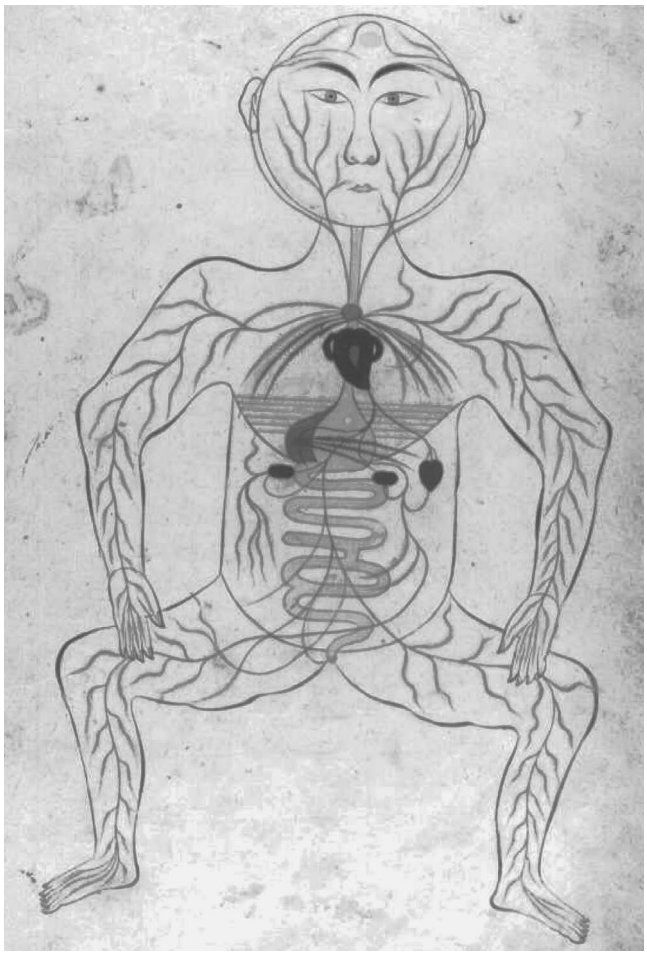


Illustration of human anatomy drawn by Persian physician Mansur ibn Mohammed in 1396. (Image courtesy of the Clendening Library at the University of Kansas Medical Center, Kansas City, MO.)

This page intentionally left blank

1 Early Research on Circadian Rhythms

1.1 REMOTE PAST

This chapter deals with the history of circadian physiology—from ancient times to the present. Before we address its history, we must define circadian physiology. *Physiology* (or "integrative biology") is the study of vital processes of living organisms, particularly at the level of organs and organ systems and at the level of the organism as a whole.[1] Because physiological processes are dependent on anatomical and biochemical factors, and because they constitute the physical basis of behavior, the discipline of physiology must incorporate the disciplines of anatomy, endocrinology, molecular biology, pharmacology, neuroscience, and psychology. *Circadian physiology* is the branch of physiology that deals with the temporal organization of vital processes in the course of a day. Thus, circadian physiology is integrative biology at its best: it deals with the integration of functions in both the spatial and temporal dimensions. A discussion of the conceptual and practical importance of circadian physiology will be initiated in Section 1.3 and will continue throughout this book.

Written records of observations in circadian physiology are by necessity limited to the few millennia since the invention of written language. However, it is very likely that early humans were aware of daily variations in physiological processes. At the very least, they must have been aware of daily rhythmicity in the environment and its impact on their own daily cycle of wake and sleep. Awareness of daily rhythmicity in the environment is evidenced by the creation of various forms of clocks and calendars. The sundial, which indicates the time of day as a function of the size and direction of the shade cast by the sun (Figure 1.1), was perhaps the first man-made clock. The Egyptians erected obelisks that were used as sundials more than 5500 years ago.[2] The Chaldeans, in Mesopotamia, created a sophisticated system of time measurement about 3000 years ago,[3] a system from which our own time measurement system is derived. In the Chaldean system, a day was divided into 12 long hours instead of into the shorter 24 hours that we adopt today, but their system was at the origin of our nondecimal division of the day. A decree issued in France in 1793 established a decimal division of the day, but it was revoked 2 years later.[4] Except for this brief diversion, the partition of a day into 24 hours, and of an hour into 60 minutes, has been a global standard for centuries. Usage of the system differs in different regions of the world up to this day, however. In the United States, only scientists and the military use a true 24-hour system; businesses and ordinary citizens use a double 12-hour system with 12 hours before noon (*ante meridiem*) and 12 hours after noon (*post meridiem*), as indicated in Figure 1.2. In many other parts of the world, official times are given in the 24-hour system (such as *20:30 hours* for a dinner invitation) but a 12-hour system is used in informal conversation (such as *8:30 at night* for an informal get-together with friends). The colon between hours and minutes is often replaced by a period in many countries (e.g., 9:14 a.m. = 9.14 hours) and is usually omitted in the American military notation (e.g., 5:35 p.m. = 1735 hours).

For longer time intervals, most contemporary human societies use the Gregorian calendar, which combines days into weeks (with 7 days in a week), weeks into months (with approximately 4 weeks in a month), months into years (with 12 months in a year), years into decades (with 10 years in a decade), decades into centuries (with 10 decades in a century), and centuries into millennia (with 10 centuries in a millennium).[5] Dates are usually expressed in terms of day, month, and year, with "year number one" being the year in which past historians believed that Jesus Christ had been born. Thus, for instance, the date of the beginning of World War II was the first day of September in 1939.[3] In the United States, the month usually precedes the day in the writing of dates, which can be very confusing if day and month are expressed numerically. Thus, for example, "9/11" in the United States refers to the 11th day of September, not the 9th day of November as it does in most of the rest of the world. To avoid confusion, some scientists express dates in the form of year-month-day (such as "2001/9/11" or "2001-9-11") to make it clear that the elements are inverted. To reference years before year 1, a regressive calendar is used with a special notation. Thus, for example, Roman leader Julius Caesar was born in the year 100 BC (100 Before Christ) or 100 BCE (100 Before the Common Era).[3] To avoid ambiguity, years after year 1 are sometimes, although not often, also expressed with a special notation. For instance, this book was published in the year AD 2016 (Anno Domini 2016) or 2016 CE (2016 of the Common Era).

1.1.1 Biological Rhythms

Jürgen Aschoff, a prominent twentieth-century circadian physiologist whose contributions will be discussed later in this chapter, believed to have identified *Archilochus* as the author of the oldest written record of observations in circadian physiology.[6] Archilochus (675–635 BC) was a Greek poet whose verses remain only as fragments today.[7] The fragment to which Aschoff alluded is shown in Figure 1.3. The critical passage is the last sentence, which can be translated as: "Recognize what sort of rhythm governs man." The historical problem here is how to determine what Archilochus meant by the term *rhythm* (ρυσμος). The fragment advises the reader to stand and fight in life, not to openly rejoice after



FIGURE 1.1 A nineteenth-century sundial in Budapest, Hungary. Sundials are one of oldest instruments devised by humankind to measure the passage of time. (Photo courtesy of Miroslav Broz, Hradec Králové, Czech Republic.)

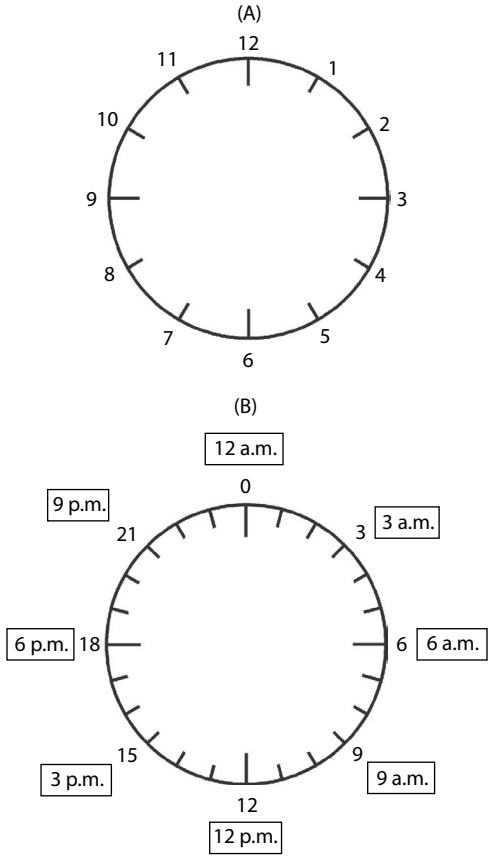


FIGURE 1.2 Diagrams of (A) a 12-hour clock and (B) a 24-hour clock. Although analog clocks almost always have 12-hour dials, a full day has twice as many hours. In most of the world, time of day is expressed according to a 24-hour clock. In the United Sates, a day is divided into 12 hours before noon (*ante meridiem*, or a.m.) and 12 hours after noon (*post meridiem*, or p.m.).

a victory or to cry after defeat, to enjoy the good times and not to regret the bad times. In this context, it would seem that *rhythm* means merely a lack of constancy, not a true recurring or oscillatory process—that is, not really a rhythm. As a matter of fact, the same verse has been translated with the word *motion* instead of *rhythm*: "A measured motion governs man."[8] Thus, it is inaccurate to identify Archilocus as the author of the oldest written record of observations in circadian physiology.

It is only three centuries later, in the fourth century BC, that we find an unambiguous written record of observations in circadian physiology. This was when *Androsthenes of Thasus*, a ship captain under the command of Alexander, the Great, (Figure 1.4), recorded his observations of daily movements in plants.[9] Androsthenes traveled through North Africa and India and had the chance to observe the daily movement of the leaves of the tamarind tree (*Tamarindus indica*). Although most of us think of plants as dull, motionless organisms, Androsthenes noticed that the tamarind leaves exhibit an impressive daily cycle of movement, wherein the leaves move up during the day and down at night. Even if not as impressive, a similar daily movement of leaves can also be seen in the common bean plant (Figure 1.5). For those interested, Exercise 1.2 (at the end of this chapter) provides instructions on how to monitor the leaf movement of the bean plant.

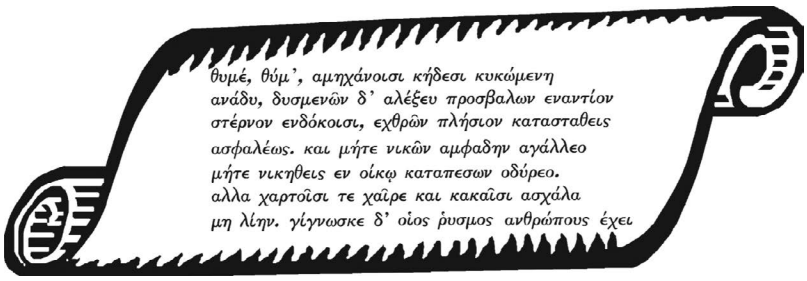


FIGURE 1.3 Is it "Greek" to you? It is Greek. This is a fragment of a poem written around 650 BC by the Greek poet Archilochus. It talks about the rhythms of life.

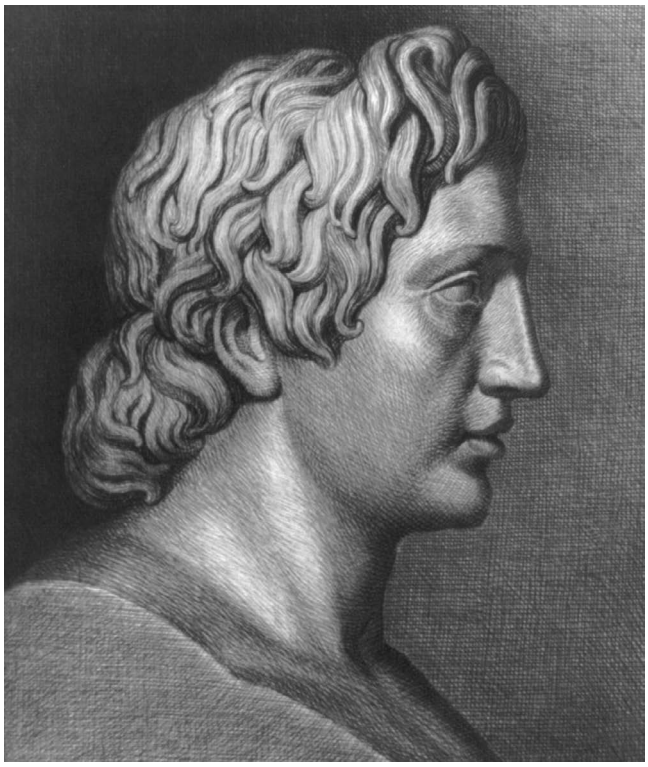


FIGURE 1.4 Alexander the Great (356–323 BC). The great Greek (Macedonian) general conquered most of the civilized world in the fourth century BC. One of the many ship captains in his fleet was Androsthenes of Thasus, who wrote the first description of daily movements in plants. (Courtesy of Library of Congress, Washington, DC.)

Other noteworthy observations of daily rhythmicity during antiquity were those of the great physicians *Hippocrates* and *Galen*.[6,10] Hippocrates (460–370 BC), the Greek healer heralded as the father of medicine, made several observations about periodic physiological processes, such as the recurrence of fever in 24-hour intervals. Galen (130–200 AC), physician to Roman emperors Marcus Aurelius and Commodus, recorded detailed descriptions of *paroxysms* (outbursts of symptoms with recurring manifestations, such as the chills of malaria). Neither Hippocrates nor Galen—nor, as far as we know, anyone in antiquity—considered that daily physiological rhythms might be caused not only by environmental factors (such as the alternation of day and night) but also by an endogenous clock (i.e., by a process that takes place inside the organism and persists in the absence of daily environmental cycles).

1.1.2 Endogenous Rhythmicity

Many commentators on the history of circadian physiology point to *Jean-Jacques de Mairan* (Figure 1.6) as the first person to demonstrate that daily rhythms may be endogenously generated.[11–13] Mairan (1678–1771) was a French astronomer and part-time botanist. He observed the *sensitive* plant (*Mimosa pudica*), which owes its name to the fact that its leaves fold up when touched. Unlike the vertical movement of the leaves of the tamarind tree, the leaves of the sensitive plant fold up along the midline at night and open up during the day. Mairan placed a sensitive plant in a totally dark place and noticed that the leaves still opened up in the morning and folded up in the evening.[14] This indicated that the daily rhythm of leaf folding does not require a daily

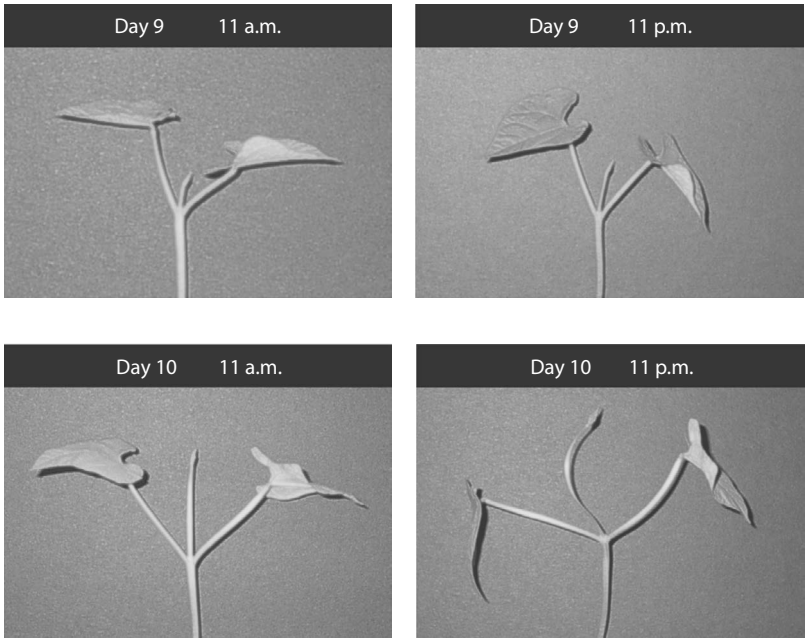


FIGURE 1.5 The "sleep" cycle of the bean plant. The leaves of the common bean plant (*Phaseolus vulgaris*) rise during the day and drop at night. (Photo and montage by R. Refinetti.)



FIGURE 1.6 Jean-Jacques Dortous de Mairan (1678–1771). This French astronomer and botanist was the first to describe the daily movement of the leaves of a plant kept in isolation from the daily cycle of light and darkness. (Courtesy of Wolfgang Steinicke, Umkirch, Germany.)

rhythm of sunlight. However, this observation alone does *not* demonstrate the existence of endogenous rhythmicity. Other environmental factors besides light might have caused the leaves to open up. As a matter of fact, Mairan's report to the French Royal Academy of Sciences concluded that "the sensitive plant perceives the sun without seeing it,"[14] thus conceding that, in his opinion, the persistent rhythmicity had an exogenous cause.

A younger contemporary of Mairan, *Henri Louis Duhamel du Monceau* (Figure 1.7), also contributed to the history of circadian physiology. A compatriot of Mairan, Monceau (1700–1782) was a botanist and naval engineer. He replicated and expanded Mairan's observations by keeping a sensitive plant inside a vault, where neither light nor temperature oscillated.[15] Like Mairan, Monceau noticed that the leaves of the plant still opened up in the morning and folded up in the evening, but he did not postulate an endogenous source of the rhythmicity.

Much more of a circadian physiologist, although also lacking an explicit notion of endogenous rhythmicity, was *Christoph Wilhelm Hufeland* (Figure 1.8). A German physician, Hufeland (1762–1836), was the creator of the discipline of *macrobiotics*, the study of the prolongation of life.[16] In his acclaimed 1797 book, *The Art of Prolonging Life*, he expressed many concepts of physiological rhythmicity and pointed out that the 24-hour period of the Earth's revolution is reflected in organic life and appears in all human diseases.[17]



FIGURE 1.7 Henri Louis Duhamel du Monceau (1700–1782). This French botanist and naval engineer repeated and expanded Mairan's observations on the daily movement of leaves. (Courtesy of Wikimedia commons.)



FIGURE 1.8 Christoph Wilhelm Hufeland (1762–1836). This German physician wrote the first systematic account of daily rhythmicity in human physiology as part of a larger book. (Courtesy of Clendening Library, University of Kansas Medical Center, Kansas City, MO.)



FIGURE 1.9 Julien Joseph Virey (1775–1846). The doctoral dissertation of this French pharmacist was the first book devoted to biological rhythms. (Courtesy of Library of the National Academy of Medicine, Paris, France.)



FIGURE 1.10 Augustin Pyramus de Candolle (1778–1841). This Swiss botanist was the first to document a circadian rhythm with a period different from 24 hours (and, therefore, not attributable to geophysical factors). (Courtesy of Library of the Russian Academy of Sciences, Moscow, Russia.)

His contemporary, *Julien Joseph Virey* (Figure 1.9), went a step further and wrote the first book (actually his doctoral dissertation in medical school) dedicated to daily rhythmicity in physiological processes.[18] A French pharmacist, Virey (1775–1846), did not defend his medical dissertation until he was 40 years old, but only a few years later, he was invited to write the entry on Periodicity for the encyclopedic *Dictionary of Medical Sciences*.[10] He believed that circadian rhythms were endogenously generated, but his actual research was restricted to the careful description of daily rhythms in diseases and mortality.[19]

The honor of first describing research that demonstrated the endogenous nature of circadian rhythms seems to belong to *Augustin de Candolle* (Figure 1.10). A renowned Swiss botanist, Candolle (1778–1841) studied the rhythm of folding and opening of the leaves of the sensitive plant, as Mairan had done a century earlier. Candolle observed that the rhythm persisted under continuous illumination, similarly to what Mairan had observed. Importantly, however, Candolle noticed that the period of the rhythm (i.e., the duration of the cycle) was shorter than 24 hours.[20] This finding was important because the period of the rhythm should have been exactly 24 hours if some uncontrolled geophysical factor had been responsible for the rhythm. A period shorter than 24 hours meant that a different clock had to be responsible for the rhythm—and, if the clock was not outside the plant, it had to be inside. Thus, Candolle effectively demonstrated the existence of an endogenous circadian clock.[21] Where exactly this endogenous clock is located and how it operates remained unknown for another 100 years.

Many other researchers investigated biological rhythms during the nineteenth century. Unfortunately, some were less trustworthy than others. One theory that deserves mention because of its surprising popularity (despite its absurdity) is that of *biorhythm*. The notion of biorhythms was developed late in the nineteenth century by two individuals working independently: German physician Wilhelm Fliess (1859–1928) and Austrian psychologist Hermann Swoboda (1873–1963). According to followers of Fliess and Swoboda, biorhythms are three natural cycles within the human body that affect us physically, emotionally, and intellectually.[22–25] The three biorhythms begin when a person is born, and they oscillate with absolute precision, as perfect sine waves, until the person dies. The *physical* rhythm regulates physical strength, energy, endurance, sex drive, confidence, and so forth. The *emotional* rhythm governs creativity, sensitivity, mood, and so on. The *intellectual* rhythm is associated with intelligence, memory, mental alertness, logical thinking, and so on. The physical rhythm is 23 days long; the emotional, 28 days long; and the intellectual, 33 days long (Figure 1.11). The different lengths of the three cycles cause them to be constantly out of phase (they coincide only at birth and every 58 years plus 66 or 67 days thereafter, depending on the number of leap years in between). Thus, a person's disposition on any given day will be a composite of the states of the three rhythms. By calculating and studying one's biorhythms, one is supposedly capable of knowing what to expect each day and, therefore, one is capable of avoiding bad experiences. Yet, neither Fliess and Swoboda nor their followers ever bothered to

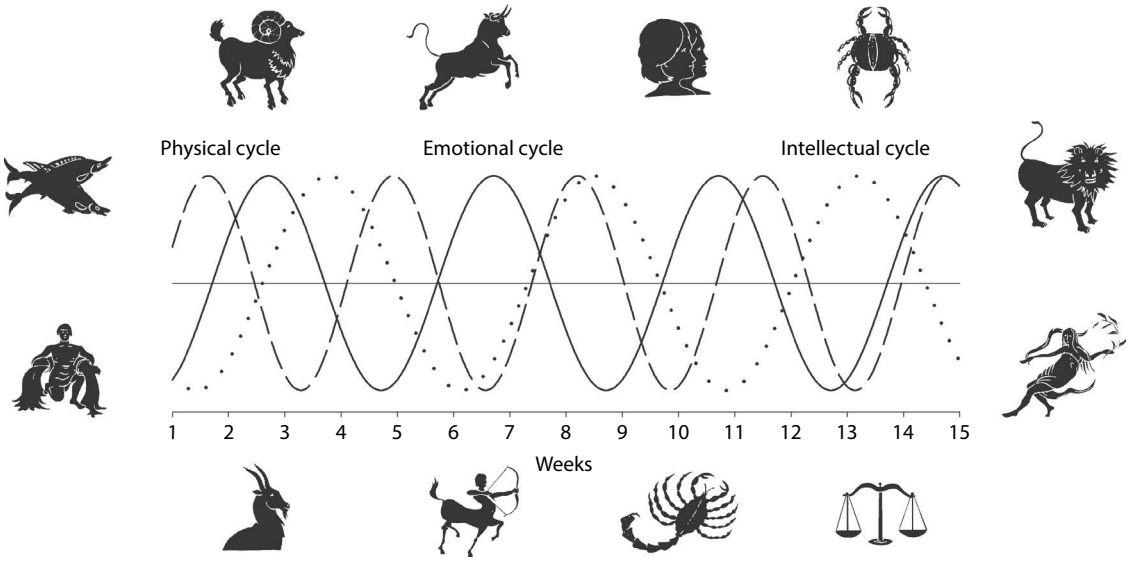


FIGURE 1.11 Biorhythms and horoscope? The concept of biorhythms was developed by W. Fliess and H. Swoboda in the late 1800s. Although they claimed no connection with the signs of the zodiac, their notion of biorhythms was just as unscientific as horoscopes are. (Data from Crawley, J., *The Biorhythm Book*, Journey, Boston, MA, 1996; Signs of the zodiac after Fisher, D. and Bragonier, R., *What's What*, Hammond, Maplewood, NJ, 1981.)

conduct actual research to verify the veracity of the theory. As a matter of fact, the essence of the theory reveals its concocted nature. As we will see throughout this book, real biological rhythms have a pattern that allows us to identify them as actual rhythms, but they are clearly subject to biological variability. Even something as mundane as one's bedtime expresses regularity with variability. You probably go to bed at about the same time each night (say, 11 o'clock or midnight), but rarely do you keep your bedtime with the accuracy of minutes (and certainly not of seconds). Variability is an essential feature of biological processes,[26] to such an extent that absence of regular variability is often a sign of disease[27] and is associated with mortality risk in middle-aged and elderly people.[28] In contrast, biorhythms are amazingly "clean" rhythms that allegedly repeat themselves for the whole life of the individual without ever deviating, even slightly, from a perfect sine wave. This extreme proposed regularity demonstrates that the theory was developed in someone's head without any observation of actual biological processes. Not surprisingly, a careful review of research on biorhythms yielded the conclusion that "biorhythm theory is not valid."[29]

In contrast to the "armchair" work of Fliess and Swoboda, physician John Davy actually recorded his own body temperature (under the tongue) in the morning and evening every day for nine consecutive months in 1844.[30] Figure 1.12 shows a 1-month segment of his data. Notice that the temperature goes up and down reliably each day but that there is also considerable variability from one day to the next. With only two measurements per day, Davy could not have a close look at the daily oscillation of his temperature. However, 22 years later, physician William Ogle recorded his own temperature several times a day for several months.[31] As can be seen in Figure 1.13, the averaged readings display clear daily rhythmicity with a peak in the evening and a trough in the early morning. Notice that even the averaged values do not form a smooth sine wave; rather, they show irregularities typical of true living beings. Many other individuals conducted empirical research on the daily rhythmicity of bodily functions in humans[32–34] and other animals[35–38] through the end of the nineteenth century.

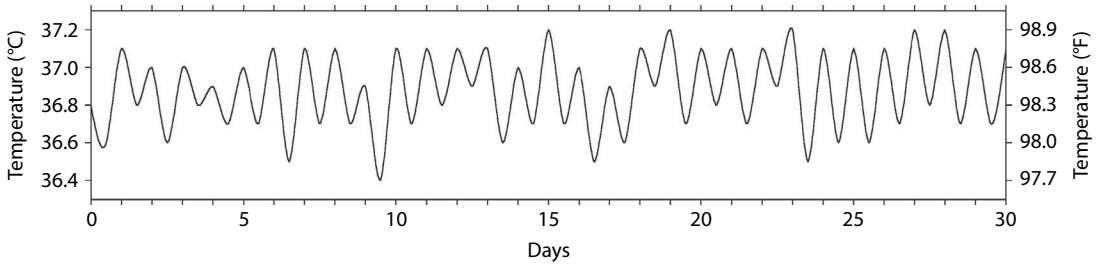


FIGURE 1.12 A real biological rhythm. In 1844, British physician John Davy made accurate measurements of the day-to-day variation of his own body temperature. (Data from Davy, J., *Philos. Trans. R. Soc. Lond.*, 135, 319, 1845.)

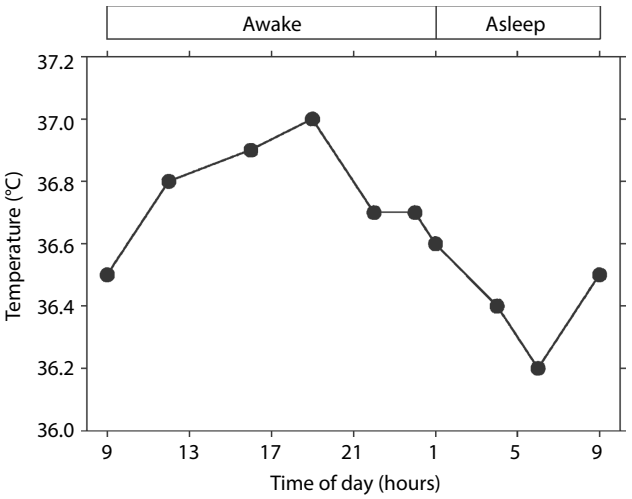


FIGURE 1.13 An early record of the daily rhythm of body temperature. In 1865, physician William Ogle conducted measurements of his own oral temperature with temporal resolution high enough to allow the characterization of a daily rhythm. (Data from Ogle, W., *St. George's Hosp. Rep*., 1, 221, 1866.)

1.2 TWENTIETH CENTURY

The twentieth century witnessed a surge in sophisticated research on circadian rhythms. Historical research is made difficult by the fact that major biomedical databases, such as the U.S. National Library of Medicine's PubMed database, have records going back only to the 1950s, but manual inspection of old library collections allows one to identify quite a few pre-1950 studies. Many of these studies, and many others published during the second half of the twentieth century, made fundamental contributions to the current knowledge in circadian physiology.

1.2.1 1901–1950

From 1902 to 1905, Sutherland Simpson and J. J. Galbraith, in Scotland, conducted detailed studies of the body temperature rhythm of monkeys maintained under light-dark cycles, constant light, and constant darkness.[39] An example of their experimental results in rhesus monkeys is shown in Figure 1.14. Body temperature clearly rises during the light phase of the daily cycle, peaks at the beginning of the dark phase, falls through the night, and then follows a similar but not identical pattern on the second day. Also at the beginning of the twentieth century, Francis Benedict, in Connecticut, and Arthur Gates, in California, conducted detailed measurements of the body temperature rhythm[40] and of daily variations in memory[41] in human subjects. None of these investigators, however, was concerned with the origin of the rhythms (i.e., whether the rhythms were caused by environmental cycles or were endogenously generated).

The first demonstration of the endogenous nature of circadian rhythms in an animal species was provided by Maynard Johnson, in Illinois, in 1926.[42] Johnson studied the rhythm of

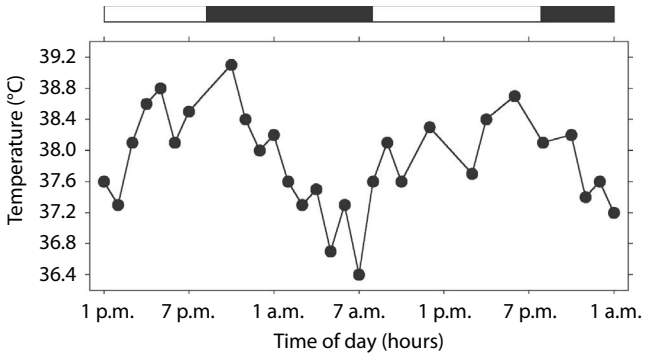


FIGURE 1.14 Old records of body temperature of a monkey. From 1902 to 1905, Simpson and Galbraith conducted numerous measurements of the body temperature of rhesus monkeys, as exemplified in these records from Monkey #31. The light and dark bars at the top of the figure indicate the approximate duration of the light and dark phases of the prevailing light–dark cycle. (Data from Simpson, S. and Galbraith, J.J., *Trans. R. Soc. Edinburgh*, 45, 65, 1906.)

locomotor activity (i.e., the rhythm of moving around) of deer mice (*Peromyscus leucopus*). He kept the mice in constant darkness in an environment without temporal cues and examined the time at which the animals became active each day (the "onset time"). As shown in Figure 1.15, the activity onsets drifted 4 hours (from 4 p.m. to 8 p.m.) in about a month—again, with some day-to-day variability. Thus, the activity onsets were delayed by about 6 minutes each day. This means that the mice were running on a 24.1-hour clock rather than on a 24.0-hour clock. Because all potential geophysical time cues would be expected to run on a 24.0-hour clock (the period of Earth's rotation), Johnson justifiably concluded that the clock responsible for the activity rhythm of the mice was

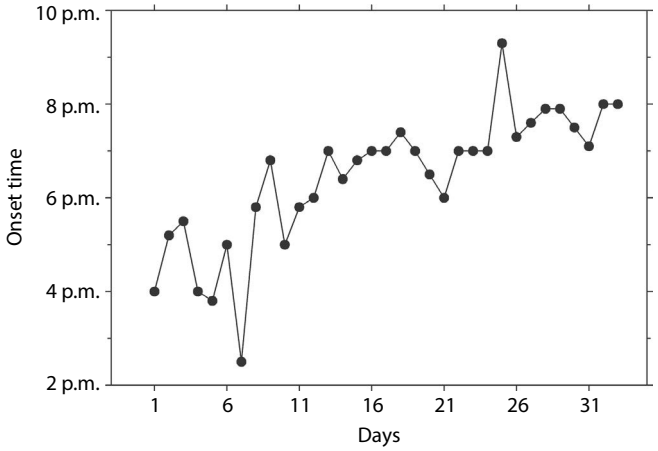


FIGURE 1.15 "Free-running" mouse. In 1925, Maynard Johnson documented a circadian rhythm of locomotor activity in deer mice (*Peromyscus leucopus*) maintained in constant darkness. The rhythm exhibited a period longer than 24 hours and, therefore, could not be attributed to geophysical factors. "Onset time" refers to the time each day when the mouse initiated activity. (Data from Johnson, M.S., *J. Mammal*., 7, 245, 1926.)

endogenous, not exogenous. This issue will be discussed in greater detail in Chapter 6.

Just 4 years later, L.A. Rogers and G.R. Greenbank reported the existence of a daily rhythm of growth in colonies of bacteria (*Escherichia coli*).[43] Representative records are shown in Figure 1.16. Despite a considerable amount of noise, clear daily rhythmicity can be seen. Rogers and Greenbank did not investigate whether the growth rhythm was endogenously generated, but their study was important because it showed daily rhythmicity in a *prokaryotic* organism (i.e., a unicellular organism without a membrane separating the nucleus from the cytoplasm), thus implying that daily rhythmicity is not restricted to more complex *eukaryotic* organisms and, therefore, is probably a characteristic of all life on Earth. We will return to this topic in Chapter 9.

Before the end of the decade of 1930, enough knowledge on daily rhythms was available to justify a literature review of the topic by John Welsh[44] and to stimulate discussion of potential medical uses of this knowledge by Arthur Jores.[45] A very influential researcher at this time was the German botanist *Erwin Bünning* (Figure 1.17). Bünning (1906–1990) worked at the universities of Jena, Königsberg, and Tübingen. His central interest was in photoperiodism (the physiological response of organisms to seasonal changes in light), but his research on the role of light in plant physiology provided several insights into circadian physiology. As we will see in Chapter 7, Bünning proposed, as early as 1936, an explanation of photoperiodism that involved a mechanism now believed to be essential for the synchronization of circadian rhythms to the environmental light–dark cycle.[46] Bünning's contribution to circadian physiology also included the writing of the first comprehensive book in the field, *The Physiological Clock*. The book was originally published in German in 1958[47] and later in three English editions, the last one of which appeared in 1973.[48]

Two other researchers who contributed significantly to the progress of circadian physiology in the early twentieth century were Curt Richter (1894–1988), a psychology professor at Johns Hopkins University who conducted extensive research on circadian rhythms in laboratory animals and human patients,[49] and Nathaniel Kleitman (1895–1999), the renowned investigator of the physiology of sleep at the University of Chicago who studied circadian rhythms in humans.[50]

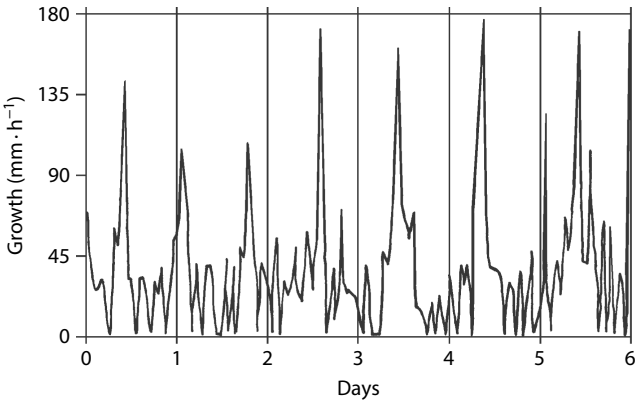


FIGURE 1.16 Daily rhythmicity in prokaryotes. In 1929, Rogers and Greenbank demonstrated the existence of daily rhythmicity in the growth of bacteria, which are prokaryotes (i.e., organisms whose cells do not have a separate nucleus). Except in the second of the 6 days shown, clear daily peaks of growth can be seen. (Data from Rogers, L.A. and Greenbank, G.R., *J. Bacteriol.*, 19, 181, 1930.)



FIGURE 1.17 Erwin Bünning (1906–1990). This German botanist, whose central research interest was in the mechanism of photoperiodism, made significant contributions to the study of circadian rhythms in the twentieth century. (Courtesy of Botanical Archive, University of Hamburg, Hamburg, Germany.)

1.2.2 1951–2000

Starting in the 1950s, many investigators concentrated their full-time efforts on research in circadian physiology, and three individuals in particular became so influential as to justify the honor of being called the *forefathers* of modern circadian physiology. They were Jürgen Aschoff, Franz Halberg, and Colin Pittendrigh.

Jürgen Aschoff (Figure 1.18) was born in Freiburg, Germany, in 1913 and spent most of his professional life at the Max Planck Institute for Behavioral Physiology, in Andechs. Originally a thermal physiologist, he gradually switched to the study of circadian rhythms.[51] He was interested in all manifestations of circadian rhythmicity, in the laboratory as well as in the field. An avid researcher, he investigated a wide variety of phenomena in a multitude of species, including humans. His discovery and interpretation of the phenomenon of *spontaneous internal desynchronization*[52,53] was a driving



FIGURE 1.18 Jürgen Aschoff (1913–1998). This German physiologist was a leader in the development of the study of circadian rhythms in the twentieth century. (Reprinted from *J. Biol. Rhythms*, 9(3), 187, Copyright 1994 by Sage Publications. With permission of Sage Publications.)

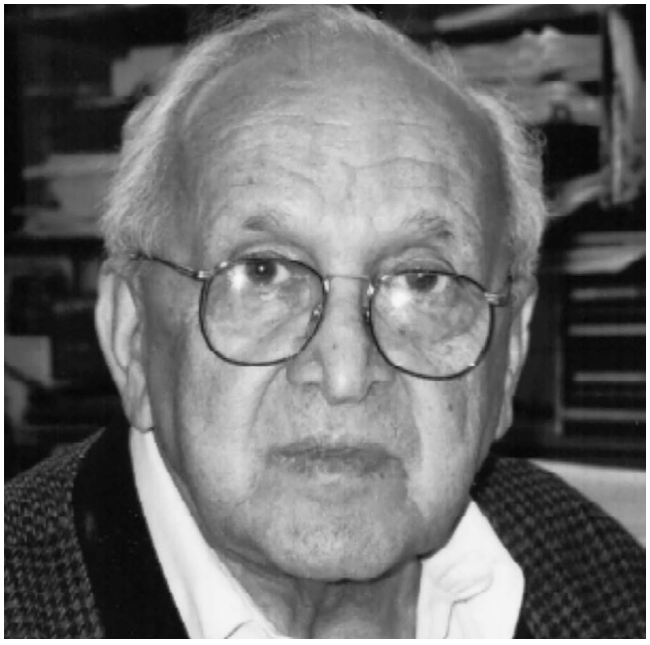


FIGURE 1.19 Franz Halberg (1919–2013). This American physician (originally from Romania) created the terms *circadian* and *chronobiology* and was a life-long advocate of the establishment of chronobiology as a separate discipline. (Photo courtesy of Franz Halberg.)

force in circadian physiology for decades. His thorough and exhaustive reviews of the literature in circadian physiology[54–56] served as invaluable guides to numerous researchers. I met Aschoff in person when he was in his 70s. He showed his age by virtue of his unsurpassed erudition in physiology, but his demeanor reflected the bursting intellectual energy of a 20-year-old. Aschoff died in 1998,[57] but his legacy lives on. Over 30 of his articles are cited in this book.

Franz Halberg (Figure 1.19) was born in Bistrita, Romania, in 1919 and moved to the United States a few years after completing medical school. He spent most of his career at the University of Minnesota. Halberg was the creator of the terms *circadian*[58] and *chronobiology*.[59] A prolific writer, he published over 2500 journal articles and books in circadian physiology, including an introductory booklet on biological rhythms for high-school students.[60] Although the medical applications of circadian physiology were his main concern,[61–63] he conducted a great deal of basic research as well.[64–67] Halberg was still alive and productive when the second edition of this book was written, and I had the chance to consult with him about historical and technical matters. He passed away in 2013.[68]

Colin S. Pittendrigh (Figure 1.20) was born in Whitley Bay, England, in 1919 and moved to the United States as a graduate student. He spent the first 20 years of his faculty career at Princeton University, in New Jersey, and then relocated to Stanford University, in California. A "clock watcher" at heart,[69] he strived to understand how the operation of a physical oscillator could explain circadian rhythmicity in animals. A great deal of our current understanding of the operation of the circadian clock is derived from his work in flies[70,71]



FIGURE 1.20 Colin Pittendrigh (1919–1996). This American biologist was a leader in the development of the study of circadian rhythms in the twentieth century. (Reprinted from Pittendrigh, C.S., *Annu. Rev. Physiol.*, 55, 16, Copyright 1993 by Annual Reviews. With permission. www.annualreviews.org.)

TABLE 1.1
Characteristics of the Two Main Factions in Chronobiology in the Twentieth Century

Faction	Leading Figure	Primary Emphasis	Central Focus	Main Tool	Favored Journal
Clocks	Pittendrigh	Basic	Mechanisms	Actogram	*Journal of Biological Rhythms* (since 1986)
Chronome	Halberg	Applied	Rhythms	Cosinor	*Chronobiologia* (1974–1994)

and rodents.[72–75] I met Pittendrigh in person very late in his life, but I was impressed by his ability to skillfully balance broad biological principles with the detailed experimental dissection of circadian rhythms. He died in 1996,[76] but his contribution to circadian physiology is everlasting, as will become evident in Chapter 7.

The personal and professional interactions between the three forefathers (and their offsprings) were not as cordial and productive as one might have expected. Aschoff did recognize both Halberg's leading role in the development of circadian physiology[6] and Pittendrigh's insights into mechanisms of circadian organization.[55] Pittendrigh acknowledged some of Halberg's contributions[69] and credited Aschoff with important discoveries.[74] On his turn, Halberg recognized the contributions of both Aschoff and Pittendrigh.[77] However, a great divide characterized the field during the second half of the twentieth century. Researchers were divided into two factions that may be referred to as the *clocks* faction and the *chronome* faction. As shown in Table 1.1, the clocks faction was headed by Pittendrigh and concerned itself mainly with the mechanisms of biological timing, whereas the chronome faction (so named because of the proposition that the temporal aspect of biological organization is as encompassing as the genome) was headed by Halberg and concerned itself mainly with the description of rhythmic processes and their relevance to medical application. Aschoff stayed neutral and interacted with both factions. The two factions avoided direct confrontation by holding separate scientific meetings, publishing their papers in separate journals, and generally ignoring each other. Although mutual criticisms were rarely put in print,[78,79] the animosity was clearly revealed by sociologists who looked at the "disciplinary stake" of chronobiology.[80] As a corollary, a textbook published by eminent members of the clocks faction in 2004 presented Pittendrigh and Aschoff as the "founders of chronobiology" with no mention of Halberg. This is especially noteworthy because the book was entitled *Chronobiology*,[12] and it was Halberg who created the term[59] and who forcefully promoted the creation of the new discipline against Pittendrigh's objections.[80]

I should hasten to point out that antagonisms are not peculiar to circadian physiology—or to science more generally. In the musical arts, for instance, the renowned classical composer and conductor Rimsky-Korsakov had this to say about the noless renowned Ludwig van Beethoven: "His music abounds in countless leonine leaps of orchestral imagination, but his technique, viewed in detail, remains much inferior to his titanic conception."[81] In plain words: "Beethoven is a bad composer!" Needless to say, I and millions of others beg to differ. In any event, small but sincere attempts at reconciliation of the two factions were made over the years. A conference held at the Cold Spring Harbor Laboratory (in Long Island, New York) in 1960 had already brought together Bünning, Aschoff, Halberg, Pittendrigh, and others under one roof,[82] although the division of the factions had not been yet strongly established at that time. Thirty-five years later, in 1995, a conference was organized at Dartmouth Medical School by members of the clocks faction (Figure 1.21). Although most participants were members of the clocks faction, members of the chronome faction were welcomed as well. A few years later, in 1999, an eclectic group of circadian physiologists organized a congress sponsored by nine different professional organizations dedicated to the study of biological rhythms. Participants in this congress—held in Washington, DC (Figure 1.22)—included basic researchers as well as medical practitioners and provided the opportunity for the exchange of ideas between individuals with quite different professional interests. After the turn of the century, in 2001, a World Federation of Societies for Chronobiology was established, bringing together 13 professional associations with diverse interests related to biological rhythms. The federation held its first congress in 2003.[83]

As for the merits of the antagonism between the two factions, something more must be said. Members of the chronome faction often felt that the clocks faction wasted time on esoteric questions instead of addressing important real-life issues. On the other side of the court, members of the clocks faction felt that the chronome faction conducted sloppy research that failed to address the intricacies of the biological clock. Because each faction judged the other by its own values, it was difficult to reach a consensus. Fortunately, members of both factions agreed that peer recognition of one's work is an objective measure of professional achievement. The extent of a researcher's *peer recognition* can be estimated by the number of times that his/her work is cited in publications by other researchers. I used the *Science Citation Index* (produced by Thomson Reuters, in New York) and truncated the search in August 2004 to avoid unfairly favoring Halberg, who lived longer than Aschoff and Pittendrigh. I found that Halberg had 9200 citations, whereas the figures were 8900 and 6800 for Aschoff and Pittendrigh, respectively. Although Halberg had more total citations than Pittendrigh, he had fewer citations per published article (11 as compared to 42), which means that he was cited more often than Pittendrigh was, but his articles were not individually considered as important as Pittendrigh's. The fact that Pittendrigh's individual articles were considered to be more important may reflect his focus on specific topics. Not a man to be content with short stories,



FIGURE 1.21 Where is Waldo? As in the popular book series *Where is Waldo?*, you may have a difficult time identifying individual circadian physiologists in this group photograph of the participants in a conference held at Dartmouth Medical School (Hanover, New Hampshire) in July 1995. (Photo courtesy of Jay Dunlap and Jennifer Loros [the meeting organizers].)

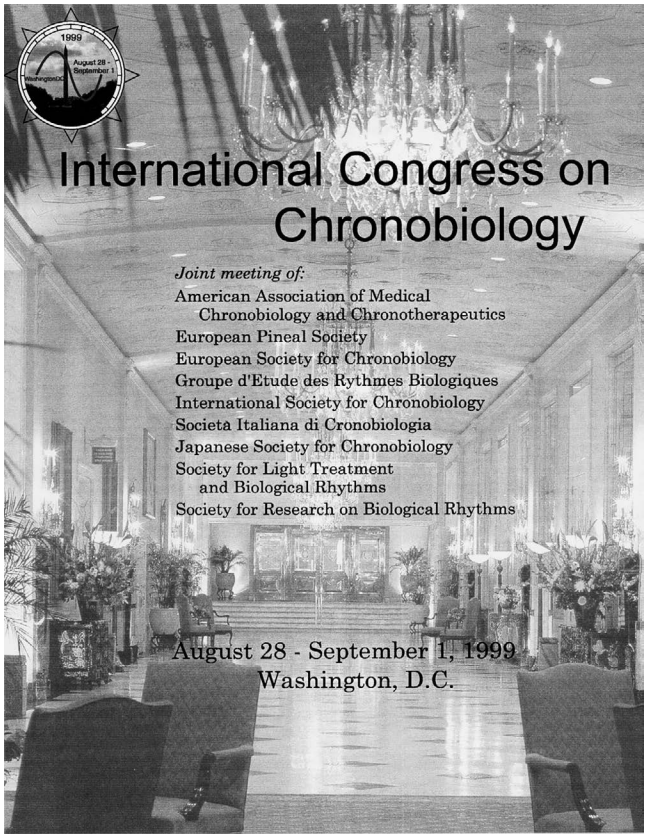


FIGURE 1.22 First major attempt to unify the field. An International Congress on Chronobiology, held in Washington, DC, in 1999, was the first major attempt to unify the field of studies of biological rhythms. (Image: Cover of the Congress' Program.)

Halberg often digressed into far-reaching topics, including the concept of "astrochronobiology."[63,84] I once told him that this reminded me of the concept of "orgasmic energy," a bizarre idea of psychoanalyst Wilhelm Reich, according to whom the energy of sexual orgasms permeates the universe.[85] Halberg's reply was not "Oops, maybe then I should be more reticent" but something like "Oh yes, poor Reich, he was ridiculed for being an open-minded scientist!" Regardless of differences in personal style, however, it is clear that the three forefathers of circadian physiology had comparable impacts on biomedical science. The chronome faction and the clocks faction were equally meritorious. They were both unjustified in degrading each other.

1.3 CURRENT TRENDS

Figure 1.23 provides a brief summary of major events in the past three centuries of the history of circadian physiology and relates them to major human inventions made at about the same time. Work in circadian physiology during the twenty-first century, a century that is still not quite two decades old, builds upon the progress made in the past. Efforts to understand organismal, cellular, and molecular processes in circadian physiology are being steadily intensified. As shown in Figure 1.24, the number of published journal articles in circadian physiology grew from fewer than 90 articles per year in 1964 to almost 3000 articles per year in the year 2014. The total number of publications in circadian physiology, as retrieved by a search of the U.S. National Library of Medicine's PubMed database using the term *circadian*, currently stands at approximately 74,000. Because the yield of database searches

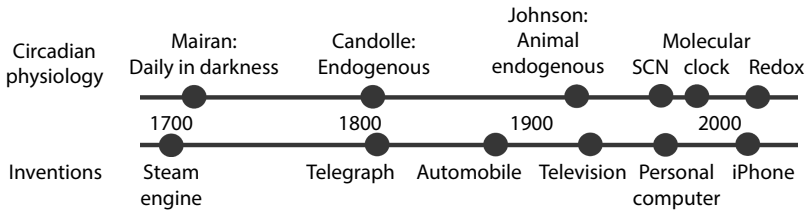


FIGURE 1.23 Circadian timeline. This diagram provides a timeline of major events in circadian physiology and major inventions in the past 300 years. Information about the suprachiasmatic nucleus (SCN), molecular clock, and redox will be provided in Chapter 12.

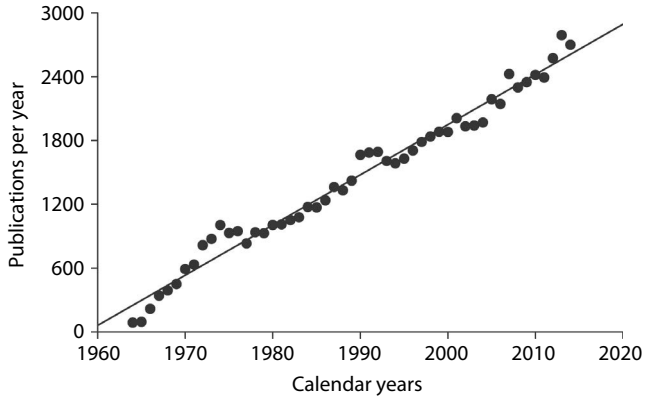


FIGURE 1.24 Growth in the number of journal articles on circadian rhythms published during the past 50 years. The number of articles on circadian rhythms catalogued by PubMed (U.S. National Library of Medicine) grew from fewer than 100 articles per year in 1964 to almost 2700 articles per year in 2014. (PubMed database searched by R. Refinetti in April 2015 targeting the term *circadian* in any searchable field year-by-year.)

is greatly reduced by limitations in coverage, indexing, and retrieval,[86–89] we can estimate the actual number of existing journal articles in circadian physiology to be around 150,000. Although this book cites only 6000 of them, I did my best to ensure that the most significant articles were included. To my help, citation analysis has shown that the most significant scientific literature appears in a small core of journals.[90] I took very seriously the task of constructing a coherent picture out of thousands of dispersed pieces of information.[91–94]

1.3.1 Circadian Physiology and the World

Any description of circadian physiology would be incomplete without a look at the "big picture." In the general scheme of human knowledge, such as we would find in the organization of colleges and departments of a contemporary university, four major categories are recognized: the humanities, the natural sciences, the social sciences, and the various graduate professional specializations (Figure 1.25). Within the natural sciences, we find biology. Subdivisions of biology are often based on the life forms under study (botany, zoology, and microbiology) or on the processes involved (anatomy, endocrinology, neuroscience, etc.). One could also subdivide biology on the basis of the duration of the phenomena under investigation (millennia, centuries, years, days, or seconds and milliseconds). Circadian physiology is that part of biology that concentrates on biological processes in the time scale of about a day. On one hand, this means that circadian physiology is a very specialized discipline, as is typical of modern sciences. On the other hand, however, findings in circadian physiology have implications for all other areas of knowledge. Within the

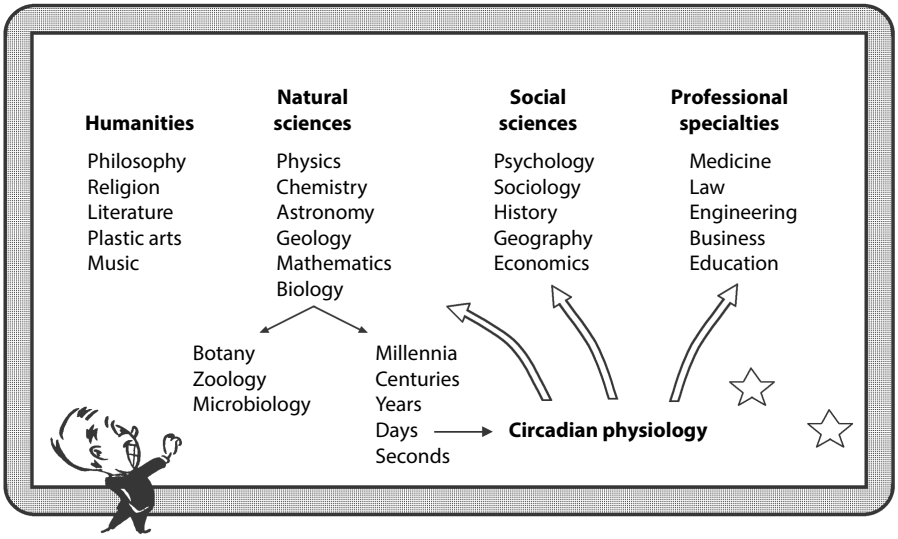


FIGURE 1.25 The "big picture." Circadian physiology has a definite place in the universe of human knowledge, and its implications are widespread.

natural sciences, circadian physiology provides essential information about the temporal structure of the processes investigated by physiologists, pharmacologists, endocrinologists, neuroscientists, and many others. In the social sciences, circadian physiology once more provides essential information about the temporal structure of the processes investigated by psychologists, sociologists, economists, and many others. As we will see in Section V of this book, knowledge of circadian physiology also has important implications for professional specialties such as business, education, and medicine. Even the humanities are affected by circadian physiology, albeit indirectly. In the area of music, one can find a rock band called Circadian Rhythm (*Internal Clock*, 1998; *Over Under Everything*, 2001), a different band called Circadian Rhythms (*A Dream or Something Else*, 2011; *In the Flowers*, 2014; *A Passing Thought*, 2015), a 1993 album by New Age musician Colin Chin called *Circadian Rhythyms* (sic!), a 1997 album by David Cohen called *Circadian Symphony*, and a 2006 album by the rock group 5th Projekt called *Circadian*. In the area of plastic arts, the "Inside Time" series of paintings by artist Julie Newdoll (www.brushwithscience.com) also serves as testimony for the influence of circadian physiology on the humanities. In the area of motion picture, a low-budget science fiction movie directed by René Besson and starring Rachel Miner was entitled *Circadian Rhythm* (Signature Pictures, 2005). In addition, a book was written about a boat called Circadian Rhythm (Robert S. Morris, *Circadian Rhythm*, CreateSpace Publishing, 2012). As shown in Figure 1.26, some of us even have a neologism for biological clock on our car's license plates!

Besides the big picture of knowledge, there is the big picture of money. In the year 2014, the federal budget of the United States reached almost four trillion dollars in expenditures.[95] As indicated in Figure 1.27, most of the expenditures were related to health care (including Medicare), social benefits (including income security and social security), and defense (including deployment of troops overseas). Less than 4% of the outlays was related to R&D (research and development), but, in such a large budget, 4% was still an enormous amount of money ($140 billion). As shown in the right side of Figure 1.27, defense (military) research accounted for half of the R&D budget. Half of the civilian research budget was allocated to biomedical research ($35 billion), a large part of it routed through the National Institutes of Health.[96] Now, what proportion of the $35 billion spent on biomedical research each year is directed to research on circadian rhythms? One way to estimate it is by calculating the proportion of published biomedical research articles that deal with circadian rhythms. The PubMed database (mentioned earlier) contained 24 million citations at the end of 2014. Of these citations, 74,000 could be retrieved by the term *circadian*. Thus, it can be conservatively estimated that 0.3% of all biomedical research deals with circadian rhythms, which implies a federal investment of $105 million in circadian research in the United States each year. That this estimate is conservative can be determined by a search of grants awarded by the National Institutes of Health, which indicates that $206 million were awarded to research on circadian rhythms in fiscal year 2013–2014 (Table 1.2).



FIGURE 1.26 Biological clock on wheels. Unorthodox spelling is needed to spell out biological clock using only the seven letters in a car's license plate. (Photo by R. Refinetti.)

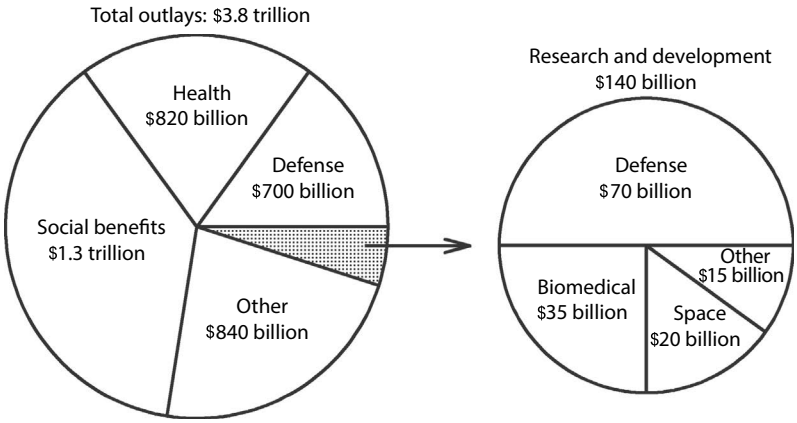


FIGURE 1.27 The money chest. Shown is the breakdown of outlays in the United States federal budget (year 2014). (From Janssen, S., ed. *The World Almanac and Book of Facts*, Simon & Schuster, New York, 2014; Mervis, J. and Malakoff, D., *Science*, 346, 1437, 2014.)

TABLE 1.2
Best-Funded Institutions Conducting Research on Circadian Rhythms[a]

Institution	Funding ($)
Brigham and Women's Hospital (Harvard)	14,094,773
University of Pennsylvania	10,050,662
University of California San Diego	7,200,478
University of Pittsburgh	6,845,856
Vanderbilt University	5,281,767
Harvard University	5,265,554
University of California Santa Barbara	5,225,198
Baylor College of Medicine	5,004,740
Stanford University	4,861,131
University of California Los Angeles	4,224,301
University of Chicago	4,128,333
Duke University	3,519,211
Scripps Research Institute	3,381,490
Washington University	3,074,736
UT Southwestern Medical Center	3,016,721
University of Washington	3,014,501
Tufts University	2,947,228
Rush University Medical Center	2,785,303
University of Southern California	2,776,642
Indiana Univ-Purdue Univ at Indianapolis	2,669,772
Oregon Health & Science University	2,578,877

[a] The data in this table refer to research grant funds awarded by the U.S. National Institutes of Health during fiscal year 2013–2014. The Research Portfolio Online Reporting Tools facility (RePORT) was searched for current grants with the term *circadian* in the Text field. There were 557 grants associated with the term *circadian*, totaling $206 million. The total amounts for the institutions that were awarded the most money are shown in the table.

Rounding up the amount to $250 million (to include other funding agencies, such as the National Science Foundation), and considering that the U.S. economy corresponds to 20% of the world's economy,[95] but also that most other countries allocate smaller proportions of their budget to R&D,[97] a rough estimate of investment in circadian research worldwide is $1 billion per year. This figure does not include funding from private sources or the salaries of investigators paid by their university employers.

1.3.2 Current Researchers

Studies of scholarly communication indicate that archival publication of research reports in peer-reviewed outlets remains as important today as it was before the Internet revolution in the late twentieth century.[98] Reports of research in circadian physiology are published in a variety of professional journals, including journals specialized in biological rhythms. Currently, there are three print journals specialized in biological rhythms: *Biological Rhythm Research*, *Chronobiology International*, and the *Journal of Biological Rhythms* (Figure 1.28). In 2003, an electronic journal specialized in circadian rhythms was created: the *Journal of Circadian Rhythms* (Figure 1.29). Two other closely related journals are *Sleep and Biological Rhythms* (published under the auspices of the Japanese Society of Sleep Research) and *ChronoPhysiology and Therapy* (an electronic journal published by Dove Press). Specialized journals that have ceased publication include the *Journal of Interdisciplinary Cycle Research* (launched in 1970 and renamed as *Biological Rhythm Research* in 1994), the *International Journal of Chronobiology* (published from 1973 to 1983), and *Chronobiologia* (published from 1974 to 1994 under Halberg's editorship).

Analysis of the scientific literature indicates that scientific research is progressively being conducted by research teams that span many universities, but, because of social stratification in multiuniversity collaborations, the current trend is toward the development of fewer, not more, research teams overall.[99] A good deal of research in circadian physiology is conducted by researchers whose main interest and expertise are in the mechanisms of biological timing. However, much research is also conducted by other life science investigators who have a secondary interest in circadian physiology. In a 12-month interval a few years ago, Thomson Reuters

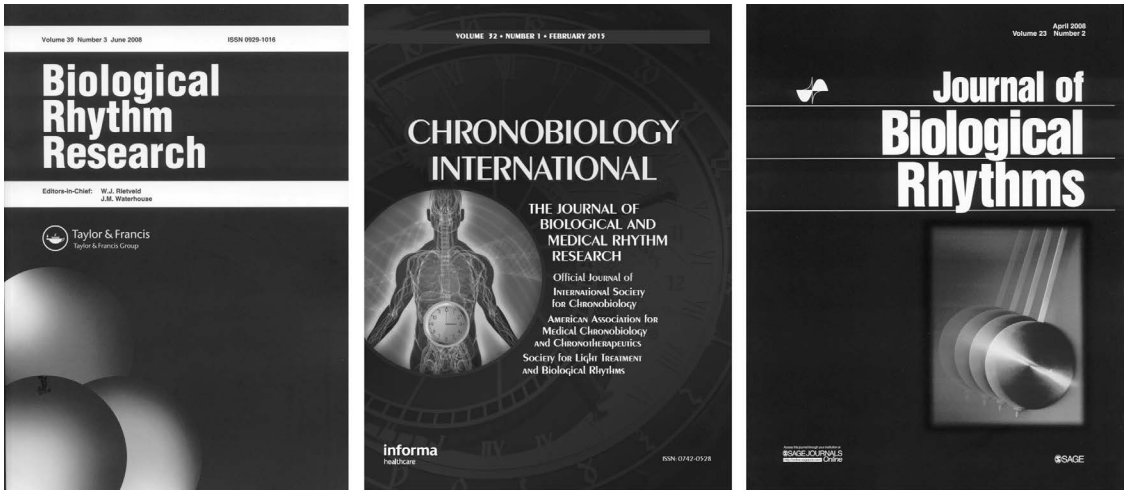


FIGURE 1.28 The three print journals specialized in biological rhythms. Currently, there are three print journals specialized in biological rhythms: *Biological Rhythm Research* (published by Taylor & Francis), *Chronobiology International* (published by Taylor & Francis), and the *Journal of Biological Rhythms* (published by Sage Science Press).

(mentioned earlier) cataloged 1227 journal articles in circadian physiology authored by 3330 researchers. As shown in Figure 1.30, 84% of these researchers published only one article during the 12-month interval. Presumably, only those who published two or more articles (the remaining 16%) are specialized in circadian physiology. To obtain a better view of the situation, I conducted a more elaborate search to identify the big players in the game of circadian physiology, as reported in Table 1.3. I ranked authors by the number of publications. Number of publications per se is not a very meaningful figure, but it has been shown that the professional prestige of a researcher is highly correlated with the number of his/her publications.[100] Thus, although Table 1.3 cannot be read as a ranking of prestige among circadian physiologists, it does provide an objective measure of the productivity of prestigious researchers in the field.

I will not talk at length about each researcher listed in Table 1.3, but I will briefly refer to four of them. The most prolific circadian physiologist at the present time is *Kazuomi Kario* (Figure 1.31). A cardiologist from Japan, Kario, works at Jichi Medical University, in Tochigi, and conducts research on the causes and prevention of human hypertension.[101–103] Fifth on the list is *Paul Pévet* (Figure 1.32), a French endocrinologist who works at the Louis Pasteur University, in Strasbourg, and conducts research on the neuroendocrinology of circadian rhythms in mammals, particularly on the role of the pineal gland.[104–106] *Urs Albrecht* (Figure 1.33), holding the 10th place on the list, is a Swiss neurobiologist who obtained his doctorate at the University of Bern and did postdoctoral work at the Baylor College of Medicine (in the United States) and the Max Planck Institute for Experimental Endocrinology (in Germany). Albrecht works at the University of Fribourg (in Switzerland), where he conducts research on the neural and molecular aspects of circadian rhythms in vertebrates.[107–109] Number 16 in the list is *Ken-ichi Honma* (Figure 1.34), a physiology professor at Hokkaido University, in Japan. He worked with Aschoff early in his career and maintained a close professional relationship with him until Aschoff's death in 1998. Honma's research involves various aspects of the neural and molecular mechanisms of circadian rhythmicity.[110–112]

Of course, many researchers in addition to those shown in Table 1.3 specialize in circadian physiology. Table 1.4 lists 355 researchers who authored 20 or more articles in the field in the past 10 years. All of the researchers listed in Table 1.4 are valuable resources for governmental agencies, health practitioners, and news media personnel seeking advice on circadian rhythms, as well as for students and postdoctoral researchers seeking experience in the field. Many of these researchers are members of one or more of the four main chronobiological research societies: the International Society for Chronobiology, the Society for Research on Biological Rhythms, the European Biological Rhythms Society, and the Japanese Society for Chronobiology.[113] Many of the researchers also attend with some regularity professional meetings dedicated to research on biological rhythms, such as the 22nd Annual Meeting of the Society for Light Treatment and Biological Rhythms held in Vienna (Austria) in 2010, the 3rd World Congress of Chronobiology held in Puebla (Mexico) in 2011, the 13th Biennial Meeting of the Society for Research on Biological Rhythms held in Destin (Florida) in 2012, the 13th Congress of the European Biological Rhythms Society held in Munich (Germany) in 2013, the 28th Conference of the International Society for Chronobiology held in Bucharest (Romania) in 2014, the 4th World Congress of Chronobiology held in Manchester (United Kingdom) in 2015, and the 15th Biennial Meeting of the Society for Research on Biological Rhythms held in Tampa (Florida) in 2016. The theme "Rhythms of Life" was chosen for the 2017 meeting of the International Union of Physiological Sciences in Rio de Janeiro (Brazil).

Efforts to access the impact that a researcher has on his or her discipline usually make use of citation statistics. Citation statistics are used because the number of times that a researcher's published work is cited by other researchers

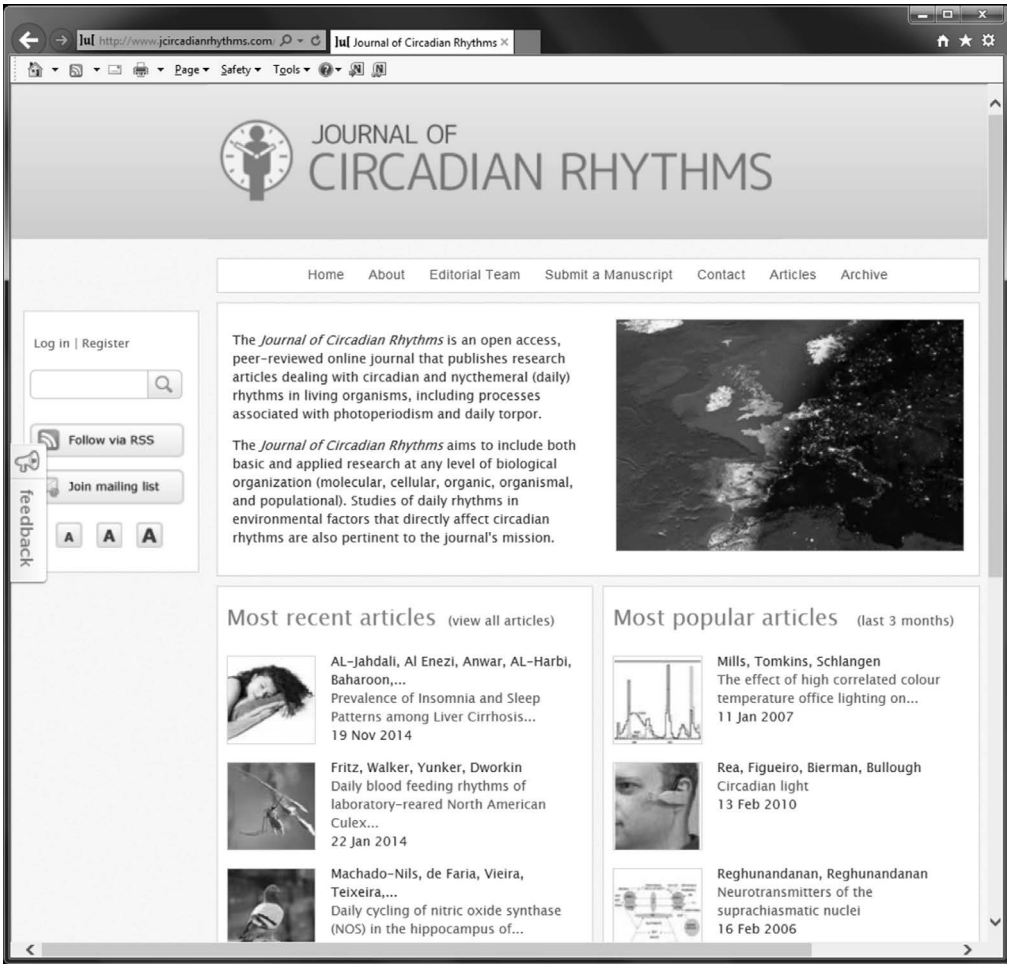


FIGURE 1.29 The *Journal of Circadian Rhythms*. The first journal specialized in circadian rhythms was launched in 2003. The *Journal of Circadian Rhythms*, published by Ubiquity Press, is an open-access electronic journal (with no print version) in which articles are freely available to readers worldwide upon publication. The URL is www.JCircadianRhythms.com.

in professional journals is an objective indicator of academic prestige. While comparison of citation statistics for a few researchers can be easily accomplished, a large-scale comparison would be extremely onerous and possibly unfeasible. First, citation data for several hundred authors (i.e., at least those listed in Table 1.4) would have to be searched to ensure that no one is inadvertently left out. Second, citations would have to be screened to separate literature review and methodological articles from research reports; this is necessary because the former items receive many more citations than the latter—and cannot be directly compared. Third, citations would have to be individually inspected to exclude articles that were written by circadian physiologists but that do not deal with circadian rhythms; this is necessary because circadian physiologists work on other topics too. An alternate approach is to identify articles that deal with circadian rhythms and that received many citations regardless of who authored them. The authors can then be identified retrospectively.

In April 2014, I searched the Web of Science database (produced by Thomson Reuters) for cited articles that had the term *circadian* in the title. Only about a quarter of articles dealing with circadian rhythms actually have the word *circadian* in the title, but this was an inevitable limitation of the search engine. The search returned 22,156 articles, most of them having fewer than two dozen citations, although 93 of them had more than 400 citations each. Seven researchers had four or more articles with 400 or more citations each: Charles A. Czeisler (Harvard Medical School), Michael H. Hastings (University of Cambridge), Steve A. Kay (University of Southern California), Michael Menaker (University of Virginia), Steven M. Reppert (University of Massachusetts Medical School), Ueli Schibler (University of Geneva), and Joseph S. Takahashi (University of Texas Southwestern Medical Center). These seven researchers are, therefore, the seven most influential circadian physiologists of our time. We do not have time to talk about each one of them in detail here, but I would like to give some detail about at least one of them. Michael Menaker (Figure 1.35) is a professor of biology at the University of Virginia. After completing his doctorate with Pittendrigh at Princeton University, he was a postdoctoral fellow at

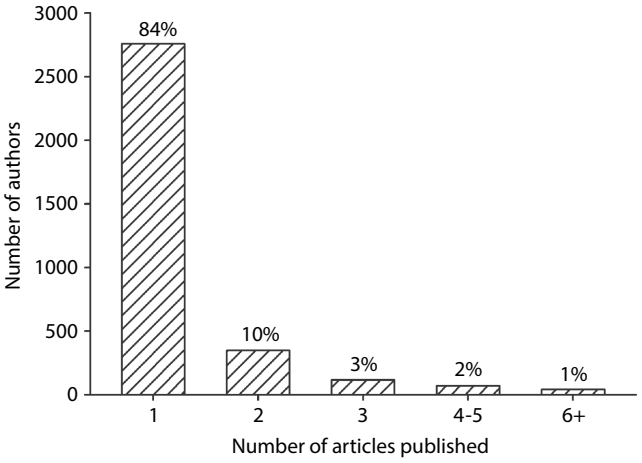


FIGURE 1.30 How many people publish research articles on circadian rhythms? In a 12-month interval not long ago, 3330 authors published journal articles containing the word *circadian* in the title or as an indexed key word. The figure shows how many of these authors published how many articles. (Data from Research Alert service of Thomson Reuters [formerly, Institute for Scientific Information]. Search conducted by R. Refinetti for the 12-month interval between November 2002 and October 2003.)

Harvard University and a professor at the University of Texas (Austin) and at the University of Oregon before being recruited to chair the biology department at the University of Virginia. He has served as doctoral and postdoctoral adviser to numerous researchers, including the author of this book. His own research involves a wide range of phenomena related to biological timing in various life forms.[114–117]

While advanced education in circadian physiology must be attained by apprenticeship at the postgraduate level, many universities currently offer undergraduate as well as graduate courses on this subject. A nonexhaustive list of such courses can be found in Table 1.5. Additionally, summer courses on biological rhythms are occasionally offered around the world. Events called Chronobiology Summer School (or a similar designation) were organized by A. Kramer and H. Herzel in Berlin (Germany) in 2012, by E. Herzog and D. McMahon in Nashville (Tennessee) in 2013, by K. Kume, H. Iwasaki, H. Ueda, and T. Yoshimura in Sapporo (Japan) in 2014, and by R. Foster, C. Espie, M. Merrow, and T. Roenneberg in Oxford (England) in 2015. A Summer Course on Chronopharmacology was organized by B. Lemmer in Heidelberg (Germany) for 14 consecutive years, from 2000 to 2014.

TABLE 1.3
The Most Prolific Circadian Physiologists Today[a]

Rank	Author	Institution	Specialty	Articles
1	Kazuomi Kario	Jichi Medical University, Japan	Circadian rhythms and human hypertension	88
2	Russell J. Reiter	University of Texas Health Science Center at San Antonio, United States	Melatonin and its role in aging and circadian organization	80
3	Daniel P. Cardinali	Pontifical Catholic University of Argentina	Human sleep and neurological disorders	77
3	Ramón C. Hermida	University of Vigo, Spain	Circadian control of blood pressure in humans	77
5	Paul Pévet	Louis Pasteur University, France	Endocrinology of circadian rhythms in mammals	76
6	Paolo Sassone-Corsi	University of California at Irvine, United States	Epigenetics of circadian clocks	75
6	Joseph S. Takahashi	University of Texas Southwestern Medical Center, United States	Physiology and molecular biology of circadian clocks in mammals	75
8	Juan A. Madrid	University of Murcia, Spain	Circadian rhythms and human health	67
9	Steve A. Kay	University of Southern California, United States	Molecular biology of circadian clocks in plants and animals	64
10	Urs Albrecht	University of Fribourg, Switzerland	Neurobiology of circadian rhythms in vertebrates	63
11	Francis Lévi	University of Paris, France	Chronotherapy of cancer (basic and applied research)	62
12	Norio Ishida	NIAIST, Japan	Molecular biology of circadian clocks in mammals	61
13	Charles A. Czeisler	Harvard University, United States	Control of sleep and circadian rhythms in humans	60
14	Andrew J. Millar	University of Edinburgh, United Kingdom	Molecular biology of circadian clocks in plants	58
14	Shigenobu Shibata	Waseda University, Japan	Nutrition and circadian rhythms in mammals	58
16	Ken-ichi Honma	Hokkaido University, Japan	Neurobiology of circadian rhythms	56
17	Sato Honma	Hokkaido University, Japan	Neurobiology of circadian rhythms	56
18	Christian Cajochen	University of Basel, Switzerland	Circadian rhythms and human physiology	55
19	Hitoshi Okamura	Kobe University, Japan	Molecular biology of circadian clocks in mammals	54
20	Etienne Challet	Louis Pasteur University, France	Endocrinology of circadian rhythms in mammals	53

[a] This table was compiled with data obtained through a search of the PubMed database (U.S. National Library of Medicine, Washington, DC) followed by analysis with proprietary software. The PubMed database was searched in April 2014 for all articles published in the preceding 10 years that had the term *circadian* in any indexed field (title, abstract, or key words). The search retrieved 23,475 articles that were authored by 59,532 authors. The authors with the highest numbers of publications were selected. A few authors were excluded because their names were not unique (and could not be distinguished from authors with the same name in another field) or because they published more than 50% of their articles with another author (and were considered to be members of that author's research team rather than independent researchers). The top 20 authors are listed in this table.



FIGURE 1.31 Kazuomi Kario (1962–). This Japanese cardiologist, who specializes in the ambulatory monitoring of blood pressure and prevention of cardiovascular disease, is currently the most prolific circadian physiologist. (Photo courtesy of Kazuomi Kario.)



FIGURE 1.33 Urs E. Albrecht (1962–). This Swiss biochemist specializes in the molecular neurobiology of circadian rhythms in vertebrates. (Photo courtesy of Urs Albrecht.)



FIGURE 1.32 Paul Pévet (1945–). This French neurobiologist specializes in the endocrinology of circadian rhythms in mammals. (Photo courtesy of Paul Pévet.)



FIGURE 1.34 Ken-ichi Honma (1946–). This Japanese medical physiologist specializes in the neurobiology of circadian rhythms in vertebrates. (Photo courtesy of Ken-ichi Honma.)

TABLE 1.4
Contemporary Circadian Physiologists[a]

Abreu-Gonzalez P
Adan A
Akerstedt T
Albrecht U
Allada R
Allen CN
Allison KC
Amir S
Ancoli-Israel S
Ando H
Antle MC
Antoch MP
Archer SN
Arendt J
Arushanian EB
Asayama K
Atkinson G
Ayala DE
Barriga C
Bass J
Beersma DG
Bertolucci C
Bjorvatn B
Blask DE
Blau J
Bloch G
Block GD
Boivin DB
Born J
Brainard GC
Bray MS
Brown SA
Brown TM
Brunner M
Buijs RM
Burgess HJ
Buysse DJ
Cajochen C
Cambras T
Caola G
Cardinali DP
Carskadon MA
Cassone VM
Cermakian N
Challet E
Chen L
Chen Y
Chen Z
Chrousos GP
Claustrat B
Colwell CS
Coogan AN
Cooper HM
Cornelissen G
Costa R
Cuspidi C
Cutolo M
Czeisler CA
Daan S
Dardente H
Davis SJ
Dawson D
de la Iglesia HO
Deboer T
Delaunay F
Diez-Noguera A
Dijk DJ
Dinges DF
Doi M
Dominguez-Rodriguez A
Doyle FJ 3rd
Duffy JF
Dumont M
Dunlap JC
Earnest DJ
Eastman CI
Edery I
Edwards B
Egli M
Eguchi K
Elliott JA
Emery P
Erren TC
Escobar C
Esquifino AI
Evans JA
Fahrenkrug J
Ferguson SA
Fernandez JR
Figueiro MG
Fliers E
Foster RG
Foulkes NS
Frolich M
Froy O
Fujimoto K
Fujimura A
Gamble KL
Garaulet M
Gimble JM
Glass JD
Golden SS
Golombek DA
Gorman MR
Gothilf Y
Grandner MA
Green CB
Grunstein RR
Halberg F
Hankins MW
Hannibal J
Hardeland R
Hardin PE
Harmer SL
Hashimoto S
Hastings MH
Hattar S
Hazlerigg DG
Helfrich-Forster C
Hermida RC
Herzel H
Herzog ED
Hida A
Hidalgo MP
Higuchi S
Hirayama J
Hogenesch JB
Honma K
Honma S
Hoshide S
Hrushesky WJ
Hut RA
Ikeda M
Imai Y
Imaizumi T
Innominato PF
Ishida N
Ishikawa J
Ishiura M
Ito S
Iuvone PM
Johnson CH
Jones H
Kalsbeek A
Kario K
Kato T
Kato Y
Kawamoto T
Kawano Y
Kay SA
Kennaway DJ
Kikuya M
Kim J
Kim JS
Kim K
Kimura G
Kirschbaum C
Klein DC
Klerman EB
Kondo T
Kondratov RV
Konstas AG
Korf HW
Koyanagi S
Kramer A
Kripke DF
Kronfeld-Schor N
Kudo T
Kumar V
Kyriacou CP
Lee C
Lee J
Lee TM
Lemmer B
Levi F
Li H
Li J
Li L
Li S
Li X
Li Y
Lightman SL
Liu J
Liu JH
Liu X
Liu Y
Lockley SW
Lopez-Olmeda JF
Loros JJ
Loudon AS
Lee J
Lee TM
Lemmer B
Levi F
Li H
Li J
Li L
Li S
Li X
Li Y
Lightman SL
Liu J
Liu JH
Liu X
Liu Y
Lockley SW
Lopez-Olmeda JF
Loros JJ
Loudon AS
Madrid JA
Mancia G
Manfredini R
Mas P
Matsunaga N
Maywood ES
Mazzoccoli G
McClung CA
McClung CR
McMahon DG
Meijer JH
Menaker M
Mendoza J
Merrow M
Metoki H
Michel S
Middleton B
Mignot E
Millar AJ
Mishima K
Mistlberger RE
Mizuno T
Mojon A
Moller M
Monk TH
Montagna P
Mori T
Munch M
Naef F
Nakamichi N
Nakamura Y
Natale V
Nelson RJ
Nishino S
Nitabach MN
Noshiro M
Numata H
Obara T
Ohdo S
Ohkubo T
Oishi K
Okamura H
O'Neill JS
Ordovas JM
Oster H
Otsuka K
Pack AI
Pallesen S
Panda S
Pandi-Perumal SR
Parati G
Partonen T
Pazienza V
Peirson SN
Pevet P
Piccione G
Pickering TG
Piggins HD
Pijl H
Portaluppi F
Rajaratnam SM
Ralph MR
Randler C
Rea MS
Reddy AB
Refinetti R
Reilly T
Reiter RJ
Reppert SM
Ripperger JA
Roach GD
Rodriguez AB
Roelfsema F
Roenneberg T
Rogers NL
Rol MA
Rosbash M
Roth T
Sancar A
Sanchez-Vazquez FJ
Sassone-Corsi P
Scheer FA
Schernhammer ES
Schibler U
Schwartz WJ
Sehgal A
Sharma VK
Shea SA
Shibata S
Shigeyoshi Y
Shimada K
Shimizu T
Silver R
Skene DJ
Sladek M
Smale L
Smolensky MH
Sothern RB
Souissi N
Srinivasan V
Staessen JA
Stanewsky R
Stengl M
Steptoe A
Stevens RG
Stewart WC
Stunkard AJ
Sumova A
Suzuki T
Takahashi JS
Takahashi M
Takumi T
Tan DX
Tanaka K
Tarquini R

(*Continued*)

TABLE 1.4 (*Continued*)
Contemporary Circadian Physiologists[a]

Todo T	Wang Y	Yamashino T
Tomioka K	Wang Z	Yamazaki S
Tosini G	Wang ZR	Yang X
Touitou Y	Watanabe T	Yoshii T
Tufik S	Waterhouse J	Yoshimura T
Turek FW	Weaver DR	Young ME
Ueda HR	Webb AA	Young MW
Umemura S	Weinreb RN	Zee PC
van der Horst GT	Welsh DK	Zeitzer JM
Van Dongen HP	White WB	Zeman M
Van Someren EJ	Wirz-Justice A	Zhang J
Veldhuis JD	Wright KP Jr	Zhang L
Wang H	Wu X	Zhang X
Wang J	Wu Y	Zhang Y
Wang L	Xu Y	Zheng T
Wang W	Yagita K	Zhu Y
Wang X	Yamamoto T	

[a] This list of contemporary circadian researchers was compiled with data obtained through a search of the PubMed database (U.S. National Library of Medicine, Washington, DC) followed by analysis with proprietary software. The PubMed database was searched in April 2014 for all articles published in the preceding 10 years that had the term *circadian* in any indexed field (title, abstract, or key words). The search retrieved 23,475 articles that were authored by 59,532 authors. Most authors published only one article, but 355 authors (0.6% of all authors) published 20 or more articles during this 10-year interval. These 355 authors, who are actively engaged in research on circadian rhythms (as evinced by an average of at least two relevant publications per year for 10 consecutive years), are listed here.

1.4 ETHICS OF ANIMAL RESEARCH

Slightly more than half of all published studies in circadian physiology today are conducted using human subjects. To protect research subjects from potential abuse by occasional "mad scientists," every research project must be preapproved by an ethics committee, which in the United States is called an Institutional Review Board (IRB).[118] A fundamental requirement for approval by the IRB is that the researcher obtain "informed consent" from every subject. Because the subject is allowed to withdraw his or her consent at any time, the subject is guaranteed not to be subjected to discomfort beyond the level that he or she is willing to endure for the good of science (or for financial incentive).

Much research in circadian physiology is conducted with nonhuman animal subjects. *Vivisection*, or experimentation with living organisms, has been practiced for thousands of years.[119] Although a small fraction of animal research conducted today is directed at improvements in veterinary care, the most common goal is to improve *human* health. Vivisection is performed in animals for the benefit of humankind.[120] Simply put, some research procedures are too harmful—or too risky, or too boring, or too painful—to be conducted on human subjects; so, we use animals instead (Figure 1.36). We also use animals (and plants and fungi, for that matter) because they provide the opportunity for the study of complex human processes in simpler, more manageable "models." However, it cannot be denied that we often use animals in research because it would be inhumane to use human subjects for the same purpose.

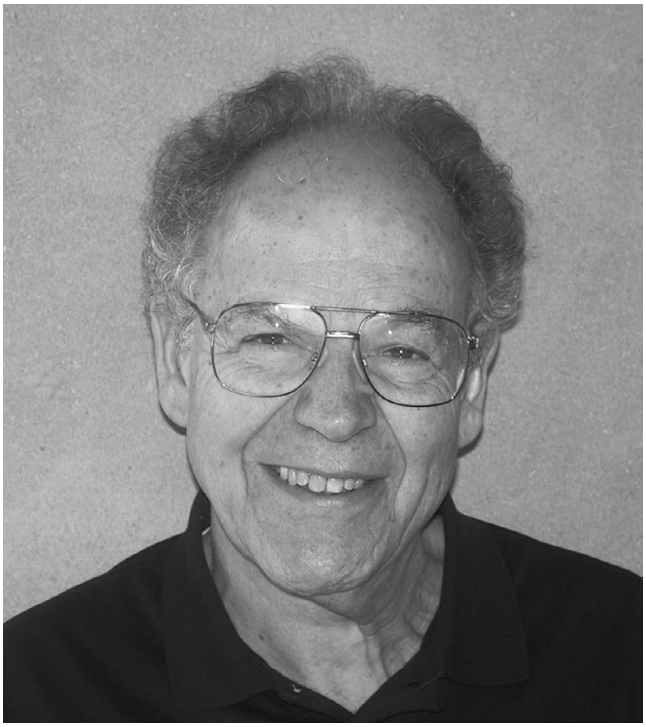


FIGURE 1.35 Michael Menaker (1934–). This American biologist specializes in the neurobiology of circadian rhythms in vertebrates. (Photo courtesy of Rebecca Arrington.)

1.4.1 Use of Animals

If we use animals as experimental subjects in biomedical research because we think that it would be inhumane to use humans, we may wonder whether the use of animals is not itself inhumane. Should we not be concerned about the *ethics* of the use of animals in research? Such wondering has been sporadically uttered throughout history, but the manifesto of the modern antivivisection movement was Peter Singer's 1975 book, *Animal Liberation*.[121] Although Singer himself was willing to defend his position against vivisection with rational arguments, a number of activists took the path of terrorism, including depredation of laboratories and attempts at murder.[122–126] Editors of biomedical journals felt the strength of the movement and wrote editorials about it.[127–130] Embarrassed by being depicted as animal torturers by the activists, biomedical researchers overreacted by imposing on themselves strict rules for the use of animals in research.[131–134] On one hand, this course of affairs was very positive because it showed that researchers

TABLE 1.5
Courses on Biological Rhythms Offered at North American Universities

Institution	Course
Alverno College	BI 443: Chronobiology
Clark University	BIOL 244: Biological Clocks
Cornell University	BIOGD 394: Circadian Rhythms
Dalhousie University	NESC 3260: Biological Rhythms
Drexel University	BMES 531: Chronobioengineering
Florida Institute of Technology	BIO 5080: Biological Clocks
Harvard University	MCB 186: Circadian Biology
Indiana University	A 501: Biological Rhythms
North Carolina State University	ZO 410: Biological Timekeeping
Northeastern University	BIO G306: Biological Clocks
Northwestern University	BIOL SCI 124: Biological Clocks
Ohio State University, Columbus	PSYC 623: Biological Clocks
Pennsylvania State University, University Park	PSY 597: Rhythms of Behavior
Simon Fraser University	PSYC 388: Biological Rhythms
Skidmore College	BI 344: Biological Clocks
State University of New York, Stony Brook	BIO 314: Biological Clocks
Texas A&M University, College Station	BIOL 601: Biological Clocks
University of California, Davis	MCP 242: Biological Rhythms
University of Connecticut, Storrs	PNB 225: Biological Rhythms
University of Houston	BIO 6213: Biological Clocks
University of Illinois, Urbana-Champaign	MCB 482: Biological Clocks
University of Massachusetts, Amherst	BIOL 571: Biological Rhythms
University of Medicine and Dentistry of New Jersey	MSBS 5050: Biological Rhythms
University of Minnesota, Morris	BIOL 1001: Biological Rhythms
University of Texas, Houston	PH 2180: Chronobiology
University of Toronto	JZP 326: Biological Rhythms
University of Virginia	BIOL 419: Biological Clocks
University of Western Ontario	PSY 734: Biological Rhythms
Vanderbilt University	BSCI 238: Biological Clocks
York University	BIOL 4310: Biological Timekeeping

were willing to compromise and also because it actually improved the quality of biomedical research by forcing scientists with sloppy animal maintenance habits to shape up. On the other hand, however, it reinforced the wrong conception that antivivisectionism is a philosophy that merely opposes the mistreatment of research animals. Once the question of mistreatment had been settled, researchers and politicians thought that all that was left to be done was to remind the public that animal research is intrinsically honorable—because it leads to the improvement of medical procedures for the treatment of diseases that afflict millions of children and adults.[135–142] This strategy failed to touch the core of the antivivisection controversy. As Singer pointed out, antivivisectionism is not restricted to the issue of liberation of laboratory animals; it encompasses the whole issue of animal rights. Although the phrase "animal rights" could refer to any set of rights attributed to animals, Singer endorsed the opinion of most antivivisectionists that animal rights are equivalent to human rights.[143] His main argument was that there is no logical reason to attribute moral rights to humans and not to animals.[144] This means that the real issue in the antivivisection controversy is not the mistreatment of research animals or the immediate usefulness of biomedical research. Well-informed individuals have no doubt that vivisection is necessary for medical progress.[135–142] The real issue in the antivivisection controversy is a conflict of values, a conflict between those who believe that animal rights are equal to human rights and those who do not.[145]

If we were all strict followers of the Judeo-Christian Bible, the issue would be solved easily. Genesis 1:28 asserts that, after having created humans on the sixth day of creation,

> God blessed them, and God said unto them, Be fruitful, and multiply, and replenish the earth, and subdue it: and have dominion over the fish of the sea, and over the fowl of the air, and over every living thing that moveth upon the earth.

For those who do not believe that humans have a divine mandate to exploit animals, we must start the discussion by looking at how many animals are used in research and what is done to them. As shown in Figure 1.37, more than half of all research in circadian physiology can be, and is, conducted on



FIGURE 1.36 Replacing animals as experimental subjects. This comic strip of the *Wizard of Id* cartoon, by Brant Parker and Johnny Hart, provides a humorous view of the importance of the use of animals in biomedical research. (Copyright 1984 by Brant Parker and Johnny Hart. Reproduced by permission of John L. Hart FLP and Creators Syndicate Inc.)

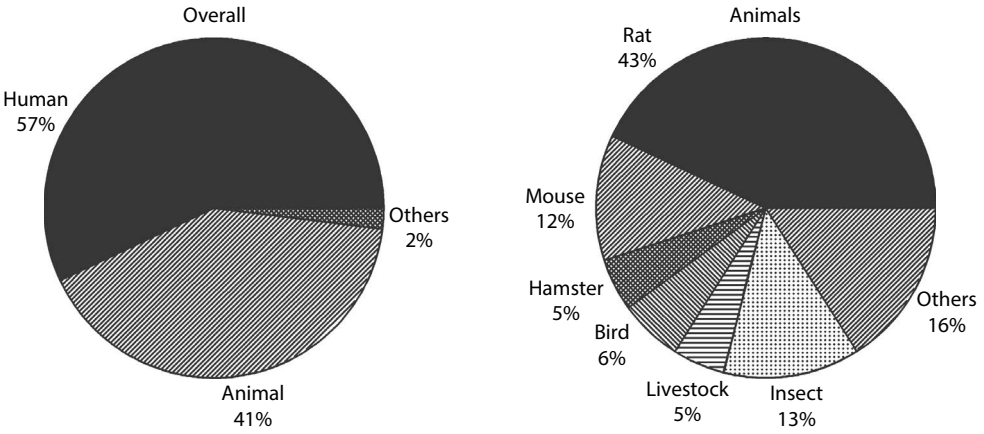


FIGURE 1.37 Proportions of experimental subjects in studies of circadian rhythms. More than half of all studies dealing with circadian rhythms are conducted on human subjects. More than half of the studies conducted in animals involve rats and mice. (PubMed database searched by R. Refinetti in October 2003 targeting the term *circadian* in any searchable field in conjunction with MeSH terms designating the various organism groups.)

human subjects. A very small fraction involves plants, fungi, bacteria, and other nonanimals, and 41% involves nonhuman animals. More than half of these nonhuman animals (59%) consists of rats and mice—two rodent species that are considered pests in most of the world.

In the United States, it is estimated that about 20 million animals are used in all of biomedical research each year,[146] even if the number of animals officially recorded has been decreasing in the past 25 years.[147] As in the particular case of circadian physiology, most of the animals are rats and mice—but 20 million is definitely a large number! If 20 million humans were decimated in any given year, we would consider it a catastrophe of unfathomable proportions. Thus, you might wonder whether scientists have gone mad. No, they have not. Surprising as it may be to some readers, 20 million animals are not too many animals in the big scheme of things. As shown in Figure 1.38, this number is lower than the number of cats and dogs euthanized in animal shelters each year (because of shortage of adoptions) and is dwarfed by the number of chickens killed each year to feed us. As a matter of fact, although some people would like to think that biomedical research is a major form of animal exploitation, a little reflection about the real world will show otherwise. Let us start with pets. We certainly love our pets and do not wish them any harm. We actually enjoy being nice to them. But one could certainly ask: Who gave us the right to purchase a pet and to keep it in our homes for as long as we want? Indeed, if your cat were a human being, you would certainly go to jail for "treating the child like an animal." Clearly, we do not treat pet animals the way we treat human beings. The abuse of animals is even clearer in industrial contexts. Farm animals are rarely "loved by their owners," and, in any case, most of us exploit animals as food—by eating their meat, drinking their milk, eating their eggs, and so on. We exploit animals as clothing—by wearing fur coats, leather jackets, and wool sweaters. We exploit them as plain entertainment—by fishing, riding a horse, and visiting the zoo. We also exploit animals as work

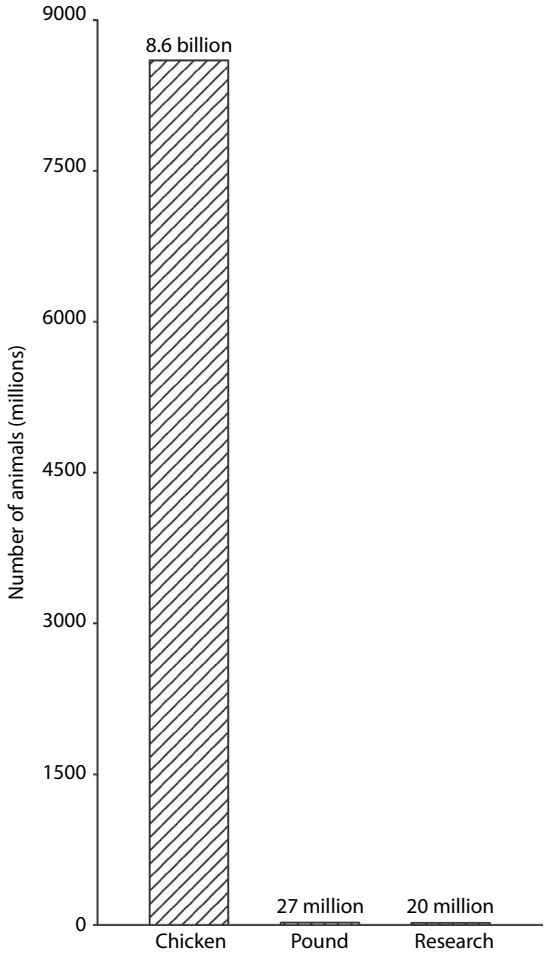


FIGURE 1.38 Comparative figures of animal use by humans. The figure shows the approximate number of animals killed each year in the United States in three sectors: chicken (used as food), pound (cats and dogs euthanized in animal shelters because of shortage of adoptions), and research (mostly rats and mice used in biomedical research). (Data from *Poultry Production and Value—2002 Summary*, National Agricultural Statistics Service, Washington, DC, 2003; Nicoll, C.S., *Physiologist*, 34(6), 303, 1991.)

force (horses, donkeys, camels) and as tools (bird feather, camel hair). With our actions, we have clearly stated that we do not think that animal rights equal human rights. Biomedical research plays no major part in this game.

Still, 20 million is 20 million, and it is fair to ask what is done to these animals. The conduct of animal research is strictly regulated in most of the world. In the United States, the use of animals in research is regulated by the Department of Agriculture (USDA)[148,149] and, for all projects that receive federal funding, it must conform to detailed guidelines set out by the Public Health Service.[150] Intentional infliction of pain is extremely rare and limited to research on the physiology of pain itself. Importantly, whether pain is expected or not, every research project must be preapproved by an ethics committee. The task of the various Institutional Animal Care and Use Committees (IACUC)[151,152] is to decide, based on the scientific and ethical values of the community, whether the discomfort caused to the animals is justified by the expected benefits of the research project. Authorization to perform the project is denied if the justification is unsatisfactory.

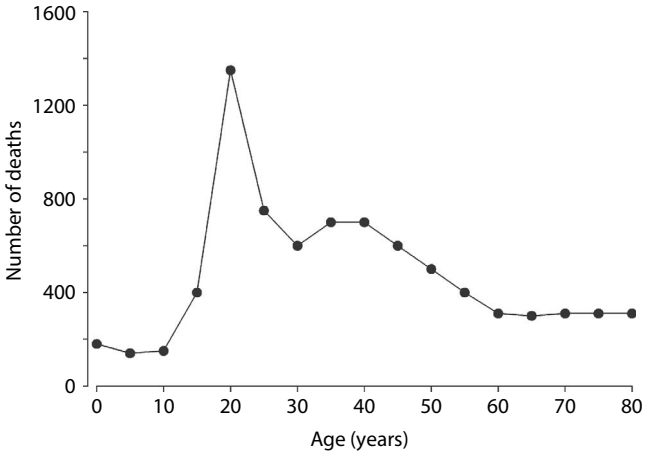


FIGURE 1.39 Deaths due to motor vehicle accidents in the United States. The figure shows the actual number of human deaths resulting from motor vehicle accidents in the year 1999 for various ages (ages between multiples of 5 are not shown). (Data from U.S. National Safety Council, *Injury Facts 2002 Edition*, U.S. National Safety Council, Itasca, IL, 2002, pp. 10–15.)

1.4.2 Ethical Issue

So, the use of animals in research is minuscule in comparison to other uses of animals by humans, and biomedical researchers are very serious about the welfare of their animals. However, we can still ask whether it is ethical to cause discomfort (and death) to a few animals to improve the lives of many humans. The moral judgments that we make about other species are often neither logical nor consistent,[153] so we may try a more generic approach. Henry Heffner, a professor of psychology at the University of Toledo, says that he asks his students if they would, hypothetically, accept a deal in which their standard of living would be raised but, as a consequence, some 30,000 people would die each year and over a million would be injured.[154] Not surprisingly, the students invariably find the deal unacceptable. Heffner then reminds them that, in their naiveté, they do not realize that they have already accepted the deal, as these are the accident statistics for passenger vehicles in the United States.[155] By choosing to drive cars on roads, we choose to kill many and to hurt many more in exchange for the convenience of moving around more easily than on foot. Thus, whether we realize it or not, we do find it ethical to sacrifice a small group for the common good, even if it is a small group of humans.

You may feel that Heffner's analogy is faulty, as people share equally the benefits and the costs of driving a car, whereas only nonhumans pay the price of research to benefit human health. This is not true, however. Not all humans are equal when it comes to traffic accidents. As shown in Figure 1.39, 20-year-olds pay a much higher price for the benefit of driving than do other members of society. As a matter of fact, motor vehicle accidents are the leading cause of death for people between 15 and 30 years of age[155] (we will see in Chapter 16 that heart disease, cancer, and other illnesses are the biggest killers of older adults). Still, you might argue that every person who does not die young gets to benefit from motor vehicles throughout his or her life, whereas a rat never becomes a human being and never benefits from advances in human medicine. Unfortunately, the accidents picture is gloomier. For instance, of the 42,401 deaths due to motor vehicle accidents in the United States in 1999, 28,552 involved male victims.[155] This means that men, who make up 50% of the human population, pay 67% of the price of the convenience of moving around in motor vehicles—and men become women about as often as rats become humans!

If you are very persistent, though, you may argue that only people who choose to get into an automobile have to share the risk of dying in an accident, whereas research animals do not choose to participate in biomedical research. In this case, I must call your attention to the left part of the curve in Figure 1.39. The curve does not go down to zero deaths at young ages. In 1999 alone, 834 children under 5 years of age (who do not choose to get into an automobile) died in motor vehicle accidents in the United States.[155] Embarrassing as these figures may be, they clearly make the point that the decision to sacrifice a number of animals in order to improve the life conditions of a larger number of humans is a moral decision at least equivalent to other moral decisions that we make in our daily lives. We may not be comfortable with some of the ethical decisions that we make—but that is the nature of ethics. As the existentialist philosopher Jean-Paul Sartre used to say, we are painfully free to choose our own destiny, and painfully responsible for each of our choices.[156]

I realize that a few readers may take my arguments backward and decide to become vegetarians and to refuse medical treatment for serious diseases (because the treatment was developed through biomedical research in animals).

They obviously have the right to do so, but I must remind them that the kingdom Animalia is only a small fraction of the diversity of life on Earth. The rebellious readers must be prepared to answer in the near future to a new generation of activists who will clamor for the end of human exploitation of all *plants*—the Vegetal Liberation movement, dedicated to the persecution of all vegetarians who exploit plants as food, decoration, clothing, and medicinal herbs.

SUMMARY

1. Jean-Jacques de Mairan (1678–1771) recorded the first observation of the persistence of daily rhythmicity in plants maintained in an environment lacking temporal cues, and Augustin de Candolle (1778–1841) noticed that the rhythmicity was endogenous because its period differed from the period of Earth's rotation.
2. Circadian physiology evolved into a structured discipline in the twentieth century, thanks especially to the research and tactical efforts of Jürgen Aschoff (1913–1998), Franz Halberg (1919–2013), and Colin Pittendrigh (1919–1996).
3. Current research in circadian physiology is a multimillion dollar enterprise with implications for all sectors of human existence, including arts and entertainment, the humanities, basic biology, business, space exploration, and human and veterinary medicine.
4. Animals are often used as experimental subjects in research in circadian physiology. This use is strictly regulated and follows universal ethical principles.

EXERCISES

1.1 Daily leaf movement of bean plant. In Section 1.1, you saw that the leaves of the bean plant rise during the day and bend down at night. Although you may take my word for it, seeing it with your own eyes may be much more convincing. Start by obtaining a dozen or so fresh beans from a grocery store or a home-and-garden center. Kidney beans are probably the easiest ones to find. You will also need a few small plant pots (thin plastic cups will not work because they will turn over when the plants grow) with some soil in them. Push the beans into the soil and water them regularly (the soil should be wet but not flooded). If you keep the pots indoors, make sure that they are close to a window so that they get light during the day but not at night. Grow the plants until the second pair of leaves is almost fully expanded (which will take 1–3 weeks depending on ambient temperature and day length). Then, choose the best plant and start the observations, for which you will need a protractor. A protractor is a plastic or cardboard semicircular instrument used for measuring angles. You can buy one at any school supply store or build it yourself. Measure the angle of the leaves every 2 hours or so from sunrise to sunset for 3 or more days (you may take measurements at night also, but make sure to use very dim light, red if possible, in order not to disturb the light–dark cycle). At the end, draw a graphic showing the leaf angle (Y-axis) as a function of time (X-axis). You should be able to observe a clear daily rhythm.

1.2 Measuring your own rhythm of body temperature. Measuring circadian rhythms in your own body is perhaps the best way to gain an intuitive feel for the ubiquity of biological rhythms. All you need is a clinical thermometer (mercury-in-glass or electronic) and a sheet of paper to record the data. Try to record your temperature every hour for two or more consecutive days. Before the first measurement, make sure to read the thermometer's instructions for proper placement of the probe. If you are taking measurements under your tongue, make sure not to eat or drink anything for at least 15 minutes before a measurement. Also, avoid measurements shortly after you take a hot shower, go for a cold swim, or do any strenuous exercise (all of these will interfere with the normal daily variation of body temperature). Occasionally, you may also use an alarm clock to briefly wake you up in the middle of the night for nocturnal measurements (but don't do this too often, otherwise you may disturb the body's clock). At the end, draw a graphic showing temperature (Y-axis) as a function of time (X-axis). You should be able to observe a clear daily rhythm.

1.3 Review crosswords. The crossword puzzle in Figure 1.40 provides the opportunity to review some of the material covered in this chapter while enjoying a popular pastime. The key appears right after the "Websites to Explore" section.

Across

4 Swiss botanist who first demonstrated circadian rhythmicity in a plant
10 German physician who created macrobiotics
12 The father of medicine
13 German botanist who wrote the first comprehensive book about research on biological rhythms
14 British physician who conducted long-term recording of his body temperature
15 One of the creators of the concept of biorhythms
16 French astronomer who first described plant rhythmicity in darkness

Down

1 Roman physician who described paroxysms
2 Most prolific current researcher in circadian physiology
3 One of the seven most influential circadian physiologists of our time
5 German forefather of modern circadian physiology

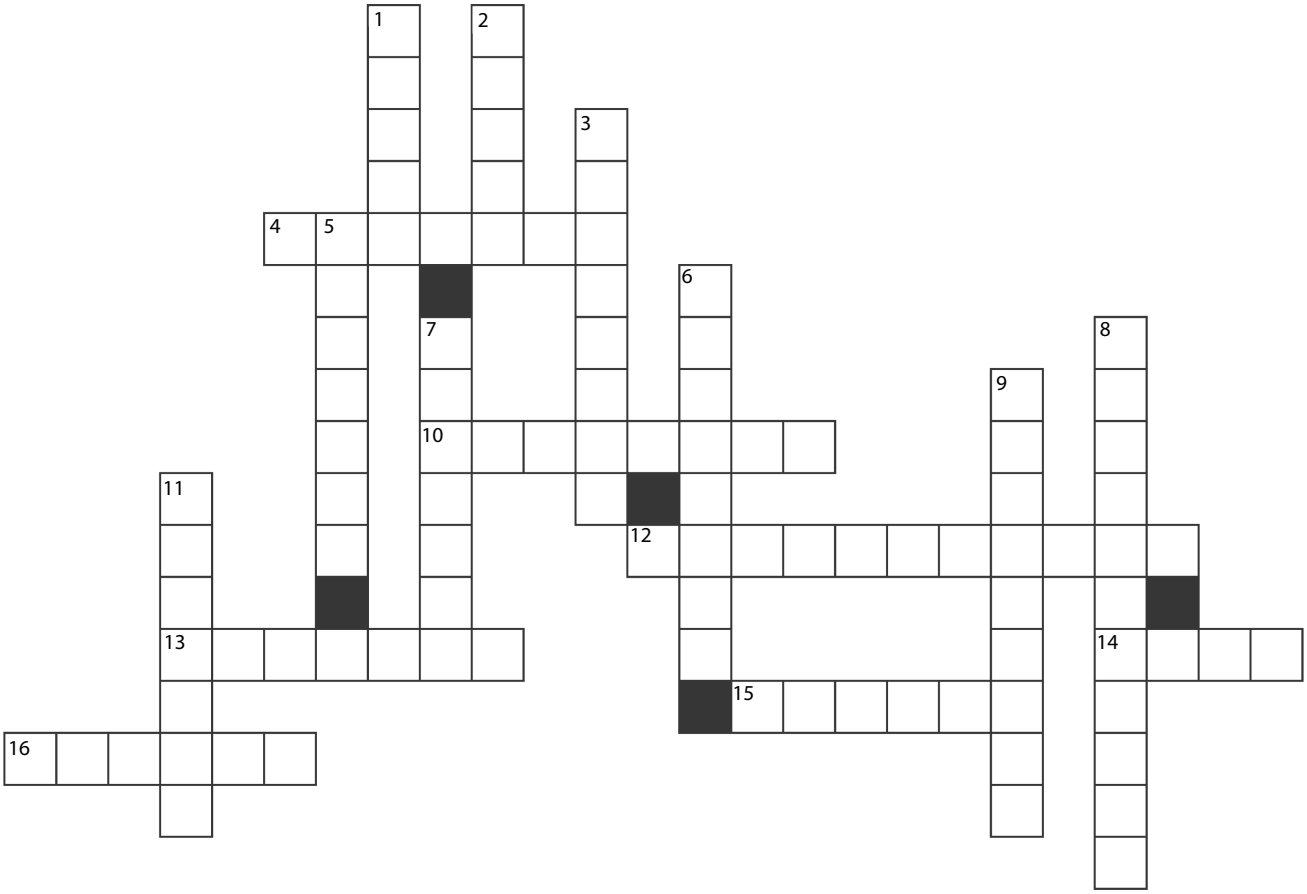


FIGURE 1.40 Crosswords. See Exercise 1.3.

6 The tree in which Androsthenes observed daily rhythmicity
7 First researcher to document circadian rhythmicity in an animal
8 British–American forefather of modern circadian physiology
9 Another one of the seven most influential circadian physiologists of our time
11 Romanian–American forefather of modern circadian physiology

SUGGESTIONS FOR FURTHER READING

There is no book dedicated specifically to the history of circadian physiology. However, information about major developments in the twentieth century can be obtained from books written by researchers who were active in the field during that time. Some of these books are listed here.

Bünning, E. (1973). *The Physiological Clock*, 3rd edn. New York: Springer.

First published in German in 1958 (*Die Physiologische Uhr*), this was probably the earliest scholarly book to summarize a large body of research on biological rhythms from a variety of investigators. This third English edition is a comprehensive account of progress in the field up to the early 1970s.

Richter, C. P. (1965). *Biological Clocks in Medicine and Psychiatry.* Springfield, IL: Charles C. Thomas.

Published a year after the first English translation of Bünning's book, this book provides a detailed description of Richter's extensive research on biological rhythmicity in health and disease.

Sweeney, B. M. (1969). *Rhythmic Phenomena in Plants.* New York: Academic Press.

As indicated by its title, this short book is restricted to biological rhythms in plants. The first chapter briefly covers research conducted in the 1800s, and the rest of the book discusses research conducted from the 1930s to the 1960s. A second edition of the book was published in 1987.

Brown Jr., F. A., Hastings, J. W., and Palmer, J. D. (1970). *The Biological Clock: Two Views.* New York: Academic Press.

A precious time capsule, this book presents a lively debate about the endogenous nature of circadian rhythms in the 1960s.

Saunders, D. S. (1977). *An Introduction to Biological Rhythms.* New York: Wiley.

Probably the first textbook on biological rhythms. It covers approximately the same material in the same epoch as Bünning's book but is more oriented toward nonspecialists.

Brady, J. (1979). *Biological Clocks*. Baltimore, MD: University Park Press.

The first book explicitly meant to be an undergraduate textbook on biological rhythms. It covers approximately the same material as Bünning's and Saunders' books but in a lighter fashion.

Moore-Ede, M. C., Sulzman, F. M., and Fuller, C. A. (1982). *The Clocks that Time Us: Physiology of the Circadian Timing System*. Cambridge, MA: Harvard University Press.

A textbook on biological rhythms that was popular in the 1980s.

Palmer, J. D. (2002). *The Living Clock: The Orchestrator of Biological Rhythms*. New York: Oxford University Press.

A short, very easy-reading book written for nonscientists. Palmer, a marine biologist who entered the field of biological rhythm research in the early 1960s, describes his own early research as well as that of others.

WEBSITES TO EXPLORE

European Biological Rhythms Society: http://www.ebrs-online.org/.
International Society for Chronobiology: http://www.ischronobiology.org/.
Japanese Society for Chronobiology: http://chronobiology.jp/.
NIH Office of Laboratory Animal Welfare: http://grants.nih.gov/grants/olaw/olaw.htm.
Search for Animal Models (LAMHDI): http://www.lamhdi.org.
Society for Research on Biological Rhythms (USA): http://www.srbr.org.
Time and Date (and Sun and Moon): http://www.timeanddate.com/astronomy/.

KEY TO CROSSWORDS (FIGURE 1.40)

Across
4 Candole
10 Hufeland
12 Hippocrates
13 Bunning
14 Davy
15 Fliess
16 Mairan

Down
1 Galen
2 Kario
3 Czeisler
5 Aschoff
6 Tamarind
7 Johnson
8 Pittendrigh
9 Takahashi
11 Halberg

REFERENCES

1. Jobe, P. C., Adams-Curtis, L. E., Burks, T. F., Fuller, R. W., Peck, C. C., Ruffolo, R. R., Snead, O. C., and Woosley, R. L. (1994). The essential role of integrative biomedical sciences in protecting and contributing to the health and well-being of our nation. *Physiologist* 37: 79–84.
2. Jespersen, J. and Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency*, 2nd edn. Mineola, NY: Dover.
3. Burns, E. M. (1973). *Western Civilizations: Their History and Their Culture*, 8th edn. New York: Norton.
4. Audoin, C. and Guinot, B. (2001). *The Measurement of Time* (Trans. by S. Lyle). Cambridge, U.K.: Cambridge University Press.
5. Blackburn, B. and Holford-Strevens, L. (2000). *The Oxford Companion to the Year: An Exploration of Calendar Customs and Time-Reckoning*. New York: Oxford University Press.
6. Aschoff, J. (1974). Speech after dinner. *Chronobiologia* 1(S): 483–493.
7. Mulroy, D. (1992). *Early Greek Lyric Poetry*. Ann Arbor, MI: University of Michigan Press.
8. Davenport, G. (1964). *The Fragments of Archilocos*. Berkeley, CA: University of California Press.
9. Bretzl, H. (1903). *Botanische Forschungen des Alexanderzuges*. Leipzig, Germany: B. G. Teubner.
10. Virey, J. J. (1819). Périodicité: recherches sur les causes des mouvemens périodiques dans l'économie animale. In: *Dictionaire des Sciences Médicales, Tome 40*. Paris, France: C. L. F. Panckoucke, pp. 419–429.
11. Bünning, E. (1964). *The Physiological Clock: Endogenous Diurnal Rhythms and Biological Chronometry*. New York: Academic Press.
12. Dunlap, J. C., Loros, J. J., and DeCoursey, P. J. (2004). *Chronobiology: Biological Timekeeping*. Sunderland, MA: Sinauer.
13. Foster, R. G. and Kreitzman, L. (2004). *Rhythms of Life: The Biological Clocks that Control the Daily Lives of Every Living Thing*. London, U.K.: Profile.
14. de Mairan, J. J. D. (1729). Observation botanique. In: *Histoire de l'Académie Royale des Sciences, Année 1729*. Paris, France: Imprimerie Royale (1731), p. 35.
15. du Monceau, H. L. D. (1758). *La Physique des Arbres*. Paris, France: Guerin & Delatour.
16. Aschoff, J. (1998). Bicentennial anniversary of Christoph Wilhelm Hufeland's *Die Kunst das menschliche Leben zu verlängern* (*The Art of Prolonging Human Life*). *Journal of Biological Rhythms* 13: 4–8.
17. Hufeland, C. W. (1797). *Die Kunst das Menschliche Leben zu Verlängern*. Jena, Germany: Akademische Buchhandlung.
18. Virey, J. J. (1814). *Éphémérides de La Vie Humaine: Recherches sur la Révolution Journalière et la Périodicité de ses Phénomènes dans la Santé et les Maladies*. Paris, France: Didot Jeune.
19. Reinberg, A. E., Lewy, H., and Smolensky, M. (2001). The birth of chronobiology: Julien Joseph Virey 1814. *Chronobiology International* 18: 173–186.
20. de Candolle, A. P. (1832). *Physiologie Végétale*. Paris, France: Béchet.
21. Roenneberg, T. and Merrow, M. (2005). Circadian clocks: The fall and rise of physiology. *Nature Reviews Molecular Cell Biology* 6: 965–971.
22. Wernli, H. J. (1960). *Biorhythm: A Scientific Exploration into the Life Cycles of the Individual*. New York: Crown.

23. Smith, R. E. (1976). *The Complete Book of Biorhythm Life Cycles*. New York: Aardvark.
24. Crawley, J. (1996). *The Biorhythm Book*. Boston, MA: Journey.
25. Gittelson, B. (1996). *Biorhythm: A Personal Science*. New York: Warner.
26. Elowitz, M. B., Levine, A. J., Siggia, E. D., and Swain, P. S. (2002). Stochastic gene expression in a single cell. *Science* 297: 1183–1186.
27. Goldberger, A. L., Rigney, D. R., and West, B. J. (1990). Chaos and fractals in human physiology. *Scientific American* 262(2): 42–49.
28. Zuurbier, L. A., Luik, A. I., Hofman, A., Franco, O. H., Van Someren, E. J. W., and Tiemeier, H. (2015). Fragmentation and stability of circadian activity rhythms predict mortality: The Rotterdam study. *American Journal of Epidemiology* 181: 54–63.
29. Hines, T. M. (1998). Comprehensive review of biorhythm theory. *Psychological Reports* 83: 19–64.
30. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
31. Ogle, W. (1866). On the diurnal variations in the temperature of the human body in health. *St. George's Hospital Reports* 1: 221–245.
32. Reeve, J. C. (1869). The course of the temperature in diseases: A guide to clinical thermometry. *American Journal of the Medical Sciences* 57: 425–447.
33. Rattray, A. (1870). On some of the more important physiological changes induced in the human economy by change of climate, as from temperate to tropical, and the reverse. *Proceedings of the Royal Society of London* 18: 513–528.
34. Féré, C. (1888). De la fréquence des accès d'épilepsie suivant les heures. *Comptes Rendus des Séances de la Société de Biologie de Paris* 40: 740–742.
35. Chossat, C. (1843). Recherches expérimentales sur l'inanition. *Annales des Sciences Naturelles, Série 2* 20: 293–326.
36. Maurel, E. (1884). Expériences sur les variations nycthémérales de la température normale. *Comptes Rendus des Séances de la Société de Biologie de Paris* 37: 588.
37. Kiesel, A. (1894). Untersuchungen zur Physiologie des facettirten Auges. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften* (*Abtheilung 3*) 103: 97–139.
38. Hobday, F. (1896). Notes on physiological temperatures. *Journal of Comparative Pathology and Therapeutics* 9: 286–314.
39. Simpson, S. and Galbraith, J. J. (1906). Observations on the normal temperature of the monkey and its diurnal variation, and on the effect of changes in the daily routine on this variation. *Transactions of the Royal Society of Edinburgh* 45: 65–104.
40. Benedict, F. G. (1904). Studies in body temperature. I. Influence of the inversion of the daily routine; the temperature of night-workers. *American Journal of Physiology* 11: 145–169.
41. Gates, A. I. (1916). Diurnal variations in memory and association. *University of California Publications in Psychology* 1: 323–344.
42. Johnson, M. S. (1926). Activity and distribution of certain wild mice in relation to biotic communities. *Journal of Mammalogy* 7: 245–277.
43. Rogers, L. A. and Greenbank, G. R. (1930). The intermittent growth of bacterial cultures. *Journal of Bacteriology* 19: 181–190.
44. Welsh, J. H. (1938). Diurnal rhythms. *Quarterly Review of Biology* 13: 123–139.
45. Jores, A. (1936). Rhythmikphysiologie und -pathologie des Menschen. *Naturwissenschaften* 24: 408–412.
46. Bünning, E. (1936). Die endonome Tagesrhythmik als Grundlage der photoperiodischen Reaktion. *Berichte der Deutchen Botanischen Gesellschaft* 54: 590–607.
47. Bünning, E. (1958). *Die Physiologische Uhr*. Berlin, Germany: Springer.
48. Bünning, E. (1973). *The Physiological Clock: Circadian Rhythms and Biological Chronometry*, 3rd edn. New York: Springer.
49. Richter, C. P. (1965). *Biological Clocks in Medicine and Psychiatry*. Springfield, IL: Charles C. Thomas.
50. Kleitman, N. (1963). *Sleep and Wakefulness*. Chicago, IL: University of Chicago Press.
51. Aschoff, J. (1990). From temperature regulation to rhythm research. *Chronobiology International* 7: 179–186.
52. Aschoff, J., Gerecke, U., and Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
53. Aschoff, J. and Wever, R. (1976). Human circadian rhythms: A multioscillatory system. *Federation Proceedings* 35: 2326–2332.
54. Aschoff, J. (1966). Circadian activity pattern with two peaks. *Ecology* 47: 657–662.
55. Aschoff, J. (1979). Circadian rhythms: Influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
56. Aschoff, J. (1981). Thermal conductance in mammals and birds: Its dependence on body size and circadian phase. *Comparative Biochemistry and Physiology A* 69: 611–619.
57. Daan, S. and Gwinner, E. (1998). Obituary: Jürgen Aschoff (1913–98). *Nature* 396: 418.
58. Halberg, F. (1959). Physiologic 24-hour periodicity: General and procedural considerations with reference to the adrenal cycle. *Zeitschrift für Vitamin-, Hormon- und Fermentforschung* 10: 225–296.
59. Halberg, F. (1969). Chronobiology. *Annual Review of Physiology* 31: 675–725.
60. Ahlgren, A. and Halberg, F. (1990). *Cycles of Nature: An Introduction to Biological Rhythms*. Washington, DC: National Science Teachers Association.
61. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., and Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *Journal-Lancet* 71: 312–319.
62. Kumagai, Y., Shiga, T., Sunaga, K., Cornélissen, G., Ebihara, A., and Halberg, F. (1992). Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 19: 43–58.
63. Halberg, F., Cornélissen, G., Watanabe, Y., Otsuka, K., Fiser, B., Siegelova, J., Mazankova, V. et al. (2001). Near 10-year and longer periods modulate circadians: Intersecting anti-aging and chronoastrobiological research. *Journal of Gerontology* 56A: M304–M324.
64. Halberg, F., Zander, H. A., Houglum, M. W., and Mühlemann, H. R. (1954). Daily variations in tissue mitoses, blood eosinophils and rectal temperatures of rats. *American Journal of Physiology* 177: 361–366.
65. Halberg, F. and Conner, R. L. (1961). Circadian organization and microbiology: Variance spectra and periodogram on behavior of *Escherichia coli* growing in fluid culture. *Proceedings of the Minnesota Academy of Science* 29: 227–239.
66. Nelson, W., Scheving, L., and Halberg, F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *Journal of Nutrition* 105: 171–184.

67. Powell, E. W., Halberg, F., Pasley, J. N., Lubanovic, W., Ernsberger, P., and Scheving, L. E. (1980). Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *Journal of Thermal Biology* 5: 189–196.
68. Refinetti, R. (2013). Franz Halberg (1919–2013). *Journal of Biological Rhythms* 28: 305.
69. Pittendrigh, C. S. (1993). Temporal organization: Reflections of a Darwinian clock-watcher. *Annual Review of Physiology* 55: 17–54.
70. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences United States of America* 40: 1018–1029.
71. Pittendrigh, C. S. (1966). The circadian oscillation in *Drosophila psudoobscura* pupae: A model for the photoperiod clock. *Zeitschrift für Pflanzenphysiologie* 54: 275–307.
72. Pittendrigh, C. S. and Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: I. The stability and lability of spontaneous function. *Journal of Comparative Physiology* 106: 223–252.
73. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology* 106: 253–266.
74. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. III. Heavy water and constant light: Homeostasis of frequency? *Journal of Comparative Physiology* 106: 267–290.
75. Pittendrigh, C. S. and Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: IV. Entrainment: Pacemaker as clock. *Journal of Comparative Physiology* 106: 291–331.
76. Menaker, M. (1996). Colin S. Pittendrigh (1918–96). *Nature* 381: 24.
77. Halberg, F., Cornélissen, G., Katinas, G., Syutkina, E. V., Sothern, R. B., Zaslavskaya, R., Halberg, F. et al. (2003). Transdisciplinary unifying implications of circadian findings in the 1950s. *Journal of Circadian Rhythms* 1: art. 2.
78. Cornélissen, G., Halberg, F., Sánchez-ed-la-Peña, S., Jinyi, W., and Carandente, F. (1988). The need for both macroscopy and microscopy in dealing with spectral structure. *Chronobiologia* 15: 323–327.
79. Turek, F. W. (1988). Do circadian biologists and chronopharmacologists talk the same "language"? *Annual Review of Chronopharmacology* 4: 205–208.
80. Cambrosio, A. and Keating, P. (1983). The disciplinary stake: The case of chronobiology. *Social Studies of Science* 13: 323–353.
81. Rimsky-Korsakov, N. (1964). *Principles of Orchestration* (Trans. by E. Agate). New York: Dover.
82. Bünning, E. (Org.) (1960). *Cold Spring Harbor Symposia on Quantitative Biology*, Vol. 25*: Biological Clocks*. Cold Spring Harbor, NY: The Biological Laboratory.
83. Honma, K. and Shibata, S. (2003). Announcement: First world congress of chronobiology. *Chronobiology International* 20: 739.
84. Nintcheu-Fata, S., Katinas, G., Halberg, F., Cornélissen, G., Tolstykh, V., Michael, H. N., Otsuka, K., Schwartzkopff, O., and Bakken, E. (2003). Chronomics of tree rings for chrono-astrobiology and beyond. *Biomedicine and Pharmacotherapy* 57: 24s–30s.
85. Reich, W. (1961). *The Function of the Orgasm*. New York: Noonday.
86. Snow, B. and Ifshin, S. L. (1984). Online database coverage of forensic medicine. *Online* 8(2): 37–43.
87. McCain, K. W., White, H. D., and Griffith, B. C. (1987). Comparing retrieval performance in online data bases. *Information Processing and Management* 23: 539–553.
88. Barber, J., Moffat, S., and Wood, F. (1988). Case studies of the indexing and retrieval of pharmacology papers. *Information Processing and Management* 24: 141–150.
89. Refinetti, R. (1990). Retrieval performance in online search in thermal physiology. *Computers and Biomedical Research* 23: 32–36.
90. Garfield, E. (1996). The significant scientific literature appears in a small core of journals. *Scientist* 10(17): 13–14.
91. Refinetti, R. (1990). In defense of the least publishable unit. *FASEB Journal* 4: 128–129.
92. Refinetti, R. (1989). Information processing as a central issue in philosophy of science. *Information Processing and Management* 25: 583–584.
93. Refinetti, R. (1999). Keeping up with the research literature through reprint requests. *Scientist* 13(12): 13.
94. Refinetti, R. (2011). Publish and flourish. *Science* 331: 29.
95. Janssen, S. (Ed.) (2014). *The World Almanac and Book of Facts*. New York: Simon & Schuster.
96. Mervis, J. and Malakoff, D. (2014). Science agencies make gains despite tight U.S. budget. *Science* 346: 1437–1438.
97. May, R. M. (1997). The scientific wealth of nations. *Science* 275: 793–796.
98. Harley, D. (2013). Scholarly communication: Cultural contexts, evolving models. *Science* 342: 80–82.
99. Jones, B. F., Wuchty, S., and Uzzi, B. (2008). Multi-university research teams: Shifting impact, geography, and stratification in science. *Science* 322: 1259–1262.
100. Simonton, D. K. (1988). *Scientific Genius: A Psychology of Science*. New York: Cambridge University Press.
101. Shimizu, M., Ishikawa, J., Yano, Y., Hoshide, S., Shimada, K., and Kario, K. (2011). The relationship between the morning blood pressure surge and low-grade inflammation on silent cerebral infarct and clinical stroke events. *Atherosclerosis* 219: 316–321.
102. Palatini, P., Reboldi, G., Beilin, L. J., Eguchi, K., Imai, Y., Kario, K., Ohkubo, T. et al. (2013). Predictive value of nighttime heart rate for cardiovascular events in hypertension: The ABP-International study. *International Journal of Cardiology* 168: 1490–1495.
103. Kario, K. and Hoshide, S. (2015). Age-related difference in the sleep pressure-lowering effect between an angiotensin II receptor blocker and a calcium channel blocker in Asian hypertensives: The ACS1 study. *Hipertension* 65: 729–735.
104. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J., and Simonneaux, V. (2003). Mechanisms regulating the marked seasonal variation in melatonin synthesis in the European hamster pineal gland. *American Journal of Physiology* 284: R1043–R1052.
105. Mendoza, J., Clesse, D., Pévet, P., and Challet, E. (2010). Food-reward signalling in the suprachiasmatic clock. *Journal of Neurochemistry* 112: 1489–1499.
106. Chakir, I., Dumont, S., Pévet, P., Ouarour, A., Challet, E., and Vuillez, P. (2015). The circadian gene *Clock* oscillates in the suprachiasmatic nuclei of the diurnal rodent Barbary striped grass mouse, *Lemniscomys barbarus*: A general feature of diurnality? *Brain Research* 1594: 165–172.

107. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U., and Nevo, E. (2004). Circadian genes in a blind subterranean mammal. III. Molecular cloning and circadian regulation of cryptochrome genes in the blind subterranean mole rat, *Spalax ehrenbergi* superspecies. *Journal of Biological Rhythms* 19: 22–34.
108. Feillet, C. A., Ripperger, J. A., Magnone, M. C., Dulloo, A., Albrecht, U., and Challet, E. (2006). Lack of food anticipation in *Per2* mutant mice. *Current Biology* 16: 2016–2022.
109. Kowalska, E., Ripperger, J. A., Hoegger, D. C., Bruegger, P., Buch, T., Birchler, T., Mueller, A., Albrecht,U., Contaldo, C., and Brown, S. A. (2013). NONO couples the circadian clock to the cell cycle. *Proceedings of the National Academy of Sciences of the United States of America* 110: 1592–1599.
110. Honma, S., Nakamura, W., Shirakawa, T., and Honma, K. (2004). Diversity in the circadian periods of single neurons of the rat suprachiasmatic nucleus depends on nuclear structure and intrinsic period. *Neuroscience Letters* 358: 173–176.
111. Abe, H., Honma, S., and Honma, K. (2007). Daily restricted feeding resets the circadian clock in the suprachiasmatic nucleus of CS mice. *American Journal of Physiology* 292: R607–R615.
112. Yamanaka, Y., Honma, S., and Honma, K. (2013). Daily exposure to a running wheel entrains circadian rhythms in mice in parallel with development of an increase in spontaneous movement prior to running-wheel access. *American Journal of Physiology* 305: R1367–R1375.
113. Honma, K. (2011). History of chronobiological societies: Chronobiologists are always looking for the best friend. *Third World Congress of Chronobiology*, Puebla, Mexico, May 5–9, p. 6.
114. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M., and Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685.
115. Davidson, A. J., Poole, A. S., Yamazaki, S., and Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain and Behavior* 2: 32–39.
116. Numano, R., Yamazaki, S., Umeda, N., Samura, T., Sujino, M., Takahashi, R., Ueda, M. et al. (2006). Constitutive expression of the Period1 gene impairs behavioral and molecular circadian rhythms. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3716–3721.
117. Murphy, Z. C., Pezuk, P., Menaker, M., and Sellix, M. T. (2013). Effects of ovarian hormones on internal circadian organization in rats. *Biology of Reproduction* 89: art. 35.
118. Bankert, E. A. and Amdur, R. J. (2005). *Institutional Review Board: Management and Function*, 2nd edn. Burlington, MA: Jones & Bartlett Learning.
119. Guerrini, A. (2003). *Experimenting with Humans and Animals: From Galen to Animal Rights*. Baltimore, MD: Johns Hopkins University Press.
120. Derbyshire, S. W. G. (2006). Time to abandon the three Rs. *Scientist* 20(2): 23.
121. Singer, P. (1975). *Animal Liberation: A New Ethics for Our Treatment of Animals*. New York: Avon.
122. Erickson, D. (1990). Blood feud: Researchers begin fighting back against animal-rights activists. *Scientific American* 262(6): 17–18.
123. Zola-Morgan, S. (1994). Animals in Research Committee monitors changing focus of activists. *Neuroscience Newsletter* 25(5): 15–16.
124. United States Department of Justice and United States Department of Agriculture (1993). Report to Congress on the extent and effects of domestic and international terrorism in animal enterprises. *Physiologist* 36: 207–259.
125. Samuels, W. M. (1990). Activist pleads Nolo Contendere to charge of attempted murder. *Physiologist* 33: 51.
126. Miller, G. (2007). Animal extremists get personal. *Science* 318: 1856–1858.
127. Korner, P. I. (1984). Medicine and the animal liberation movement. *Medical Journal of Australia* 141: 773–775.
128. Koshland Jr., D. E. (1989). Animal rights and animal wrongs. *Science* 243: 1253.
129. White, R. J. (1988). Animal rights versus human rights. *Surgical Neurology* 30: 410–411.
130. Conn, P. M. and Parker, J. (1998). Animal rights: Reaching the public. *Science* 282: 1417.
131. Bulger, R. E. (1987). Use of animals in experimental research: A scientist's perspective. *Anatomical Record* 219: 215–220.
132. Dresser, R. (1988). Standards for animal research: Looking at the middle. *Journal of Medicine and Philosophy* 13: 123–143.
133. Johnson, D. (1990). Animal rights and human lives: Time for scientists to right the balance. *Psychological Science* 1: 213–214.
134. McCance, I. (1989). The number of animals. *News in Physiological Sciences* 4: 172–176.
135. Kaplan, J. (1988). The use of animals in research. *Science* 242: 839–840.
136. Nicoll, C. S. and Russell, S. M. (1991). Mozart, Alexander the Great, and the animal rights/liberation philosophy. *FASEB Journal* 5: 2888–2892.
137. Hatch, O. G. (1987). Biomedical research. *American Psychologist* 42: 591–592.
138. Maas, G. A. (1994). Public relations and animal research. *Lab Animal* 23(4): 28–31.
139. Nicoll, C. S. and Russell, S. M. (1989). Animal research vs. animal rights. *FASEB Journal* 3: 1668–1671.
140. Botting, J. H. and Morrison, A. R. (1997). Animal research is vital to medicine. *Scientific American* 276(2): 83–85.
141. Epstein, A. (2005). The "animal rights" movement's cruelty to humans. *Physiologist* 48: 223–225.
142. Ringach, D. L. and Jentsch, J. D. (2009). We must face the threats. *Journal of Neuroscience* 29: 11417–11418.
143. Plous, S. (1991). An attitude survey of animal rights activists. *Psychological Science* 2: 194–196.
144. Singer, P. (1990). The significance of animal suffering. *Behavioral and Brain Sciences* 13: 9–12.
145. Refinetti, R. (1990). The real issue in the antivivisection controversy. *Science, Technology, and Human Values* 25: 122–123.
146. Shalev, M. (1997). Animals used in research, 1973–1995. *Lab Animal* 26(1): 14–16.
147. Kulpa-Eddy, J., Snyder, M., and Stokes, W. (2008). A review of trends in animal use in the United States (1972–2006). *Alternatives to Animal Testing and Experimentation* 14: 163–165.
148. United States Congress (1966). Animal Welfare Act. United States Code, Title 7, Sections 2131–2156.
149. Animal and Plant Health Inspection Service (2006). Code of Federal Regulations, Title 9, Chapter 1, Subchapter A. Riverdale, MD: United States Department of Agriculture.
150. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press.

151. Podolsky, M. L. and Lukas, V. S. (1999). *The Care and Feeding of an IACUC*. Boca Raton, FL: CRC Press.
152. Silverman, J., Suckow, M. A., and Murthy, S. (2014). *The IACUC Handbook*, 3rd edn. Boca Raton, FL: CRC Press.
153. Herzog Jr., H. A. (1988). The moral status of mice. *American Psychologist* 43: 473–474.
154. Heffner, H. (2001). Animal research in the college classroom. *APS Observer* 14(3): 5, 31.
155. U.S. National Safety Council (2002). *Injury Facts 2002 Edition*. Itasca, IL: U.S. National Safety Council, pp. 10–15.
156. Sartre, J. P. (1948). *Existentialism and Humanism* (Trans. by P. Mairet). London, U.K.: Methuen.

2 Research Methods in Circadian Physiology

2.1 SCIENTIFIC METHOD

Research in circadian physiology is, of course, conducted according to the scientific method. But what is the scientific method and why should it be used? Answering this question is the first step in the study of research methods. The answer is particularly important for academic scientists and university students in the United States in the early twenty-first century. These individuals will most likely be confronted with a philosophical movement referred to as *constructivism*, which is presented as a facet of *postmodernism* and is often associated with various versions of *feminism*. As pointed out by concerned scholars, this philosophical movement poses a threat to the progress of science and the preservation of social order.[1–3] Thus, awareness of the constructivist movement may be necessary for the advancement of scientific research, including research in circadian physiology. Because the term *constructivism* has been used in many different contexts with many different connotations,[4–7] we must have a closer look at the issue.

2.1.1 Philosophy and Science

Since at least the late 1800s, the view of science espoused by most scientists and lay citizens has been what in philosophy is called a *positivist* view. The name derives from *positivism*, a philosophical system developed by the French philosopher *Auguste Comte* (Figure 2.1). Comte reflected the worldview of his time, which, as far as science is concerned, can be characterized by three fundamental assumptions:

1. The world is "out there" (it exists independently of us), and it is our job to go out and learn about it.
2. Knowledge is cumulative, and each generation is closer to the eventual full knowledge of the world.
3. There is a hierarchy that unites the various sciences, a hierarchy that runs up from mathematics and physics to chemistry, to biochemistry, to cell biology, and to physiology.

Comte had a relatively idiosyncratic view in which *sociology* (the science that he himself created) should be at the top of the hierarchy,[8] similarly to how Plato, thousands of years earlier, had felt that *philosophy* was at the top of the hierarchy of knowledge.[9] Although this aspect of Comte's thought did not gather many adepts (except maybe among sociologists), positivism in general became a very influential philosophy around the world. The influence was so strong that the positivist motto ("Order and Progress") even made it into a national flag (Figure 2.2). As a matter of fact, if you felt that the three assumptions listed earlier were rather obvious (as you probably did), you are living proof of the pervasiveness of the positivist thought.

The first of the three assumptions (namely, that the world exists on its own) is common to many philosophies and is called "realism." An alternative to realism is "relativism." As diagrammed in Figure 2.3, realism assumes that we can look at the world and can get to know it (Panel A). Relativism, on the other hand, asserts that our view of the world (and, therefore, our knowledge of it) depends on how we look at it (Panel B). If we look at the world from one side, we will think that it is one thing; if we look at it from the other side, we will think that it is something else. How, then, can we tell which one is the *real* world? Of course, we could look from both sides and then combine the information into a single real world. But, if there are two possible worldviews, how can we be sure that there aren't more than two views? And what if there are infinite views? If there are infinite views, we cannot possibly find out what the *real* world is. We are forced to admit that the doctrine of realism is untenable![10]

If this reasoning is starting to sound too abstract to you, a simple but concrete example should solve the problem. Figure 2.4 is a classical depiction of the figure–background ambiguity in visual perception. If you choose the black color as the background, you can clearly see a white goblet. On the other hand, if you choose the white color as the background, you will see two silhouetted faces staring at each other. What is the *true* content of the figure? Is it the goblet or the faces? In answering this question, the realist would make the assumption that the true content of the figure lies somewhere beyond our sensory experiences—but how can we know it, if it is beyond our sensory experiences? The relativist would simply accept the ambiguity of the figure.

Realism has been an assumption of scientists for centuries. *Galileo Galilei* (Figure 2.5), universally recognized as the father of modern science, implicitly indicated in his book *Assayer*, published in 1623, that he believed that nature is sitting *out there*, like an open book from which science extracts knowledge.[11] *Albert Einstein* (Figure 2.6), the best known scientist of the twentieth century, was also a realist. Although he became famous for his work on *relativity*, he had no penchant for *relativism*. For instance, in enunciating the "principle of special relativity," which deals with the relative movements of "inertial systems," he emphasized not the fact that different inertial systems provide alternative worldviews, but that "the



FIGURE 2.1 Auguste Comte (1798–1857). This French philosopher was the creator of the doctrine of positivism as well as of the discipline of sociology. (Courtesy of Maison d'Auguste Comte, Paris, France.)

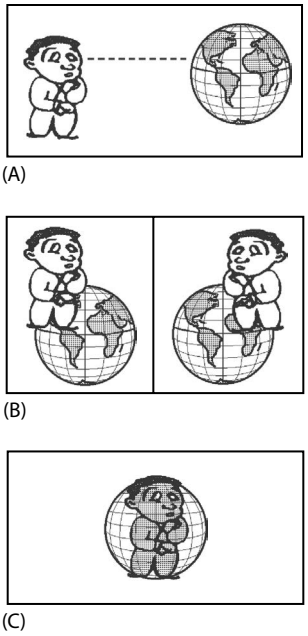


FIGURE 2.3 Three different worldviews. The drawings symbolize the three main epistemological perspectives: realism (A), relativism (B), and dialectics (C).

laws of nature are in concordance for all inertial systems."[12] That is, he didn't emphasize the *relative*; he emphasized the *absolute*. Likewise, a panel of late-twentieth-century scientists assembled by the U.S. National Academy of Sciences expressed its adoption of the realist perspective in sentences such as "New observations and theories survive the scrutiny of scientists and earn a place in the edifice of scientific knowledge because they describe the physical or social world more completely or more accurately."[13]

Many philosophers, Comte among them, were also realists, but it is among philosophers that most relativists can be found. One can find relativism as far back as 500 BC in Heraclitus' famous verses asserting that no man can bathe in the same river twice (because the water keeps flowing, and the river is thus never the same).[14] The verses are generally understood as an assertion of the relativity of knowledge resulting from the absence of an immutable world waiting to be known. There have been many relativist philosophies over the centuries, but a major resurgence took place in the 1960s. One of



FIGURE 2.2 The national flag of the Republic of Brazil. The Brazilian flag, which was created at the height of the positivist movement in the late 1800s, bears the positivist motto "Order and Progress." (From Pauwels, G.J., *Atlas Geográfico Melhoramentos*, 50th edn., Melhoramentos, São Paulo, Brazil, 1987.)

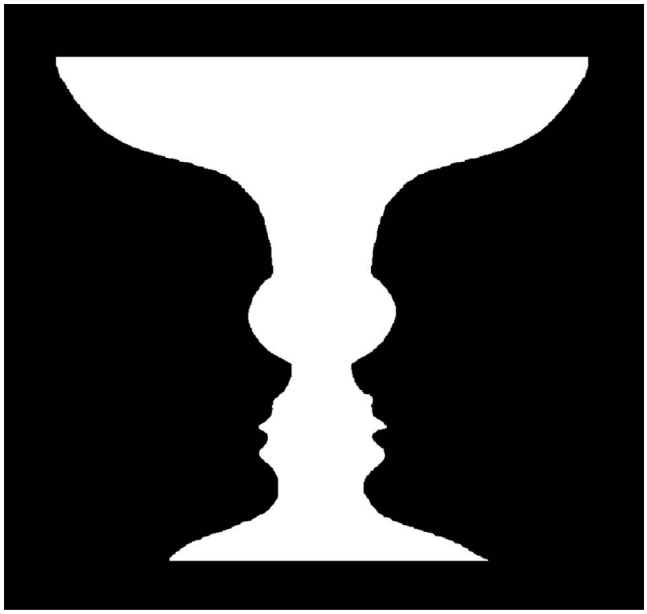


FIGURE 2.4 A goblet or two faces? This figure shows that the perception of an image may depend on how one looks at it. (Adapted from Levine, M.W. and Shefner, J.M., *Fundamentals of Sensation and Perception*, Addison-Wesley, Reading, MA, 1981.)

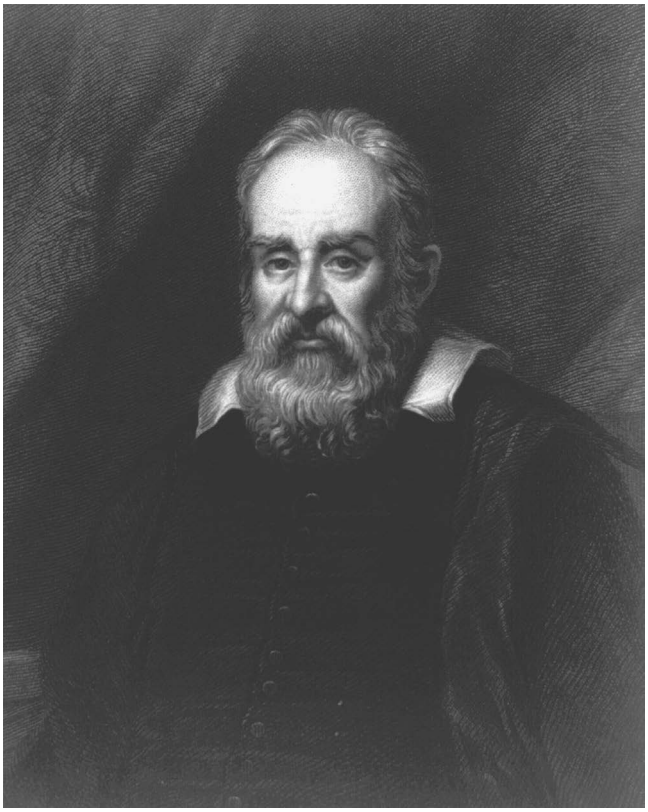


FIGURE 2.5 Galileo Galilei (1564–1642). This famous Italian astronomer and physicist endorsed epistemological realism. (Courtesy of Library of Congress, Washington, DC.)

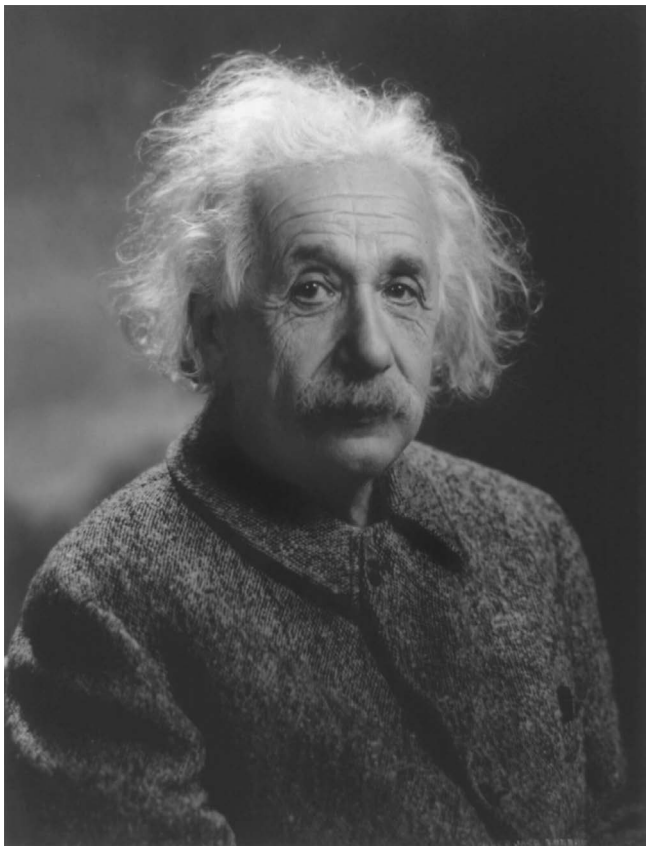


FIGURE 2.6 Albert Einstein (1879–1955). This German theoretical physicist, the most famous scientist of the twentieth century, is widely known for his theory of relativity. Nonetheless, he was an epistemological realist. (Courtesy of Library of Congress, Washington, DC.)

the main characters in the play was the American historian of science *Thomas Kuhn* (Figure 2.7). Kuhn did not mean to be a relativist, but his analysis of how progress is attained in science led him to question the doctrine of realism. As described in his 1962 book, *The Structure of Scientific Revolutions* (Figure 2.8), Kuhn introduced a new way of thinking about science by claiming that current scientific knowledge is part of a transitory *paradigm* that, by necessity, must eventually be discarded in order for scientific progress to take place.[15] In contrast to the positivist belief in cumulative knowledge perfected by successive improvements in experimental methods, Kuhn claimed that progress is a discontinuous process that involves many arbitrary decisions along the way. For example, the transition from Lamarckism to Darwinism was not the result of a gradual improvement in evolutionary research but the result of a *revolutionary* change from one paradigm to another. Now, since scientific truths are, by necessity, restricted to their paradigms, one must conclude that there is no *real* truth. Scientific truths are always *relative* to the paradigms in which they are enunciated. Thus, we can never know what the *real* world is.

Kuhn himself was not quite ready to go "all the way." Although reluctantly, he retained the epistemological perspective that an empirical world that can be effectively known lies

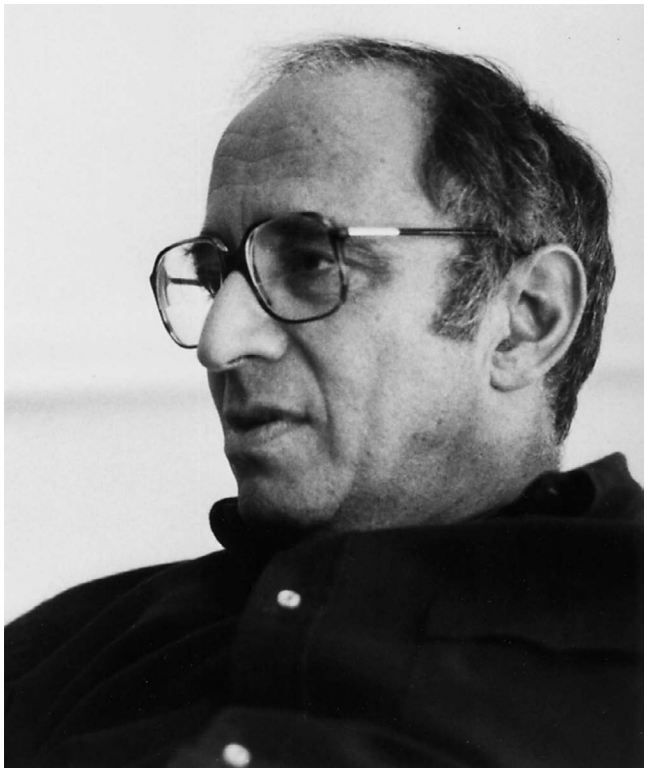


FIGURE 2.7 Thomas Kuhn (1922–1996). This American philosopher of science was greatly influential in the resurgence of epistemological relativism in the late twentieth century. (Courtesy of MIT Museum, Massachusetts Institute of Technology, Cambridge, MA.)

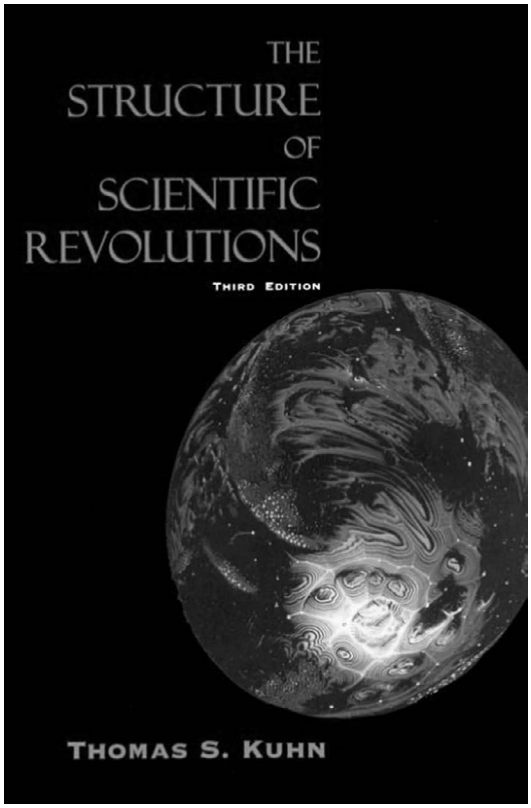


FIGURE 2.8 Cover of the 3rd edition of Kuhn's *The Structure of Scientific Revolutions*. This book by Thomas Kuhn, first published in 1962, is probably the best known philosophy of science book ever published. The third edition was published by the University of Chicago Press in 1996.

beyond the "incommensurability" of scientific paradigms.[15] That is, there were two Kuhns: the Kuhn who was a Kuhnian and the Kuhn who was a realist.[16] Across the Atlantic, several French philosophers of science were much bolder, however. Their ideas eventually crossed the ocean and took over a large sector of American academia. Three French authors were particularly influential: Lyotard, Derrida, and Foucault.

Jean François Lyotard (1924–1998) was the one who created the term *postmodernism*.[17] By *postmodernism*, he meant a worldview distinct from the *modern* view characterized by "grand theories" of religion, politics, and culture in general. The postmodern view asserts the *incommensurability* of various forms of discourse (or, in plain English, the arbitrary nature of established knowledge). Thus, Lyotard did to cultural values what Kuhn did to scientific paradigms.

Jacques Derrida (1930–2004) was the one who developed the notion of *deconstruction*,[18] which eventually led to the term *constructivism*. To *deconstruct* a theory is to bring to light its assumptions and, therefore, to show that the value of the theory is limited to the universe of its assumptions. Conversely, scientific knowledge, as a form of human activity, is molded by the cultural forces that affect every form of human activity, so that scientific truths are presumably *made up* (*constructed*) by cultural forces rather than *discovered* by objective research.

Michel Foucault (1926–1984) was, in my opinion, the most interesting of the three philosophers. Some of his work can be classified as belonging to the doctrine of *structuralism* that characterized the linguistic research of Ferdinand de Saussure, the psychological research of Jean Piaget, and the anthropological research of Claude Lévi-Strauss.[19,20] Foucault's 1969 book, *The Archeology of Knowledge* (Figure 2.9), is essentially a manual on how to conduct good research from a structuralist perspective,[21] even though Piaget felt that Foucault missed the main point of structuralism.[19] In any case, Foucault's connection with postmodernism derives from the fact that the structures that he identified (the *epistemes*)[22] resemble Kuhn's paradigms and Lyotard's "language games." One episteme succeeds another, but there is no actual progress.

When American authors in the *science studies* field (mostly people in sociology, education, and women's studies) embraced the writings of Lyotard, Derrida, and Foucault, they rapidly started to question the legitimacy of traditional science. Locally, they had the partial support of Kuhn's writings as well as of newcomer Austrian psychologist *Ernst von Glasersfeld* (1917–2010), who joined the faculty of the University of Georgia in 1970. Glasersfeld, whose original interest was in cybernetics, went on to propose *radical constructivism*, an explicit antirealism enunciation of constructivism.[23]

To the extent that erosion of public trust in science could lead to reduced federal funding of scientific research and,

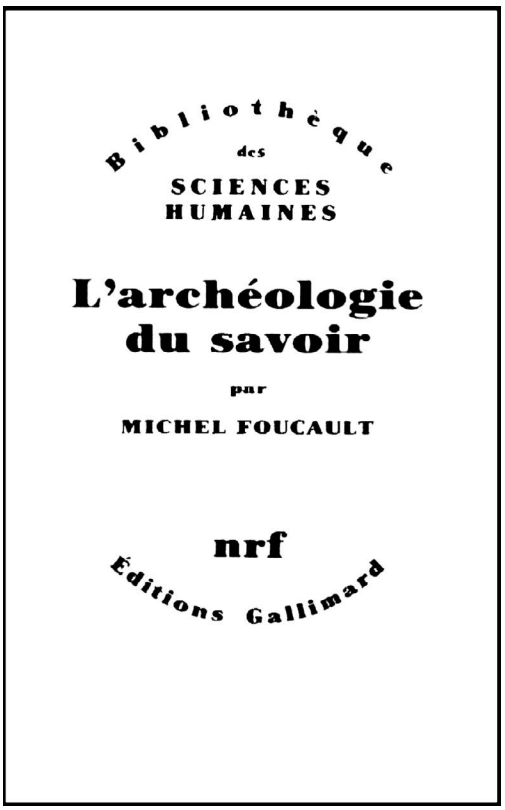


FIGURE 2.9 Cover of the original edition of Michel Foucault's *The Archeology of Knowledge*. This book by French philosopher Michel Foucault, published by Éditions Gallimard in 1969, delineated the method that leads to relativism through structuralism.

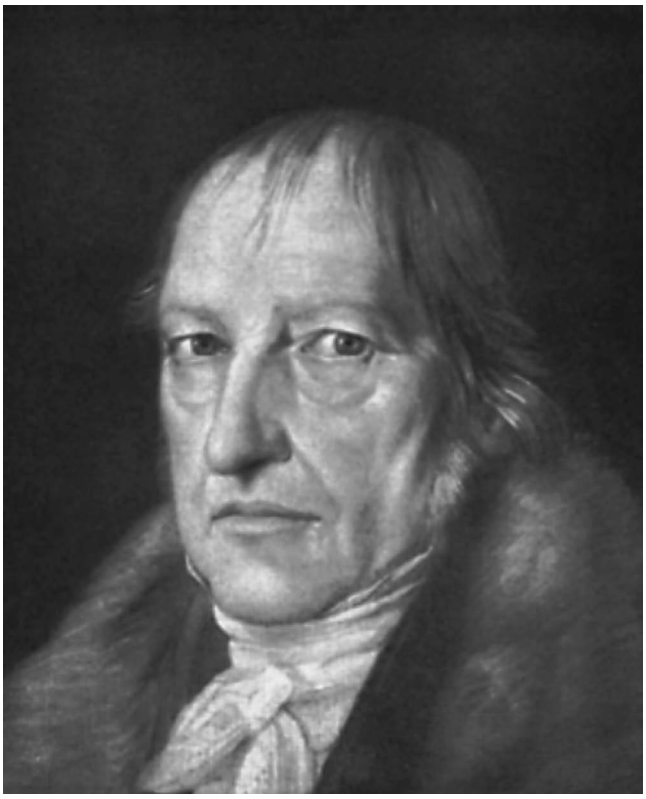


FIGURE 2.10 Georg Wilhelm Friedrich Hegel (1770–1831). This German philosopher is considered by many as the greatest philosopher of modern times. He was the father of modern dialectics. (Courtesy of The North American Fichte Society, University of Pennsylvania, Philadelphia, PA.)

consequently, to a derailment of the scientific enterprise, scientists were justified in being infuriated by the constructivists' attacks on realism.[24–28] However, it should be pointed out that the constructivist philosophy itself is quite sensible, and that it cannot be blamed for its misuse by *science studies* authors.[29] As pointed out by serious philosophers and scientists, the criticism of realism does not imply the criticism of science.[30–32] After all, *absolute* realism is a metaphysical principle that has very little to do with science. Although most of us assume that there is a *real* world lying behind our experiences, this assumption is not necessary and is not even consistent with our actual experience of the world. Let us look at Figure 2.3 again. Panels A and B depict the realist and relativist perspectives. Now notice that it is irrelevant whether our experience of the world involves only one view (realism) or multiple views (relativism). In either case, we and the world (or worlds) are separate entities. That is, we, as observers and possessors of knowledge, are not included in the attained knowledge, so that our total knowledge is necessarily incomplete. The only way that we could have *absolute* knowledge would be if we were one with the world (Panel C). This is a central element of the *dialectical* perspective elaborated in the early 1800s by the German philosopher *Georg Wilhelm Friedrich Hegel* (Figure 2.10). In the long, dense preface to his book *Phenomenology of the Spirit*, he encapsulated his thoughts in the sentence "Das Wahre ist das Ganze,"[33] which can be translated as "The truth is in the whole, not in any of its individual parts" (Figure 2.11). That is, knowledge can never be complete if the knower is not integrated with the known. Absolute knowledge can be attained only if the "subject–object dichotomy" is surpassed through a dialectical synthesis. As you can see, the notion of absolute truth is an *idea* that may capture the imagination of philosophers but that has little to do with the work of scientists or the lives of ordinary people.

Too much philosophical talk? Let me try a different version of the same argument: Even if you are a realist, you can *conceive of* the existence of alternate worlds. You may feel that the knowledge obtained through science is knowledge of the *real* world, but you are certainly capable of *imagining* that the real world could be different from what you believe it to be. In fact, you do this every time you watch a science fiction movie. Now, because you cannot *prove* that alternate worlds do not exist, you must accept them as hypothetical possibilities—no matter how unlikely you believe them to be. And that's all. If uncorrupted by *science studies* activists, this is the essence of constructivism—and, as a matter of fact, of philosophy in general.[34] As a scientist, you have nothing to fear from philosophers, just as you have nothing to fear from movie directors. Science describes the world in which we live; what lies beyond this world is as meaningful

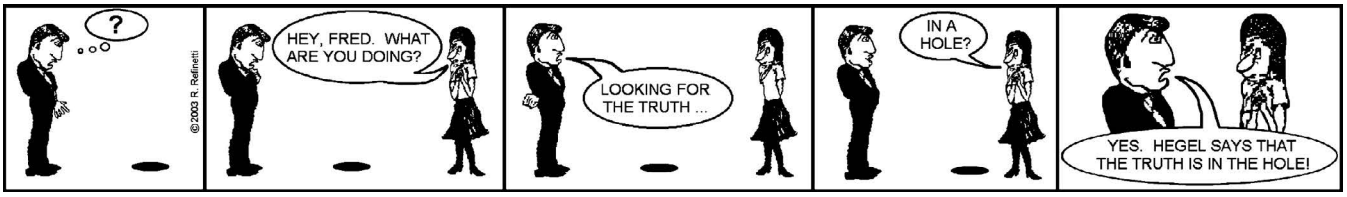


FIGURE 2.11 Looking for the truth. This comic strip is a pun upon Hegel's dialectics.

to scientists as the hypothetical knowledge of the genome of angels. If you don't think so, it is you who is out of touch with reality. According to the American Association for the Advancement of Science,[35] anyone who finished high school should know that "In science, the testing, revising, and occasional discarding of theories, new and old, never ends. This ongoing process leads to an increasingly better understanding of how things work in the world but not to absolute truth." Philosopher of science Peter Lipton put it perhaps even more clearly: "Nobody thinks current science is the complete truth; nobody thinks current science is just a story unconstrained by evidence. But almost every intermediate position has its supporters."[36]

2.1.2 Rules of the Method

After a long preamble, readers may now be expecting an extensive list of the rules of the scientific method. Ironically, the scientific method is rather simple. To this day, learning the scientific method involves not the reading of voluminous books but the practical experience of conducting original research under the guidance of a mentor. From a pragmatic perspective, as well as from a philosophical one, it has been argued that there is no such thing as the scientific method.[37,38] Of course, there are published books on the scientific method,[39–42] but their message is usually that common sense is all there is to it. One will not find in science the sort of formal precepts that one finds, for example, in logics. Knowing how to handle a simple syllogism (Figure 2.12) is probably as sophisticated as one must get in general research methods. Specific methods applied to particular research questions are another story, and we will deal with them in Sections 2.2 through 2.4.

So, the scientific method consists of applying common sense to scientific problems. But common sense is rather broad. Can't we have some advice on how to optimize the use of common sense? In fact, many scientists have provided written advice to beginners. We can start with René Descartes in the seventeenth century. In his *Discourse on Method*,[43] he suggested four rules:

1. Never accept anything for true if you are not certain about it.
2. Divide each of the difficulties under examination into as many parts as necessary to facilitate their understanding.
3. Always start with the simplest and easiest problems, and then proceed step-by-step to the more complex ones.
4. Make enumerations so complete, and reviews so general, that you can be confident of not having forgotten anything.

John Platt, a biophysicist at the University of Chicago in the 1960s, recommended four steps in the path to "strong inferences" in scientific research[44]:

1. Devise alternative hypotheses to explain the phenomenon that you are investigating.
2. Devise a crucial experiment (or several of them).
3. Carry out the experiment so as to get a clean result.
4. Repeat the aforementioned steps to refine the possibilities that remain.

A panel of scientists assembled by the American Association for the Advancement of Science in the late 1980s agreed on five criteria to determine whether an investigation can be considered to be scientific[45]:

1. Science demands evidence.
2. Science is a blend of logic and imagination.
3. Science explains and predicts.
4. Scientists try to identify and avoid bias.
5. Science is not authoritarian.

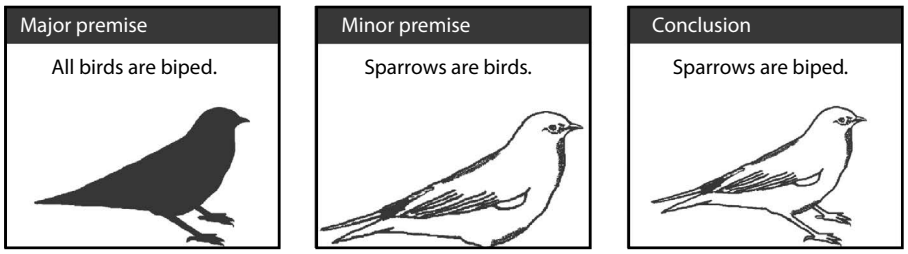


FIGURE 2.12 Syllogism. A basic syllogism consists of deriving a conclusion from a major premise and a minor premise.

More recently, David Paydarfar and William Schwartz, professors at the University of Massachusetts Medical School, offered five principles for the conduct of successful scientific research[46]:

1. Don't rush; explore all possible alternatives.
2. Read the pertinent literature, but do not allow it to stifle your imagination.
3. Pursue quality for its own sake.
4. Always look at the raw data.
5. Cultivate smart friends.

In my opinion, there is one commonsense principle that surpasses all others in its importance for scientific research: the *principle of determinism*. This principle can be stated simply as "every effect has a cause" (Figure 2.13). The water for your coffee will not boil unless you light the stove (or provide heat by means of an electric heater, a microwave oven, etc.). A female dog will not become pregnant unless she has sex with a male dog (or is artificially inseminated). You will not get to your in-laws' house unless you drive there (or walk, or fly, etc.). The principle of determinism may not apply in full to the most fundamental level of reality involved in quantum mechanics,[47,48] but it is a very safe guiding principle in all other areas of scientific inquiry. As statistician Bradley Efron puts it, "a scientist at work relies on the assumption that nature has no will and runs by rules that make no exceptions: no magic, no miracles, no answered prayers, no appeal to higher authority."[49] Accordingly, the principle of determinism is considered to be a *core principle* of physiology.[50]

Naturally, an effect may have more than one cause. This is why serious research always involves a *control group.* If you want to find out whether sex causes pregnancy, it is not enough to pair, say, six male dogs with six female dogs; you must also have six female dogs that are not paired with males. The fact that the six paired dogs get pregnant, while none of the unpaired dogs does, allows you to conclude that sex is the cause of pregnancy. Without the control group, you would not be able to exclude an infinite number of alternative explanations, such as "the spirit of pregnancy fell upon the female dogs at the same time as they were paired with the males." If the "spirit of pregnancy" did fall upon the dogs, it should have fallen upon all 12 bitches, not just upon the 6 paired ones. Of course, the pairing with a male (without actual sex) could be the stimulus for the fall of the spirit. If you had this suspicion, your control group would consist of female dogs paired with infertile male dogs. Clearly, the idea is to have a control group that is identical to the experimental group except for the element that you are studying. Since the control group is not exposed to the cause, it does not display the effect. No cause, no effect. No heat, no hot coffee.

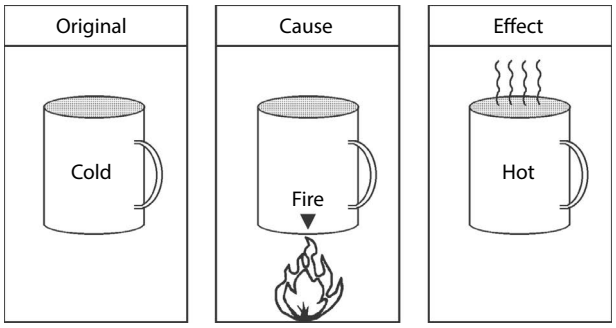


FIGURE 2.13 The principle of determinism. The principle of determinism asserts that every change in nature requires a cause. To warm up some coffee, heat is needed. No heat, no hot coffee.

In clinical studies, experimental control involves one additional element: the *placebo* control. Placebo, as you probably know, refers to fake medication—sugar pills intended to please the patient without having any real pharmacological effect. There is much more to it, though.[51,52] It has long been known that placebo medication actually improves the condition of a small but significant number of patients. This means that the mere belief that one is receiving adequate medication may improve one's condition. It is a *psychological* cure, in the sense that some unknown process in the brain has the same effect on the target organ as the intended drug has. Consequently, if we want to know what the effects of the actual drug are, our control group must be a placebo group. Any changes observed in the placebo group will be due to *psychological* processes, whereas changes in the experimental group will be the result of the combination of psychological processes and the specific effect of the drug. Thus, if the results indicate improvement in 40% of the patients in the placebo group, any improvement below 40% in the experimental group will be meaningless (and may even indicate that the drug is actually hurting the patients).

As a side note, I must point out that the notion of determinism (and causality) discussed earlier is only one of the several rival notions adopted by various philosophers along the millennia of civilization—even if it is a predominant one. The ancient Greek philosopher Aristotle, for example, recognized not one but four types of *causes*, usually designated as material, formal, efficient, and final.[53] Biologist Nikolaas Tinbergen, a 1973 Nobel Prize laureate, adapted the Aristotelian classification and proposed that biological phenomena can have not one but four causes: physiological, ontogenetic, functional, and evolutionary.[54] In modern science, it is common to recognize only one type of cause (which does not correspond exactly to any of Aristotle's four causes), but there is not a universal consensus on it. This lack of consensus becomes apparent sometimes when people disagree about whether a causal explanation is indeed a causal explanation.

An important point to be made is that believing in the principle of determinism is of no help if one does not look for the opportunity to apply it. As a matter of fact, professional magicians (entertainers) often take advantage of people's tendency to ignore the principle of determinism through what is called "survivorship bias," as exemplified in the following passage about an anecdotal Greek skeptic visiting a monastery[55]:

> He sees a row of tributes given by sailors who had faced fearsome storms, prayed to the gods, and survived. "How can you doubt the gods when all these sailors have testified that they prayed and were saved?" he is asked. "Simple," he replies, "the ones who prayed and drowned aren't around to tell us about it."

Moral of the story: If you don't demand a causal explanation, you will be fooled by hidden evidence. The use of *horoscope* is an even better example of failure to apply the principle of determinism. There is no scientific evidence whatsoever that the alignment of planets in the solar system has an effect on the behavior of human beings on Earth (except, of course, that awareness of the alignment causes superstitious people to behave differently). Yet, hundreds of thousands of people read their horoscopes each day. Some do it just for entertainment, but many do it because they believe that the particular alignment of planets will actually affect their daily lives. They probably never thought about testing whether horoscopes actually work. By chance (if not by suggestion), some of the predictions of the horoscope do turn out to be true once in a while, and the credulous reader seems to be satisfied with that. However, should the credulous reader bother to write down all the predictions each day and have somebody count how many predictions turned out to be correct and how many turned out to be wrong, he or she would soon realize that the predictions are no better than random guessing. Let us say that all that the astrologer had to do would be to predict whether the horoscope reader will have a good day or a bad day. Obviously, the odds that the astrologer would guess correctly 100 times out of 100 are very small—but even the credulous reader does not expect such an outstanding performance. What about 10 out of 10? The odds of succeeding by guessing are not that small in this case, but the situation is still unrealistic. More likely, the astrologer would predict correctly perhaps 7 out of 10 times. The credulous reader would consider this outcome very good and would not even bother to consider the odds of this outcome happening by chance (that is, by pure guessing). In actuality, standard statistical procedures allow us to calculate that the odds of this event are higher than 1 in 20—and I doubt that this same person would ever drive on a highway if the odds of having a fatal accident were 1 in 20. Thus, if the horoscope reader would seriously put to test the hypothesis of a causal link between planet alignment and human predisposition, he or she would refute the hypothesis.

A less mundane and more instructive case is that of *therapeutic touch*. No more than a few years ago, therapeutic touch was a widely used procedure employed by professional nurses who claimed to heal (or at least to improve) medical conditions by moving their hands around the patient to affect the patient's *energy field*. Because the ability to heal depends on the ability to perceive (and then to manipulate) the patient's energy field, a simple test of the procedure can be—and was—devised. Using a young child as a member of the research team, researchers were able to convince 21 practitioners of therapeutic touch to participate in a study. The practitioner sat down with his or her hands palms-up resting on a table that had a tall opaque divider blocking the view of the child sitting across the table. In 10 trials at a time, the child would place her hand palm down about 9 cm above either the left or the right hand of the practitioner. All the practitioner had to do was to identify which hand had been *stimulated* by the child's energy field. When all the data were collected and analyzed, it became clear that the practitioners did not perform better than chance.[56] Despite their claims, they were not able to detect the child's *energy field*.

The moral of this actual story is, again, that believing in the principle of determinism is of no help if one does not look for the opportunity to apply it. To use the terminology of the renowned logician Karl Popper,[57] scientific statements (as opposed to superstitious ones) are *refutable*—although not actually *refuted*—by experimentation. A *refuted* hypothesis is, of course, of no use. However, a hypothesis that is not *refutable* is also useless. Consider the following hypothesis: "Angels have 25 pairs of chromosomes." This hypothesis is useless because it is not refutable. Since angels are immaterial, we cannot test the hypothesis. Now consider another hypothesis: "All sparrows are gray" (Figure 2.14). We can test this hypothesis by going outside and looking for sparrows. If we see only gray sparrows, we will feel like "accepting" the hypothesis, even though we will not be really certain about how many gray sparrows we need to see before we actually accept the hypothesis. However, if we see just one white sparrow (see the rightmost rectangle in Figure 2.14), we can be confident that the hypothesis is wrong—and that it should be refuted. Popper used this argument to defend his view that there is no *inductive logic* (and that scientists use *deductive*

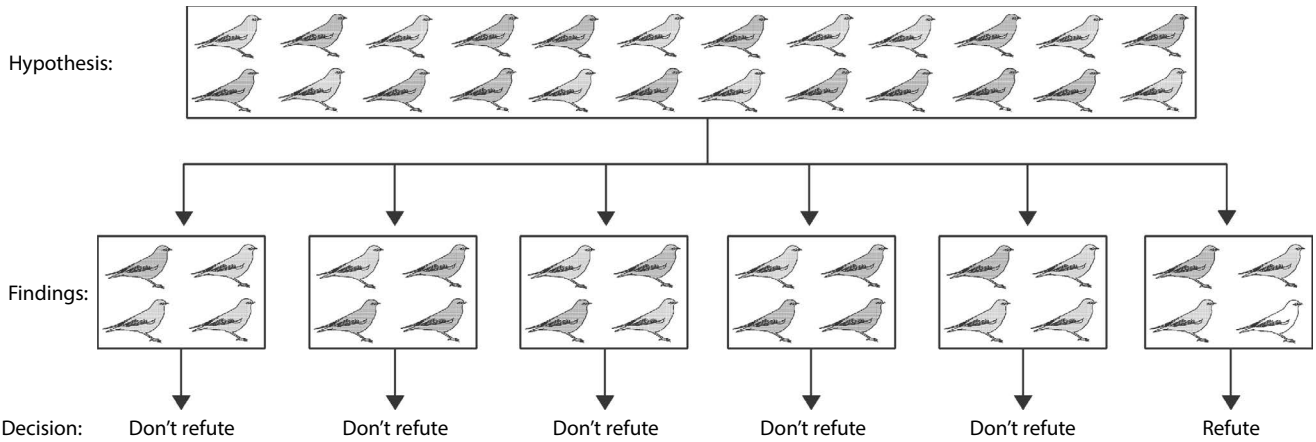


FIGURE 2.14 The refutability of hypotheses. If one has the hypothesis that all sparrows are gray, one can hold on to the hypothesis so long as one sees only gray sparrows. However, the sight of a single white sparrow is enough to refute the hypothesis.

logic), but we need not get into this issue here. It is sufficient to emphasize that the principle of determinism provides no help if we don't bother to test our hypotheses. To do science, we need refutable hypotheses.

It must be noted that disregard for hypothesis testing is not restricted to the realm of superstition. As a matter of fact, it is very common even among scientists. Many scientists seem to inadvertently disregard Platt's advice mentioned earlier (namely, "Devise alternative hypotheses to explain the phenomenon that you are investigating"). The scientific literature is filled with reports that postulate a causal relationship between variables when all that was determined was a *correlation* between them. Yet, a correlation between variables does not prove anything about the nature of cause and effect. Consider Figure 2.15. It has been reported that children who are breast-fed as infants are more intelligent when they become adults (Panel A). That is, there is a correlation between breast-feeding in infancy and intelligence in adulthood. A common, although erroneous, inference is that the mother's milk contains some substance that enhances intelligence. The inference is erroneous because the correlation itself does not specify a causal link. Two possible alternatives for the causal link are that mothers who are more intelligent tend to breast-feed their infants more often than other mothers do (and, of course, intelligent mothers tend to have intelligent children) or that infants who are breast-fed spend more time in close contact with their mothers and receive more stimulation (so that the stimulation, not the milk, would be the cause of the greater intelligence). The existence of the correlation says nothing about cause and effect. In order to find out if the mother's milk does contain some substance that enhances intelligence, one would need to set up an appropriate experiment. For instance, one could take a sample of babies and randomly assign them to two groups: one to receive mother's milk and one to receive cow's milk (or infant formula), but both being bottle fed (Panel B in Figure 2.15). If, in this case, the babies who were fed mother's milk grew up to be more intelligent than the babies who were fed cow's milk, then the researcher would be justified in speaking of a causal link (that is, a refutable hypothesis was tested, and it was not refuted). Introductory statistics textbooks always warn readers that "correlation does not imply causation,"[58–61] but it would seem that many students forget about it by the time they finish graduate school and become research scientists.

I must note that the urge to infer causation from correlation has led recently to strong efforts to make such an inference legitimate. For example, Judea Pearl, a computer science professor at the University of California in Los Angeles, has for many years defended the idea that cause-and-effect inferences can be made from correlational data as long as one can develop "path diagrams" that are complex enough to incorporate all possible connections between the correlated variables.[62] The main obstacle, of course, is to determine what a sufficiently complex model is.[63] More radically, scientists involved in the analysis of very complex systems (such as ecosystems) have proposed that causation can be established *even in the absence of correlation*. They claim that changes in predictability of temporal patterns of one variable when another variable is removed from the system can be used instead of measurable correlation (and path diagrams) to identify causation between time series variables.[64]

Finally, as the great French mathematician Pierre Simon de Laplace wisely pointed out in 1814, there is a grandiose philosophical implication of the principle of determinism: if we really believe in it, then we must consider the current state of the universe to be the effect of its past state as well as the cause of its future state. Consequently, someone who knew all the forces of nature at a given time would be cognizant of nothing less than the past and the future of the Universe.[65]

2.1.3 Science and Religion

When one emphasizes the essential role of refutable hypotheses in the advancement of knowledge, one cannot avoid contrasting science and religion. Requiring refutable hypothesis is akin to favoring *reason* over *faith*—as in *religious faith*. Because most nonscientists (and many scientists as well) have a religious faith, it is important to ask whether religions can be scientific, whether scientists can be religious, and whether any of this matters. These questions deserve careful examination.

On one hand, traditional religions have no interest in being scientific. They encourage man's search for knowledge about the world, but their mission is of a moral nature. Religious faith is not scientific, but it usually does not wish to be.

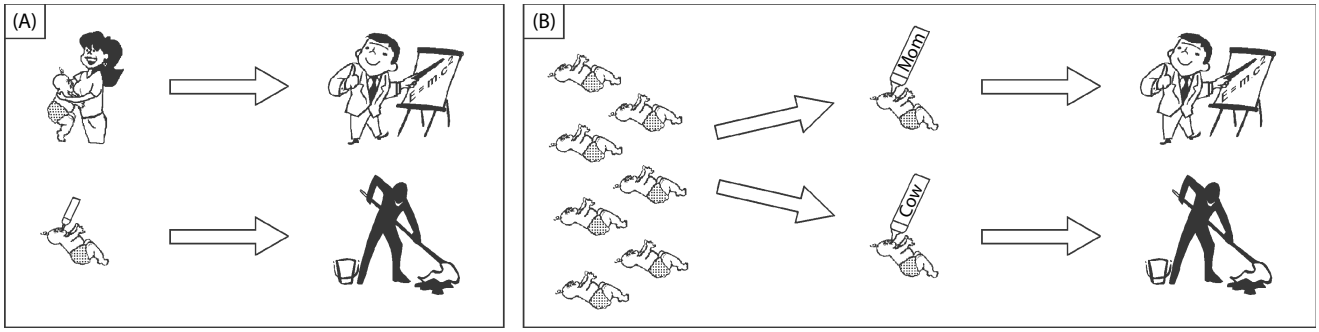


FIGURE 2.15 Correlation and causation. Although a correlation is often suggestive of causation (A), only controlled experiments allow reliable inferences about causality (B). See text for details.

Jesus Christ's Apostle Paul believed that faith is a gift from God (*Ephesians* 2: 8–9), and the great philosopher Immanuel Kant pondered that accepting a religious doctrine against rational arguments is a demonstration of greater faith (and, therefore, of greater religiosity).[66] On the other hand, dogmatic faith is incompatible with scientific inquiry, and it has been argued convincingly that religious education can be detrimental to science education[67] and that religious thinking can be detrimental to scientific research.[68–71]

Some basic statistics will help us start the conversation: Among the general public, 91% of people worldwide believe in some form of God or belong to a formal religious organization,[72] even if textual analysis of over five million books shows that use of the word "God" in print declined significantly from the 1800s to the 2000s,[73] and analysis of census data indicates that religious affiliation is plummeting in developed countries.[74] Among university professors, 77% of people believe in some form of God.[75] Among scientists, the figure is lower at 61%, and it goes down to 7% among the very top scientists.[76]

If most nonscientists are religious and most top scientists are nonreligious, one wonders how scientists become nonreligious. It is unlikely that scientists actually become nonreligious. Much more likely, people with nonreligious inclinations are attracted to science. Why are people with nonreligious inclinations attracted to science? Probably because the thought processes required for scientific research are discordant from those required for religious faith. It is likely that what brings one to science—and away from religion—is a belief in the primacy of tangible evidence and in the ultimate reality of matter. If one is a *materialist*, one does not believe in spirits, and certainly not in the Supreme Spirit—that is, God.

Let us briefly review some of the reasons why people who were raised to believe in God end up becoming atheists (or *agnostics*, which is simply a euphemism used by those who feel coerced by the religious majority) and later on become scientists:

Many of us remember when we were children and were excited about the Tooth Fairy, the Easter Bunny, and Santa Claus (Father Christmas)—or equivalent fictitious entities in other cultures. Most of us remember how we found out that the Tooth Fairy was not real. It was quite simple: the Tooth Fairy was too fallible to be an actual supernatural entity. That is, our parents did not take the Tooth Fairy legend seriously enough to make sure that every lost tooth without exception was rewarded by money under our pillow, much less to make sure that more painful teeth were rewarded with larger sums of money. But how would the real Tooth Fairy not be aware that we lost another tooth on that night when our parents were too busy entertaining guests? Or how would the Tooth Fairy not know that this one tooth was really painful and worthy of much more money than the easy tooth that we lost last month?

After the Tooth Fairy, it was time for the Easter Bunny to be debunked. As we started to learn the difference between mammals, reptiles, and birds, it became impossible to believe that a rabbit would lay eggs—chocolate eggs, no less! By the time we were teenagers, Santa Claus also started to abound in inconsistencies: How come there was a different Santa Claus in each shopping mall? How could Santa Claus deliver toys to billions of children all over the world in one night? How could Santa Claus have accidentally taped the label from a local store on a present built by elves in the North Pole? And so on.

For the majority of us who grew up to become scientists, God was the next supernatural being to be debunked. The fact that there were at least five major religions in the world (Buddhism, Christianity, Hinduism, Islam, and Judaism—see Figure 2.16), and that their teachings were in many respects incompatible, was a major blow to the notion of absolute truth that we had been taught to associate with God. It was certainly possible that one of these religions was correct and the others were wrong, but how would we know which one was the correct one? If religions were compilations of truths, one would expect them to convince and recruit converts more or less uniformly all over the world. Yet, most people believe in the religion that they were taught when they were children, which indicates that belief in a given religion is not the result of reasoning but the consequence of indoctrination into the religion of our particular region of the world. Indeed, examination of a map of the world according to the language spoken in each region (Figure 2.17) makes clear that there is a close connection between language and religion. But, if people do not claim that one language is superior to another, why do they claim that a religion is superior to another? And, if one religion is not better than another, why would anyone believe that any religion is right?

Still, because we all grew up in a world where most people are religious, we scientists-to-be wondered whether our scientific way of thinking might somehow be misguided. If God is a delusion,[77] why do so many people believe in God? Many "atheist" philosophers, scientists, and artists have suggested explanations for it. Nietzsche explained religion as an effort of miserable people to console themselves with the idea that they would have a much better existence in the afterlife.[78]



FIGURE 2.16 Diversity of religions. These five symbols represent the five major religions in the world today: the Buddhist Wheel of Dharma, Christian Cross, Hindu Aumkar, Islamic Star and Crescent, and Jewish Star of David. (From Wikimedia commons.)

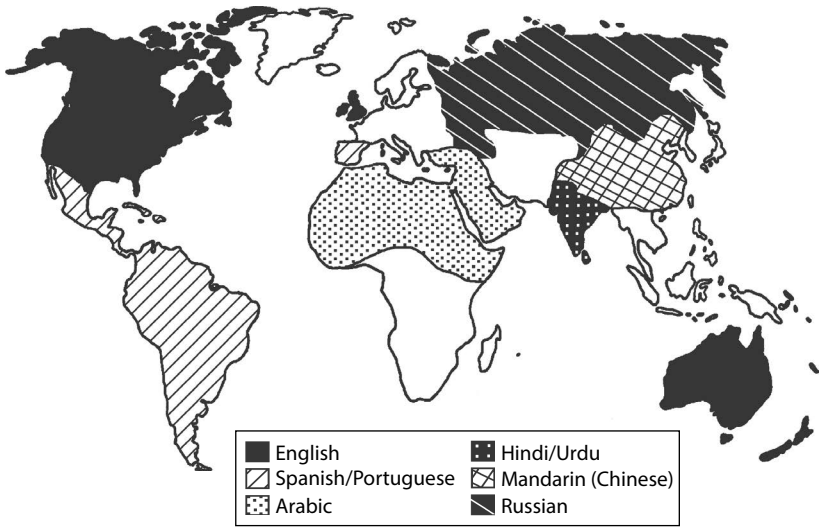


FIGURE 2.17 Languages of the world. The world distribution of languages is very similar to the world distribution of religions. The predominant language and predominant religion of different parts of the world are paired thus: English with Christian Protestantism, Spanish/Portuguese with Christian Catholicism, Arabic with Islam, Hindi/Urdu with Hinduism, Mandarin with Buddhism, and Russian with Orthodox Christianity. (Data from *TIME Almanac*, TIME Books, Des Moines, IA, 2009.)

Freud considered religion to be a form of collective neurosis.[79] Marx called religion the opium of the people.[80] Comte suggested that individual humans (and human societies) progress through three stages of thinking, with religious thinking being the first stage, later replaced by metaphysics and then finally by "positive" thinking.[81] Making use of parody instead of philosophical argumentation, musician Ian Anderson from the British rock band Jethro Tull suggested that Man created God, and gave God a multitude of names, and fantasized that God might be Man's Lord (*Aqualung*, Island Records, 1971). Similarly, British comedian Ricky Gervais suggested in his movie *The Invention of Lying* (Warner Brothers Pictures, 2009) that religion was invented to help people deal with the pain of losing loved ones and the agony of not knowing the answer to the perennial questions regarding the origin of the universe, the nature of good and evil, the meaning of life, and so on. Paleontologist Gregory Paul suggested that religiosity relies on dysfunctional psychosociological conditions, so that religious beliefs tend to be discarded as human societies improve their socioeconomic conditions.[82] Consistently with this latter suggestion, survey data across 132 nations indicated that religion is less important to people of wealthier countries.[83] Psychological research provided suggestive evidence that, when personal control is threatened, people try to preserve a sense of order by believing in an interventionist God.[84,85]

Whatever the reasons were for the creation and permanence of religions, there is no arguing with the fact that most people alive today are religious to some extent. Although it would not be correct to say that humans have a mind for religion, it does seem to be the case that religious concepts fit particularly well with the minds that humans have.[86] Sacred values associated with religions act as moral imperatives that facilitate adherence to societal rules and promote cooperative endeavors that generate commitment in low-power groups to face off (and often prevail over) stronger enemies.[87] Importantly, religious faith seems to be helpful to people irrespective of what exactly they have faith in. Psychological research on depression in terminally ill patients showed that well-structured religious worldviews effectively help people handle death concerns near life's end.[88] Notably, the thoughts need not even be religious: plain superstition (such as crossing one's finger for luck) can improve performance in various tasks, presumably because it boosts the participant's confidence in his or her ability to complete the task.[89]

Now, if, as I suggested, the real issue is the choice of materialism over spiritualism (or vice versa), then we must address what is perhaps the most effective "recruiting tool" of religions. Just like the relativist scared the realist by forcing him or her to admit at least the possibility of alternate worlds (see Section 2.1.1), spiritualists often force materialists to admit at least the possibility that there may be something beyond matter. Just in case there is eternal life after death, isn't it worth wasting a few hours on Earth fearing God and preparing for the afterlife? This thought has shaken the confidence of many a materialist over the centuries. Ironically, the table has now been turned. The materialist can now scare the spiritualist by telling him or her that he or she is throwing away the only chance of eternal life precisely by believing in a spiritual illusion. Because of advances in neuroscience and, especially, in computer science, it may very soon be possible to "download" one's mind to a computer, thus ensuring immortality of the mind.[90] If this does happen, then materialists will have eternal life, while the minds of all of those who prayed for God and believed in a spiritual soul will simply decompose along with their bodies after death.

And yet, despite the ultimate incompatibility of science and religion, it often happens that a religious scientist can appeal to faith in religious matters but resort to scientific procedures in empirical matters. In such cases, religion and science can coexist peacefully in the same individual—or at

least so claimed a few well-known scientists and philosophers who had religious faith.[91,92] In other instances—such as that of members of some Protestant denominations who perceive a conflict between *creationism* and *evolution*—religious faith requires abandonment of the scientific approach. In these cases, religious and scientific interests have irreconcilable differences.[93–95]

Various individuals and religious organizations in the United States and other countries around the world have attempted, sometimes successfully, to introduce creationism in the biology curriculum of elementary and secondary education. Creationism is the Biblical mythology of the instantaneous creation of all animals by God, as described in the book of *Genesis*. Creationism is material extraneous to the biology curriculum. It is supported by political groups rather than by experts in the biological sciences and is inconsistent with fundamental principles of evolutionary biology.[96–98]

Although most of the world's religions do not adopt a creationist perspective, various Protestant Christian sects in the United States see creationism as a fundamental element of their faith. For the members of these sects, the Bible must be interpreted literally and, of course, the word of God is much more reliable than the word of human scientists. Thus, if evolutionary biology says that organisms progressively evolved from simple life forms into complex organisms such as mammals (including humans), whereas the Bible says that God created all animals at once (and created humans as distinct, superior beings), then a man of faith should reject biology and trust divine knowledge (Figure 2.18).

Protestants (that is, Baptists, Episcopalians, Evangelicals, Lutherans, Methodists, and Presbyterians) make up slightly less than half of the adult population of the United States,[99] and, consistently, almost half of all adults in the United States are sympathetic to creationism.[100,101] Although technically a minority, this group of individuals has historically played a major role in American politics. Its members feel that their religious faith is closely connected to their patriotism and see their own efforts to introduce creationism in the biology curriculum as a legitimate strategy to preserve the American way of life. This is all understandable, but Protestantism is, and has always been, only one of many elements of the American culture. More fundamental than Protestantism in the American culture is, and has always been, the pursuit of liberty and justice. The First Amendment to the U.S. Constitution, enacted in 1791, warns that "Congress shall make no law respecting an establishment of religion," which means that citizens shall be entitled to freedom *from* religion just as much as they shall be entitled to freedom *of* religion. The conservative Protestant groups claim that, because early European colonizers of North America were Protestants, the United States is a "Christian nation." However, as early as 1797, the U.S. Congress ratified the statement that "the Government of the United States of America is not, in any sense, founded on the Christian religion."[102] Thus, the United States is a country with strong Christian history, but it is not a Christian nation.[103,104]

Legally, when the Protestant belief in creationism conflicts with the constitutional principle of the separation of church and state, creationism must be the one to retreat. The judicial system of the United States, including the Supreme Court, has repeatedly stated so in cases such as Epperson v. Arkansas (1968), McLean v. Arkansas (1981), Segraves v. California (1981), Edwards v. Aguillard (1987),

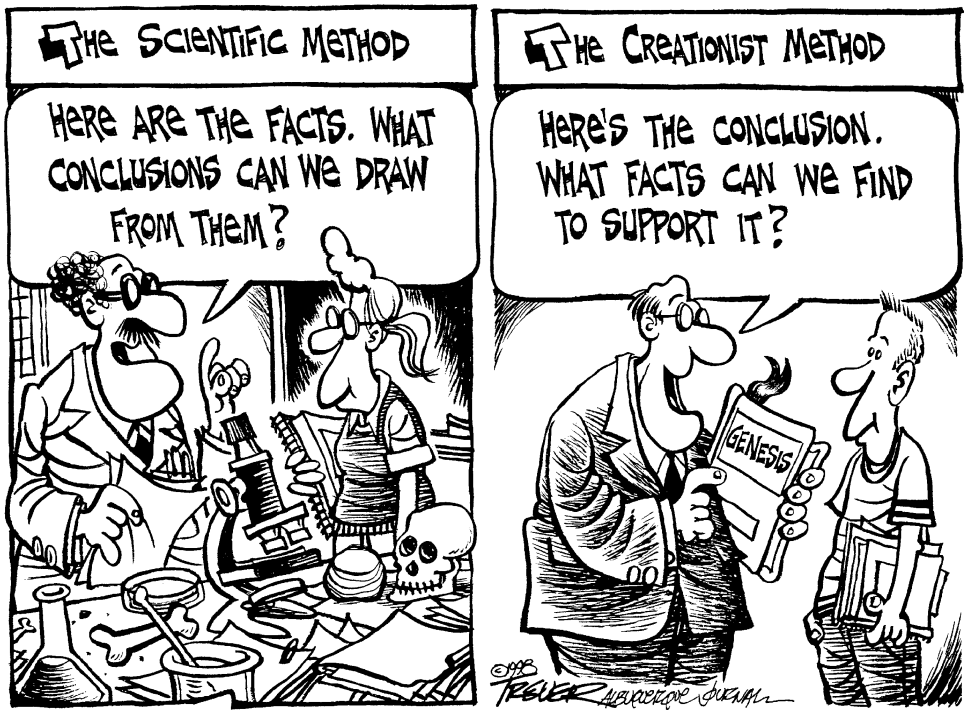


FIGURE 2.18 Different approaches to learning. This cartoon by John Trever characterizes well the difference in the approaches that scientists and creationists use to learn about the world. (Copyright 1998 by John Trever. Reproduced by permission of PoliticalCartoons.com.)

Webster v. New Lenox (1990), Peloza v. Capistrano (1994), Freiler v. Tangipahoa (1999), LeVake v. Independent School District (2001), and Kitzmiller et al. v. Dover Area School District (2005). Since the September 11 massacre in 2001, many Americans have decried the behavior of religious fanatics who placed their religious faith above the fundamental values of life. Creationists do not seem to realize that, by eroding the teaching of modern biology, they may one day succeed in aborting progress in biomedical research altogether and eventually be responsible for the deaths of many more Americans than those achieved by the al-Qaeda terrorists—all in the name of religion.

It should be emphasized that the same constitutional principles that prevent the teaching of creationism under the guise of science stand to guarantee the rights of religious groups to profess their faith in private and in public and even to teach it openly in public schools in courses on comparative religion, philosophy, or history. Evidently, some religious groups are not content with these rights. They wish to place their literal interpretation of the Bible at the same epistemological level as science. Having realized that progress in scientific knowledge is slow and imperfect, they feel that they may have a chance to disguise creationism under the cloth of a science, or at least of a scientific theory. They call it the *theory of intelligent design* and ask state school systems to incorporate the theory in the biology curriculum. According to the theory, living beings are too exquisitely complex to have evolved by natural selection acting on random mutations; they must instead have been abruptly created by an intelligent agent.[105,106]

In very general terms, a request to make additions to the educational curriculum should always be welcome—and intelligent design is no exception. All requests, however, must be evaluated by a qualified committee. When your car breaks down, you don't ask a religious leader to fix it. Instead, you take it to a mechanic. Likewise, when a new scientific theory emerges, it should be scientists, not religious advocates or politicians, who decide whether the theory should be added to the curriculum. If scientists judged that intelligent design is a legitimate scientific theory, then it would certainly be considered for inclusion in the curriculum. However, intelligent design is such a poor theory that it cannot even disguise its main purpose of sneaking religion into the lay curriculum.

Intelligent design is not a scientific theory because it rejects the very first requirement of a scientific theory, namely, that it be open to refutation by repeated experimentation. Scientists do not hesitate to refer to evolution as a *theory*, and creationists often use this technical label as an argument to question the validity of evolutionary principles. What eludes creationists is the fact that scientists use the word *theory* precisely to emphasize that what we believe to be the truth today is open to refutation by continuing experimentation. There is no safe place for dogma in science. Yet, dogma is all there is in the theory of intelligent design. Even for a nonscientific theory, intelligent design is a poor theory for two reasons: (1) there are plenty of cases in which explanations of complexity in the world *do not require* an intelligent designer and (2) the world is filled with evidence of *stupid* (rather than *intelligent*) design.



FIGURE 2.19 Intelligent alien design. These lenticular clouds over the Rocky Mountains in Colorado look like they could be part of a fleet of alien flying saucers. (Courtesy of UCAR Digital Image Library, National Center for Atmospheric Research, Boulder, CO.)

It is logically possible that an intelligent designer created the whole world, including the organisms that populate it. But what is logically possible is not always true or necessary. Look at Figure 2.19, which is a genuine photograph of clouds above the Front Range of the Rocky Mountains in Colorado.[107] The objects above the mountains on the left side of the picture look very much like flying saucers and, consequently, could be explained as the result of the intelligent design of extraterrestrial beings. Yet, the mysterious objects are simply *lenticular clouds* that form downwind from an obstacle in the path of a strong air current. The extraterrestrial explanation is logically plausible but totally unnecessary. According to the principle of Ockham's Razor, which has guided the human quest for knowledge for centuries, one should choose the simpler theory whenever two theories of different complexities can equally explain a phenomenon.[108] If intelligent design wishes to be considered a scientific theory, the first thing it must do is to discard the concept of intelligent design itself!

The proponents of intelligent design correctly point out that many biological structures and processes are so elaborate that one may feel inclined to infer the existence of an intelligent designer. However, many other structures and processes are so awkwardly arranged that, if we were to use the same reasoning, we would be inclined to infer the existence of a stupid designer. Of course, there is no conceptual impediment to a *theory of stupid design*. However, such theory would be of no use to religious groups, as it would imply a blasphemy: that the creator of the world did not always act intelligently. The very idea of intelligent design requires the complementary idea of stupid design, which ruins the strategy of sneaking religion into science.

Examples of stupid design are well known to school children. They include the presence of the appendix in the human digestive tract, the temporary presence of a tail in human fetuses, the presence of eyes in subterranean animals that are never exposed to light, the presence of penis-like vaginas in female spotted hyenas, and many others. These senseless structures are much more easily explained as mere remnants

in a haphazard evolutionary process than as blunders of an intelligent designer.

In conclusion, the theory of intelligent design is not only nonscientific but also plainly a bad theory. It is a poor attempt to disguise the intrusion of religion in the educational arena. It does not belong in science classes.

It is understandable that citizens whose religious beliefs are incompatible with the biological sciences feel threatened by science and try to subvert it. They have a constitutional right to do so, as long as they honestly admit that their arguments are based on religious faith rather than on scientific evidence. Placing religious mythology above intellectual honesty and respect for life may be acceptable for members of some religious sects, but it violates the fundamental principles on which civilization stands.

2.2 RESEARCH ON POPULATIONS AND ORGANISMS

As in many other fields of biology, research in circadian physiology usually involves one or more of four major categories of experimental procedures: recording, stimulation, lesioning, and transplantation (Figure 2.20). Recording a physiological variable is the simplest and usually least invasive of the four procedures. It is also, by far, the most widely used procedure in circadian physiology. The specific instruments and methods that are used for recording are generally the same as those used in traditional biological research, except that adjustments are needed to ensure long-term monitoring of the processes under study. For the study of circadian rhythms, data must be recorded for at least several consecutive days. The adequate temporal resolution of data acquisition depends on the variable being measured. Generally, one data point every 6 minutes (0.1 hour) is adequate, but higher temporal resolution may be needed in some applications, and lower resolution may be imposed by methodological limitations in other applications.

Circadian physiologists seldom study actual populations, such as a group of horses in the wild (Figure 2.21). When they do study populations, they may make use of *satellite telemetry* (Figure 2.22). In this case, a signal emitter is attached to one or more of the subjects, and the location of the emitter is tracked by an orbiting satellite.[109] More commonly, individual subjects are studied via land-based *radiotelemetry*[110] (Figure 2.23) or by the satellite-assisted Global Positioning System (GPS).[111,112] Manufacturers of equipment for satellite telemetry or radiotelemetry for use in the wild include Lotek Wireless Co. (Newmarket, Canada), North Star Science and Technology (Baltimore, Maryland), Sirtrack Ltd. (Havelock North, New Zealand), Telenax Ltda. (Playa del Carmen, Mexico), Telemetry Solutions Inc. (Concord, California), and Telonics Inc. (Mesa, Arizona).



FIGURE 2.21 Horses in the wild. The study of behavior and physiology of populations of organisms is one of many facets of circadian physiology. (Photo courtesy of Tim McCabe, United States Department of Agriculture's Photography Center, Washington, DC.)

In the laboratory, social interaction is usually studied by visual inspection of video recordings, often using time-lapse photography.[113–115] Recent advances in video-tracking software have made it possible to computerize the video analysis of social interactions, at least in mice.[116]

Locomotor activity of individual animals in the laboratory is most often monitored by *infrared motion detectors* or *running wheels*. Traditionally, miniature motion detectors have been used for very small animals, such as insects,[117–119] whereas running wheels have been used for rodents[120–122] (Figure 2.24). The use of infrared detectors is predicated on the assumption that the organism under study cannot see

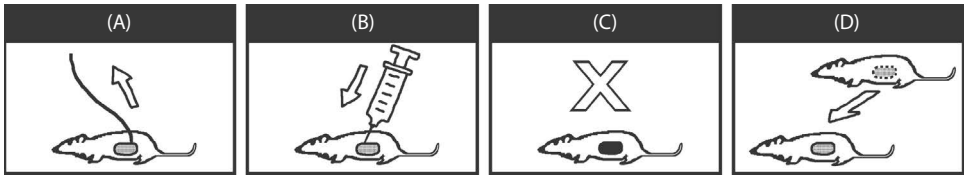


FIGURE 2.20 Research methods in physiology. Research on the vital processes of living organisms always involves one or more of four basic methods: (A) recording, (B) stimulation, (C) lesioning, and (D) transplantation.

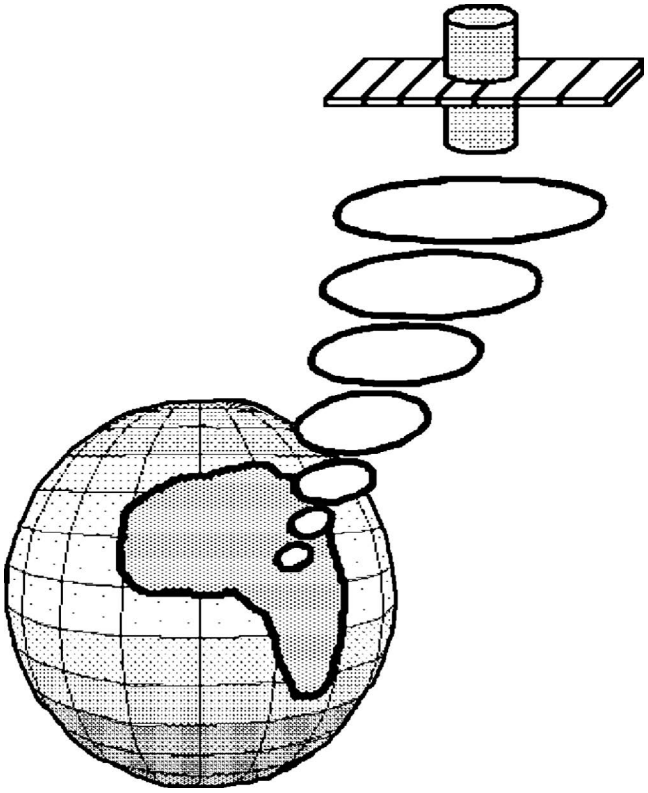


FIGURE 2.22 Satellite telemetry. Signals traced by satellite can be used for the study of populational rhythms in natural settings.

electromagnetic radiation in the infrared range, which is generally true for many but not all animals (see Chapter 11). Much less common is the use of a microwave radar system[123] or of a force transducer system.[124]

Running wheels are the "gold standard" for the monitoring of locomotor activity in rodents, although many laboratories have recently switched to infrared motion detectors or implantable telemetry devices. As discussed in greater detail below (regarding the monitoring of body temperature), implantable telemetry devices have the disadvantage of requiring surgical implantation but the advantage of being nondisruptive after the unit has been implanted. Infrared motion detectors are totally nondisruptive.

When first introduced to running wheels, rodents may take a couple of weeks to reach top performance,[125] but performance is stable afterward, until impairment due to aging occurs (see Chapter 9). Running wheels are the gold standard not only for historical reasons but also because rhythm robustness is much greater in running-wheel activity than in general activity (which provides greater sensitivity to the effects of experimental manipulations). In golden hamsters, for example, running-wheel activity is almost three times as strong as general activity.[126] Two issues deserve special attention in this matter:

1. It could be claimed that running-wheel activity is an arbitrary subset of general activity, because animals only run in the wheel after they are awake and alert and because they stop running before they wind down and go to sleep. This would justify a preference for the recording of general activity. However, the onset of activity is not the result of an arbitrary cut in the activity records. Instead, running-wheel activity commences each day at the time when the animal chooses to jump on the wheel and run. The activity onset is the expression of a natural physiological process. Because running-wheel activity is more robust than general activity, it is sensible to use the former instead of the latter.
2. It is a fact that the determination of free-running period of an animal may depend on whether the animal is allowed to exercise in a running wheel.[127–135] Some researchers have interpreted this fact as an indication that access to running wheels artificially

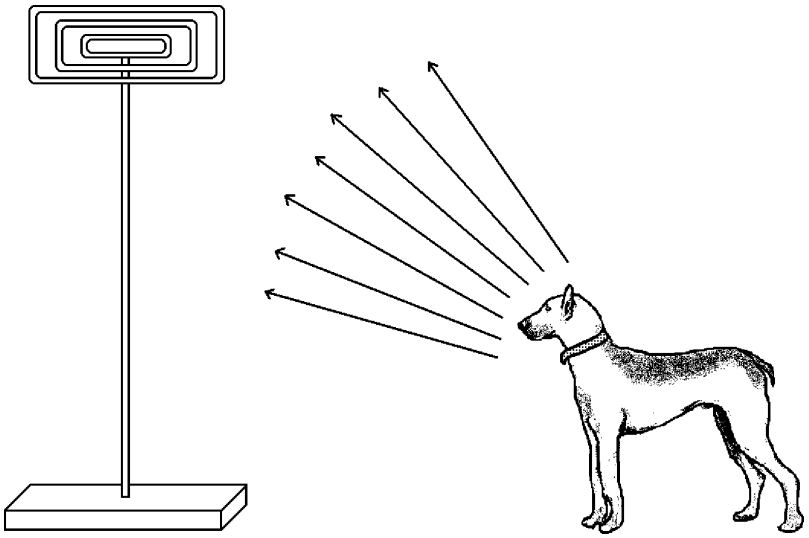


FIGURE 2.23 Radiotelemetry. Radio signals emitted by a transmitter attached to an organism (such as a collar worn by a dog) allow for the monitoring of rhythms over relatively wide areas.

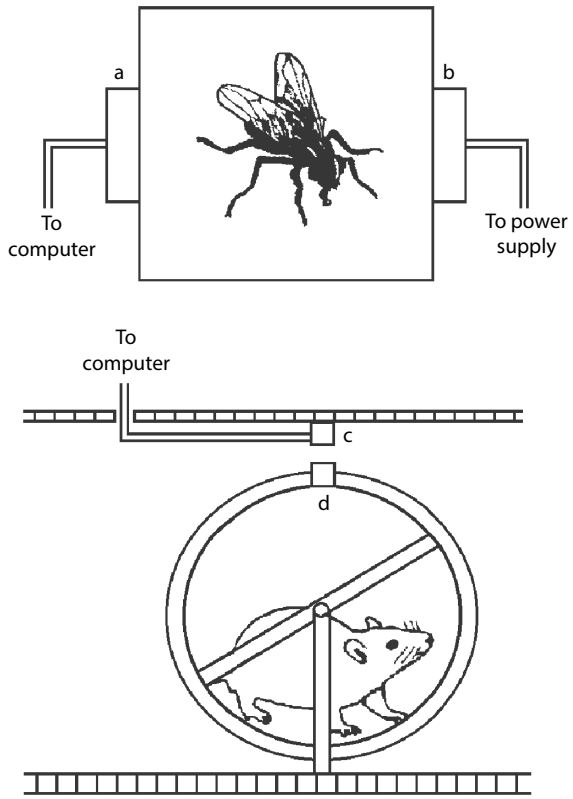


FIGURE 2.24 Monitoring activity rhythms in the laboratory. In laboratory settings, locomotor activity of individual organisms is often monitored by infrared movement detectors (a, sensor; b, emitter) or by mechanic or magnetic switches attached to running wheels (c, magnetic switch; d, magnet).

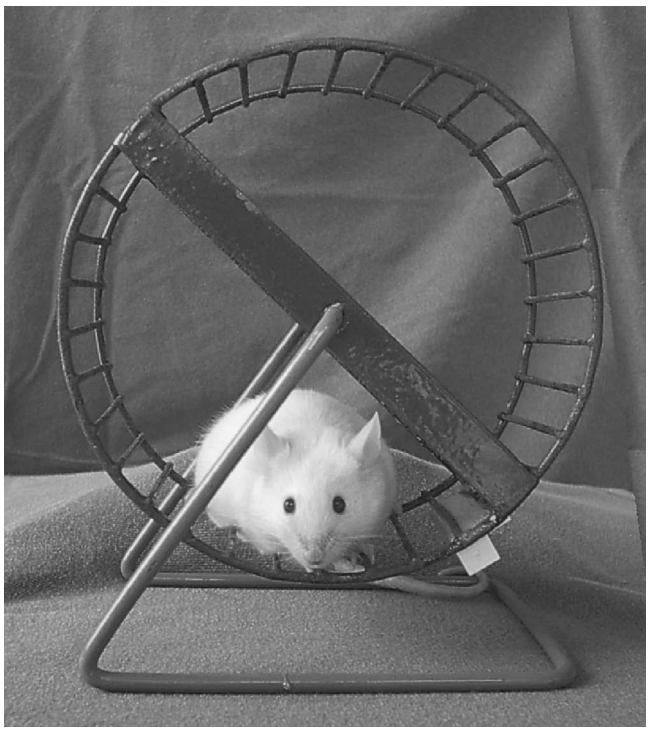


FIGURE 2.25 Mouse on wheels. An albino mouse stares at you from his running wheel.

shortens the free-running period, and these researchers prefer to use general activity instead of running-wheel activity. However, these researchers make the unjustified assumption that exercising in a wheel is an unnatural activity. Man-made wheels are not available in the wild, of course, but animals in the wild have plenty of opportunities for exercising. What is certainly unnatural is to be locked in a 30 cm × 15 cm (12 in. × 6 in.) cage for one's whole life with no opportunity for exercising. It is quite possible that the free-running period determined without running wheels reflects the unnatural sedentary life of a laboratory rodent rather than the natural circadian period of the animal. If so, then the availability of a wheel may provide the animal with the opportunity to have a more natural lifestyle and to exhibit its natural free-running period. This issue has not been definitively settled.

Commercial firms sell systems for the monitoring of activity in insects (TriKinetics Inc., Waltham, Massachusetts) and rodents (Actimetrics, Wilmette, Illinois; Columbus Instruments, Columbus, Ohio; Lafayette Instrument, Lafayette, Indiana; TSE Systems Inc., Chesterfield, Missouri), although the technical simplicity of the methods usually does not justify the high purchase price of commercial systems. Investigators can easily assemble their own data acquisition system instead. For instance, a running-wheel system can be assembled using running wheels sold at pet stores (Figure 2.25), magnetic switches sold at home improvement stores, and a personal computer. Personal computers have many interface ports that can be used for monitoring the closure status of the switches (Figure 2.26). Several simple applications have been described,[136–138] and Exercise 2.3 at the end of this chapter describes an additional one. Because computer interfaces have been undergoing standardization in recent years, with most ports now using a USB connector, researchers may find it convenient to use USB data acquisition boards such as those manufactured by LabJack Corp. (Lakewood, Colorado) or National Instruments Corp. (Austin, Texas).

Infrared motion detectors with a switch output, which can be used instead of running wheels for the monitoring of locomotor activity of mid- to large-sized animals, are available at many home and office security stores. Two such products are the CX-502 passive infrared detector manufactured by Optex Inc. (Rancho Dominguez, California) and the AD-2 activity detector manufactured by Sable Systems International (North Las Vegas, Nevada). Special circuits for the monitoring of drinking and feeding are also available commercially (e.g., Columbus Instruments, Columbus, Ohio; Research Diets Inc., New Brunswick, New Jersey; TSE Systems Inc., Chesterfield, Missouri).

For research involving human subjects (or animals equal or larger in size), wristwatch accelerometers are often used. These units can record activity unobtrusively over long

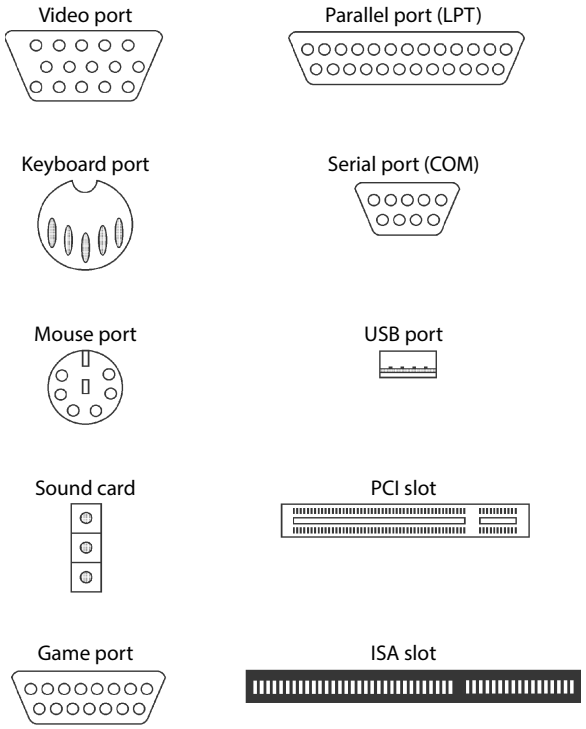


FIGURE 2.26 Standard computer interfaces. These diagrams identify the 10 interface connectors available in most personal computers. Access to PCI slots and ISA slots usually requires opening of the computer cover. In recent years, there has been a trend to replace the directly accessible interfaces with USB ports.

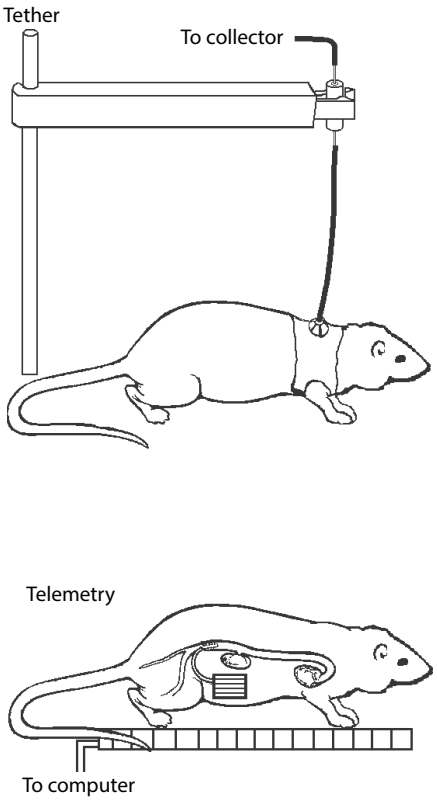


FIGURE 2.27 Monitoring physiological variables: tether and telemetry. Monitoring of physiological variables such as body temperature, heart rate, and concentration of hormones in the blood requires tether or short-range telemetry devices.

periods of time. Traditional brands include the Actigraph (Ambulatory Monitoring, Ardsley, New York), Actiwatch (Philips Respironics, Bend, Oregon), ActTrust (Condor Instruments, São Paulo, Brazil), GeneActiv (Activinsights, Kimbolton, England), and MotionWatch (CamNtech Inc., Boerne, Texas). The physical exercise fad of the mid-2010s brought to market numerous wristwatch accelerometers at a price less than a tenth that of traditional brands. These "step-tracking bands" (branded as Fitbit Charge, Garmin Vivofit, Microsoft Band, Nike FuelBand, Samsung Gear Fit, etc.) did not initially provide full access to the raw data, but they are likely to change the accelerometer market forever.

Monitoring of physiological variables such as body temperature, heart rate, blood pressure, and sleep stages (electroencephalogram) in humans can be easily accomplished with commercially available biomonitoring systems such as those marketed by AD Instruments Inc. (Colorado Springs, Colorado), Biopac Systems Inc. (Goleta, California), MindWare Technologies Ltd. (Gahanna, Ohio), and Noldus Information Technology (Wageningen, Netherlands). Similar measurements in animals require either a *tether* system[139–142] or a short-range *radiotelemetry* system[143–146] (Figure 2.27). Tethering is less expensive than telemetry, but it has the disadvantage of restricting the animal's movement in its cage. On the other hand, telemetry requires surgery for implantation of the radio transmitter, which, in some species, can cause a blunting of daily rhythmicity for up to a week.[147–150] The recently introduced BioTherm13 "passive integrated transponder" radio frequency identification device (Biomark Inc., Boise, Idaho) is slim and sturdy enough to be injected subcutaneously in rodents for continuous measurement of body temperature without the need for surgery, although the emitted signal is rather weak and provides a very limited monitoring range. In studies using human subjects and limited to temperature measurements, radio transmitters may be swallowed instead of surgically implanted,[151,152] although they stay in the digestive system for only a few days. Gut temperature (measured with a swallowed sensor–transmitter) correlates better with rectal temperature than axillary temperature (measured under the arm) does.[153] For women, an intravaginal wireless sensor similar in appearance to a contraceptive diaphragm (Figure 2.28) has been recently introduced by Prima-Temp Inc. (Boulder, Colorado). This core temperature monitoring device sends signals directly to a smartphone.

Tethering equipment is marketed by most specialized suppliers of equipment for animal laboratory research, including Harvard Apparatus (Holliston, Massachusetts), Kent Scientific (Torrington, Connecticut), and Stoelting (Wood Dale, Illinois). For biotelemetry equipment, the major manufacturers in the United States are Data Sciences Inc. (St. Paul, Minnesota), the Stellar Telemetry branch of TSE Systems (Chesterfield, Missouri), the Implantable Telemetry branch of Millar Inc. (Houston, Texas), and the E-Mitter Telemetry branch of Starr Life Sciences (Oakmont, Pennsylvania). The first three of

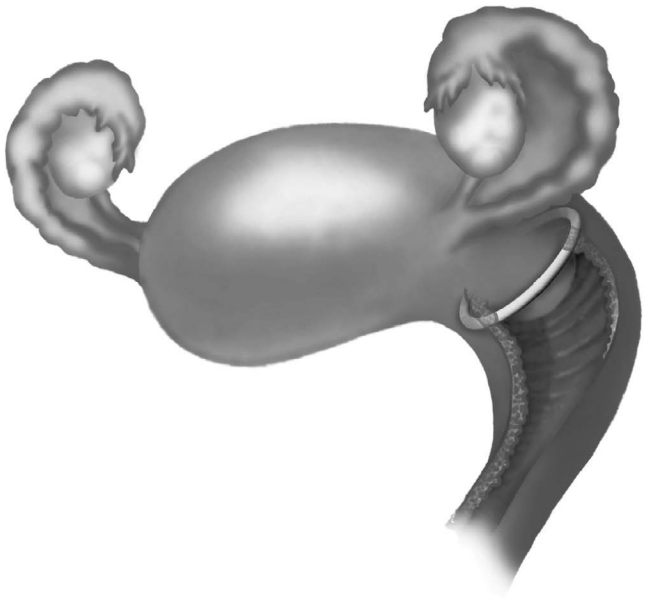


FIGURE 2.28 OvuRing. This wireless temperature sensor was developed to help women monitor body temperature changes along the menstrual cycle. (Image courtesy of Prima-Temp Inc., Boulder, CO.)

these four companies offer radio transmitters capable of measuring body temperature, blood pressure, electrocardiogram (heart), electroencephalogram (brain), and electromyogram (muscle), as well as locomotor activity. Starr Life's E-mitters can measure only body temperature, heart rate, and locomotor activity, but users have the choice of purchasing simplified transmitters that are smaller and less expensive if there is need only for the monitoring of body temperature and activity. Starr Life's E-mitters and Millar's Telemeters are *transponder* transmitters (that is, transmitters that are teleenergized by the radio receiver). This feature is especially convenient in long-term studies in which traditional transmitters will run out of battery, although transponders usually require maintenance after about 2 years of operation (thus reducing their advantage over battery-based transmitters). Worthy of mention is also the extremely small LT6 transmitter manufactured by Titley Scientific (Lawnton, Australia). These transmitters, which were designed for temperature measurements in very small animals in field studies, weigh as little as 470 mg, including the battery (whereas usual radio transmitters weigh over 2 g without the battery). On the other end of the body size continuum, TekVet Technologies (Palmetto, Florida) manufactures temperature transmitters for use in livestock. The transmitters are placed in the animal's ear, close to the tympanic membrane, thus allowing measurement of core temperature without the need for surgical intervention.

For researchers with limited budgets, I should add that, when only temperature measurement is needed, it is possible to assemble one's own telemetry system at a fraction of the cost of commercially available ones.[137,154–160] A major caveat is that considerable knowledge of electronics and substantial debugging time are usually required.

An alternative to telemetry, particularly for use with larger animals, is the *data logger*[161–164] (Figure 2.29). Data loggers (such as the actigraphy equipment described earlier) are devices that can record and *store* data. The advantage over telemetry is that the experimental subjects can move freely over large distances without causing a loss of signal (because the "receiver" moves along with them). The disadvantage is that the experimenter cannot access the data until the logger is retrieved. Manufacturers of data loggers include DataTaker Ltd. (Rowville, Australia), Onset Computer Corp. (Bourne, Massachusetts), and Pico Technology Ltd. (St. Neots, United Kingdom). A very convenient data logger for research in rodents when only temperature measurements are needed is the iButton temperature logger (Maxim Integrated Products, San Jose, California). These miniature loggers (16 mm diameter) can be surgically implanted like radio transmitters. Like larger loggers, iButtons have the advantage of not requiring a separate receiver and the disadvantage of not allowing online access to the data. The price of an iButton is less than 10% of the price of a transponder radio transmitter, and it can be

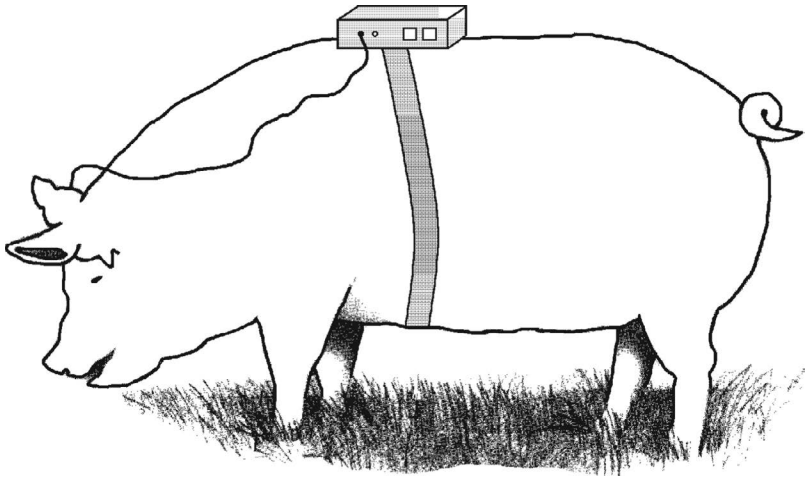


FIGURE 2.29 Monitoring physiological variables: data loggers. Data loggers are an alternative for the use of tether and telemetry devices in the monitoring of physiological variables.

modified to become small enough for implantation in mice,[165] but there are two downsides: iButtons have a resolution of only about 0.5°C and are limited to 2048 data points between downloads, which means that data can be collected for fewer than 10 consecutive days if a 6-minute resolution is used. Available at a higher price but having better resolution and being able to record data for much longer are the miniature data loggers marketed by SubCue Dataloggers (Calgary, Canada) and Star-Oddi Ltd. (Gardabaer, Iceland). Perhaps because of its low price, the iButton has been used in human studies for the ambulatory monitoring of skin temperature (usually at the wrist).[166–168]

Not widely used for long-term recording in animal research despite having been commercially available for many years is *infrared thermography*, which involves the measurement of infrared radiation emitted by warm-bodied animals. The advantage of this technology is that body temperature measurements can be conducted without touching or disturbing the animal, from close by or from as far away as a kilometer or more. Recent advances in thermal imaging technology have made possible the production of small instruments that have large storage capacity of digital thermal images with high temperature and spatial resolution.[169]

An ingenious approach for electrocardiograms in rodents that does not use either tethers or telemetry is the ECGenie system manufactured by Mouse Specifics Inc. (Framingham, Massachusetts). This noninvasive system records cardiac electrical activity through the paws of freely moving animals. The researcher places an animal onto the recording platform, and an electrocardiogram signal is acquired when the paws of the animal are in contact with the electrodes.

Monitoring of hormones and metabolites in the general circulation requires blood sampling, which is relatively simple in large domestic animals and humans. In smaller animals, human intervention can interfere with the rhythm being measured, so that tethering equipment is necessary. For rodents being monitored in running wheels, the tubes exiting the catheters must not be blocked. Instead of full wheels, flat wheels (or discs) are used in such cases. Med Associates (St. Albans, Vermont) manufactures wireless low-profile wheels suitable for mice (Figure 2.30).

Some hormones and electrolytes can be measured in urine or saliva, which simplifies sample collection. Measurement of the hormone melatonin is commonly used to determine the phase of the circadian system in humans, and saliva samples can produce results as good as blood samples.[170]

For the monitoring of energy metabolism, traditional methods of *indirect calorimetry*[171,172] are sufficient, provided that a computer is used to activate the air-switch valve and to collect the data (Figure 2.31). Indirect calorimetry is based on the measurement of oxygen consumed by the organism and on the chemical properties of oxidation. A few years after Joseph Priestley identified the oxygen gas (or "dephlogisticated air," as he called it) in the mid-1700s, the legendary chemist Antoine Lavoisier observed that oxygen is equally necessary for combustion and respiration and that both processes release carbon dioxide and heat.[173,174] As knowledge of the stoichiometric properties of oxidative processes increased, it became possible to calculate the amount of nutrient being used and the amount of heat being released, by measuring only the amount of oxygen being consumed. As illustrated in Figure 2.32 for the particular case of glucose, 6 mol of oxygen are necessary to fully oxidize 1 mol of glucose, which results in 6 mol of water, 6 mol of carbon dioxide, and 673 kcal of free energy. The free energy is either incorporated into molecules of ATP (adenosine triphosphate, the energy currency in the body) or lost as heat. Thus, if we measure how much oxygen is consumed by an organism, we can calculate how much nutrient was used and how much of the end products was produced. Of course, most organisms do not use only glucose as the source of energy, so we cannot rely solely on the equation for glucose. However, an "average" equation for the three main nutrients (carbohydrates, lipids, and proteins) can be used as a very reasonable approximation, and even more precise results can be obtained if the ratio of oxygen consumed to carbon



FIGURE 2.30 Low-profile wheel. Flat running wheels (or discs) must replace standard running wheels when research is conducted on tethered rodents. (Photo by R. Refinetti.)

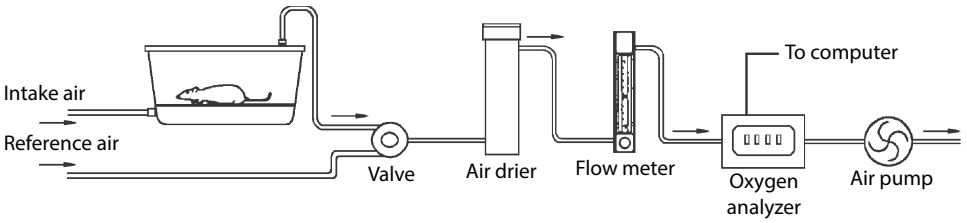


FIGURE 2.31 Indirect calorimetry. This diagram shows a typical setup for "open system" indirect calorimetry. The concentration of oxygen in the air that passes through the organism is compared with the concentration in the air that does not pass through the organism. The difference between the two concentrations reflects the fraction of oxygen consumed by the organism.

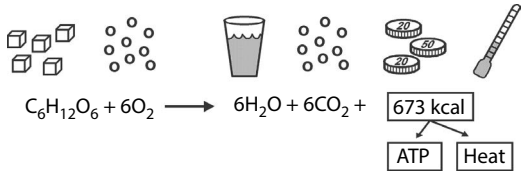


FIGURE 2.32 Why indirect calorimetry works. Measurement of oxygen consumption allows the computation of metabolic heat production because the stoichiometry of chemical reactions is independent of the steps taken to achieve the final result. As depicted here, the oxidation of one mole of glucose releases 673 kcal, and this is true whether the glucose is metabolized by an organism or burnt in a chemist's calorimeter.

dioxide produced (which is called the "respiratory quotient" [RQ]) is used. It is well established that the RQ value varies with nutritional parameters and ambient temperature.[175–183] To measure the concentration of oxygen (and carbon dioxide, if needed) in the air used by the organism, gas analyzers are employed (see Figure 2.31). Suppliers of gas analyzers for biomedical research include Servomex (Crowborough, England), Columbus Instruments (Columbus, Ohio), Sable Systems International (North Las Vegas, Nevada), and Qubit Systems (Kingston, Canada). By determining the difference in the concentration of oxygen in the air that enters the chamber (which is constant at 20.95% in atmospheric air) and in the air that leaves the chamber, one can determine the percentage of oxygen consumed by the organism. The percentage can then be converted into actual amount of oxygen if the exact flow of air through the chamber is known.[184,185]

Another instrument that can be easily adapted for circadian physiology research is the *temperature gradient device*. This device, used for invertebrates[186,187] as well as for rodents,[188,189] allows the animal to choose its preferred environmental temperature. By adding motion detectors monitored by a computer, a continuous record of the animal's behavior can be obtained. As shown in Figure 2.33, a temperature gradient can be generated in the animal's cage by the heating and cooling of the opposite ends of a surrounding copper pipe. The position of the animal along the gradient (and, therefore, its temperature choice) is determined by the computer through multiple infrared motion detectors. Body temperature can be simultaneously monitored by telemetry. To facilitate visual inspection of the diagram, some details were omitted: food and water are provided at equally spaced locations along the animal's chamber, which has a transparent Plexiglas top and a perforated bottom (to allow the disposal of urine and feces); coated solid wire is wound around the animal's chamber and serves as an antenna to pick up the signal from the radio transmitter implanted in the animal's abdomen.[190,191]

In simpler life forms, such and fungi and bacteria, *race tubes* (Figure 2.34) are standard equipment in circadian research.[192–194] Colonies are placed at one end of a glass tube with plenty of nutritive medium and are allowed to grow along the tube. The circadian pattern of growth can be seen as light bands on a dark background, or vice versa. A computerized optical-density reader can be used to quantify the growth pattern once the tube is filled. On the other end of the evolutionary spectrum, paper-and-pencil tests are often used in research with human subjects. One test that has been widely

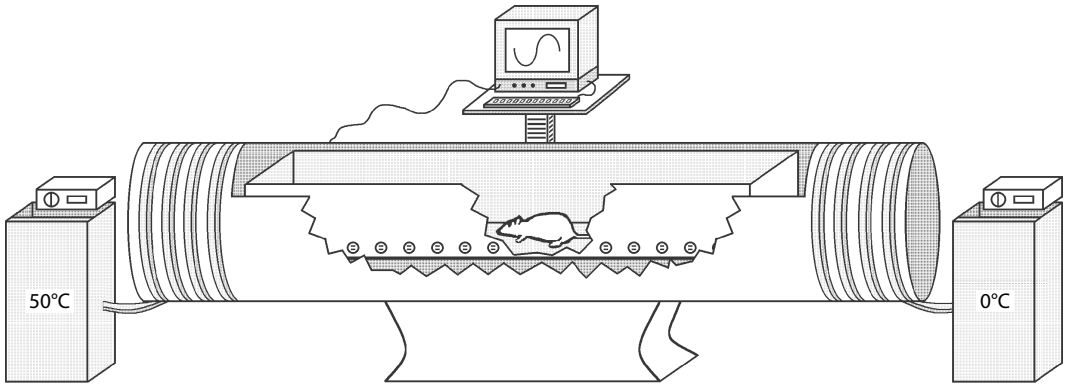


FIGURE 2.33 A temperature gradient device. By heating one end of a copper pipe and cooling the other end, one can generate a temperature gradient in the animal's cage, thus allowing it to choose its preferred temperature.

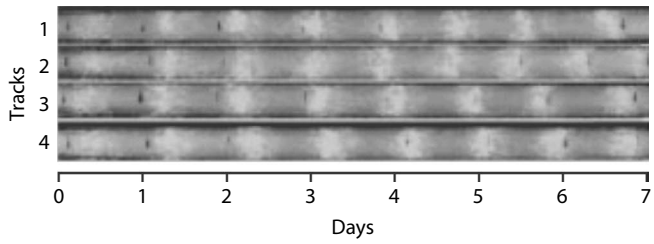


FIGURE 2.34 Race tubes. In microorganisms, growth rhythms are studied in *race tubes*. The colony grows along a small glass tube, and new bands can be seen each day. The growth of four colonies of bread mold over 7 days is shown here. (Adapted from Cheng, P. et al., *Proc. Natl. Acad. Sci. USA*, 98, 7408, 2001.)

used in circadian research is the *morningness–eveningness inventory*. Like other paper-and-pencil tests, this one assumes that the subjects are willing to respond truthfully to the questions and that their honest recollections are accurate. The answers to various questions are used to classify the individuals along a scale of early risers to late risers. A very popular morningness–eveningness inventory was developed by Horne and Östberg in 1976[195] (Table 2.1), and similar inventories have been developed since then.[196–201] We will cover the morningness–eveningness typology in Chapter 14.

TABLE 2.1
Sample Questions of the Morningness–Eveningness Inventory

Question	Possible Answers
3. If there is a specific time at which you have to get up in the morning, to what extent are you dependent on being woken up by an alarm clock?	[] Not at all dependent [] Slightly dependent [] Fairly dependent [] Very dependent
5. How alert do you feel during the first half hour after having woken up in the morning?	[] Not at all alert [] Slightly alert [] Fairly alert [] Very alert
8. When you have no commitments the next day, at what time do you go to bed compared to your usual bedtime?	[] Seldom or never later [] Less than 1 hour later [] 1–2 hours later [] More than 2 hours later
12. If you went to bed at 11 p.m., at what level of tiredness would you be?	[] Not at all tired [] A little tired [] Fairly tired [] Very tired
19. One hears about "morning" and "evening" types of people. Which one of these types do you consider yourself to be?	[] Definitely a "morning" type [] More a "morning" than an "evening" type [] More an "evening" than a "morning" type [] Definitely an "evening" type

Source: Horne, J.A. and Östberg, O., *Int. J. Chronobiol.*, 7, 97, 1976. Copyright Taylor & Francis (www.tandf.co.uk). Reproduced with permission.

2.3 RESEARCH ON ORGANS, CELLS, AND MOLECULES

Research at the level of whole organisms is necessary but not sufficient for the understanding of mechanisms of circadian rhythmicity. A great deal of research is conducted at the level of individual organs, cells, and even molecules inside a cell. Before we look at some of the techniques used in the study of circadian physiology at the level of organs and cells, we must briefly review some commonly used anatomical terms. The proverb says that "there are many ways to skin a cat," and there are also many ways to dissect a body or an individual organ. The three main planes of slicing (coronal, horizontal, and sagittal) are indicated in Figure 2.35. Knowledge of these names will be very helpful when we discuss actual experiments in later chapters. Also important will be knowledge of the names of positions along the three axes. Just like we need to know north, south, east, and west when looking at a map, we need to know the anatomical terms shown in Figure 2.36. For instance, the head of a pigeon is located in the anterior–medial–dorsal region of its body, just like Alaska is located in the northwest region of the world. In addition, it is often very convenient to use terms that denote relative, rather than

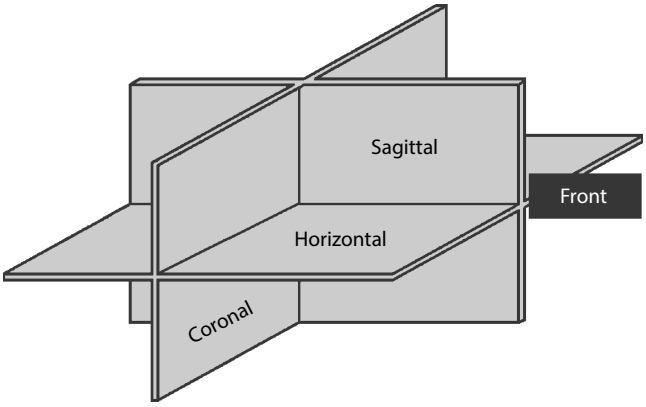


FIGURE 2.35 Anatomical planes. Research dealing with specific body organs often requires knowledge of basic anatomical terms, such as the three spatial planes.

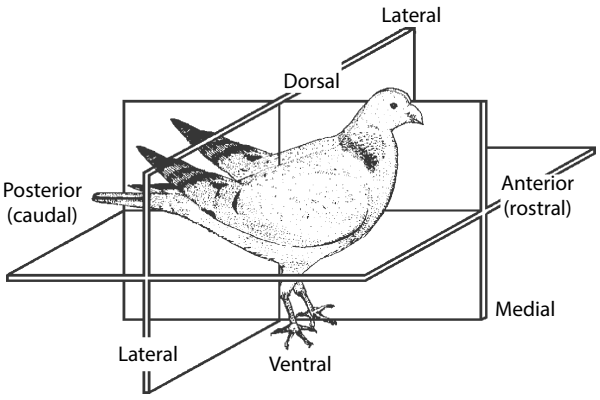


FIGURE 2.36 Anatomical directions. Directions along the three anatomical planes have special names that must be learned by beginners in physiological research.

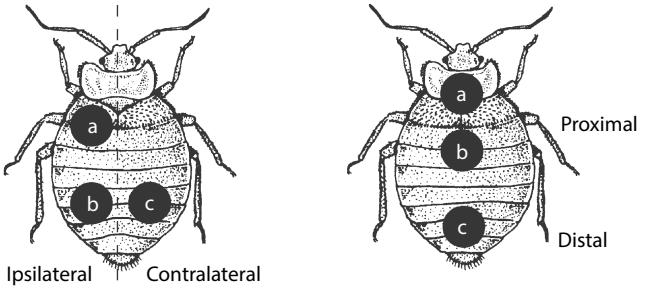


FIGURE 2.37 More anatomical terms. In addition to the absolute terms presented in Figures 2.35 and 2.36, there are relational anatomical terms used to specify a location (b or c) not in reference to the whole body but in reference to another location within the body (a).

absolute, position. Thus, in reference to a given location (say, point a in the left drawing in Figure 2.37), locations on the same side of the body (point b) are said to be *ipsilateral* to the reference location, whereas locations on the opposite side of the body (point c) are said to be *contralateral* to the reference location. Similarly, when we pick a reference location in the body (point a in the right drawing), areas in the proximity of this location (point b) are called "proximal," whereas areas that are more distant from the reference location (point c) are called "distal." When a reference location is not specified, it is usually assumed to be the central nervous system (or the body core in general).

Physiologists who study vertebrate animals (thus including birds and mammals) usually concentrate their efforts in one of various *systems*, as shown in Figure 2.38. The *nervous system* controls most of the other systems and is composed of the (1) central nervous system (brain and spinal cord) and (2) peripheral nervous system (the nerves that take information into and out of the brain and spinal cord). The *endocrine system* controls specific functions and is composed of various endocrine glands, including (1) pineal gland, (2) hypothalamus, (3) pituitary gland, (4) thyroid gland, (5) parathyroid glands, (6) adrenal glands, (7) kidneys, (8) pancreas, (9) ovaries (in females), and (10) testes (in males). The *cardiovascular system* takes oxygen, nutrients, and hormones to the whole body through the blood stream and is composed mainly of the (1) heart, (2) arteries, and (3) veins. The *respiratory system* is responsible for the acquisition of oxygen and the excretion of carbon dioxide by breathing and is composed of the (1) trachea, (2) lungs, and (3) diaphragm. The *skeletomuscular system* provides structure, protection, and movement by means of thousands of bones, muscles, and tendons. The *digestive system* extracts energy from ingested food and is composed mainly of the (1) mouth, (2) salivary glands, (3) esophagus, (4) liver, (5) stomach, (6) small intestine, and (7) large intestine. The *urinary system* assists in the excretion of unused and toxic substances and involves the (1) kidneys and (2) bladder. The *thermoregulatory system* is responsible for the maintenance of a constant body temperature and utilizes three main

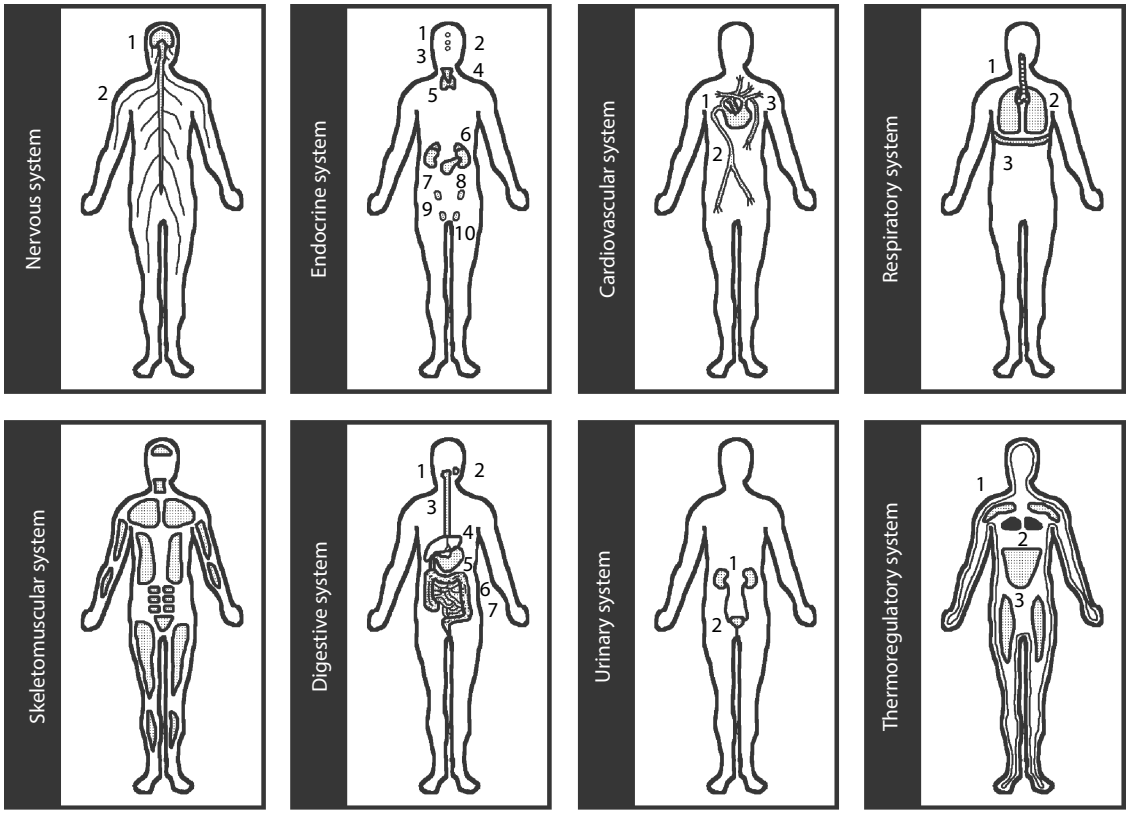


FIGURE 2.38 The major physiological systems. Those who conduct research at the level of body organs often specialize in one of the eight major physiological systems. See text for details.

organs: (1) the skin (for vasomotor control of heat transfer and for sweating), (2) brown adipose tissue (for regulatory thermogenesis), and (3) muscles (for shivering and behavioral responses). Not shown in the figure is the *immune system*, which is responsible for immunity against infectious diseases.

2.3.1 Research on Organs

To study organ function, physiologists have utilized several methods that can be applied to research on circadian rhythms with greater or lesser success. The activity of secretory organs can be studied by the monitoring of the secretion in the blood, digestive tract, or other body compartments, whereas the activity of excitable tissue (muscles and nerve cells) can be studied by the monitoring of changes in voltage or electric current. The activity of any organ can also be studied by the monitoring of how much nutrient or oxygen the organ consumes, and—because nutrients and oxygen are carried to the organ in the blood—by the monitoring of how much blood flows to the organ. In the last 10–20 years, organ activity has also been studied by the monitoring of the activation of particular genes or the synthesis of the proteins for which they code.

When secretions in the blood are monitored, it is important to know what it is that we are calling "blood." As shown in Figure 2.39, blood analysis may be conducted on full blood, on plasma (full blood minus red cells, white cells, and platelets), or on serum (plasma minus fibrinogen and clotting factors). Blood samples collected at regular intervals throughout the day can be analyzed for the concentration of hormones and drugs using standard laboratory techniques.[202–209] In some cases, the less invasive procedures of urine or saliva collection can be used.[210–216] On the other hand, special invasive techniques are required when measurements must be made *in vivo* (that is, inside the live animal). These are usually based on the process of *dialysis* (Figure 2.40). Dialysis is the diffusion of liquid solutions across a semipermeable membrane. Figure 2.40B shows a push–pull cannula inserted into an organ, blood vessel, or other body compartment. Saline (or other neutral solution) is pushed in through the outside tube and pulled out through the inside tube. If a membrane of appropriate porosity is placed at the end of the cannula, hormones and drugs can be captured and then analyzed.[217–221] Figure 2.40A shows a dialysis system based on passive diffusion, which is used in the construction of electrodes for the measurement of local concentrations of selected substances, such as glucose, lactate, and oxygen.[222] Although this type of electrode could be used for measurements of oxygen consumption of individual organs, other techniques are more frequently used for the monitoring of activity of nonsecretory organs. A fully implantable electrode for the monitoring of oxygen in brain tissue by telemetry in freely moving rodents has been recently described,[223] although it is not clear yet whether it can measure the minute changes in oxygen concentration associated with normal brain function, which would be needed for studies in circadian physiology.

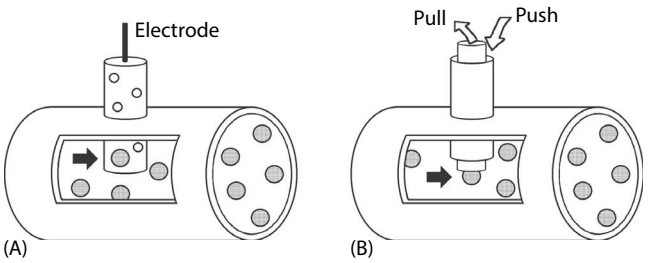


FIGURE 2.40 Dialysis. The procedure of dialysis has many applications in physiological research, such as in the operation of membrane electrodes (A) and push–pull cannulae (B). Dark arrows depict the movement of a substance into the electrode or cannula.

Techniques that monitor tissue blood flow (as a measure of metabolic activity) include *radioactive microspheres*[224] and the high-budget procedures of *positron emission tomography* (PET) and *functional magnetic resonance imaging* (*f*MRI).[225] The microsphere technique requires euthanasia of the experimental subjects and, therefore, can be used only in cross-sectional studies. PET and *f*MRI require only temporary anesthesia and can be used in longitudinal studies. However, they are prohibitively expensive for most researchers, and the scope of their application in neural tissue has been reduced by the finding that the responses of nerve cells and adjacent glial cells cannot be adequately differentiated.[226]

For the monitoring of glucose utilization, the 2-deoxyglucose (2-DG) methodology can be used. The 2-DG methodology was developed by Louis Sokoloff in the 1970s.[227,228] The methodology involves the use of radioactively labeled 2-DG, which is an analog of glucose. Brain cells utilize glucose as their main substrate for energy metabolism, so that cells that are more active utilize more glucose. While 2-DG is utilized

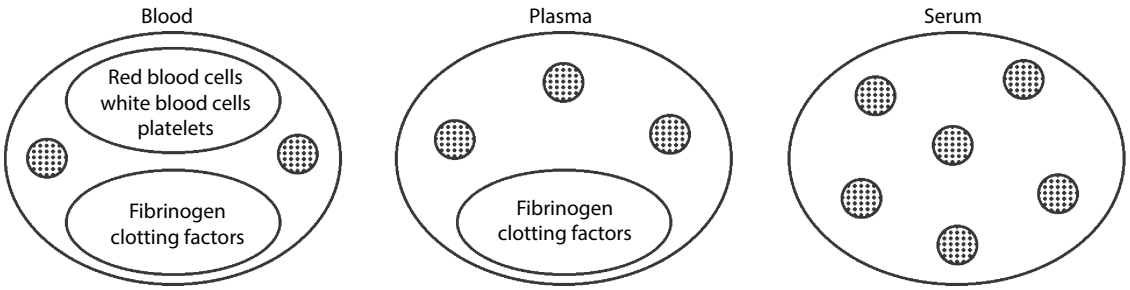


FIGURE 2.39 What's in your blood? Blood, plasma, and serum are terms used to refer to different levels of integrity of the fluid that circulates through the heart, arteries, capillaries, and veins.

similarly to glucose, it is not fully metabolized and gets trapped in the tissues. Therefore, more active cells accumulate more 2-DG. By radiographic techniques, one can determine what parts of the brain were more active following a 2-DG injection (because they accumulate more radioactive molecules). This technique has been successfully used in circadian research,[229–231] but it requires euthanasia of the experimental subjects and, therefore, can be used only in cross-sectional studies. In addition, the scope of its application in neural tissue has been reduced by the uncertainty about the roles played by nerve cells and glial cells.[232] An alternative technique is that of *Fos immunocytochemistry*. Fos is the protein produced by the c-*fos* (or just *fos*) gene, a gene widely used in cells in the process of activation of other genes. The amount of Fos produced is a reliable marker of overall gene activation and, therefore, of cell and organ function.[233,234] This technique has been widely used in circadian research[235–241]; however, like the 2-DG technique, it requires euthanasia of the animals and, therefore, cannot be used in longitudinal studies.

For longitudinal studies of organs *in vitro* (that is, organs isolated from the body and cultured in a dish), optical imaging of *bioluminescence* has been successfully used in circadian research. Of course, most animals do not naturally exhibit bioluminescence; however, modern techniques of genetic engineering allow the construction of transgenic animals in which a luciferase gene (responsible for bioluminescence in fireflies) is linked to the promoter region of a circadian clock gene in the mammalian animal model. Cultured explants of organs from the transgenic animals can then be studied longitudinally by optical imaging of the light emission.[242–246] For a few years, the technology for luminescence produced signals strong enough for *in vitro* studies, or *in vivo* studies of small organisms,[247,248] but the signal was too weak to allow *in vivo* measurements in vertebrates. Recent methodological improvements have allowed measurement of organ activity of whole mice without any incisions,[249–251] as exemplified for kidney activity in Figure 2.41. In this example, the degree of noninvasiveness is such that the temporal progression of kidney activity can be studied at several time points during a day. The noninvasiveness is relative, however. The mice had to be anesthetized and injected with luciferin at each time point,[251] a procedure that could have many unplanned side effects. A research team led by Hitoshi Okamura at Kobe University (in Japan) succeeded in recording rhythmicity of luminescence from the brain of freely moving mice,[252] and the research team led by Ueli Schibler at the University of Geneva (in Switzerland) developed a procedure for real-time recording of gene expression in the liver of freely moving mice.[253] Toshiyuki Hamada, from Hokkaido University (in Japan), has developed a new imaging system to measure gene expression from multiple organs simultaneously in freely moving mice, although only a brief description of the system is available at this time.[254] An important footnote here, which will be expanded in Chapter 12, is that these imaging procedures track the expression of the *per* gene, a gene that is a central component of the molecular mechanism of the circadian clock but whose role in the specific operation of different organs is unknown.

Optical imaging can also be used for *phosphorescence* instead of luminescence, without the requirement for the development of transgenic animals. In this case, a phosphorescent substance that is inhibited by the presence of oxygen is injected into blood vessels that irrigate the explanted organ. The greater the consumption of oxygen by the organ, the greater is the depletion of oxygen in the fine vasculature and, consequently, the greater is the phosphorescence when the tissue is illuminated.[255] A promising new application of optical imaging techniques for the longitudinal study of organ function *in vivo* is that of *quantum dots*, although this technique has not been used in circadian research despite having been available for over a decade. Quantum dots, also called "nanocrystals," are microscopic semiconductor particles that glow when illuminated. They can be attached to proteins and antibodies that move into target tissues, thus allowing optical imaging of cells and organs deep inside live animals.[256–259] Because fluorescence is obtained by external photic stimulation, the signal can be made strong enough to cross several layers of tissue and, therefore, to be observed in intact animals under anesthesia (Figure 2.42). For applications targeted at synaptic processes in nerve cells, a new technique based on the expression of a pH-sensitive protein involved in synaptic vesicle fusion has also been developed.[260]

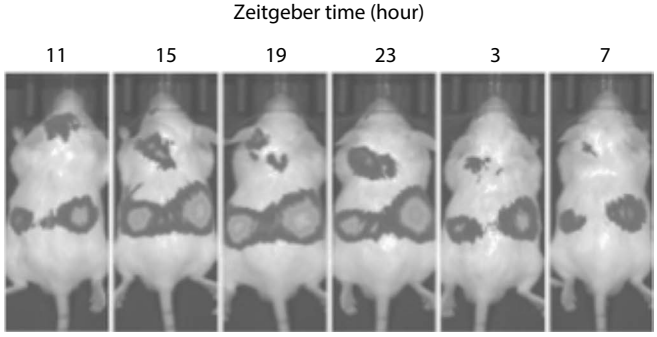


FIGURE 2.41 A glowing mouse. In this example of the use of the PER2:LUCIFERASE imaging methodology, temporal changes in the production of luciferase by the kidneys can be measured through the skin of anesthetized mice. (Adapted from Kuroda, H. et al., *Sci. Rep.*, 2, art. 711, 2012.)

2.3.2 Research on Cells

Although some of the techniques described earlier have enough resolution for the study of individual cells, research on cells of excitable tissue (muscle and nerve) has traditionally relied on the recording of electrical activity (*electrophysiology*). Before we discuss this technique, however, a brief review of neurobiology is needed. We should start by recalling that communication between different organs in the body is accomplished by two different systems: the nervous system and the endocrine system. Both systems use chemicals: *neurotransmitters* in the nervous system and *hormones* in the endocrine system (Figure 2.43). The main difference between the two classes of chemicals is that neurotransmitters are released at nerve terminals exactly where they are meant to go, whereas hormones are released in the circulating blood and go everywhere in the body. Nevertheless,

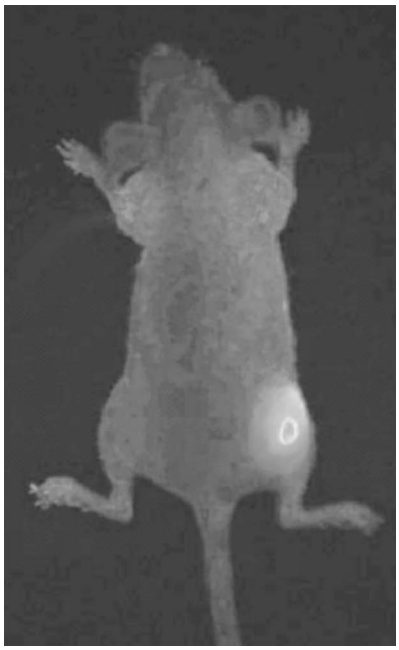


FIGURE 2.42 A different glowing mouse. In this example of the use of quantum dot methodology, alloyed semiconductor quantum dots injected into a live mouse mark the location of a tumor. (Photo courtesy of Shuming Nie, Department of Biomedical Engineering, Emory University, Atlanta, GA.)

both neurotransmitters and hormones act only on destinations that have the appropriate receptor structures. Thus, although hormones go everywhere through the blood, they only activate organs that possess the particular humoral (hormonal) receptor.

The critical cells in the nervous system are the *neurons* (Figure 2.44). The semipermeable cell membrane of neurons restricts the flow of ions into and out of the cell, resulting in a polarized condition (the *resting potential*). That is, neurons are like tiny batteries, with a positive pole outside and a negative pole inside. Neurons in the peripheral nervous system usually exhibit a voltage gradient of 70 mV, or about one-twentieth of a standard 1.5 V battery used in radios, flashlights, and electronic toys. Conduction of information along an axon (the long arm of a neuron) is accomplished by successive depolarizations of segments of the membrane, and this is why the activity of a nerve cell can be monitored by the recording of changes in voltage and current. When the propagated depolarization (called an "action potential") reaches the end of the axon, it causes the release of a neurotransmitter (Figure 2.45). Not all neurons use neurotransmitters, but many do—especially in the peripheral nervous system. After the neurotransmitter is released, it crosses the space that separates a neuron from another (called a "synapse") and attaches to a postsynaptic receptor, thus initiating a process of depolarization or hyperpolarization in the postsynaptic neuron. Different neuronal circuits usually employ different neurotransmitters. Some well-known neurotransmitters are as follows: *acetylcholine* (used in all synapses between nerves and skeletal muscles), *norepinephrine* (used in synapses between nerves of the sympathetic nervous system and its target organs), *dopamine* (used in numerous circuits in the brain), and *nitric oxide* (a peculiar neurotransmitter, as it is found as a gas, not as a liquid, in the central and peripheral nervous system). Each neurotransmitter can attach only to its corresponding receptor, although many neurotransmitters are capable of attaching to a class of similar receptors. For instance, norepinephrine (also called "noradrenaline") can attach to at least five noradrenergic receptors designated as α_1, α_2, β_1, β_2, and β_3.

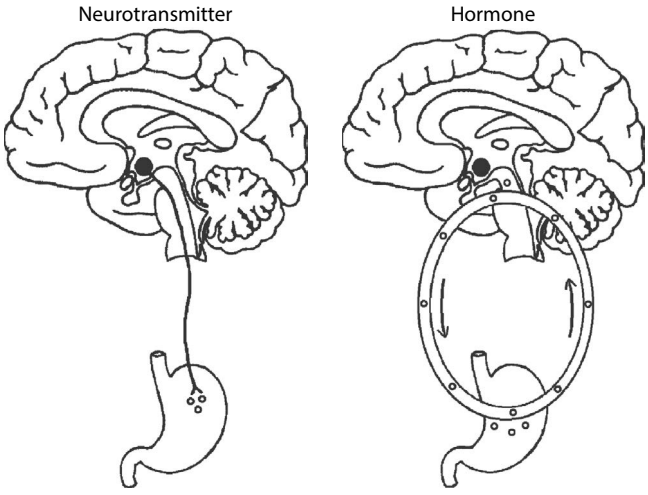


FIGURE 2.43 Two major information highways. Information transmitted in the body flows through two main highways: the nervous system and the endocrine system. The nervous system often utilizes neurotransmitters released at nerve terminals, whereas the endocrine system utilizes hormones released into the bloodstream.

Although luminescence and phosphorescence techniques can be used for the study of individual cells,[261–265] electrophysiological recording has been the most commonly used technique for the study of muscle and nerve cells for decades. Electrophysiology has a tradition of over a century. As early as 1905, Keith Lucas showed that muscle cells operate on the principle of "all or none" (that is, each cell either fires with full strength or does not fire at all, so that contraction in the whole muscle is graded by the number of single cells called into play, not by a gradation in the response of each cell).[266] In 1914, Edgar Adrian showed that the same principle applies to nerve cells,[267] and 25 years later, Alan Hodgkin and Andrew Huxley performed the first intracellular recording of an action potential.[268] A typical experimental setup for electrophysiological recording from nerve cells *in vitro* is shown in Figure 2.46. For recordings *in vivo*, electrodes can be surgically implanted. Manufacturers of microelectrodes, amplifiers, and other equipment for electrophysiological recording include Grass Instrument (West Warwick, Rhode Island) and World Precision Instruments (Sarasota, Florida). Circadian physiologists have recorded both single-cell and multiunit activity, both *in vitro* and *in vivo*.[269–275]

2.3.3 Research on Molecules

Before we look at some of the techniques used in the study of circadian physiology at the molecular level, we must briefly

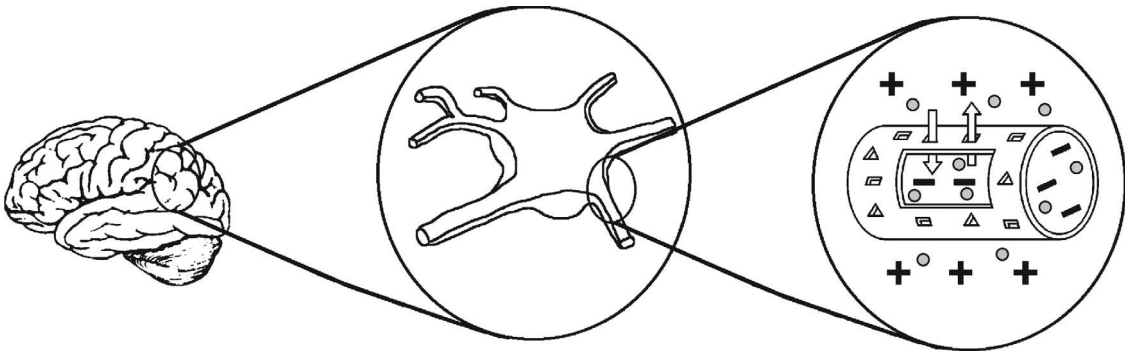


FIGURE 2.44 All about nerve cells. The main constitutive cells of the nervous system are neurons. Neurons are specialized cells that are polarized like a battery because of selective permeability of the cell membrane.

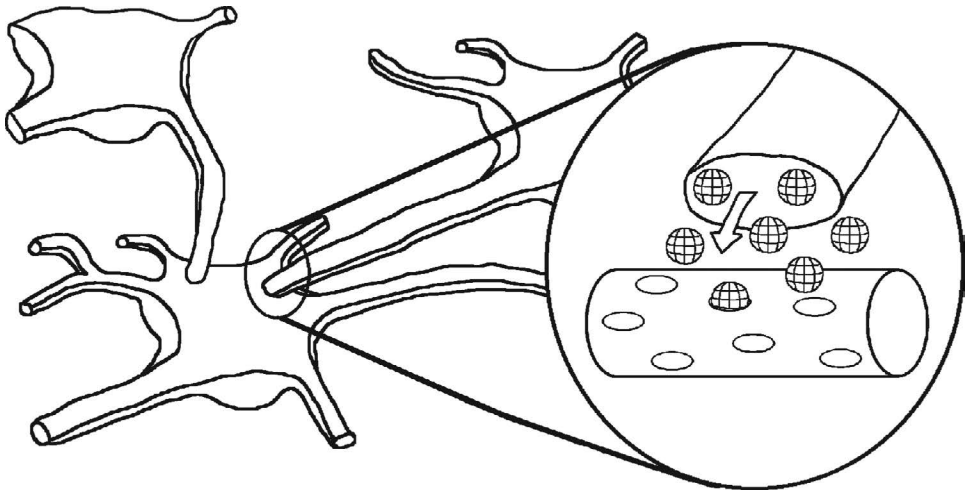


FIGURE 2.45 A chemical synapse. Neurons communicate with each other and with muscles and glands through synapses. Many synapses rely on the release of neurotransmitters from the presynaptic terminal and the binding of the transmitters to the postsynaptic terminal.

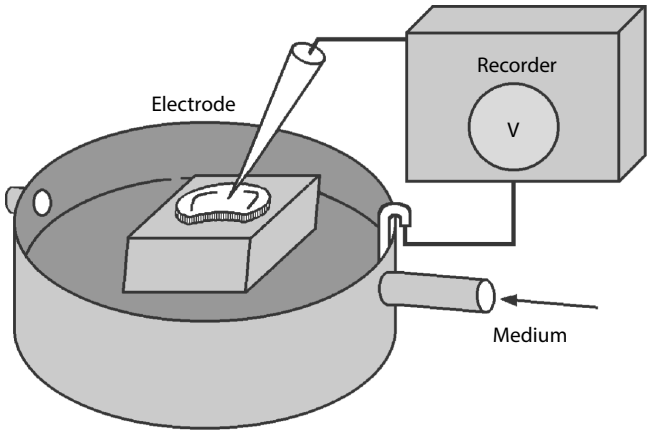


FIGURE 2.46 Electrophysiological recording. This diagram shows the main elements of an electrophysiological setup for the recording of activity of nerve cells *in vitro*.

review some basic principles of molecular biology. As summarized in Figure 2.47, the body is made up of billions of cells, and genetic information is contained in *chromosomes* in the nucleus of each cell. Chromosomes contain deoxyribonucleic acid (DNA) molecules, segments of which constitute *genes*. In eukaryotes (organisms whose cells have nuclei), genes are located in the nucleus of the cell and are normally inactive (Figure 2.48). When a gene is activated, it *transcribes* its double-stranded DNA sequence into a single-stranded ribonucleic acid (RNA) sequence, which is called the messenger RNA (mRNA). The mRNA molecule leaves the cell nucleus and attaches to ribosomes with the assistance of transport RNA (tRNA). In the ribosome, mRNA is *translated* into a protein. Not long ago, it was thought that each gene carried information concerning the production of one protein. Currently, it is believed that the information carried by the gene can be modified by several mechanisms, including alternative splicing of RNA and interference by micro-RNAs, and that the protein produced may be further modified by phosphorylation, methylation, acetylation, and other processes.[276] As a consequence, it is impossible to know *a priori* if a gene produces only one protein or thousands of them. Curiously, although the total number of proteins that can be produced is astronomical, all proteins are combinations of one or more of only 20 amino acids. Table 2.2 lists the names, abbreviations, and codons (triplets of bases in the mRNA) of the 20 amino acids. It is not known why nature picked these particular 20 amino acids. Other amino acids could be used in principle, and organisms

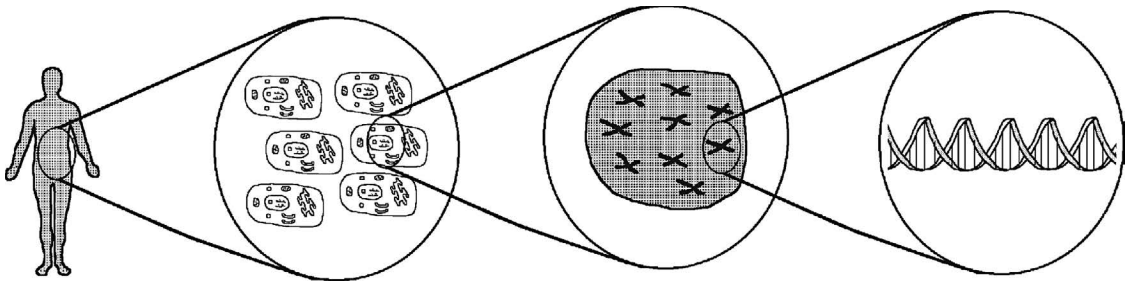


FIGURE 2.47 DNA: the blueprint of life. The body is made up of billions of cells. In the nucleus of each cell, genetic information is contained in chromosomes. Chromosomes contain DNA molecules, the segments of which constitute genes.

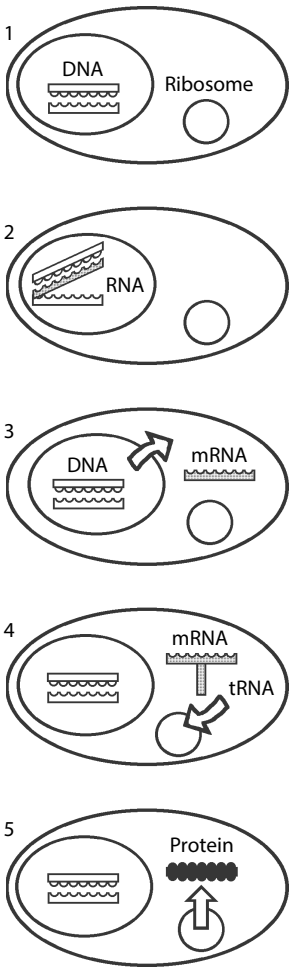


FIGURE 2.48 Gene expression. In eukaryotes (organisms whose cells have nuclei), genes are located in the chromosomes in the nucleus of the cell and are normally inactive. When a gene is activated, it transcribes its double-stranded DNA sequence into a single-stranded RNA sequence (the messenger RNA). The mRNA molecule leaves the cell nucleus and attaches to ribosomes with the assistance of transport RNA. In the ribosome, mRNA is translated into a protein.

can be artificially induced to use them.[277] Ten of the 20 amino acids are called "essential" because they cannot be synthesized by the human body and must, therefore, be obtained in the diet.

An important fact about DNA is that it is normally wrapped around histone molecules, thus forming *nucleosomes* (Figure 2.49). Although almost all cells in the body have the same genes (the same DNA sequence), the cells are differentiated into skin, bone, muscle, heart, and so on. It is believed that modifications in the relationship between DNA and histones determine what genes are expressed in which cells, thus allowing *epigenetic* differentiation of cell types.[278,279]

Except for the bioluminescence technique previously described, molecular techniques are incompatible with longitudinal studies of circadian rhythmicity in organs or cells, unless small biopsies or fluid secretions are used. However, cross-sectional studies are feasible with traditional techniques of molecular biology, such as gel electrophoresis, Southern blotting, Northern blotting, Western blotting, or column chromatography. Column chromatography and gel electrophoresis are the two traditional methods for the identification (separation) of proteins (Figure 2.50). *Column chromatography* (Panel A) relies on the movement of protein samples through a porous solid material. The sample is placed in the column (a) and, as the proteins move down, they are retarded to different degrees because of their different sizes, binding activities, and other properties (b). In *gel electrophoresis* (Panel B), a polyacrylamide gel slows the migration of proteins in an electric gradient (a) in approximate proportion to their charge-to-mass ratios. Usually, several lanes are run simultaneously, resulting in a characteristic pattern of horizontal bands (b). In the last decade or so, *mass spectrometry* has become a viable alternative for the identification and characterization of proteins.[280,281]

Because animals have several tens of thousands of genes, which may produce more than a million proteins, one has to be very lucky to identify a few genes or proteins of interest. Thus, the technique of *DNA microarrays* has provided great hopes for a better understanding of the mechanism of gene expression. DNA microarrays rely on the hybridization (base pairing) of nucleic acid samples (RNA) to a nucleic acid (DNA) with known sequence.[282] Thousands of minuscule DNA spots are deposited on a solid surface such as a glass slide or a nylon membrane, and the RNA sample is then added. Since only active genes contribute RNA to the sample, inspection of the hybridized array allows the identification of genes that were active when the sample was collected, and the magnitude of gene expression can be estimated by the abundance of the RNA. Circadian physiologists have used DNA microarrays in studies on fungi,[283] insects,[284–287]

TABLE 2.2
The 20 Amino Acids

Essential			Nonessential		
Name	**Abbr.**	**Codons**	**Name**	**Abbr.**	**Codons**
Arginine	Arg (R)	CGU, CGC, CGA, CGG, AGA, AGG	Alanine	Ala (A)	GCU, GCC, GCA, GCG
Histidine	His (H)	CAU, CAC	Asparagine	Asn (N)	AAU, AAC
Isoleucine	Ile (I)	AUU, AUC, AUA	Aspartate	Asp (D)	GAU, GAC
Leucine	Leu (L)	UUA, UUG, CUU, CUC, CUA, CUG, UCA, UCG	Cysteine	Cys (C)	UGU, UGC
Lysine	Lys (K)	AAA, AAG	Glutamate	Glu (E)	GAA, GAG
Methionine	Met (M)	AUG	Glutamine	Gln (Q)	CAA, CAG
Phenylalanine	Phe (F)	UUU, UUC, UCU, UCC	Glycine	Gly (G)	GGU, GGC, GGA, GGG
Threonine	Thr (T)	ACU, ACC, ACA, ACG	Proline	Pro (P)	CCU, CCC, CCA, CCG
Tryptophan	Trp (W)	UGG	Serine	Ser (S)	AGU, AGC
Valine	Val (V)	GUU, GUC, GUA, GUG	Tyrosine	Tyr (Y)	UAU, UAC

Sources: Clark, D.P. and Russell, L.D., *Molecular Biology*, Cache River Press, Vienna, IL, 1997; Nelson, D.L. and Cox, M.M., *Lehninger Principles of Biochemistry*, 3rd edn., Worth Publishers, New York, 2000.

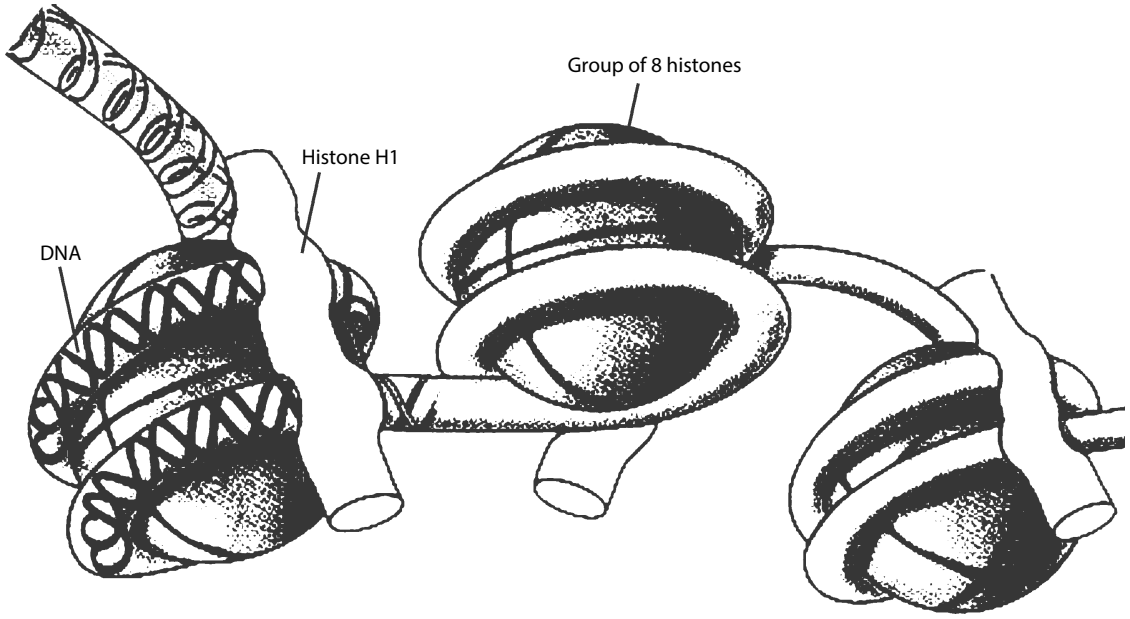


FIGURE 2.49 The nucleosome. Nucleosomes are basic units of DNA packaging in eukaryotes, consisting of a segment of DNA wound around eight histone protein cores.

and mammals[288–292] (Figure 2.51). Protein microarrays are in development,[293–296] and their utilization in circadian research can be expected to follow soon. A search of the U.S. National Library of Medicine's PubMed database for "microarray" in early 2015 showed a rapid growth in the number of publications from 1998 to 2004 followed by a constant rate of about 6000 publications per year in recent years.

2.4 RESEARCH ON THE ENVIRONMENT

Circadian physiologists do not routinely conduct research on environmental variables. This type of research is in the purview of meteorologists. However, circadian physiologists often need to monitor the environment in which their subjects are maintained. In addition, they often need to control environmental variables in their experiments.

2.4.1 MONITORING

Two important environmental variables that the circadian physiologist very often needs to monitor are illumination and temperature.

Illumination is provided by light, which is a form of *electromagnetic radiation*. Three concepts are particularly important in the measurement of the intensity of illumination: radiant flux, radiance, and irradiance[297–299] (Figure 2.52).

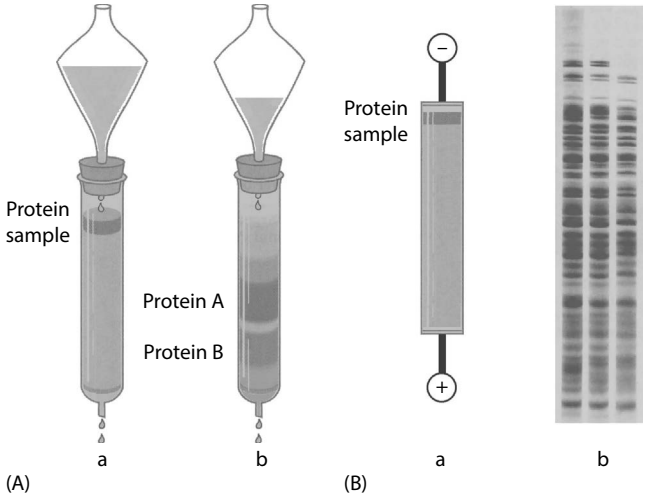


FIGURE 2.50 The two traditional methods of protein identification. Proteins can be separated by column chromatography (A) or gel electrophoresis (B). See text for details. (Adapted from Nelson, D.L. and Cox, M.M., *Lehninger Principles of Biochemistry*, 3rd edn., Worth Publishers, New York, 2000.)

Radiant flux refers to the power of the light source and, accordingly, is expressed in watts (W). Notice that this refers to the power of the emitted light (radiant power). For many decades, commercial light bulbs were rated according to their consumption of energy, not by the emitted light, and those ratings are still displayed in the packages today to help people who grew up using the old ratings. Typically, radiant power is about 1/4 of the consumed power, so that a 100 W bulb puts out only 25 W (the rest is lost as heat). Now, unless you swallow a light bulb, you will never experience all of the radiant power of the bulb. Two other concepts are needed: radiance and irradiance. *Radiance*, which is more commonly used by vision researchers, is a measure of the fraction of radiant power that can reach you. It indicates the radiant power per unit area per unit solid angle (see Figure 2.52) and, therefore, is expressed in watts per square meter per steradian ($W \cdot m^{-2} \cdot sr^{-1}$). The inclusion of unit solid angle ensures that the measured radiant intensity is the same regardless of how close to or far from the light source the observer is. *Irradiance*, which is more commonly used by circadian researchers, is a measure of radiant power from the point of view of the perceiver, who is at a specified distance from the light source. Thus, irradiance indicates the radiant power per unit area only—that is, watts per square meter ($W \cdot m^{-2}$).

A problem related to light measurement is that human vision is extremely limited. What we call light is a very narrow range of electromagnetic radiation. As shown in Figure 2.53, we are sensitive only to wavelengths between approximately 400 and 700 nm (1 nm = 10^{-9} m). Regardless of the intensity of illumination, we see no light below 400 nm or above 700 nm. Therefore, a set of *photometric* units has been created to supplement the *radiometric* units that we have just discussed.[298,300] The photometric units "correct" the radiometric units according to the human eye. Radiometric and photometric names and units of measurement are shown in Table 2.3.

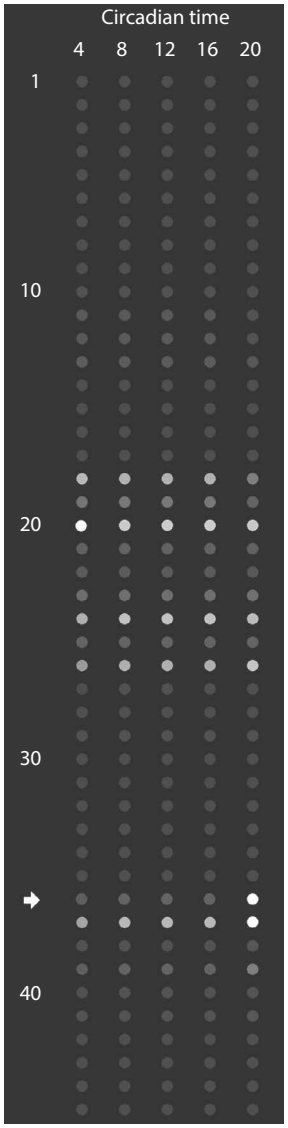


FIGURE 2.51 Tracking thousands of genes at the same time. DNA microarrays make it possible to track the expression of thousands of genes at the same time. In this short segment of a much larger array, expression of 45 genes in cells of the fruit fly (one gene per line) is tracked over five circadian times. Greater expression levels are indicated by whiter shades of gray. The arrow points to a gene that showed greater expression at circadian time 20 than at other times of the daily cycle. (Data from *Drosophila* Microarray Database at Washington University School of Medicine, St. Louis, MO.)

At our peak sensitivity (555 nm), an irradiance of 1 $W \cdot m^{-2}$ corresponds to an illuminance of 683 lux. (The abbreviation for lux is lx but, because only one letter is saved by the abbreviation, most people do not bother to use it.) At shorter and longer wavelengths, 1 $W \cdot m^{-2}$ corresponds to less than 683 lux—and, as a matter of fact, it corresponds to 0 lux in the ultraviolet and infrared ranges. Figure 2.54 shows an approximate illuminance guide for the lux scale when a full-spectrum light source ("white light") is used.

There is no consensus about whether one should use radiometric or photometric units when dealing with

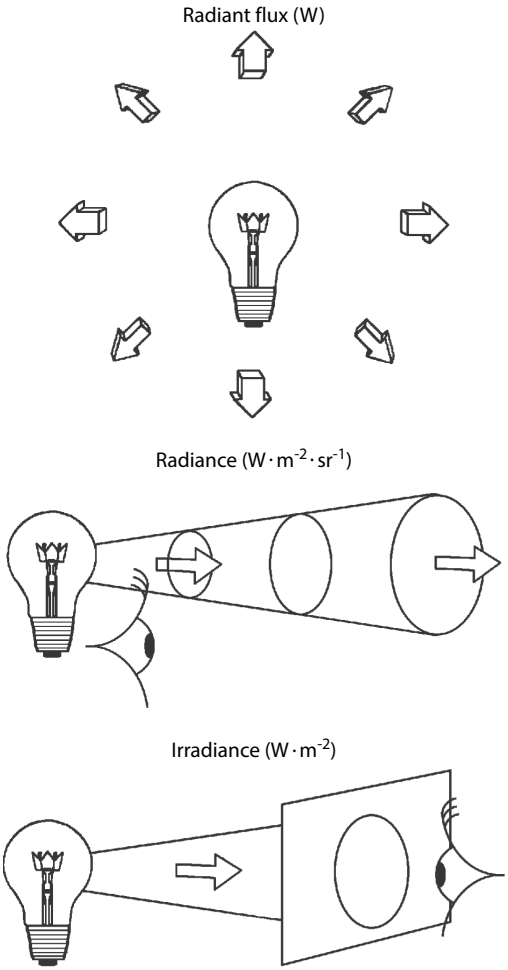


FIGURE 2.52 Measuring illumination. Three concepts are particularly important in the measurement of illumination (electromagnetic radiation): radiant flux, radiance, and irradiance.

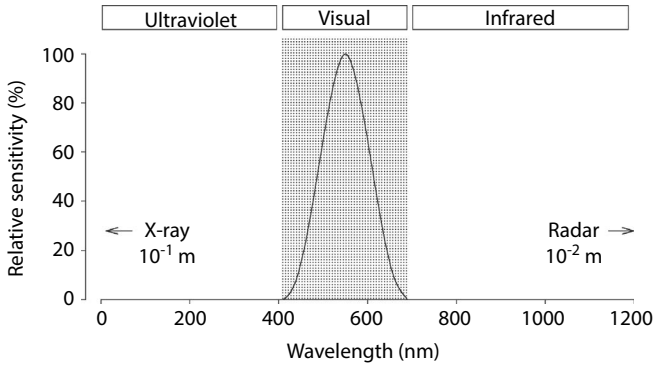


FIGURE 2.53 The narrow range of visual sensitivity. Vision is elicited by electromagnetic radiation. However, we are blind to all radiation outside the narrow range of wavelengths from 400 to 700 nm.

nonhuman subjects. Photometric units are obviously biased toward humans, but so is science. Science is conducted *by* humans *for* humans. However, because the spectral sensitivity of the circadian system seems to be different from that of the visual system (as will be discussed in Chapter 11), it would seem prudent to use radiometric units most of the time, or even to develop the concept of *circadian light*.[301] It should also be pointed out that, because electromagnetic radiation can be considered either as a wave phenomenon or as a quantum phenomenon, irradiance is sometimes expressed in number of photons per unit time per unit area.[299] At 555 nm, 1 $W \cdot m^{-2}$ corresponds to approximately 2.79×10^{18} photons $\cdot s^{-1} \cdot m^{-2}$.

TABLE 2.3
Radiometric and Photometric Units[a]

Radiometric		Photometric	
Process	**Unit**	**Process**	**Unit**
Radiant flux	W	Luminous flux	lm
Radiance	$W \cdot m^{-2} \cdot sr^{-1}$	Luminance	$lm \cdot m^{-2} \cdot sr^{-1}$
Irradiance	$W \cdot m^{-2}$	Illuminance	$lm \cdot m^{-2}$ (=lx)

[a] For more details on units of measurement, see the last section of the Dictionary of Circadian Physiology at the end of the book. Abbreviations used in this table are W, watt; m, meter; sr, steradian; lm, lumen; and lx, lux.

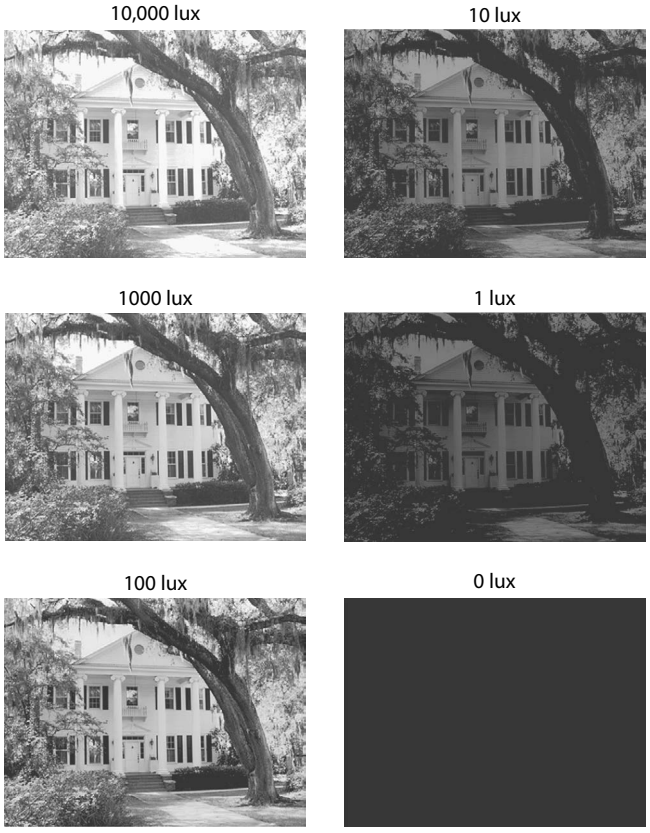


FIGURE 2.54 Quantifying the intensity of visual light. The intensity (brightness) of visual light is measured in lux. This series of photographs serves as a guide to the lux scale. Notice that our perception of brightness follows a logarithmic function (e.g., 1000 lux is twice as bright as 100 lux).



FIGURE 2.55 A photometer. Photometers are instruments used to measure illuminance (light intensity in lux). (Image courtesy of the Cooke Corporation, Auburn Hills, MI.)

Instruments used to measure light intensity provide either photometric measurements (*photometers*) or radiometric measurements (*radiometers*), or both (*photometer–radiometers*). Because photometers are widely used in nonscientific applications (such as photography and civil engineering), they are easily available at moderate prices. Figure 2.55 shows a precise but inexpensive photometer manufactured by the Cooke Corporation (Auburn Hills, Michigan). Photometers of varying precision and cost are manufactured by a variety of companies and are sold by suppliers of scientific equipment as well as by optical and photographic stores.

In contrast to photometers, radiometers are available only from specialized suppliers and can cost over 10 times as much as ordinary photometers. Manufacturers of radiometers include International Light (Newburyport, Massachusetts), Eppley Laboratory (Newport, Rhode Island), United Detector Technology (Baltimore, Maryland), and Macam Photometrics (Livingston, Scotland). It should be pointed out that photometers and radiometers measure the incident light regardless of its composition, under the assumption that you know what kind of light source you have. To measure the spectral composition of light, spectroradiometers or spectrophotometers are needed. Manufacturers include International Light (Newburyport, Massachusetts), Beckman Coulter (Fullerton, California), Apogee Instruments (Logan, Utah), Gretag Macbeth (Regensdorf, Switzerland), Glen Spectra (Stanmore, England), and Instrument Systems (Munich, Germany).

Temperature is another very important environmental variable that the circadian physiologist often must monitor. Temperature is a measure of the average kinetic energy of the molecules of a substance.[297,302,303] The instrument used to measure temperature is the *thermometer*, which was invented in the 1600s and has evolved considerably since then.[304–306] To simply monitor the temperature of the environment over extended periods of time, a standard *thermograph* (temperature chart recorder) can be used. Figure 2.56 shows a thermograph manufactured by Dickson Instruments (Addison, Illinois). Other manufacturers of ambient temperature recorders include Yellow Springs Instruments (Yellow Springs, Ohio), Omega Engineering (Stamford, Connecticut), and Hanna Instruments (Woonsocket, Rhode Island). Many thermographs have a digital output, so that the temperature data can be simultaneously transferred to a computer. For environmental monitoring in deep sea (temperature and depth), data loggers are manufactured by Vemco (Bedford, Canada) and Star-Oddi Ltd. (Gardabaer, Iceland).

The official unit of measurement for temperature is the Kelvin. However, in everyday life, and even in many scientific applications, other units are used. In most countries, temperature is measured in degrees Celsius (°C), which are the same as Kelvin minus 273—that is, °C = K − 273 (actually, 273.15). In the United States, except among scientists, temperature is measured in degrees Fahrenheit (°F). The relationship between the Fahrenheit scale and the Celsius scale is °F = 1.8 × °C + 32. The temperatures of some important phenomena in the two scales are shown in Figure 2.57.

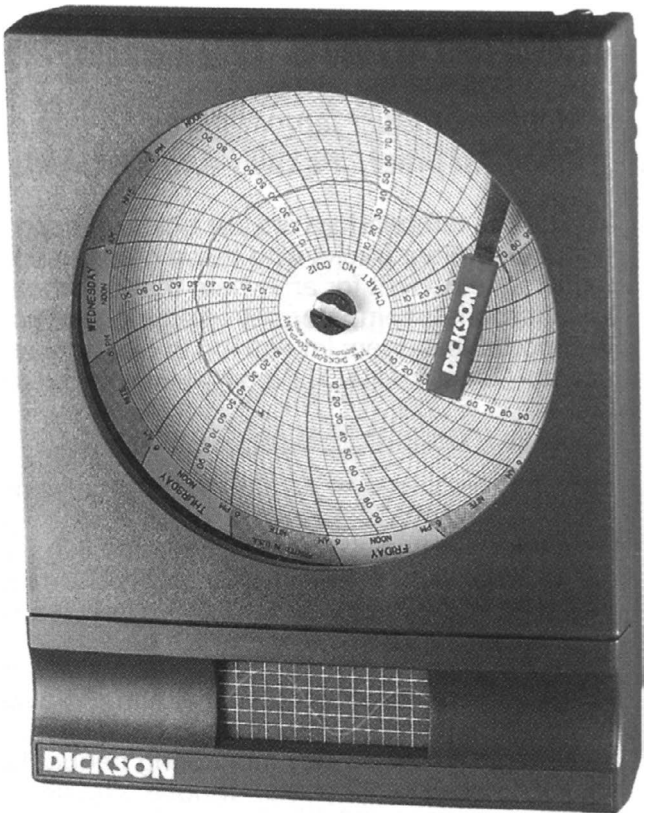


FIGURE 2.56 A thermograph. Thermographs provide a convenient means of recording ambient temperature continuously for many days. (Image courtesy of Dickson Instruments, Addison, IL.)

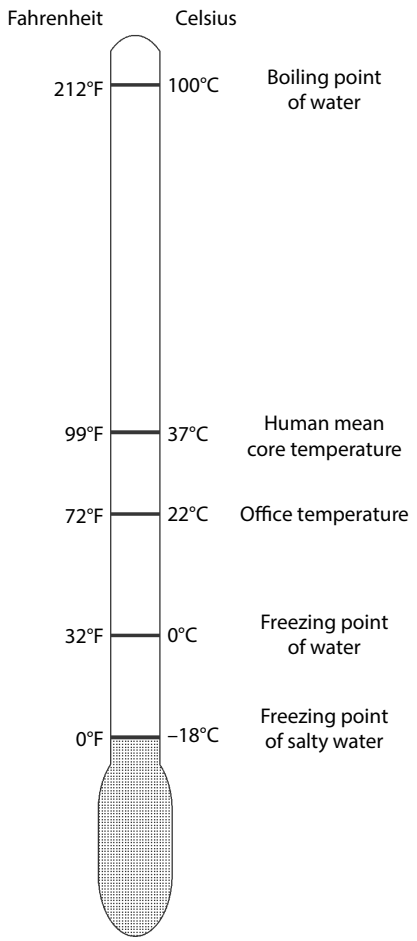


FIGURE 2.57 Temperature scales: Celsius and Fahrenheit. In most of the world, temperature is measured in degrees Celsius (°C). In the United States, the Fahrenheit scale (°F) is used. The diagram shows the correspondence of the two scales.

2.4.2 Control

Besides monitoring environmental variables, the circadian physiologist often needs to manipulate environmental variables in his or her research. Three very important variables are illumination, temperature, and food availability. Sources of illumination include sunlight, incandescent light bulbs (using tungsten or tungsten–halogen filaments), fluorescent bulbs, xenon arc lamps, light-emitting diodes (LEDs), and lasers.[299,307] Solid-state light sources such as LEDs are much more efficient than incandescent or fluorescent lamps and can provide radiation confined to a very narrow spectral region, which allows the generation of monochromatic stimuli without the need for special filters.[308] In circadian research, the goal is usually to simulate sunlight, so that light sources with a broader spectral radiant power distribution are desirable. Xenon arc lamps are excellent daylight simulators, but fluorescent ("cool") lamps provide a reasonable approximation and are the lamps most commonly used by circadian physiologists. The preferable illuminance level varies according to the species used and to the goals of the research. For general animal housing during experiments, values usually range from 10 to 1000 lux. (For reference, full moon is about 0.1 lux, average human indoor working space is 200 lux, and full daylight on a clouded day is 15,000 lux.[307])



FIGURE 2.58 Controlling the light–dark cycle. An electronic controller timer such as this one provides a simple way to turn lights on and off at desired times in a laboratory setting. (Photo by R. Refinetti.)

In circadian research, the timing of light is just as important as its brightness. Therefore, some sort of device is needed to automate the process of turning on and off the lights. Figure 2.58 shows an inexpensive controller timer. Such timers, which are marketed by all major suppliers of scientific equipment as well as by retail electronics stores, provide the ability to turn lights on and off according to the requirements of most research designs. If the researcher wishes to vary the period of the light–dark cycle (that is, to have days shorter or longer than 24 hours), a more sophisticated timer (such as ChronTrol XT, manufactured by the ChronTrol Corporation in San Diego, California) is needed. Of course, maximal flexibility in the control of illumination can be attained by the use of a computer fitted with interface boards. In this case, even modulation of brightness to simulate natural dawn and dusk can be attained with the appropriate hardware and software. Computer interface boards for data acquisition and control are marketed by a large number of companies, including IOtech (Cleveland, Ohio), Measurement Computing (Middleboro, Massachusetts), Microstar Laboratories (Bellevue, Washington), National Instruments (Austin, Texas), National Semiconductors (Santa Clara, California), and United Electronic Industries (Canton, Massachusetts).

The dark phase of a light–dark cycle is, of course, supposed to be *dark*. However, *dark* is a relative term. As will be discussed in Chapter 11, the spectral sensitivity of the circadian system (as well as that of the visual system) varies from one organism to another. Thus, in laboratory situations

that require human visual inspection of experimental subjects in darkness, it is possible to provide illumination for human vision without introducing photic stimulation to the subjects. Some researchers utilize infrared viewers ("night-vision goggles"),[309–314] although dim red illumination in the normal human visual range (1 lux, $\lambda > 600$ nm) is often used in studies involving rodents.[315–322] Unfortunately, enough illumination to allow good human visual resolution (for instance, for reading or performing surgery) cannot be attained by these means. It has been recently reported that sodium lamps can produce adequate illumination for human vision without disturbing the normal behavior of mice,[323] although thorough tests have not been conducted to ascertain that the murine circadian system is indeed unaffected by the illumination.

Most of the time, control of ambient temperature is not a concern in circadian physiology. The standard heating and air-conditioning system of modern buildings is capable of maintaining a stable thermal environment with daily oscillations not exceeding 1°C or 2°C. In some instances, however, precise control of ambient temperature is needed. Control of ambient temperature in the laboratory is most effectively achieved by the use of environmental chambers (often called "refrigerated incubators"). Figure 2.59 shows a refrigerated incubator with controller timers that allow the programming of cycles of both light and ambient temperature. Manufacturers of environmental chambers include Revco (Asheville, North Carolina), New Brunswick Scientific (Edison, New Jersey), and VWR International (West Chester, Pennsylvania). For simpler applications requiring a cycle of ambient temperature, a small timer-controlled heater inside a cabinet kept in an air-conditioned room may provide an adequate daily temperature cycle at a fraction of the cost of an environmental chamber.

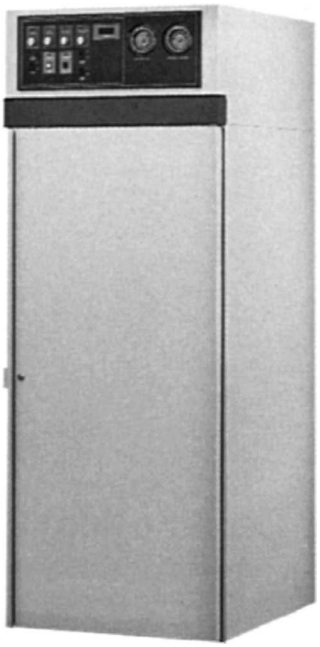


FIGURE 2.59 Controlling ambient temperature. Environmental chambers (ventilated heated incubators) such as this one provide a reliable way to control ambient temperature in a laboratory setting. Some models have programmable timers that allow the generation of daily cycles of ambient temperature. (Photo by R. Refinetti.)

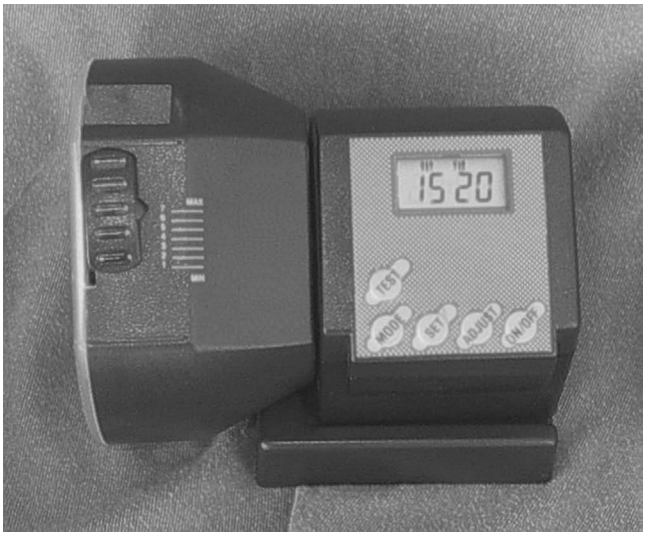


FIGURE 2.60 Controlling food availability. Automated fish feeders such as this one provide a simple way to control feeding time for small animals in a laboratory setting. (Photo by R. Refinetti.)

In most research in circadian physiology, food is freely available to the experimental subjects at all times (*ad libitum* feeding). However, sometimes a schedule of restricted feeding is required. A simple way to automate daily feeding is to use automated fish feeders, which are available at most aquatic pet stores (Figure 2.60). The very popular NutraMatic feeder (marketed by Role C. Hagen Corp., Mansfield, Massachusetts) can hold about 120 g of small-pellet food, which will feed a mouse for over 20 days. In some cases, duration of access to food (rather than timed delivery of a standard amount of food) may be desired. In these cases, a custom-built feeder with a solenoid-activated access door may be required. Although expensive, such a system for rodents is available from Research Diets Inc. (New Brunswick, New Jersey) and from TSE Systems Inc. (Chesterfield, Missouri), whereas feeders for large animals are manufactured by Sweeney Enterprises (Boerne, Texas). An alternative (for rodents) would be the use of operant conditioning apparatuses, such as those manufactured by Lafayette Instrument (Lafayette, Indiana) and Med Associates (St. Albans, Vermont). The system can be easily programmed to deliver rewards only to lever presses performed during a predefined interval of time each day.

SUMMARY

1. Research in circadian physiology is conducted according to the scientific method, which consists of the systematic application of commonsense

principles, particularly the principle of determinism. Although absolute truths come within the realms of metaphysical speculation, knowledge generated by scientific research is rigorous and progressive.

2. The variables most frequently measured at the organism level are locomotor activity, body temperature, and blood pressure. Locomotor activity is often measured with infrared motion detectors or running wheels. The two other variables require tethering or telemetry techniques.
3. The activity of secretory organs can be studied by the monitoring of secretion in the blood or other body fluid, whereas the activity of muscles and nerve tissue can be studied by the monitoring of changes in voltage or electric current. Organ activity can also be studied by the monitoring of how much nutrient or oxygen the organ consumes and of how much blood flows to the organ. Techniques for studies at the cellular and subcellular levels include optical imaging of phosphorescence/luminescence and DNA microarrays.
4. Circadian physiologists often need to *monitor* the environment in which their subjects are maintained. Two very important environmental variables are illumination and temperature. Circadian physiologists often also need to *manipulate* environmental variables. Three very important variables are illumination, temperature, and food availability.

EXERCISES

2.1 Plotting Data (Equally Spaced Data Points). Visual inspection of the data is the first step in any data analysis procedure. In this exercise, we will use the program **Plot** to inspect a number of data files with equally spaced data points. The next exercise will deal with the case of files containing unequally spaced data points (which includes files containing data collected at regular intervals but missing several points).

1. Assuming that you have already installed the software package that accompanies this book, double-click on the **Circadian** icon to open the program banner.
2. Select the **Plot** program (the first icon on the left). Notice that the program window contains three main panels: a *Source* panel, a *Data* panel, and a display panel. For now, you may leave all the values in the *Data* panel at their default values (which means that the *Unequally spaced* checkbox should not be checked).
3. In the *Source* panel, double-click on the Data subfolder to access the folder and then click on the file **A01**. This file contains body temperature measurements of a squirrel collected by telemetry every 6 minutes for 7 days.
4. Click on the Cartesian plot button (the purple button). You can see a nice daily oscillation. Use the horizontal scroll bar (under the display panel) to see the next 6 days.
5. When you have seen enough, load the file **A02** (select the file in the *Source* panel and then click on the Cartesian plot button). This file contains body temperature measurements of a degu (a South American rodent) collected by telemetry every 6 minutes for 8 days.
6. Unlike the squirrel data, these data are rather noisy because of equipment problems. Browse the whole file (using the horizontal scroll bar) and notice that any daily rhythmicity that may exist has been masked by the recording noise.
7. In the next chapter, we will look at ways to filter out noise, but we can improve the plot right away with a few tricks. First, click on the *Dots* option button at the bottom right of the program window. Browse the whole file and notice that you can easily distinguish apparently genuine data points from bad data points.
8. Notice that the range of oscillation of the data points is indicated above the display panel (in this case, the range is from 30.06°C to 39.06°C). Because most noise seems to be below 35.5°C, we may discard these points in order to see the genuine points better. At the bottom of the *Data* panel, there are three *Filter* boxes. Click on the *Low* box, delete the default **0**, and type **35.5**.
9. Click on the Cartesian plot button to refresh the image. The program will warn you that you are choosing to ignore some data points. Just click on *OK*. Browse the whole data set and notice that, although the plot is still not very good, you can now have a better view of the data.
10. Finally, let us plot a data set with much lower temporal resolution. Select the file **A29**. This file contains measurements of plasma urea concentration of a goat (in mmol per liter) taken at 3-hour intervals over 8 days.
11. Before plotting the data, we need to set the *Low Filter* back to **0**. Do so now. We also need to adjust the bin size in the *Data* panel. Set the bin size to **180** (i.e., 180 minutes or 3 hours). Finally, click on the Cartesian plot button.
12. Browse the various days, both in *Dots* and *Lines* mode. Even though the temporal resolution is much lower than in the previous data sets (180 minutes instead of 6 minutes), you can observe clear daily rhythmicity.

2.2 Plotting Data (Unequally Spaced Data Points).

1. Start the program **Plot**.
2. All steps here will be the same as those in Exercise 2.1 except that the checkbox *Unequally spaced* (in the *Data* panel) should be checked. To check the box, simply click on it.

3. Select the sample data file **A19**. This file contains measurements of oral temperature of a human subject over a day. Although the measurements were taken at regular intervals, some data points are missing (and, therefore, the file contains time tags to specify the correct time for each data point).
4. Click on the Cartesian plot button. If you failed to follow step 2, you will receive an error message; otherwise, you will see a clear daily rhythm with lower values during the early morning.
5. In order to see the individual data points, click on the *Dots* option button at the bottom right of the program window (if you have not done so previously).
6. After you have seen enough, load the file **A14**. This file contains body temperature measurements of a laboratory rat collected by telemetry almost every 6 minutes for 7 days. Because some data points are missing, the file must contain time tags (as it does).
7. As you can see by browsing the full data set, there is a reasonable daily rhythm that peaks around noon each day. The rhythm will be more obvious if you switch to *Lines* instead of *Dots* mode.
8. Load another file now: sample data file **A15**. This file is identical to file **A14** except that even more data points are missing. In this case, the plot looks better in *Dots* mode than in *Lines* mode.
9. For a final example, load file **A28** now. This file contains measurements of air relative humidity taken at irregular intervals over 2 days in Walterboro, South Carolina. Because the data were not collected very often, the graph does not look very good in either *Dots* or *Lines* mode. However, you can see clear daily rhythmicity with values around 50% in the early afternoon and around 80% during the rest of the day. South Carolina is not a dry place!
10. You may also use **Plot** to graph the data that you obtained in the exercises in Chapter 1 (angles of bean leaves and your own body temperature). To do so, you must first create text files with time tags. Use your word processor to create a simple document that contains one time point per line. Each line must contain two values (separated by a space): a time tag and the value to be plotted. The time tag must be in 24-hour clock mode (e.g., 22.5 for 10:30 p.m.). If the file contains more than 1 day, the clock must be reset to 0 every day at midnight. Make sure to save the file as "text only" with an appropriate file name. (If you are unsure about the file format, open the sample data file **A15** or **A28** in your word processor and inspect the sample file before creating your own file.)

2.3 Setting up a Simple Data Acquisition System. This exercise is not for everyone. However, if you are an electronically inclined individual, or a researcher with scarce research funds, you will find it very interesting. The goal is to build a running-wheel data acquisition system using any Windows-based (or even DOS-based) computer and less than $100 in parts.

1. Let us start with the wheels. You can buy a running wheel at almost any pet store for about $6. Metallic wheels are slightly more expensive than plastic wheels but are much more durable and well worth the extra cost. Choose the size of the wheel according to the size of the animal that you will use.
2. Next, you will need to visit an electronics shop (or a home improvement store) and obtain a small magnetic switch. Magnetic switches are commonly used in home alarm systems for windows or doors. Radio Shack sells a magnetic switch (Product No. 54-630) for about $4. To avoid further trips later, you may want to buy 4 or 5 switches right away.
3. Aside from the cage in which you will keep the animal, the only other piece of equipment needed is a desktop personal computer. There is no need for a fancy computer. As a matter of fact, older computers running Windows 95 or DOS are preferable because they are more likely to have easily accessible interface ports. The easiest setup uses the game port. If you don't know what the game port looks like, look back at Figure 2.26 (the port access is a female DB-15 connector). If your computer does not have a game port, you can use a USB port instead, but you will need an adapter. USB Gear (www.usb-gear.com) sells a USB to game port adapter for $20.
4. Before we proceed to assembling the system, you should obtain some wire to make the necessary connections (black AWG 26 insulated wire would be fine). Also, although you may insert wires directly into the game port's holes, it would be wise to purchase a male DB-15 connector to be attached to the female connector in the computer (or in the USB adapter). You can purchase one at Radio Shack or any other electronics store.
5. Now look at Figure 2.61. It shows how the magnetic switch should be attached to the wheel and provides the pin numbers of the game port connector. The figure assumes that you will mount the wheel on the floor of the cage. If possible, you should mount the wheel on the cage top instead, so that the animal cannot reach the wires. If the wires stay inside the cage, you will need to glue them to the wheel frame with strong glue; otherwise, the animal will chew the wires in no time!
6. You can connect up to eight wheels to the game port, although the simplest setup uses only four channels (which correspond to the fire buttons in joysticks). One of the wires from each magnetic switch should be attached to pin 4 (Ground). The other wire from the magnetic switch should be attached as follows: Wheel A = Pin 2, Wheel B = Pin 7, Wheel C = Pin 10, and Wheel D = Pin 14. Again, you may insert the wires directly into the holes in the game port, but it is much neater to solder them to the pins of

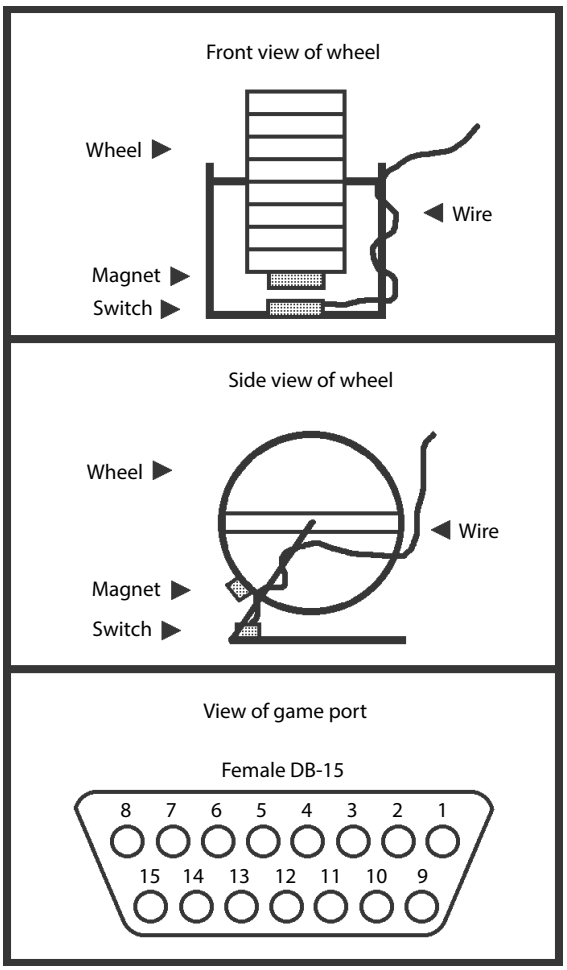


FIGURE 2.61 Running-wheel setup. These diagrams will help you set up your running-wheel data acquisition system. See Exercise 2.3 for details.

a male connector and then attach the male connector to the computer's female connector. *Warning: Make sure to use the correct pin numbers. You may damage your computer if you use the wrong pins!*

7. The CD that comes with the book contains a short DOS program that monitors the status of the four wheels and saves the data to disk every 6 minutes. Although the program does not appear in the Circadian banner, it should have been copied to your hard drive when the software package was installed. Look for it (**Collect.exe**) in the folder where the other programs are ("\Program Files\Circadian") and check it out. Although it is a DOS program, it runs like any Windows program. The only difference is that it does not have a fancy icon.
8. If you like computer programming, you can write your own data collection program and utilize all 8 channels of the game port. How you access the status of the game port depends on the language that you use, so you need to consult the appropriate reference manual. The old **QuickBasic** language that was included in the distribution CD for Windows 95 and Windows 98 provides easy access to the game port channels through the STRIG and STICK functions. Recent versions of major programming languages (Visual C++, Visual Basic, or Java) do not provide direct access to the game port. In these languages, you will need to make use of API calls.
9. After you have made sure that the system is working properly and recording data, put some animals in (don't forget food and water!) and leave them under a light–dark cycle for at least a week. Then look at the data files using the **Plot** program described in Exercise 2.1. The data files will be in the same folder in which you placed **Collect** and will be named in the format HYYMMDD.txt, where H is the channel (A, B, C, or D), YY is the last two digits of the year when data collection started, MM is the month, and DD is the day. Thus, a data file for channel A starting on December 25, 2009 would be named A091225.txt.

SUGGESTIONS FOR FURTHER READING

There are only two books dedicated specifically to research methods in circadian physiology. They are listed first. Other books listed here deal with the fundamentals of the various disciplines pertinent to the study of circadian rhythms.

Young, M. (Ed.) (2005). *Circadian Rhythms* (*Methods in Enzymology*, Vol. 393). San Diego, CA: Academic Press.

An edited book with chapters written by experts in behavioral, genetic, cellular, and molecular research methods in circadian physiology. Volumes 551 and 552 of *Methods in Enzymology* are also about circadian rhythms, but their contents are more about research findings than about research methods, so these two volumes are listed under Suggestions for Further Reading in Chapter 12.

Rosato, E. (Ed.) (2007). *Circadian Rhythms: Methods and Protocols*. Totowa, NJ: Humana Press.

This is also an edited book with chapters written by experts in behavioral, genetic, cellular, and molecular research methods in circadian physiology. There is some overlap with the book edited by Young, but both books are excellent reference books.

Schmidt-Nielsen, K. (1997). *Animal Physiology*, 5th edn. New York: Cambridge University Press.

An excellent introductory physiology textbook, even if almost 20 years old. Schmidt-Nielsen knew how to keep the reader interested. I used an earlier edition when I was a student and loved it.

Kalat, J. W. (2016). *Biological Psychology*, 12th edn. Belmont, CA: Wadsworth.

In my opinion, the best biological psychology textbook in the market. Kalat covers all the important topics and does so with style. The book is very well written, and the production is impeccable.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Morgan, D., Roberts, K., and Walter, P. (2014). *Molecular Biology of the Cell*, 6th edn. New York: Garland Science.

A thorough introductory textbook on cell biology, including molecular genetics. With over 1,500 pages, the book provides the fundamentals essential for the understanding of current developments in molecular biology.

Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S. A., and Hudspeth, A. J. (2013). *Principles of Neural Science*, 5th edn. Columbus, OH: McGraw-Hill.

An upper-level neuroscience textbook. Specialists in different fields contribute chapters from cell biology of neurons to cortical function and everything in between.

Curd, M., Cover, J. A., and Pincock, C. (2012). *Philosophy of Science: The Central Issues*, 2nd edn. New York: W. W. Norton.

A wonderful introduction to philosophy of science in the twentieth century. The book is actually an anthology, but the editors tie the various articles together very well with introductory essays. Among the various authors included in the anthology are Karl Popper, Thomas Kuhn, W. V. Quine, Carl Hempel, and Larry Laudan. (Unfortunately, important French authors such as Gaston Bachelard and Michel Foucault are not included.)

WEBSITES TO EXPLORE

Center for Biological Clocks Research at Texas A&M University: http://clocks.tamu.edu/.

Center for Chronobiology at the University of Basel (Switzerland): http://www.chronobiology.ch.

Center for Circadian Biology at the University of California, San Diego: http://ccb.ucsd.edu/.

Center for Sleep and Circadian Biology at Northwestern University: http://www.cscb.northwestern.edu/.

Center for Study and Treatment of Circadian Rhythms (Canada): http://www.douglas.qc.ca/page/circadian-rhythms-study-centre/.

Jackson Laboratory's Mouse Gene Expression Database: http://www.informatics.jax.org/mgihome/GXD/aboutGXD.shtml.

Rhythms Research Group at Michigan State University: http://rhythms.psy.msu.edu/.

REFERENCES

1. Koertge, N. (Ed.) (1998). *A House Built on Sand: Exposing Postmodernist Myths about Science*. New York: Oxford University Press.
2. Patai, D. (1998). *Heterophobia: Sexual Harassment and the Future of Feminism*. Lanham, MD: Rowman and Littlefield.
3. Gross, P. R. and Levitt, N. (1994). *Higher Superstition: The Academic Left and Its Quarrels with Science*. Baltimore, MD: Johns Hopkins University Press.
4. Golinski, J. (1998). *Making Natural Knowledge: Constructivism and the History of Science*. New York: Cambridge University Press.
5. Fosnot, C. T. (Ed.) (1996). *Constructivism: Theory, Perspectives, and Practice*. New York: Teachers College Press.
6. Mahoney, M. J. (2003). *Constructive Psychotherapy: A Practical Guide*. New York: Guilford.
7. Green, D. M. (Ed.) (2002). *Constructivism and Comparative Politics*. Armonk, NY: M. E. Sharpe.
8. Comte, A. (1877). *Cours de Philosophie Positive*. Paris, France: J. B. Baillière.
9. Plato (1985). *The Republic* (Trans. by R. W. Sterling and W. C. Scott). New York: Norton.
10. Refinetti, R. (2010). Relativism. In: Leeming, D. A., Madden, K., and Marlan, S. (Eds.), *Encyclopedia of Psychology and Religion*. New York: Springer, pp. 762–764.
11. Galilei, G. (1933). Il saggiatore. In: *Le Opere di Galileo Galilei*, Vol. 6. Firenze, Italy: G. Barbèra.
12. Einstein, A. (1955). *The Meaning of Relativity*, 5th edn. Princeton, NJ: Princeton University Press.
13. Committee on the Conduct of Science (1989). *On Being a Scientist*. Washington, DC: National Academy Press.
14. Waterfield, R. (2000). *The First Philosophers: The Presocratics and Sophists*. New York: Oxford University Press.
15. Kuhn, T. S. (1962). *The Structure of Scientific Revolutions*. Chicago, IL: University of Chicago Press.
16. Bellone, E. (1978). Thomas Kuhn and the Book of Nature. *Scientia* 113: 683–688.
17. Lyotard, J. F. (1979). *La Condition Postmoderne: Rapport sur le Savoir*. Paris, France: Minuit.
18. Derrida, J. (1967). *La Voix et le Phénomène*. Paris, France: Presses Universitaires de France.
19. Piaget, J. (1968). *Le Structuralisme*. Paris, France: Presses Universitaires de France.
20. Auzias, J. M. (1971). *Clefs pour le Structuralisme*. Paris, France: Seghers.
21. Foucault, M. (1969). *L'Archéologie du Savoir*. Paris, France: Gallimard.
22. Foucault, M. (1966). *Les Mots et les Choses*. Paris, France: Gallimard.
23. von Glasersfeld, E. (1984). An introduction to radical constructivism. In: Watzlawick, P. (Ed.), *The Invented Reality*. New York: Norton, pp. 17–40.
24. Klotz, I. M. (1996). Postmodernist rhetoric does not change fundamental scientific facts. *Scientist* 10(15): 9.
25. Levitt, N. and Gross, P. R. (1996). Academic anti-science. *Academe* 82(6): 38–42.
26. Hodgkin, P. (1996). Medicine, postmodernism, and the end of certainty. *British Medical Journal* 313: 1568.
27. Horowitz, I. L. (1995). Searching for enemies. *Society* 33: 42–50.
28. Koertge, N. (1998). Postmodernists and the problem of scientific literacy. In: Koertge, N. (Ed.), *A House Built on Sand: Exposing Postmodernist Myths about Science*. New York: Oxford University Press, pp. 257–271.
29. Refinetti, R. (1997). Philosophy of science and physiology education. *American Journal of Physiology* 272: S31–S35.
30. Nickles, T. (1987). The reconstruction of scientific knowledge. *Philosophy and Social Action* 13: 91–104.
31. Miller, D. (1999). Being an absolute skeptic. *Science* 284: 1625–1626.
32. Gould, S. J. (2000). Deconstructing the "science wars" by reconstructing an old mold. *Science* 287: 253–261.
33. Hegel, G. W. F. (1964). *Phänomenologie des Geistes*. Stuttgart, Germany: F. Frommann.
34. Fougeyrollas, P. (1960). *La Philosophie en Question*. Paris, France: Denoël.
35. American Association for the Advancement of Science (1994). *Benchmarks for Science Literacy*. New York: Oxford University Press.

36. Lipton, P. (2007). The world of science. *Science* 316: 834.
37. Medawar, P. B. (1979). *Advice to a Young Scientist*. New York: Basic Books.
38. Feyerabend, P. (1975). *Against Method: Outline of an Anarchistic Theory of Knowledge*. London, U.K.: NLB.
39. Gauch, H. G. (2003). *Scientific Method in Practice*. Cambridge, U.K.: Cambridge University Press.
40. Carey, S. S. (2004). *A Beginner's Guide to Scientific Method*, 3rd edn. Belmont, CA: Wadsworth.
41. Gower, B. (1997). *Scientific Method: A Historical and Philosophical Introduction*. London, U.K.: Routledge.
42. Chang, M. (2014). *Principles of Scientific Methods*. Boca Raton, FL: CRC Press.
43. Descartes, R. (2001). *Discourse on Method* (Trans. by J. Veitch). London, U.K.: J. M. Dent.
44. Platt, J. R. (1964). Strong inference. *Science* 146: 347–353.
45. Rutherford, F. J. and Ahlgren, A. (1990). *Science for All Americans*. New York: Oxford University Press.
46. Paydarfar, D. and Schwartz, W. J. (2001). An algorithm for discovery. *Science* 292: 13.
47. D'Espagnat, B. (1979). The quantum theory and reality. *Scientific American* 241(5): 128–140.
48. Hilborn, R. C. (2000). *Chaos and Nonlinear Dynamics*, 2nd edn. New York: Oxford University Press.
49. Efron, B. (2004). Statistics and the rules of science. *American Statistical Association News* 325: 2–3.
50. Michael, J., Modell, H., McFarland, J., and Cliff, W. (2009). The "core principles" of physiology: What should students understand? *Advances in Physiology Education* 33: 10–16.
51. Bunk, S. (1998). Ethical debate on placebo use may prompt new trial designs. *Scientist* 12(8): 1, 7, 14.
52. Brown, W. A. (1998). Alternative medicine: It's time to get smart. *Scientist* 12(24): 13.
53. Aristotle (1933). *Metaphysics* (Trans. by H. Tredennick). Cambridge, MA: Harvard University Press.
54. Tinbergen, N. (1951). *The Study of Instinct*. Oxford, U.K.: Claredon.
55. Bram, U. (2015). A statistical kind of magic. *Significance* 12: 6.
56. Rosa, L., Rosa, E., Sarner, L., and Barrett, S. (1998). A close look at therapeutic touch. *Journal of the American Medical Association* 279: 1005–1010.
57. Popper, K. (1959). *The Logic of Scientific Discovery*. New York: Harper & Row.
58. Hurlburt, R. T. (2012). *Comprehending Behavioral Statistics*, 5th edn. Dubuque, IA: Kendall Hunt.
59. Sprinthall, R. C. (2011). *Basic Statistical Analysis*, 9th edn. Boston, MA: Allyn and Bacon.
60. Moore, D. S., Notz, W. I., and Fligner, M. A. (2013). *The Basic Practice of Statistics*, 6th edn. New York: Freeman.
61. Weiss, N. A. (2011). *Elementary Statistics*, 8th edn. Reading, MA: Addison-Wesley.
62. Pearl, J. (2009). *Causality: Models, Reasoning, and Inference*, 2nd edn. New York: Cambridge University Press.
63. Dawid, A. P. (2010). Beware of the DAG!. *JMLR: Workshop and Conference Proceedings* 6: 59–86.
64. Sugihara, G., May, R., Ye, H., Hsieh, C., Deyle, E., Fogarty, M., and Munch, S. (2012). Detecting causality in complex ecosystems. *Science* 338: 496–500.
65. de Laplace, P. S. (1840). *Essai Philosophique sur les Probabilités*, 6th edn. Paris, France: Bachelier.
66. Kant, I. (1933). *Critique of Pure Reason* (Translated by N. K. Smith). London, U.K.: Macmillan.
67. Mahner, M. and Bunge, M. (1996). Is religious education compatible with science education? *Science and Education* 5: 101–123.
68. Kurtz, P. (2007). Is religion compatible with science and ethics? In: Kurtz, P. (Ed.), *Science and Ethics*. Amherst, NY: Prometheus, pp. 258–265.
69. Ray, D. W. (2009). *The God Virus: How Religion Infects Our Lives and Cultures*. Bonner Springs, KS: IPC Press.
70. Eller, D. (2010). The cultures of Christianity. In: Loftus, J. W. (Ed.), *The Christian Delusion*. Amherst, NY: Prometheus, pp. 25–46.
71. Gervais, W. M. and Norenzayan, A. (2012). Analytic thinking promotes religious disbelief. *Science* 336: 493–496.
72. Janssen, S. (Ed.) (2014). *The World Almanac and Book of Facts*. New York: Simon & Schuster.
73. Michel, J. B., Shen, Y. K., Aiden, A. P., Veres, A., Gray, M. K., Google Books Team, Pickett, J. P. et al. (2011). Quantitative analysis of culture using millions of digitized books. *Science* 331: 176–182.
74. Abrams, D. M., Yaple, H. A., and Wiener, R. J. (2011). A mathematical model of social group competition with application to the growth of religious non-affiliation. *Cornell University Library* arXiv: 1012.1375.
75. Gross, N. and Simmons, S. (2007). *How Religious are America's College and University Professors*? Social Science Research Council, Brooklyn, NY, http://ssrc.org.
76. Larson, E. J. and Witham, L. (1998). Leading scientists still reject God. *Nature* 394: 313.
77. Dawkins, R. (2006). *The God Delusion*. Boston, MA: Houghton Mifflin.
78. Nietzsche, F. W. (1918). *The Genealogy of Morals* (Trans. by H. B. Samuel). New York: Boni and Liveright.
79. Freud, S. (1928). *The Future of an Illusion* (Trans. by W. D. Robson-Scott). New York: H. Liveright.
80. Marx, K. (1970). *Critique of Hegel's Philosophy of Right* (Trans. by A. Jolin and J. O'Malley). Cambridge, U.K.: Cambridge University Press.
81. Comte, A. (1908). *A General View of Positivism* (Trans. by J.H. Bridges). London, U.K.: Routledge.
82. Paul, G. S. (2009). The chronic dependence of popular religiosity upon dysfunctional psychosociological conditions. *Evolutionary Psychology* 7: 398–441.
83. Oishi, S. and Diener, E. (2014). Residents of poor nations have greater sense of meaning in life than residents of wealthy nations. *Psychological Science* 25: 422–430.
84. Kay, A. C., Whitson, J. A., Gaucher, D., and Galinsky, A. D. (2009). Compensatory control: achieving order through the mind, our institutions, and the heavens. *Current Directions in Psychological Science* 18: 264–268.
85. Kay, A. C., Moscovitch, D. A., and Laurin, K. (2010). Randomness, attributions of arousal, and belief in God. *Psychological Science* 21: 216–218.
86. Gervais, W. M. (2013). Perceiving minds and gods: How mind perception enables, constrains, and is triggered by belief in gods. *Perspectives on Psychological Science* 8: 380–394.
87. Atran, S. and Ginges, J. (2012). Religious and sacred imperatives in human conflict. *Science* 336: 855–857.
88. Edmondson, D., Park, C. L., Chaudoir, S. R., and Wortmann, J. H. (2008). Death without god: Religious struggle, death concerns, and depression in the terminally ill. *Psychological Science* 19: 754–758.
89. Damisch, L., Toberock, B., and Mussweiler, T. (2010). Keep your fingers crossed! How superstition improves performance. *Psychological Science* 21: 1014–1020.

90. Katz, B. F. (2008). *Neuroengineering the Future: Virtual Minds and the Creation of Immortality*. Hingham, MA: Infinity Science Press.
91. Gould, S. J. (1997). Nonoverlapping magisteria. *Natural History* 106: 16–22.
92. Ruse, M. (2010). *Science and Spirituality: Making Room for Faith in the Age of Science*. New York: Cambridge University Press.
93. Stenger, V. J. (2007). *God: The Failed Hypothesis*. Amherst, NY: Prometheus.
94. Malko, J. A., Hutton, W. C., and Fajman, W. A. (2002). An in vivo MRI study of the changes in volume (and fluid content) of the lumbar intervertebral disc after overnight bed rest and during an 8-hour walking protocol. *Journal of Spinal Disorders and Techniques* 15: 157–163.
95. Brown, S. (2012). Misguided state lawmakers, school officials launch attacks on evolution instruction in public school science classes. *Church & State* 65 (2): 4–5.
96. Leshner, A. I. (2005). Redefining science. *Science* 309: 221.
97. Wallis, C. (2005). The evolution wars. *Time Magazine* 166(7): 26–35.
98. Berkman, M. B. and Plutzer, E. (2011). Defeating creationism in the courtroom, but not in the classroom. *Science* 331: 404–405.
99. U.S. Census Bureau (2012). *Statistical Abstract of the United States*. Washington, DC: U.S. Department of Commerce.
100. Miller, J. D., Scott, E.C., and Okamoto, S. (2006). Public acceptance of evolution. *Science* 313: 765–766.
101. Coalition of Scientific Societies (2008). Evolution and its discontents: A role for scientists in science education. *FASEB Journal* 22: 1–4.
102. Congress of the United States (1832). *Treaty of Peace and Friendship between the United States of America and the Bey and Subjects of Tripoli* (American State Papers: 1789–1815, Vol. 2). Washington, DC: Gales and Seaton, pp. 18–19.
103. Tomasin, J. (2008). Clear proof America is not a 'Christian nation'. *Free Inquiry* 28(2): 27–29.
104. Whitten, M. W. (2009). Manufactured myth: America isn't a 'Christian Nation' as the religious right claims and the constitutional convention proves it. *Church & State* 62(2): 20–21.
105. Behe, M. (1996). *Darwin's Black Box: The Biochemical Challenge to Evolution*. New York: Free Press.
106. Davis, P. and Kenyon, D. (1989). *Of Pandas and People: The Central Question of Biological Origins*. Dallas, TX: Haughton.
107. Calvin, C. (2001). Lenticular Cloud (DI00141). In: *UCAR Digital Image Library*. Bolder, CO: National Center for Atmospheric Research.
108. Hughes, G. (1997). Ockham's razor. In: Mautner, T. (Ed.), *Dictionary of Philosophy*. New York: Penguin.
109. Bennett, K. A., McConnell, B. J., and Fedak, M. A. (2001). Diurnal and seasonal variations in the duration and depth of the longest dives in southern elephant seals (*Mirounga leonina*): Possible physiological and behavioral constraints. *Journal of Experimental Biology* 204: 649–662.
110. Berger, A., Scheibe, K. M., Michaelis, S., and Streich, W. J. (2003). Evaluation of living conditions of free-ranging animals by automated chronobiological analysis of behavior. *Behavior Research Methods, Instruments, and Computers* 35: 458–466.
111. Merrill, S. B. and Mech, L. D. (2003). The usefulness of GPS telemetry to study wolf circadian and social activity. *Wildlife Society Bulletin* 31: 947–960.
112. Ensing, E. P., Ciuti, S., de Wijs, F. A. L. M., Lentferink, D. H., ten Hoedt, A., Boyce, M. S., and Hut, R. A. (2014). GPS based daily activity patterns in European red deer and North American elk (*Cervus elaphus*): Indication for a weak circadian clock in ungulates. *PLOS ONE* 9: e106997.
113. Van Bergeijk, W. A. (1967). Anticipatory feeding behaviour in the bullfrog (*Rana catesbeiana*). *Animal Behaviour* 15: 231–238.
114. Refinetti, R., Rissman, E., and Menaker, M. (1991). Circadian organization of sex behavior in pairs of tau-mutant hamsters. *FASEB Journal* 5(5): A1126.
115. Kuijper, B. and Morrow, E. H. (2009). Direct observation of female mating frequency using time-lapse photography. *Fly* 3(2): 1–3.
116. de Chaumont, F., Coura, R. D., Serreau, P., Cressant, A., Chabout, J., Granon, S., and Olivo-Marin, J. C. (2012). Computerized video analysis of social interactions in mice. *Nature Methods* 9: 410–417.
117. Meyer-Peters, H. (1993). Seasonality of circadian locomotor activity in an insect. *Journal of Comparative Physiology A* 171: 713–724.
118. Shimizu, I., Kawai, Y., Taniguchi, M., and Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
119. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., and Tomioka, K. (2004). *Drosophila cry^b* mutation reveals two circadian clocks that drive locomotor rhythm and have different responsiveness to light. *Journal of Insect Physiology* 50: 479–488.
120. Albers, H. E., Gerall, A. A., and Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
121. Boulos, Z., Macchi, M., Houpt, T. A., and Terman, M. (1996). Photic entrainment in hamsters: Effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
122. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
123. Pasquali, V., Scannapieco, E., and Renzi, P. (2006). Validation of a microwave radar system for the monitoring of locomotor activity in mice. *Journal of Circadian Rhythms* 4: art. 7.
124. Chiesa, J. J., Araujo, J. F., and Díez-Noguera, A. (2006). Method for studying behavioural activity patterns during long-term recordings using a force-plate actometer. *Journal of Neuroscience Methods* 158: 157–168.
125. Basu, P., Singaravel, M., and Refinetti, R. (2015). Experimental quantification of improvement during circadian wheel running in the Indian field mouse, *Mus terricolor*: Theoretical uses. *Biological Rhythm Research* 46: 173–180.
126. Refinetti, R., Kaufman, C. M., and Menaker, M. (1994). Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters. *Journal of Comparative Physiology A* 175: 223–232.
127. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S., and Takahashi, K. (1988). Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiology and Behavior* 42: 87–91.
128. Edgar, D. M., Martin, C. E., and Dement, W. C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *Journal of Biological Rhythms* 6: 185–199.

129. Edgar, D. M., Kilduff, T. S., Martin, C. E., and Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior* 50: 373–378.
130. Kuroda, H., Fukushima, M., Nakai, M., Katayama, T., and Murakami, N. (1997). Daily wheel running activity modifies the period of free-running rhythms in rats via intergeniculate leaflet. *Physiology and Behavior* 61: 633–637.
131. Mistlberger, R. E., Bossert, J. M., Holmes, M. M., and Marchant, E. G. (1998). Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. *Behavioural Brain Research* 96: 93–99.
132. Mrosovsky, N. (1999). Further experiments on the relationship between the period of circadian rhythms and locomotor activity levels in hamsters. *Physiology and Behavior* 66: 797–801.
133. Mistlberger, R. E. and Holmes, M. M. (2000). Behavioral feedback regulation of circadian rhythm phase angle in light–dark entrained mice. *American Journal of Physiology* 279: R813–R821.
134. Deboer, T. and Tobler, I. (2000). Running wheel size influences circadian rhythm period and its phase shift in mice. *Journal of Comparative Physiology A* 186: 969–973.
135. Koteja, P., Swallow, J. G., Carter, P. A., and Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.
136. Antinuchi, C. D., Luna, F., and Busch, C. (1999). Automatic data recording of circadian rhythms. *Journal of Biological Education* 33: 220–222.
137. Oshima, I. and Ebihara, S. (1988). The measurement and analysis of circadian locomotor activity and body temperature rhythms by a computer-based system. *Physiology and Behavior* 43: 115–119.
138. Sharma, V. K. (2003). A simple computer-aided device for monitoring activity of small mammals and insects. *Biological Rhythm Research* 34: 3–12.
139. Smith, T. L., Coleman, T. G., Stanek, K. A., and Murphy, W. R. (1987). Hemodynamic monitoring for 24 h in unanesthetized rats. *American Journal of Physiology* 253: H1335–H1341.
140. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M., and Loudon, A. S. I. (1999). Free running circadian rhythms of melatonin, luteinizing hormone, and cortisol in Syrian hamsters bearing the circadian *tau* mutation. *Endocrinology* 140: 758–764.
141. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D., and Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
142. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P., and Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
143. Refinetti, R. and Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
144. Kramer, K., Grimbergen, J., Brockway, B., Brockway, B., and Voss, H. P. (1999). Circadian respiratory rate rhythms in freely moving small laboratory animals using radio telemetry. *Lab Animal* 28(4): 38–41.
145. Ishii, K., Uchino, M., Kuwahara, M., Tsubone, H., and Ebukuro, S. (2002). Diurnal fluctuations of heart rate, body temperature and locomotor activity in the house musk shrew (*Suncus murinus*). *Experimental Animals* 51: 57–62.
146. Ray, B., Mallick, H. N., and Kumar, V. M. (2004). Changes in thermal preference, sleep-wakefulness, body temperature and locomotor activity of rats during continuous recording for 24 hours. *Behavioural Brain Research* 154: 519–526.
147. Refinetti, R. (1990). Experimentally induced disruption of the diurnal rhythm of body temperature of the rat. *Biotemas* 3(2): 47–58.
148. Tornatzky, W. and Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior* 53: 983–993.
149. Weinert, D., Uhlemann, S., and Eimert, H. (1994). Age-depending effects of narcosis and transmitter implantation on circadian body temperature and activity rhythms in mice. *Biological Rhythm Research* 25: 203–211.
150. Leon, L. R., Walker, L. D., DuBose, D. A., and Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: Impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
151. Kolka, M. A., Levine, L., and Stephenson, L. A. (1997). Use of ingestible telemetry sensor to measure core temperature under chemical protective clothing. *Journal of Thermal Biology* 22: 343–349.
152. Byrne, C. and Lim, C. L. (2007). The ingestible telemetric body core temperature sensor: A review of validity and exercise applications. *British Journal of Sports Medicine* 41: 126–133.
153. Edwards, B., Waterhouse, J., Reilly, T., and Atkinson, G. (2002). A comparison of the suitabilities of rectal, gut, and insulated axilla temperatures for measurement of the circadian rhythm of core temperature in field studies. *Chronobiology International* 19: 579–597.
154. Rawson, R. O., Stolwijk, J. A. J., Graichen, H., and Abrams, R. (1965). Continuous radio telemetry of hypothalamic temperatures from unrestrained animals. *Journal of Applied Physiology* 20: 321–325.
155. Spencer, H. (1968). Thermally stable telemeter for thermoregulation studies. *Science* 161: 574–575.
156. Pivorun, E. B. (1976). A biotelemetry study of the thermoregulatory patterns of *Tamias striatus* and *Eutamias minimus* during hibernation. *Comparative Biochemistry and Physiology A* 53: 265–271.
157. Thornhill, J. A., Hirst, M., and Gowdey, C. W. (1978). Measurement of diurnal core temperatures of rats in operant cages by AM telemetry. *Canadian Journal of Physiology and Pharmacology* 56: 1047–1050.
158. Cunningham, C. L. and Peris, J. (1983). A microcomputer system for temperature biotelemetry. *Behavior Research Methods and Instrumentation* 15: 598–603.
159. Varosi, S. M., Brigmon, R. L., and Besch, E. L. (1990). A simplified telemetry system for monitoring body temperature in small animals. *Laboratory Animal Science* 40: 299–302.
160. Chesmore, E. D., Yoon, C. S., Talling, J. C., van Driel, K. S., and Lake, M. (2003). Non-invasive sensors for monitoring the body temperature and heart rate of pigs during transport. In: Cox, S. (Ed.), *Precision Livestock Farming*. Wageningen, the Netherlands: Wageningen Academic Publishers, pp. 33–37.
161. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A., and Littledike, E. T. (1990). Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. *Journal of Animal Science* 68: 2658–2665.
162. Moore, R. J., Watts, J. T., Hood, J. A., and Burritt, D. J. (1999). Intra-oral temperature variation over 24 hours. *European Journal of Orthodontics* 21: 249–261.

163. Nocek, J. E., Allman, J. G., and Kautz, W. P. (2002). Evaluation of an indwelling ruminal probe methodology and effect of grain level on diurnal pH variation in dairy cattle. *Journal of Dairy Science* 85: 422–428.
164. Hermida, R. C., Fernández, J. R., Ayala, D. E., Mojón, A., Alonso, I., and Calvo, C. (2004). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiology International* 21: 147–160.
165. Lovegrove, B. G. (2009). Modification and miniaturization of Thermochron iButtons for surgical implantation into small animals. *Journal of Comparative Physiology B* 179: 451–458.
166. Romeijn, N. and Van Someren, E. J. W. (2011). Correlated fluctuations of daytime skin temperature and vigilance. *Journal of Biological Rhythms* 26: 68–77.
167. Kolodyazhniy, V., Späti, J., Frey, S., Götz, T., Wirz-Justice, A., Kräuchi, K., Cajochen, C., and Wilhelm, F. H. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. *Journal of Biological Rhythms* 26: 55–67.
168. Martinez-Nicolas, A., Ortiz-Tudela, E., Rol, M. A., and Madrid, J. A. (2013). Uncovering different masking factors on wrist skin temperature rhythm in free-living subjects. *PLOS ONE* 8: e61142.
169. McCafferty, D. J. (2007). The value of infrared thermography for research on mammals: Previous applications and future directions. *Mammal Review* 37: 207–223.
170. Benloucif, S., Burgess, H. J., Klerman, E. B., Lewy, A. J., Middleton, B., Murphy, P. J., Parry, B. L., and Revell, V. L. (2008). Measuring melatonin in humans. *Journal of Clinical Sleep Medicine* 4: 66–69.
171. Adolph, E. F. (1950). Oxygen consumption of hypothermic rats and acclimatization to cold. *American Journal of Physiology* 161: 359–373.
172. Lindmark, L., Rudholm, L., and Lundholm, K. (1986). A method for measuring energy metabolism and whole body oxidation in small animals with special reference to experimental metabolism and nutrition. *Scandinavian Journal of Clinical and Laboratory Investigation* 46: 273–281.
173. Lavoisier, A. L. (1777). Expériences sur la respiration des animaux et sur les changements qui arrivent a l'air en passant par leur poumon (*Mémoires de l'Académie des Sciences*, 1777, p. 185). In: *Oeuvres de Lavoisier, Tome II*. Paris, France: Imprimerie Impériale (1862), pp. 174–183.
174. Lavoisier, A. L. and de Laplace, P. S. (1780). Mémoire sur la chaleur (*Mémoires de l'Academie des Sciences*, 1780, p. 355). In: *Oeuvres de Lavoisier, Tome II*. Paris, France: Imprimerie Impériale (1862), pp. 283–333.
175. Du Bois, E. F. (1924). A graphic representation of the respiratory quotient and the percentage of calories from protein, fat, and carbohydrate. *Journal of Biological Chemistry* 59: 43–49.
176. Horst, K., Mendel, L. B., and Benedict, F. G. (1930). The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. *Journal of Nutrition* 3: 177–200.
177. Kayser, C. (1937). Variations du quotient respiratoire en fonction de la température du milieu chez le rat, le pigeon et le cobaye. *Comptes Rendus des Séances de la Société de Biologie de Strasbourg* 126: 1219–1222.
178. Swift, R. W. and Forbes, R. M. (1939). The heat production of the fasting rat in relation to the environmental temperature. *Journal of Nutrition* 18: 307–318.
179. Pagé, E. and Chénier, L. P. (1953). Effects of diets and cold environment on the respiratory quotient of the white rat. *Revue Canadienne de Biologie* 12: 530–541.
180. Nakatsuka, H., Shoji, Y., and Tsuda, T. (1983). Effects of cold exposure on gaseous metabolism and body composition in the rat. *Comparative Biochemistry and Physiology A* 75: 21–25.
181. Diamond, P. and LeBlanc, J. (1987). Hormonal control of postprandial thermogenesis in dogs. *American Journal of Physiology* 253: E521–E529.
182. Refinetti, R. (1989). Effect of ambient temperature on respiratory quotient of lean and obese Zucker rats. *American Journal of Physiology* 256: R236–R239.
183. McKechnie, A. E. and Lovegrove, B. G. (2003). Facultative hypothermic responses in an Afrotropical arid-zone passerine, the red-headed finch (*Amadina erythrocephala*). *Journal of Comparative Physiology B* 173: 339–346.
184. Livesey, G. and Elia, M. (1988). Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: Evaluation of errors with special reference to the detailed composition of fuels. *American Journal of Clinical Nutrition* 47: 608–628.
185. Mansell, P. I. and Macdonald, I. A. (1990). Reappraisal of the Weir equation for calculation of metabolic rate. *American Journal of Physiology* 258: R1347–R1354.
186. Barlow, C. A. and Kuenen, D. J. (1957). A new thermopreferendum apparatus for terrestrial isopods. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Ser. C* 60: 240–250.
187. Krafft, B. (1967). Thermopreferendum de l'araignée sociale *Agelena consociata* Denis. *Insectes Sociaux* 14: 161–182.
188. Laughter, J. S. and Blatteis, C. M. (1985). A system for the study of behavioral thermoregulation of small animals. *Physiology and Behavior* 35: 993–997.
189. Gordon, C. J., Lee, K. A., Chen, T. A., Killough, P., and Ali, J. S. (1991). Dynamics of behavioral thermoregulation in the rat. *American Journal of Physiology* 261: R705–R711.
190. Gordon, C. J. (1994). 24-Hour control of body temperature in rats. I. Integration of behavioral and autonomic effectors. *American Journal of Physiology* 267: R71–R77.
191. Refinetti, R. (1995). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behavioral Neuroscience* 109: 523–527.
192. Cheng, P., Yang, Y., and Liu, Y. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences United States of America* 98: 7408–7413.
193. Froehlich, A. C., Liu, Y., Loros, J. J., and Dunlap, J. C. (2002). White collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. *Science* 297: 815–819.
194. Vitalini, M. W., Morgan, L. W., March, I. J., and Bell-Pedersen, D. (2004). A genetic selection for circadian output pathway mutations in *Neurospora crassa*. *Genetics* 167: 119–129.
195. Horne, J. A. and Östberg, O. (1976). A self-assessment questionnaire to determine morningness–eveningness in human circadian rhythms. *International Journal of Chronobiology* 4: 97–110.
196. Adan, A. and Almirall, H. (1991). Horne & Östberg morningness–eveningness questionnaire: A reduced scale. *Personality and Individual Differences* 12: 241–253.
197. Koscec, A., Radosevic-Vidacek, B., and Kostovic, M. (2001). Morningness–eveningness across two student generations: Would two decades make a difference? *Personality and Individual Differences* 31: 627–638.

198. Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M., and Tisak, J. (2002). Investigation of morning–evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.
199. Roenneberg, T., Wirz-Justice, A., and Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
200. Di Milia, L., Smith, P. A., and Folkard, S. (2004). Refining the psychometric properties of the circadian type inventory. *Personality and Individual Differences* 36: 1953–1964.
201. Miguel, M., de Oliveira, V. C., Pereira, D., and Pedrazzoli, M. (2014). Detecting chronotype differences associated to latitude: A comparison between Horne-Östberg and Munich Chronotype questionnaires. *Annals of Human Biology* 41: 105–108.
202. Shanahan, T. L. and Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *Journal of Clinical Endocrinology and Metabolism* 73: 227–235.
203. Kennaway, D. J. and Van Dorp, C. F. (1991). Free-running rhythms of melatonin, cortisol, electrolytes, and sleep in humans in Antarctica. *American Journal of Physiology* 260: R1137–R1144.
204. Guerin, M. V., Deed, J. R., Kennaway, D. J., and Matthews, C. D. (1995). Plasma melatonin in the horse: Measurements in natural photoperiod and in acutely extended darkness throughout the year. *Journal of Pineal Research* 19: 7–15.
205. Irvine, C. H. G. and Alexander, S. L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology* 11: 227–238.
206. Thrun, L. A., Moenter, S. M., O'Callaghan, D., Woodfill, C. J. I., and Karsch, F. J. (1995). Circannual alterations in the circadian rhythm of melatonin secretion. *Journal of Biological Rhythms* 10: 42–54.
207. Piccione, G., Caola, G., and Refinetti, R. (2003). Circadian rhythms of body temperature and liver function in fed and food-deprived goats. *Comparative Biochemistry and Physiology A* 134: 563–572.
208. Wright, K. P., Hughes, R. J., Kronauer, R. E., Dijk, D. J., and Czeisler, C. A. (2001). Intrinsic near-24-h pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. *Proceedings of the National Academy of Sciences United States of America* 98: 14027–14032.
209. Ruiter, M., La Fleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A., and Buijs, R. M. (2003). The daily rhythm in plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. *Diabetes* 52: 1709–1715.
210. Aschoff, J., Gerecke, U., and Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
211. Hack, L. M., Lockley, S. W., Arendt, J., and Skene, D. J. (2003). The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects. *Journal of Biological Rhythms* 18: 420–429.
212. Moore-Ede, M. C., Kass, D. A., and Herd, J. A. (1977). Transient circadian internal desynchronization after light–dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
213. Roelfsema, F., van der Heide, D., and Smeenk, D. (1980). Circadian rhythms of urinary electrolyte excretion in freely moving rats. *Life Sciences* 27: 2303–2309.
214. Voultsios, A., Kennaway, D. J., and Dawson, D. (1997). Salivary melatonin as a circadian phase marker: Validation and comparison to plasma melatonin. *Journal of Biological Rhythms* 12: 457–466.
215. Jelínková-Vondrasová, D., Hájek, I., and Illnerová, H. (1999). Adjustment of the human circadian system to changes of the sleep schedule under dim light at home. *Neuroscience Letters* 265: 111–114.
216. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C., and Weber, J. M. (2004). Evening melatonin and bright light administration induce additive phase shifts in dim light melatonin onset. *Journal of Pineal Research* 36: 192–194.
217. Hasegawa, M. and Ebihara, S. (1992). Circadian rhythms of pineal melatonin release in the pigeon measured by in vivo microdialysis. *Neuroscience Letters* 148: 89–92.
218. Nakahara, D., Nakamura, M., Iigo, M., and Okamura, H. (2003). Bimodal circadian secretion of melatonin from the pineal gland in a living CBA mouse. *Proceedings of the National Academy of Sciences United States of America* 100: 9584–9589.
219. Maywood, E. S., Hastings, M. H., Max, M., Ampleford, E., Menaker, M., and Loudon, A. S. I. (1993). Circadian and daily rhythms of melatonin in the blood and pineal gland of free-running and entrained Syrian hamsters. *Journal of Endocrinology* 136: 65–73.
220. Sun, X., Liu, T., Deng, J., and Borjigin, J. (2003). Long-term in vivo pineal microdialysis. *Journal of Pineal Research* 35: 118–124.
221. Liu, T. and Borjigin, J. (2006). Relationship between nocturnal serotonin surge and melatonin onset in rodent pineal gland. *Journal of Circadian Rhythms* 4: art. 12.
222. Schneider, B. H., Hill, M. R. S., and Prohaska, O. J. (1990). Microelectrode probes for biomedical applications. *American Biotechnology Laboratory* 8(2): 17–23.
223. Russell, D. M., Garry, E. M., Taberner, A. J., Barrett, C. J., Paton, J. F. R., Budgett, D. M., and Malpas, S. C. (2012). A fully implantable telemetry system for the chronic monitoring of brain tissue oxygen in freely moving rats. *Journal of Neuroscience Methods* 204: 242–248.
224. Foster, D. O. and Frydman, M. L. (1979). Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by non-shivering thermogenesis. *Canadian Journal of Physiology and Pharmacology* 57: 257–270.
225. Klaunberg, B. A. and Lizak, M. J. (2004). Considerations for setting up a small-animal imaging facility. *Lab Animal* 33(3): 28–34.
226. Kasischke, K. A., Vishwasrao, H. D., Fisher, P. J., Zipfel, W. R., and Webb, W. W. (2004). Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science* 305: 99–103.
227. Sokoloff, L. (1981). Localization of functional activity in the central nervous system by measurement of glucose utilization with radioactive deoxyglucose. *Journal of Cerebral Blood Flow and Metabolism* 1: 7–36.
228. Borowsky, I. W. and Collins, R. C. (1989). Metabolic anatomy of brain: A comparison of regional capillary density, glucose metabolism, and enzyme activities. *Journal of Comparative Neurology* 288: 401–413.
229. Schwartz, W. J., Davidsen, L. C., and Smith, C. B. (1980). *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *Journal of Comparative Neurology* 189: 157–167.

230. Room, P., Tielemans, A. J. P. C., De Boer, T., Tonnaer, J. A. D. M., Wester, J., Van den Broek, J. H. M., and Van Delft, A. M. L. (1989). Local cerebral glucose uptake in anatomically defined structures of freely moving rats. *Journal of Neuroscience Methods* 27: 191–202.
231. Servière, J., Gendrot, G., LeSauter, J., and Silver, R. (1994). Host resets phase of grafted suprachiasmatic nucleus: A 2-DG study of time course of entrainment. *Brain Research* 655: 168–176.
232. Pellerin, L. and Magistretti, P. J. (2004). Let there be (NADH) light. *Science* 305: 50–52.
233. Morgan, J. I. and Curran, T. (1989). Stimulus-transcription coupling in neurons: Role of cellular immediate-early genes. *Trends in Neural Science* 12: 459–462.
234. Dragunow, M. and Faull, R. (1989). The use of c-*fos* as a metabolic marker in neuronal pathway tracing. *Journal of Neuroscience Methods* 29: 261–265.
235. Prosser, R. A., Macdonald, E. S., and Heller, H. C. (1994). c-*fos* mRNA in the suprachiasmatic nuclei in vitro shows a circadian rhythm and responds to a serotonergic agonist. *Molecular Brain Research* 25: 151–156.
236. Krajnak, K., Dickenson, L., and Lee, T. M. (1997). The induction of Fos-like proteins in the suprachiasmatic nuclei and intergeniculate leaflet by light pulses in degus (*Octodon degus*) and rats. *Journal of Biological Rhythms* 12: 401–412.
237. Novak, C. M. and Nunez, A. A. (1998). Daily rhythms in Fos activity in the rat ventrolateral preoptic area and midline thalamic nuclei. *American Journal of Physiology* 275: R1620–R1626.
238. Sumová, A., Trávnícková, Z., Mikkelsen, J. D., and Illnerová, H. (1998). Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research* 801: 254–258.
239. Caldelas, I., Salazar-Juárez, A., Granados-Fuentes, D., Escobar, C., and Aguilar-Roblero, R. (1998). Circadian modulation of c-Fos expression occurs only in the SCN and not in other visual projection areas in the rat. *Biological Rhythm Research* 29: 494–500.
240. Novak, C. M., Smale, L., and Nunez, A. A. (1999). Fos expression in the sleep–active cell group of the ventrolateral preoptic area in the diurnal murid rodent, *Arvicanthis niloticus*. *Brain Research* 818: 375–382.
241. Guido, M. E., de Guido, L. B., Goguen, D., Robertson, H. A., and Rusak, B. (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *Journal of Biological Rhythms* 14: 275–280.
242. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M., and Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685.
243. Asai, M., Yamaguchi, S., Isejima, H., Janouchi, M., Moriya, T., Shibata, S., Kobayashi, M., and Okamura, H. (2001). Visualization of *mPer1* transcription in vitro: NMDA induces a rapid shift of *mPer1* gene in cultured SCN. *Current Biology* 11: 1524–1527.
244. Izumo, M., Johnson, C. H., and Yamazaki, S. (2003). Circadian gene expression in mammalian fibroblasts revealed by real-time luminescence reporting: Temperature compensation and damping. *Proceedings of the National Academy of Sciences United States of America* 100: 16089–16094.
245. Terazono, H., Mutoh, T., Yamaguchi, S., Kobayashi, M., Akiyama, M., Udo, R., Ohdo, S., Okamura, H., and Shibata, S. (2003). Adrenergic regulation of clock gene expression in mouse liver. *Proceedings of the National Academy of Sciences United States of America* 100: 6795–6800.
246. Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka et al. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences United States of America* 101: 5339–5346.
247. Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S., and Johnson, C. H. (1993). Circadian rhythms in prokaryotes: Luciferase as a reporter of circadian gene expression in cyanobacteria. *Proceedings of the National Academy of Sciences United States of America* 90: 5672–5676.
248. Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-ike, N., and Ishiura, M. (2004). Large-scale screening of *Arabidopsis* circadian clock mutants by a high-throughput real-time bioluminescence monitoring system. *Plant Journal* 40: 1–11.
249. Collaco, A. M. and Geusz, M. E. (2003). Monitoring immediate-early gene expression through firefly luciferase imaging of HRS/J hairless mice. *BMC Physiology* 3: art. 8.
250. Collaco, A. M., Rahman, S., Dougherty, E. J., Williams, B. B., and Geusz, M. E. (2005). Circadian regulation of a viral gene promoter in live transgenic mice expressing firefly luciferase. *Molecular Imaging and Biology* 7: 342–350.
251. Kuroda, H., Tahara, Y., Saito, K., Ohnishi, N., Kubo, Y., Seo, Y., Otsuka, M. et al. (2012). Meal frequency patterns determine the phase of mouse peripheral circadian clocks. *Scientific Reports* 2: art. 711.
252. Yamaguchi, S., Kobayashi, M., Mitsui, S., Ishida, Y., van der Horst, G. T. S., Suzuki, M., Shibata, S., and Okamura, H. (2001). View of a mouse clock ticking. *Nature* 409: 684.
253. Saini, C., Liani, A., Curie, T., Gos, P., Kreppel, F., Emmenegger, Y., Bonacina, L. et al. (2013). Real-time recording of circadian liver gene expression in freely moving mice reveals the phase-setting behavior of hepatocyte clocks. *Genes and Development* 27: 1526–1536.
254. Hamada, T., Ishikawa, M., Sutherland, K., Miyamoto, N., Shirato, H., Honma, S., and Honma, K. (2014). In vivo whole body 4D imaging by tracking clock gene expression in multiregions of freely moving mice. *Sapporo Symposium on Biological Rhythms* 15: 98.
255. Rumsey, W. L., Vanderkooi, J. M., and Wilson, D. F. (1988). Imaging of phosphorescence: A novel method for measuring oxygen distribution in perfused tissue. *Science* 241: 1649–1651.
256. Seydel, C. (2003). Quantum dots get wet. *Science* 300: 80–81.
257. Larson, D. R., Zipfel, W. R., Williams, R. M., Clark, S. W., Bruchez, M. P., Wise, F. W., and Webb, W. W. (2003). Water-soluble quantum dots for multiphoton fluorescence imaging in vivo. *Science* 300: 1434–1436.
258. Bailey, R. E. and Nie, S. (2003). Alloyed semiconductor quantum dots: Tuning the optical properties without changing the particle size. *Journal of the American Chemical Society* 125: 7100–7106.
259. Michalet, X., Pinaud, F. F., Bentolila, L. A., Tsay, J. M., Doose, S., Li, J. J., Sundaresan, G., Wu, A. M., Gambhir, S. S., and Weiss, S. (2005). Quantum dots for live cells, in vivo imaging, and diagnostics. *Science* 307: 538–544.
260. Bozza, T., McGann, J. P., Mombaerts, P., and Wachowiak, M. (2004). In vivo imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* 42: 9–21.
261. Stephens, D. J. and Allan, V. J. (2003). Light microscopy techniques for live cell imaging. *Science* 300: 82–86.

262. Akemann, W., Mutoh, H., Perron, A., Rossier, J., and Knöpfel, T. (2010). Imaging brain electric signals with genetically targeted voltage-sensitive fluorescent proteins. *Nature Methods* 7: 643–649.
263. Berning, S., Willig, K. I., Steffens, H., Dibaj, P., and Hell, S. W. (2012). Nanoscopy in a living mouse brain. *Science* 335: 551.
264. Enoki, R., Ono, D., Hasan, M. T., Honma, S., and Honma, K. (2012). Single-cell resolution fluorescence imaging of circadian rhythms detected with a Nipkow spinning disk confocal system. *Journal of Neuroscience Methods* 207: 72–79.
265. Packer, A. M., Russell, L. E., Dalgleish, H. W. P., and Häusser, M. (2015). Simultaneous all-optical manipulation and recording of neural circuit activity with cellular resolution in vivo. *Nature Methods* 12: 140–146.
266. Lucas, K. (1905). On the gradation of activity in a skeletal muscle-fibre. *Journal of Physiology* 33: 125–137.
267. Adrian, E. D. (1914). The all-or-none principle in nerve. *Journal of Physiology* 47: 460–474.
268. Hodgkin, A. L. and Huxley, A. F. (1939). Action potentials recorded from inside a nerve fibre. *Nature* 144: 710–711.
269. Meijer, J. H., Schaap, J., Watanabe, K., and Albus, H. (1997). Multiunit activity recordings in the suprachiasmatic nuclei: In vivo versus in vitro models. *Brain Research* 753: 322–327.
270. Prosser, R. A. (1998). In vitro circadian rhythms of the mammalian suprachiasmatic nuclei: Comparison of multi-unit and single-unit neuronal activity recordings. *Journal of Biological Rhythms* 13: 30–38.
271. Mrugala, M., Zlomanczuk, P., Jagota, A., and Schwartz, W. J. (2000). Rhythmic multiunit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *American Journal of Physiology* 278: R987–R994.
272. Colwell, C. S. (2000). Rhythmic coupling among cells in the suprachiasmatic nucleus. *Journal of Neurobiology* 43: 379–388.
273. Saeb-Parsy, K. and Dyball, R. E. J. (2003). Defined cell groups in the rat suprachiasmatic nucleus have different day/night rhythms of single-unit activity in vivo. *Journal of Biological Rhythms* 18: 26–42.
274. Burgoon, P. W., Lindberg, P. T., and Gillette, M. U. (2004). Different patterns of circadian oscillation in the suprachiasmatic nucleus of hamster, mouse, and rat. *Journal of Comparative Physiology A* 190: 167–171.
275. Van Diepen, H. C., Ramkisoensing, A., Peirson, S. N., Foster, R. G., and Meijer, J. H. (2013). Irradiance encoding in the suprachiasmatic nuclei by rod and cone photoreceptors. *FASEB Journal* 27: 4204–4212.
276. Silverman, P. H. (2004). Rethinking genetic determinism. *Scientist* 18(10): 32–33.
277. Chin, J. W., Cropp, T. A., Anderson, J. C., Mukherji, M., Zhang, Z., and Schultz, P. G. (2003). An expanded eukaryotic genetic code. *Science* 301: 964–967.
278. Kubicek, S. (2011). Epigenetics: A primer. *Scientist* 25(3): 32–33.
279. Bonasio, R., Tu, S., and Reinberg, D. (2010). Molecular signals of epigenetic states. *Science* 330: 612–616.
280. DePalma, A. (2003). Mass spectrometry and proteomics. *Scientist* 17(16): 44–46.
281. Tabb, D. L. (2012). Evaluating protein interactions through cross-linking mass spectrometry. *Nature Methods* 9: 879–881.
282. Grigorenko, E. V. (2002). *DNA Arrays: Technologies and Experimental Strategies*. Boca Raton, FL: CRC Press.
283. Correa, A., Lewis, Z. A., Greene, A. V., March, I. J., Gomer, R. H., and Bell-Pedersen, D. (2003). Multiple oscillators regulate circadian gene expression in *Neurospora*. *Proceedings of the National Academy of Sciences United States of America* 100: 13597–13602.
284. McDonald, M. J. and Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* 107: 567–578.
285. Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N., and Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32: 657–671.
286. Ceriani, M. F., Hogenesch, J. B., Yanovsky, M., Panda, S., Straume, M., and Kay, S. A. (2002). Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *Journal of Neuroscience* 22: 9305–9319.
287. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N., and Taghert, P. H. (2002). Influence of the *period*-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences United States of America* 99: 9562–9567.
288. Kita, Y., Shiozawa, M., Jin, W., Majewski, R. R., Besharse, J. C., Greene, A. S., and Jacob, H. J. (2002). Implications of circadian gene expression in kidney, liver and the effects of fasting on pharmacogenomic studies. *Pharmacogenetics* 12: 55–65.
289. Grundschober, C., Delaunay, F., Pühlhofer, A., Triqueneaux, G., Laudet, V., Bartfai, T., and Nef, P. (2001). Circadian regulation of diverse gene products revealed by mRNA expression profiling of synchronized fibroblasts. *Journal of Biological Chemistry* 276: 46751–46758.
290. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H., and Kyriacou, C. P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Current Biology* 12: 540–550.
291. Duffield, G. E., Best, J. D., Meurers, B. H., Bittner, A., Loros, J. J., and Dunlap, J. C. (2002). Circadian programs of transcriptional activation, signaling, and protein turnover revealed by microarray analysis of mammalian cells. *Current Biology* 12: 551–557.
292. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H., and Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.
293. Lane, L. (2003). Protein microarrays at the cusp. *Scientist* 17(14): 42–44.
294. Melton, L. (2004). Proteomics in multiplex. *Nature* 429: 101–107.
295. Ramachandran, N., Hainsworth, E., Bhullar, B., Eisenstein, S., Rosen, B., Lau, A. Y., Walter, J. C., and LaBaer, J. (2004). Self-assembling protein microarrays. *Science* 305: 86–90.
296. Zhu, H. and Qian, J. (2012). Applications of functional protein microarrays in basic and clinical research. *Advances in Genetics* 79: 123–155.
297. Uttal, W. R. (1973). *The Psychobiology of Sensory Coding*. New York: Harper & Row.
298. Zaha, M. A. (1972). Shedding some needed light on optical measurements. *Electronics* Nov. 6: 91–96.
299. Wyszecki, G. and Stiles, W. S. (1982). *Color Science: Concepts and Methods*, 2nd edn. New York: Wiley.

300. Johnston, S. F. (2001). *A History of Light and Colour Measurement: Science in the Shadows*. Bristol, U.K.: Institute of Physics.
301. Rea, M. S., Figueiro, M. G., Bierman, A., and Bullough, J. D. (2010). Circadian light. *Journal of Circadian Rhythms* 8: art. 2.
302. Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. London, U.K.: Chapman and Hall.
303. Lide, D. R. (Ed.) (2002). *Handbook of Chemistry and Physics*, 83rd edn. Boca Raton, FL: CRC Press.
304. Middleton, W. E. K. (1966). *A History of the Thermometer and Its Use in Meteorology*. Baltimore, MD: Johns Hopkins Press.
305. Christian, K. A. and Tracy, C. R. (1985). Measuring air temperature in field studies. *Journal of Thermal Biology* 10: 55–56.
306. Walsberg, G. E. and Weathers, W. W. (1986). A simple technique for estimating operative environmental temperature. *Journal of Thermal Biology* 11: 67–72.
307. Rea, M. S. (Ed.) (2000). *The IESNA Lighting Handbook*, 9th edn. New York: Illuminating Engineering Society of North America.
308. Schubert, E. F. and Kim, J. K. (2005). Solid-state light sources getting smart. *Science* 308: 1274–1278.
309. Nelson, D. E. and Takahashi, J. S. (1991). Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *Journal of Physiology* 439: 115–145.
310. Ralph, M. R. and Mrosovsky, N. (1992). Behavioral inhibition of circadian responses to light. *Journal of Biological Rhythms* 7: 353–359.
311. Shimomura, K. and Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
312. Challet, E., Losee-Olson, S., and Turek, F. W. (1999). Reduced glucose availability attenuates circadian responses to light in mice. *American Journal of Physiology* 276: R1063–R1070.
313. Boulos, Z., Macchi, M. M., and Terman, M. (2002). Twilights widen the range of photic entrainment in hamsters. *Journal of Biological Rhythms* 17: 353–363.
314. Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Lelièvre, V., Hu, Z., and Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology* 287: R1194–R1201.
315. Marchant, E. G. and Mistlberger, R. E. (1997). Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6J mice. *Brain Research* 765: 273–282.
316. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1998). Ultraviolet-light-evoked phase shifts in the locomotor activity rhythm of the field mouse *Mus booduga*. *Journal of Photochemistry and Photobiology B* 45: 83–86.
317. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M., and Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
318. Best, J. D., Maywood, E. S., Smith, K. L., and Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *Journal of Neuroscience* 19: 828–835.
319. Canal-Corretger, M. M., Vilaplana, J., Cambras, T., and Díez-Noguera, A. (2001). Functioning of the rat circadian system is modified by light applied in critical postnatal days. *American Journal of Physiology* 280: R1023–R1030.
320. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2004). Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. *American Journal of Physiology* 286: R539–R546.
321. Schwartz, M. D., Nunez, A. A., and Smale, L. (2004). Differences in the suprachiasmatic nucleus and lower subparaventricular zone of diurnal and nocturnal rodents. *Neuroscience* 127: 13–23.
322. Hofstetter, J. R., Hofstetter, A. R., Hughes, A. M., and Mayeda, A. R. (2005). Intermittent long-wavelength red light increases the period of daily locomotor activity in mice. *Journal of Circadian Rhythms* 3: art. 8.
323. McLennan, I. S. and Taylor-Jeffs, J. (2004). The use of sodium lamps to brightly illuminate mouse houses during their dark phases. *Laboratory Animals* 38: 384–392.

This page intentionally left blank

3 Analysis of Circadian Rhythmicity

3.1 DATA ANALYSIS

Data analysis in circadian physiology is mostly a task of identifying circadian rhythmicity in data sets that naturally contain many rhythmic and nonrhythmic components. Because the data points refer to successive observations made over time, the data set is often called a "time series." Many books on time series analysis are available,[1–7] although most of them deal with economic issues, such as fluctuations in stock-market prices, and none of them deals in detail with circadian rhythms. These books make a distinction between analysis in the "time domain" and analysis in the "frequency domain." The distinction has to do with the methods used for analysis: methods in the "time domain" look for regularities in a time series itself, whereas methods in the "frequency domain" treat the time series as a composite of underlying oscillatory processes. Both classes of methods are used in the analysis of circadian rhythms, and the distinction will become clearer as we proceed.

Consider the data on the body temperature of a female golden hamster shown in Panel A of Figure 3.1. These are *idealized* data that I generated with the help of a computer for didactical purposes. Notice that the time series has a clear pattern: body temperature rises early each day, then falls slightly before rising to a midday peak, then falls again, rises again (but not too much), and finally falls again at the end of the day. The oscillatory pattern repeats itself day after day, except that a higher midday peak is reached every 4 days. This description of the data is a simple form of analysis in the "time domain."

Because I generated the data set myself, I know how I generated it. The four Panels (a) through (d) in Panel B of Figure 3.1 show the four components that I used to build the data set in Panel A. I added four distinct components: (a) a sinusoidal oscillation that repeats itself every 24 hours, (b) another sinusoidal oscillation that repeats itself every 8 hours, (c) an intermittent oscillation that recurs at intervals of 96 hours, and (d) a random pattern of high-frequency, low-amplitude oscillation ("biological noise"). As we will see later, these four components simulate four processes that are known to affect the body temperature of a female hamster, respectively: (a) circadian rhythmicity, (b) ultradian rhythmicity, (c) estrous rhythmicity, and (d) biological noise. Interestingly, even if I had not created the data set artificially, we could still find out that it includes the four components if we conducted "spectral analysis" (i.e., analysis in the "frequency domain"). All of this will be discussed in detail in this chapter. For now, the take-home message is a warning, or a reminder, that, when we analyze circadian rhythms, we are concentrating on a temporal window that most likely is only one dimension of a more complex time series. As briefly mentioned in Chapter 1, Halberg created the term *chronome* exactly to emphasize that circadian rhythmicity is only one of many rhythmicities in living organisms.[8,9]

Data analysis in general can be of two basic types: graphical or numerical. Graphical analysis relies on the observation of a graphical display of the data, whereas numerical analysis involves the computation of one or more "statistic" derived from the raw data. For instance, I asked the students in my human sexuality class to keep records of the times at which they had sex over several weeks. I obtained usable data sheets from 11 students (ranging in age from 18 to 51 years), which documented 71 sexual encounters in 2 weeks. A small sample of these sheets is shown in Figure 3.2. Now, a simple way of analyzing the data is to plot the total number of sexual encounters initiated at each hour of the day for the whole group, as shown in Figure 3.3. Inspection of the figure immediately reveals the existence of a daily rhythm of sexual activity. Even though the students seemed to find opportunities for sex at practically any time of the day, most sexual acts occurred around bedtime (11 p.m. to 1 a.m.). A smaller peak in sexual activity occurred around wake time.[10]

Although graphical analysis may suffice in certain situations, numerical quantification often is necessary for a more detailed evaluation of the data (Figure 3.4). For instance, the graphs in Figure 3.5 show the mean values of rectal temperature, plasma concentration of urea, and plasma concentration of cholesterol measured at 3-hour intervals in five goats, as well as the time of daily feeding. The graphs do suggest the presence of daily rhythmicity, but it is difficult to determine at what time of day each variable reaches its daily peak or how the variables relate to each other. One way to improve the analysis is to numerically calculate parameters of each rhythm and to express them as vectors in a circumference, as shown in Figure 3.6. In this case, the angle of each vector indicates the time of the daily peak (as calculated by one of the procedures that will be described in Section 3.2), whereas the length of the vector indicates the strength of rhythmicity (its "robustness," as defined in Section 3.2.4). It can be easily seen, for instance, that the rhythm of blood cholesterol (C) is weaker, and occurs later in the day, than the rhythm of rectal temperature (T). Thus, numerical analysis provides more useful information about the data than graphical analysis alone. Of course, even when numerical analysis is performed, basic graphical analysis should be performed first. As Chris Chatfield—a statistics professor at the University of Bath (in England)—puts it,[2] "anyone who tries to analyze a time series without plotting it first is asking for trouble." To help you stay out of trouble, the software package that accompanies this book contains a program (**Plot**) for quick and simple

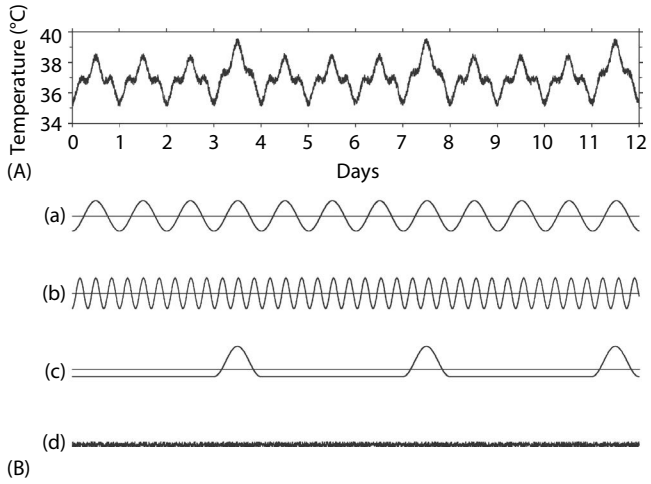


FIGURE 3.1 Composite rhythmicity. This 12-day-long data set with 6-minute resolution depicts an *idealized* record of the body temperature of a female golden hamster. The composite oscillatory pattern includes periodicities of 8, 24, and 96 hours, as well as high-frequency noise. Panel A shows a composite rhythm. Panel B shows the components of the composite rhythm.

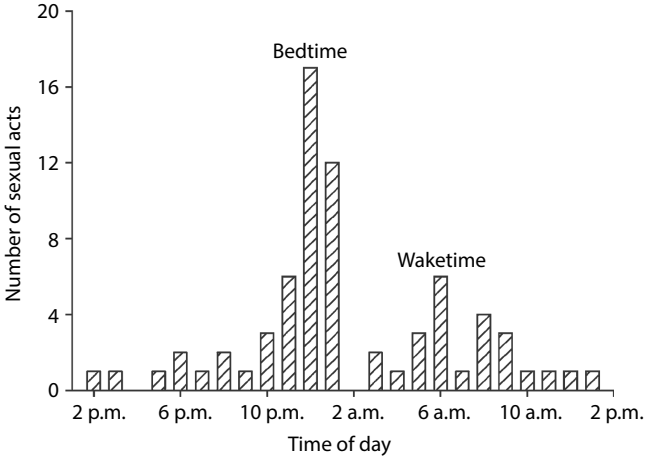


FIGURE 3.3 Time for sex. According to the results of a study conducted by the author, bedtime is the most popular time for sex. (Data from Refinetti, R., *J. Circadian Rhythms*, 3, art. 4, 2005.)

inspection of data sets as Cartesian plots (i.e., as values plotted on the Y-axis against time in the X-axis). You may have already used this program if you did Exercise 2.1 in the preceding chapter.

When we analyze a time series to detect the presence of circadian rhythmicity, we look for a periodical pattern that recurs at approximately 24-hour intervals. At least four words in English refer to periodic events: rhythm, oscillation, cycle, and wave (Figure 3.7). The four terms have equivalent, but not identical, meanings. "Rhythm" is commonly used to refer to a repetitive pattern of sounds, such as that represented in Panel A of Figure 3.7. "Oscillation" is commonly used to refer to the swinging of a pendulum (Panel B), whereas "cycle" is associated with a circular pattern (Panel C), and "wave" refers either to a swell moving along the surface of a body of water or to a regular repetitive process (Panel D). The computer program **Wave**, which is a component of the software package that accompanies this book, provides a brief tutorial on the four terms (see Exercise 3.1). In circadian physiology, "rhythm" is usually applied to variables that can be measured (e.g., the rhythm of body temperature), whereas "oscillation" is usually—although not exclusively—applied to theoretical variables (e.g., the oscillation of the circadian pacemaker) or to periodic events outside the circadian range (e.g., high-frequency oscillations). "Cycle" is used with a meaning very similar to that of "rhythm" but is frequently restricted to the reproductive system (e.g., the menstrual cycle). "Wave" is not generally used in circadian physiology, although it can be very helpful in the formal analysis of rhythmic processes. For instance, if you throw a rock in a pond, you will generate a wave pattern (Figure 3.8). If you were to submerge yourself in the water, leaving only your eyes above the surface, you would observe a pattern of deformations of the water surface that can be diagrammed as in Figure 3.9. Although it may not be evident at first, the rhythmic pattern can be fully described by four parameters: period, mean level, amplitude, and phase. "Period" is the distance between two consecutive peaks—or, more generally, the duration of each wave. If the duration of each wave is 2 seconds, then the period is 2 seconds. You could also express this parameter as its reciprocal (that is, "frequency"). If the period is 2 seconds, then the frequency

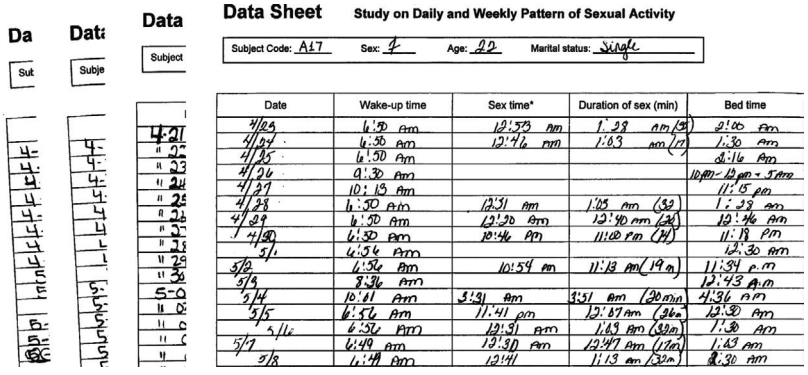


FIGURE 3.2 Have you had sex today? These are four representative data sheets from a study on the daily and weekly patterns of sexual activity of university students.

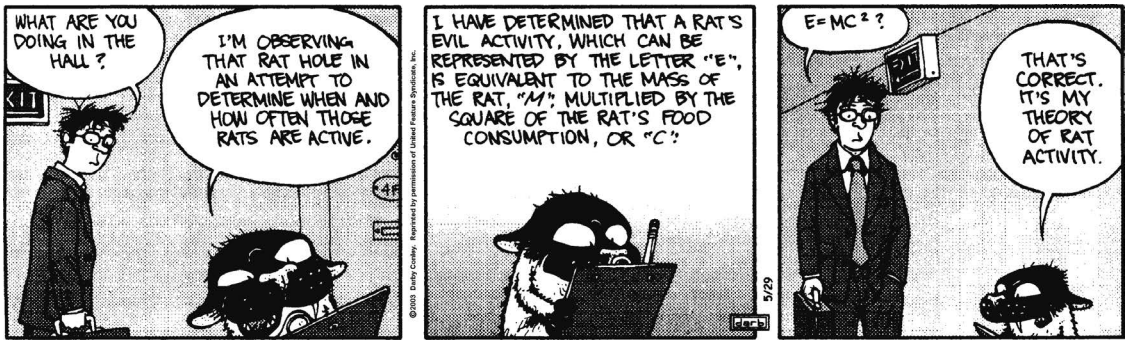


FIGURE 3.4 Importance of quantitative data analysis. As suggested by this comic strip from the cartoon *Get Fuzzy*, by Darby Conley, rigorous data collection can lead to detailed numerical analysis. (Copyright 2003 Darby Conley. Get Fuzzy reprinted by permission of United Feature Syndicate, Inc., New York.)

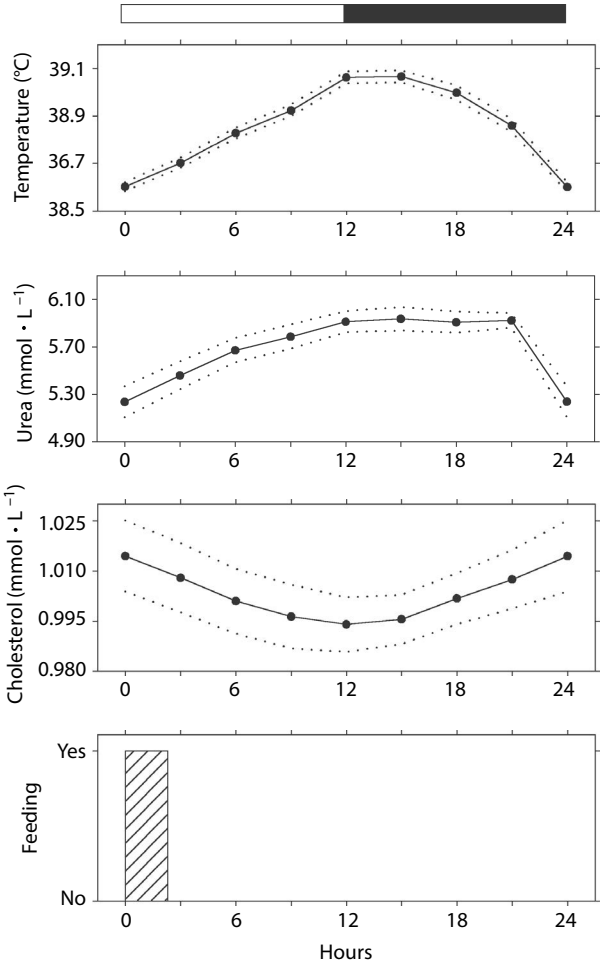


FIGURE 3.5 Daily rhythms in goats. Shown are the mean values of rectal temperature, plasma concentration of urea, and plasma concentration of cholesterol measured at 3-hour intervals in goats, as well as the time of daily feeding. The data points are the mean values for 5 goats, each averaged over 10 days. The dotted lines denote the boundaries of the 95% confidence intervals of the means. The white and dark horizontal bars at the top indicate the duration of the light and dark phases of the light–dark cycle, respectively. (Data from Piccione, G. et al., *Comp. Biochem. Physiol. A*, 134, 563, 2003.)

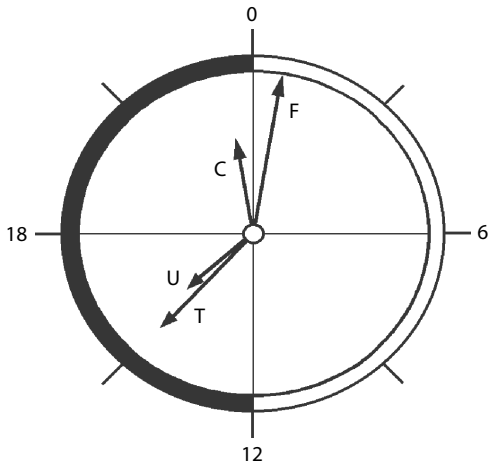


FIGURE 3.6 Vectors in a circle. This figure presents the results of numerical data analysis (time and magnitude of the daily peaks) of the four rhythms shown in Figure 3.5. *Abbreviations*: F, feeding; C, cholesterol; T, temperature; U, urea.

is 2^{-1} Hz (or 0.5 Hz)—which, of course, means that there is half a wave each second, or one wave each 2 seconds. "Mean level" is, well, the mean level—the water level around which the wave undulates. "Amplitude" is half the range of oscillation of the wave. "Phase" is a relative term used to indicate the displacement between a chosen point (say, the peak) and a reference point (say, the rock). The wave may be close to the rock, far from the rock, or anywhere in between.

The analogy between circadian rhythms and waves in the pond is not perfect. In the analysis of circadian rhythms, we need to consider two additional parameters: waveform and robustness. "Waveform," not surprisingly, refers to the form of the wave. The wave in Figure 3.9 had a sinusoidal form, but circadian rhythms rarely are this elegant. Also, we assumed that you would not stay submerged in water for too long and, therefore that you would not notice that, after some time, the waves did not look exactly like they had looked in the beginning. That is, we took it for granted that the waves were "stationary" (which is a technical term

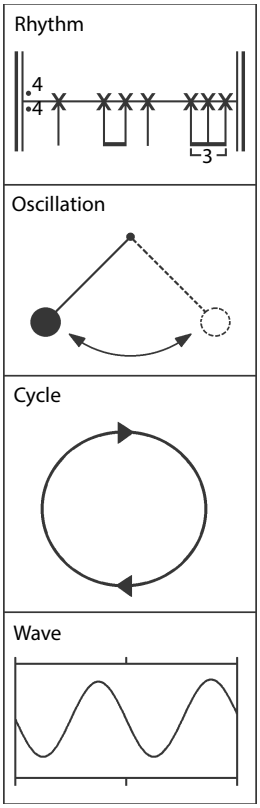


FIGURE 3.7 Periodic events. At least four words in English refer to periodic events: rhythm, oscillation, cycle, and wave.

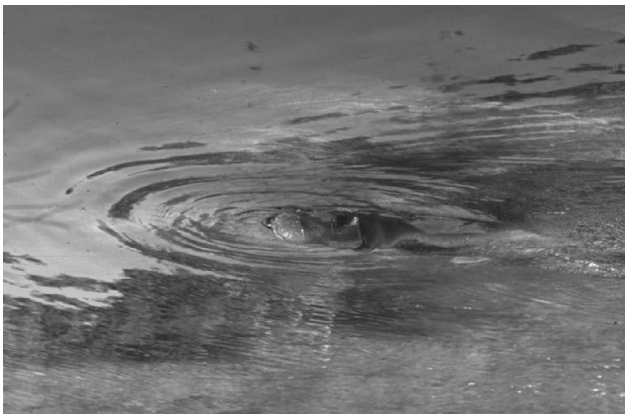


FIGURE 3.8 Throw a rock in the pond. When one taps the surface of a calm body of water at regular intervals, a concentric series of waves is produced. (Copyright ArtToday, Tucson, AZ.)

in time series analysis). When we say that the waves are stationary (or that they exhibit "stationarity"), we do not mean that they do not move (they obviously do) but simply that they are all identical, so that it does not matter whether we analyze the 1st wave, the 10th wave, or the millionth wave. In other words, stationary waves are stationary because they remain always the same. As we will see in Section 3.2.4, circadian rhythms are not strictly stationary. The closer a rhythm is to stationarity, the greater is its "robustness."

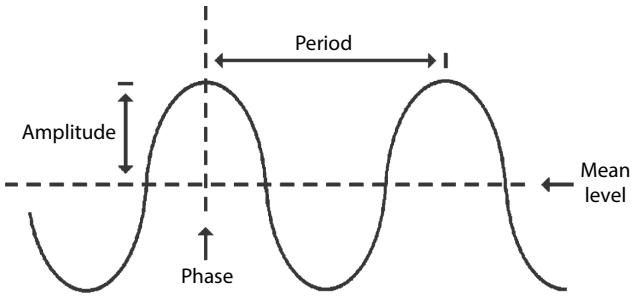


FIGURE 3.9 Rhythmic parameters. A stationary sinusoidal wave can be fully characterized by four parameters: mean level, amplitude, period, and phase.

3.2 PARAMETERS OF CIRCADIAN RHYTHMS

Before we start examining the six parameters that characterize circadian rhythms, it will be helpful to briefly discuss procedures for filtering data—under the reasonable assumption that your data may not be as clean as you would like them to be. Consider the data set in Panel A of Figure 3.10.

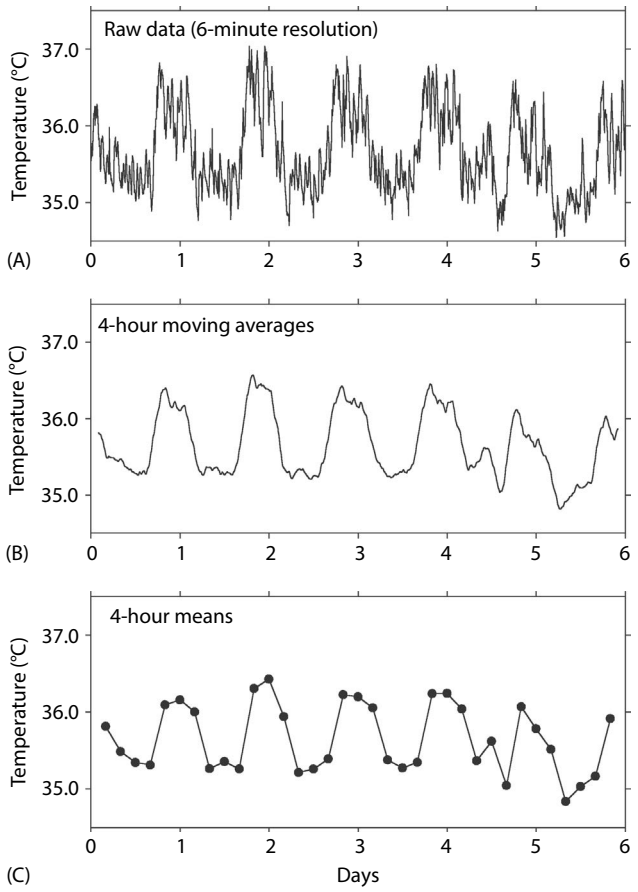


FIGURE 3.10 Smoothing things out. As shown here for a data set containing the records of body temperature of a golden hamster taken by telemetry every 6 minutes for 6 consecutive days, raw data can be smoothed by a moving averages procedure (with a 4-hour window size in this case) or by the plain averaging of adjacent data points (also with a 4-hour window size in this case). (Data from Refinetti, R., *J. Therm. Biol.*, 19, 269, 1994.)

TABLE 3.1
Data Analysis Programs in the Circadian Physiology Software Package[a]

Topic	Program	Method	Exercises
Visual inspection of data (Cartesian plot)	Plot		2.2, 2.3
Visual inspection of data (actogram)	Plot		3.3
Data filtering	Moving	Moving averages	3.2
General detection of rhythmicity (full data sets)	Rhythm	Chi-square periodogram	4.2
Detection of rhythmicity in a single cycle	Onecycle	Kolmogorov–Smirnov test	4.3
Detection of periodicity in a sequence of infrequent events	Rayleigh	Rayleigh test	4.4
Computation of mean level, amplitude, and acrophase	Acro	Best-fit cosine wave	5.2
Computation of circadian period (actogram)	Plot	Modulo adjustment	3.4
Computation of circadian period (equally spaced data)	Tau	Chi-square periodogram	5.3
Computation of circadian period (unequally spaced data)	LSP	Lomb–Scargle periodogram	5.4
Computation of circadian period, mean level, amplitude, and acrophase	Cosinor	Cosinor rhythmometry	5.5
Calculation of phase shifts (actogram)	Plot	Regression of onsets	7.1
Analysis of ultradian rhythms	Fourier	Fourier analysis	4.5

[a] The principle of operation of each program is described in this chapter. Tutorials on the use of the programs are provided in the exercises at the end of several chapters, as indicated in the right column of the table. Additional information about the programs appears in the "Software Installation" section at the beginning of the book.

It consists of body temperature measurements from a male golden hamster made every 6 minutes (0.1 hour) by telemetry. Although daily rhythmicity is evident, there seems to be also a great deal of noise (high-frequency oscillations). Thus, in order to observe the daily rhythmicity more clearly, we may want to filter out the high-frequency oscillations. Two very simple and popular procedures for data filtering are the moving averages procedure and the plain averaging of two or more contiguous data points. Plain averaging is just plain averaging: instead of using all data points, we use the arithmetic means of groups of contiguous data points. For instance, Panel C in Figure 3.10 shows 4-hour means. Because the original data set had 10 values per hour, the 4-hour means were based on 40 data points. Notice that the resulting curve is much smoother than the original one. Of course, the smoothness was obtained at the cost of the loss of temporal resolution: we now have only one data point each 4 hours. To avoid this problem, a "moving averages" procedure can be used (Panel B in Figure 3.10). In this case, each data point is replaced by the mean of the 40 data points around it (i.e., the 20 data points that precede it and the 20 data points that follow it). Thus, the original 6-minute resolution is maintained, while the high-frequency oscillations are filtered out. As indicated in Table 3.1, the software package that accompanies this book includes a program to perform the moving averages procedure (**Moving**), as just described.

Sometimes, malfunction of the recording equipment may generate spurious data points. If the spurious data points are few, and the errors are of small magnitude, they can be filtered out by the moving averages procedure. However, if the errors are large (say, a body temperature reading of 100°C when all other readings are in the 35°C–39°C range), the single spurious value may skew the mean. In this case, it may be necessary to accept the fact that errors do occur and, accordingly, to reject the spurious value. To preserve the integrity of the time series, the spurious value may be replaced by the value of the mean of the whole time series, or by the value immediately preceding it, etc. If the data set is *very* bad (with many spurious values, missing values, baseline drifts, etc.), the best approach is to discard the data and redo the experiment. If collecting new data is not an option, sophisticated methods of data conditioning may be employed.[11,12]

3.2.1 Mean Level and Amplitude

Let us start with the analysis of the "mean level" of a rhythm (Figure 3.11). The mean of a time series, as the mean of any set of data, is a measure of the "central tendency" of the data—that is, a measure of the point of balance of the distribution of values. Consider Figure 3.12. The distribution of tiny squares in Panel A *tends* to the left, whereas that in Panel C *tends* to the middle. Three traditional indexes of central tendency are the "mode," the "median," and the "mean," as shown in the figure and as described in introductory statistics textbooks.[13–18] The three indices are based on different properties of the distribution and are numerically distinct unless the distribution is symmetrical (as in Panel C). The mean—more specifically, the "arithmetic mean"—is by far the most commonly used measure of central tendency. No special statistical training is needed to know that, for instance, the mean of 2, 3, and 7 is 4—that is, $(2 + 3 + 7) \div 3 = 4$. Let us go back to the pond where we threw a rock earlier, and this time let us

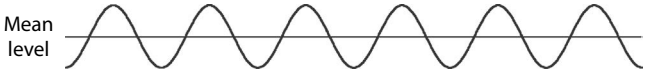


FIGURE 3.11 Mean level. The mean level is one of the parameters that characterize a circadian rhythm.

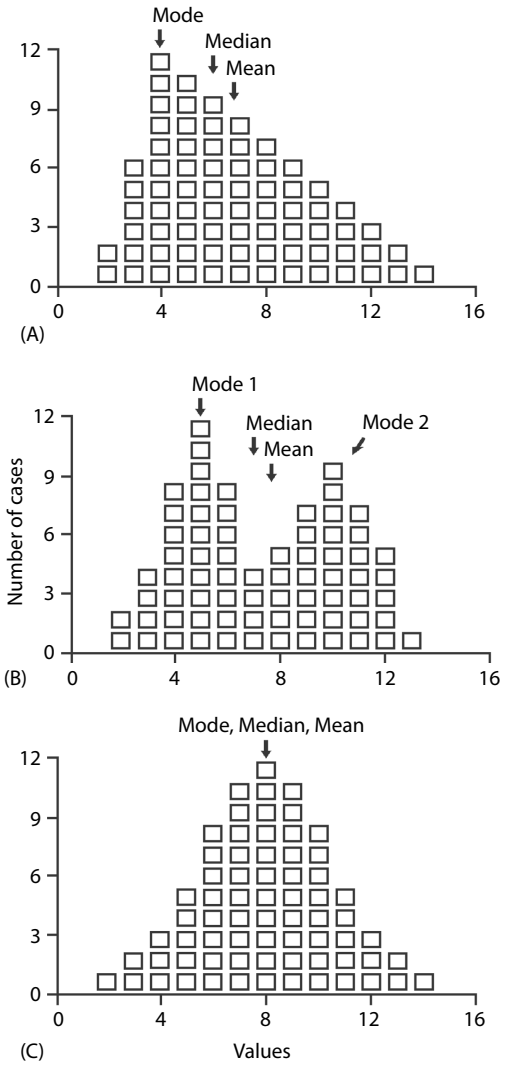


FIGURE 3.12 Measures of central tendency. Three common measures of the central tendency of a distribution are the mode, median, and mean. They may be numerically distinct (A, B) or identical (C), depending on the shape of the distribution.

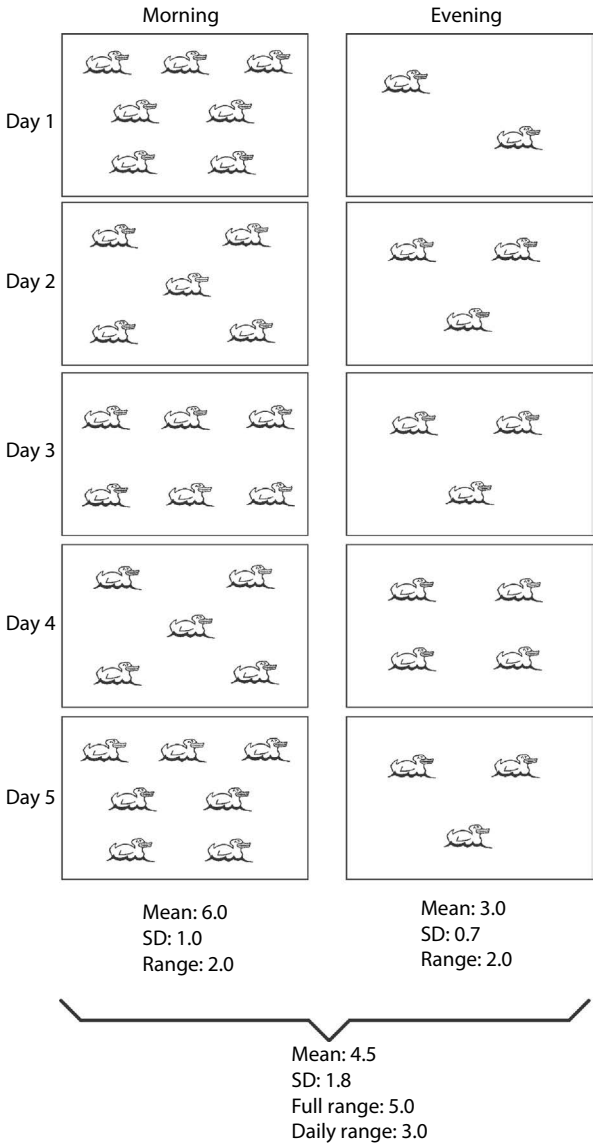


FIGURE 3.13 Mean, standard deviation, range, and amplitude. This simple example of counting the number of ducks in a pond at two times of the day allows for easy computation of measures of central tendency and variability.

count the number of ducks that we see in the morning and in the evening. Using the diagrams in Figure 3.13, we notice that more ducks can be found in the morning than in the evening on each of the 5 days. The mean number of ducks found in the morning is 6, whereas the mean number in the evening is 3—and the mean number for all 10 observations (i.e., the "mean level" of our time series) is 4.5. Of course, the mean is slightly misleading, as we did not ever actually observe 4.5 ducks in the pond (to start with, there was never a half-duck floating around), but we understand that 4.5 is an average value. Our observations ranged from 2 ducks (in the evening of Day 1) to 7 ducks (in the morning of Days 1 and 5)—thus, a range of 5 ducks. The mean daily range (morning minus evening each day) was 3 ducks. Instead of the range, statisticians normally use another index of variability: the "standard deviation" (abbreviated as SD). We need not discuss the concept of SD at this time, but the importance of having some index of variability for the mean (whether it is the range or the SD) must be emphasized. Suppose that there were two men who lived in a faraway land (Figure 3.14). For a month, we fed a whole chicken every day to one of the men and gave nothing to the other man (Panel B). Mathematically, it is correct to say that, on average, each man was fed half a chicken each day (Panel C). In reality, however, the man who was fed the whole chicken gained weight, whereas the other man died of starvation (Panel D)!

For reasons that will become evident as we proceed, some circadian physiologists do not calculate the mean level of a rhythm by simply computing the arithmetic mean of all values in the time series. Instead, they use a procedure developed by Halberg and called "cosinor rhythmometry."[19] Halberg's reasoning was that, since circadian rhythms can be thought of as smooth rhythms with added noise, a cosine wave could be

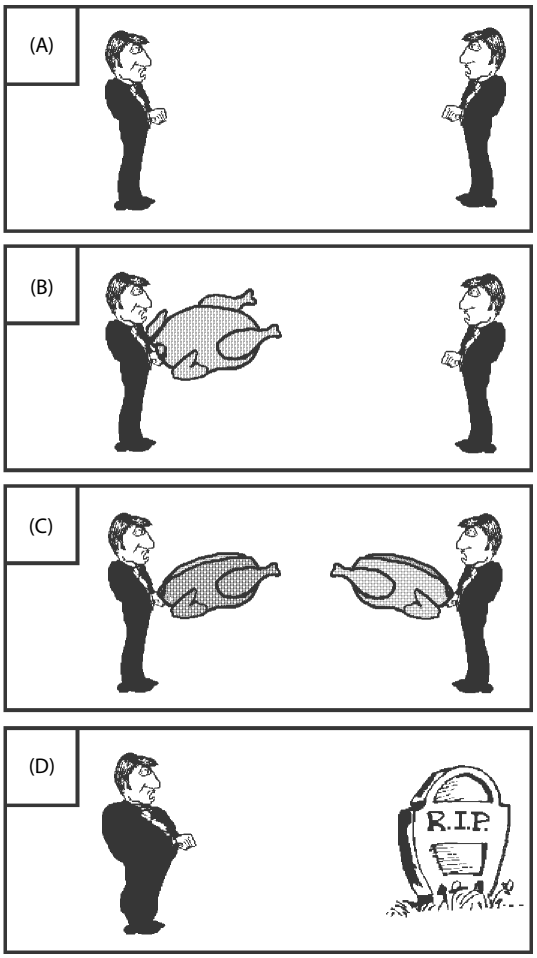


FIGURE 3.14 Means can be deceiving. If two men (A) are fed a chicken each day, they will, on average, be fed half a chicken a day. This average food intake may indicate either that one man eats a whole chicken and the other starves (B) or that each man eats half a chicken (C). Unfortunately, if situation B is true, one of the men will gain weight whereas the other will die (D).

fitted to the data as an estimate of the pattern of the smooth rhythm. Two examples of cosine waves fitted to actual rhythms are shown in Figure 3.15. Notice that the fit is very good for the squirrel data (A) but not as good for the tree shrew data (B). Of course, more complex mathematical procedures could be used to improve the fit, and some circadian physiologists have followed this route.[20–25] In these cases, Akaike's method can help the researchers to select the number of harmonics to incorporate in their models.[26] However, the physiological meaning of complex models is not clear at all. The addition of harmonics to the single cosinor improves the fit, but it also moves us closer to a spectral analysis of the time series. The analysis will necessarily identify multiple oscillatory processes, but we will not be able to determine whether these oscillatory processes are mere mathematical descriptions of noise inherent in the time series or a reflection of actual biological oscillations. The cosinor method with one component may have its limitations, but it is intuitive and has the advantage of simplicity. Thus, if "modeling" is to be used, I favor the single cosinor model.

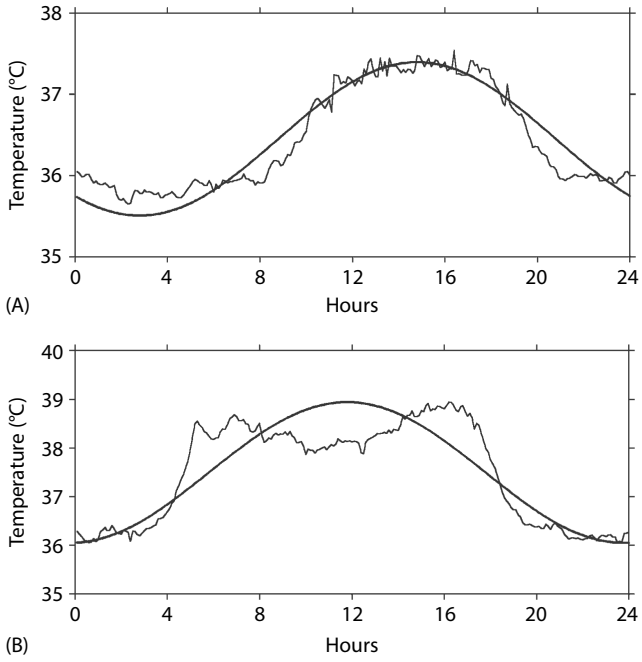


FIGURE 3.15 Cosinor. The cosinor method relies on the fitting of a cosine wave to the raw data set. This figure shows cosine waves fitted to the body temperature rhythms of (A) a Richardson's ground squirrel and (B) a tree shrew. The mean level of a rhythm calculated by the cosinor method is a rhythm-adjusted mean called "mesor." (Data from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999.)

When the single cosinor is employed, the mean level of the fitted curve is used as the "mean level" of the rhythm. Halberg called this the "mesor" (an abbreviation of *m*idline-*e*stimating *s*tatistic *o*f *r*hythm).[27] When the data points are equidistant in time, the mesor is equal to the arithmetic mean. If the data points are distributed over time in a biased manner, the mesor provides a better estimate of the mean level of the rhythm than the arithmetic mean does.

We now turn to the computation of the "amplitude" of a rhythm (Figure 3.16). Technically, the amplitude of a smooth function is the distance between the mean value and the peak (or between the mean value and the trough, as we assume that peak and trough are equidistant from the mean level). Therefore, the "amplitude" is half the "range of oscillation". It should be pointed out, however, that the range of oscillation is more meaningful than the amplitude for circadian physiologists. It is more meaningful primarily because it tells us what the boundaries of the oscillation are, whereas the amplitude is a concept that may facilitate computations in engineering but only serves to confuse physiologists and clinicians. In addition, the range of oscillation is more meaningful than the amplitude because real circadian rhythms are not necessarily

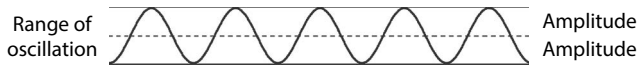


FIGURE 3.16 Amplitude. Amplitude is one of the parameters that characterize a circadian rhythm. For a sinusoidal wave, the amplitude is half the range of oscillation.

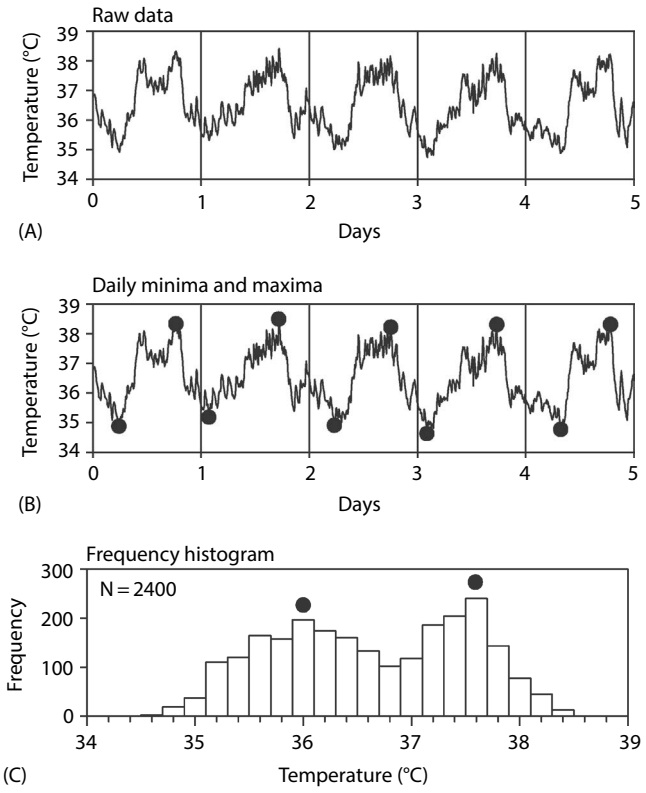


FIGURE 3.17 Standard methods for the calculation of rhythm amplitude. Panel A shows raw data depicting the body temperature rhythm of a fat-tailed gerbil. Panel B illustrates the maxima-minus-minima method of computing rhythm amplitude, whereas Panel C illustrates the frequency histogram method. (Data from Refinetti, R., *Comp. Biochem. Physiol.*, 119A, 295, 1998.)

symmetrical, so that the amplitude below the mean level may be different from the amplitude above the mean level (thus rendering the notion of amplitude useless).

How do we calculate the amplitude (or the range of oscillation) of a rhythm? As we did for the mean level, we can use the actual data or a cosine function fitted to the data. Figure 3.17 shows a 5-day segment of the records of body temperature of a fat-tailed gerbil (a small rodent). The raw data, collected in 6-minute intervals, is shown in Panel A. With 240 data points per day, we would not want to search for the daily peaks and troughs by hand; however, we could easily program a computer to do it for us, as shown in Panel B. If the rhythm were noisy, we could instruct the computer to filter the data prior to computing the daily minima and maxima. To calculate the "range of oscillation" for the 5-day interval, we could simply average the five daily ranges of oscillation. To obtain the "amplitude," we would just divide the range of oscillation by 2. For the data in Figure 3.17, the amplitude calculated by this method is 1.6°C. An alternative method is that of the "frequency histogram,"[28] as shown in Panel C of Figure 3.17. This method consists simply of building a frequency histogram of all data points (in this case, 2400 points [10 days]) and locating the two modal peaks that correspond to daytime temperatures and nighttime temperatures. The difference between the temperatures associated with the two peaks is the amplitude of the rhythm (in this case, 1.6°C, which is the same amplitude as that found by the previous method). Computation of the amplitude by the "cosinor" method is also very simple once the cosine wave has been fitted to the data. Because the cosine wave is symmetrical, the amplitude is simply half the range of oscillation. However, it should be pointed out that, depending on the procedure used to fit the cosine curve, the range of oscillation of the fitted curve may be smaller than the range of oscillation of the actual rhythm. The amplitude and the range of oscillation (which is sometimes called "double amplitude") that are computed by the cosinor method as described by Halberg[19,29] are always smaller than the values computed by the other two methods discussed here.

3.2.2 Phase

Computation of the *phase* of a rhythm (Figure 3.18) can also be conducted by several methods. The most traditional method in circadian physiology is one based on a graphical display called an "actogram." The first actogram was drawn by Maynard Johnson in 1926,[30] many decades before the "computer revolution." Today, actograms can be easily generated from data automatically collected by a computer. Let us start with records of locomotor activity plotted in the familiar Cartesian style (Panel A in Figure 3.19). The use of Cartesian plots has two disadvantages: (1) each day is plotted to the right of the preceding day, which makes it difficult to compare the temporal distribution of activity on different days, and (2) the temporal resolution of the plot is rather low, as several days are plotted on the same line. These two disadvantages can be overcome by *cutting out* the data segment for each day, *stretching* it to the full width of a page, and *pasting* it below the preceding day. The resulting graph shows data for 1 day per line, with successive days appearing on successive lines (Panel B). The vertical alignment of the data provides instant information about the duration of the circadian cycle (i.e., its period): drifts to the left indicate that the cycle is shorter than 24 hours (the onsets of activity are earlier each day), whereas drifts to the right indicate that the cycle is longer than 24 hours (the onsets are later each day). The drift to the left in Figure 3.19 indicates that the cycle is shorter than 24 hours. Notice that a very compact actogram (Panel C) can be obtained by digitizing the activity values so that data points are plotted on a yes-or-no basis. This is especially helpful when one needs to inspect many days worth of data or when a "double plot" is desirable.

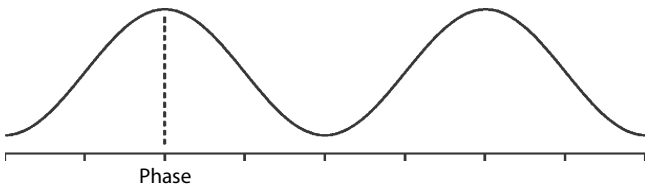


FIGURE 3.18 Phase. Phase is one of the parameters that characterize a circadian rhythm.

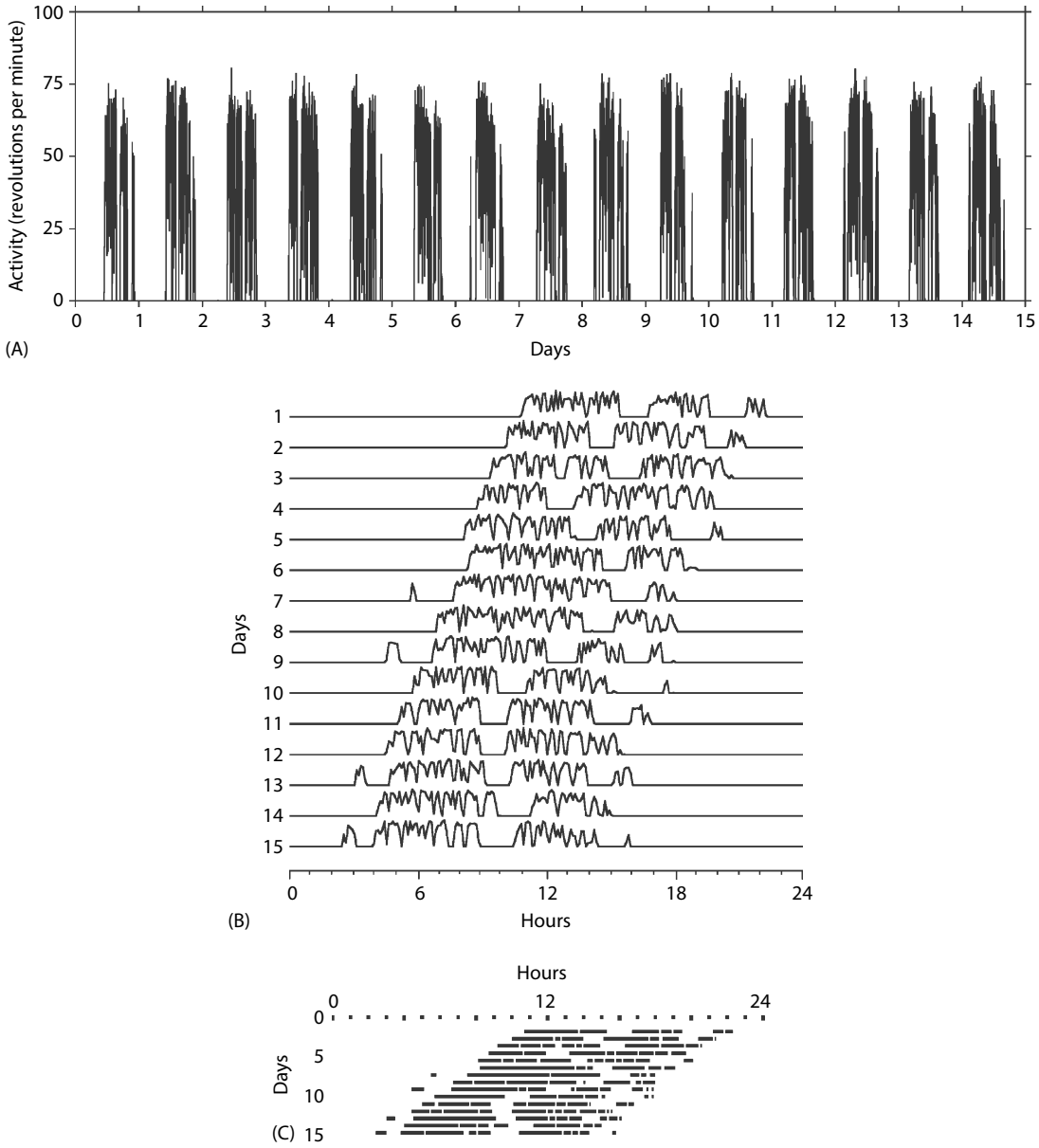


FIGURE 3.19 How an actogram is built. Construction of an actogram can be thought of as a process that starts with a regular Cartesian plot of the running-wheel activity of a mouse (A). Each successive 24-hour segment of the data set is then cut out, stretched, and pasted below the preceding one (B). A very compact actogram (C) can be obtained by digitizing the activity values (i.e., by plotting each point on a yes-or-no basis). (Data from Refinetti, R., *Integr. Physiol. Behav. Sci.*, 37, 114, 2002.)

A "double plot" is simply an actogram that is double-plotted to facilitate inspection of records in which the activity pattern would run out of the page because of drifts in the activity onsets (Figure 3.20). In a double plot, each line contains data for the current day as well as for the following day, so that two repeated bands of activity can be seen instead of the single one seen in a single-plotted actogram. The program **Plot** (see Table 3.1) can be used to construct simple actograms. More sophisticated programs are commercially available, such as Chronobiology Kit Analysis (Stanford Software Systems, Santa Cruz, California) and ClockLab Analysis (Actimetrics Software, Wilmette, Illinois). A good, freely available program is ActogramJ.[31]

Actograms provide an easy way to determine the phase of the rhythm. Traditionally, the daily onsets of activity are used as phase markers,[32–35] although the "offsets" can be used as well.[36–38] As a matter of fact, actograms can be used for the determination of several parameters of circadian rhythms, as shown in Figure 3.21 and discussed in detail later on. Notice that lower case Greek letters are used to identify the various parameters. The band of activity (which occurs during the dark phase of the light–dark cycle in nocturnal organisms) is designated as α (*alpha*). The rest interval between consecutive intervals of activity is designated as ρ (*rho*). The phase of the rhythm in relation to a constant *external* reference point (such as the time of lights-off) is called ψ (*psi*). The duration

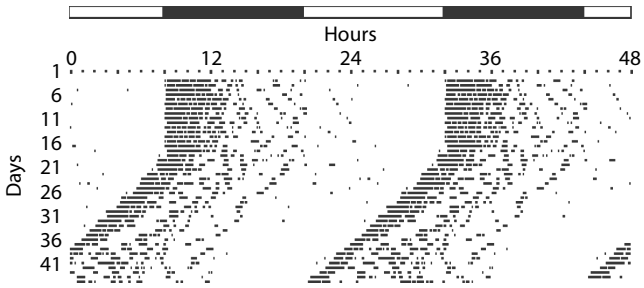


FIGURE 3.20 A double plot. Actograms are often double-plotted to facilitate the inspection of activity records that drift over many days. In a double plot, the second day worth of data is plotted not only under the first day but also to its right (so that there are 2 days per line). Similarly, the third day is plotted not only under the second day but also to its right. And so forth. The white and dark horizontal bars at the top are used to indicate the duration of the light and dark phases of the prevailing light–dark cycle, respectively. The mouse whose records are shown in this figure was under a light–dark cycle for the first 17 days and in constant darkness afterward. (Data from Refinetti, R., *Physiol. Behav.*, 74, 101, 2001.)

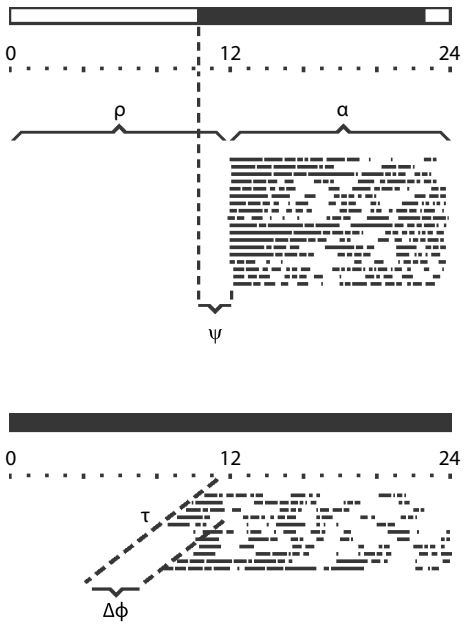


FIGURE 3.21 A Greek alphabet soup. Several letters of the Greek alphabet are used to denote properties of circadian rhythms: the duration of the daily rest interval (ρ), the duration of the active interval (α), the phase angle of entrainment (ψ), the period (τ), and a phase shift of the rhythm (Δϕ). Notice that a completely dark horizontal bar at the top of an actogram denotes constant darkness (i.e., the dark phase of the light–dark cycle lasts the whole day).

of the cycle (its *period*) is called τ (*tau*). Phase shifts caused by endogenous or exogenous factors are designated as Δϕ (*delta phi*). Not shown in the figure is the designation ϕ (without the Δ preceding it), which usually refers to the phase of a rhythm in relation to an *internal* reference point. If you need assistance remembering the meaning of the various Greek letters, you may consult the *Dictionary of Circadian Physiology* at the end of this book.

Two other methods that are commonly used for the determination of the phase of circadian rhythms are the cosinor method and the method of identification of the peak of smoothed rhythms. Consider Figure 3.22. Panel A shows raw data concerning the locomotor activity of a golden hamster, as determined every 6 minutes by telemetry. Panel B shows a cosine wave fitted to the raw data (although the data are omitted for clarity). The peak of the cosine wave provides a suitable phase marker: the "acrophase."[19] Alternatively, the raw data may be smoothed by a moving averages procedure (Panel C). In this case, two potential phase markers are evident: the threshold of the daily elevation (which is similar, although not identical, to the onset of activity determined in

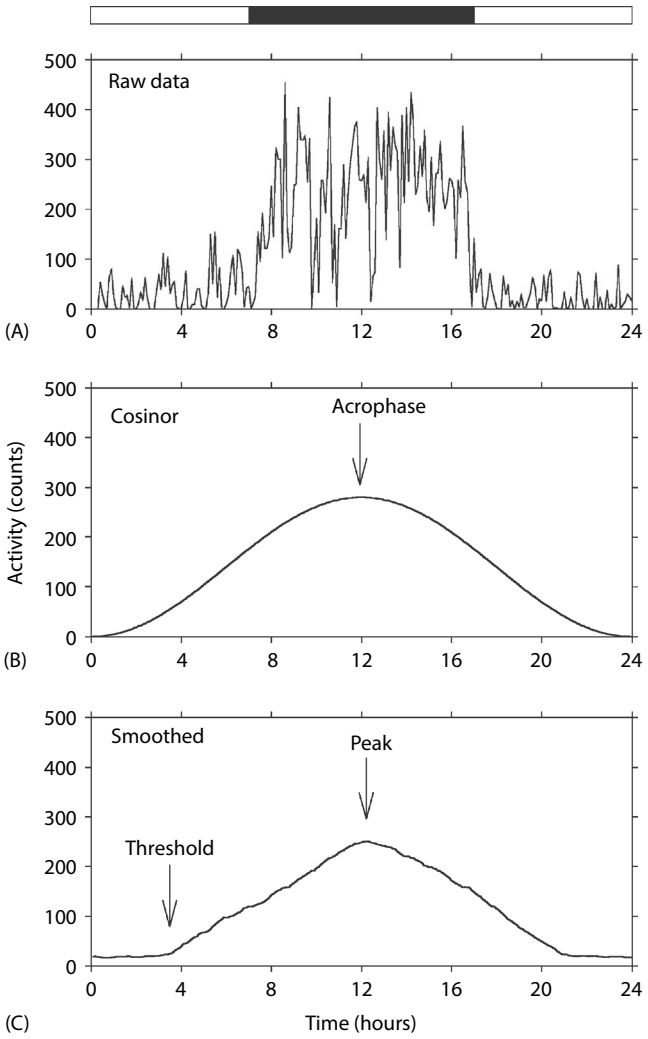


FIGURE 3.22 Other methods to determine the phase of a rhythm. Panel A shows raw data depicting the locomotor activity of a golden hamster monitored by telemetry. Panel B illustrates the computation of the acrophase of the rhythm by the cosinor method (the acrophase is the time of the peak of the cosine wave fitted to the data). Panel C illustrates the computation of phase by the identification of the peak of a smoothed curve derived from the raw data by the use of an 8-hour moving averages filter. A threshold of the nocturnal elevation in the level of activity can also be identified. (Data from Refinetti, R., *Physiol. Behav.*, 56, 829, 1994.)

an actogram) and the peak (which is similar to the acrophase determined by the cosinor method).[39]

I should point out at this time that the cosinor method is based on a mathematical model that allows the computation of mean level, amplitude, and phase all at once. The best-fitting cosine wave can be described by the function:

$$f(t) = M + A\cos(\omega t + \phi)$$

where

- f(t) denotes the value of the function at time t
- M is the mesor
- A is the amplitude
- ω is the angular frequency (i.e., 360°/24 if the cycle is 24 hours long and t is measured in hours)
- ϕ is the acrophase (in degrees)

Notice that here ϕ designates the phase in relation to either an internal or external reference.

A system of three equations with three unknowns can be derived from the cosine function and be solved in algebraic form.[19,40,41] The accuracy of the computed acrophase depends on the strength of the oscillatory process, the amount of embedded biological noise, the sampling rate, the duration of data collection, and other factors. If the oscillatory process is very strong and the signal-to-noise ratio is high, an accuracy better than 1 hour can be obtained with as few as three data points collected over a single day.[42]

The program **Acro** (see Table 3.1) calculates the mean level, amplitude, and acrophase of rhythms. However, in order to stay loyal to the actual data, the mean level is computed as the arithmetic mean of all values in the data set, and the amplitude is calculated as half the range of oscillation, which on its turn is computed as the mean daily difference between peaks and troughs. The acrophase is calculated through the fitting of a cosine wave, but not according to the single cosinor method. For consistency with the calculations of mean level and amplitude based on the raw data, the cosine function is not computed by the formal system of equations. Instead, M and A are taken from the raw data, and ϕ is determined by iteration: the true value of ϕ is considered to be the one that produces the smallest sum of squares of the deviations between iterated cosine functions and the raw data. An index of goodness of fit is computed as the ratio of the sums of squares of the best fit and the worst fit. In contrast to **Acro**, the program **Cosinor** (see Table 3.1) performs the single cosinor by solving the formal system of equations. As previously mentioned, the computation of amplitude based on this procedure yields smaller values than those obtained by half the mean difference between peaks and troughs. The mean level (mesor) and acrophase may also differ slightly between the two programs.

Other procedures besides the ones described here may be used for the computation of phase. The three procedures that were covered (inspection of actograms, computation of the peak of the smoothed rhythm, and acrophase of the fitted cosine curve) are the most commonly used ones but not the only possible ones. As a matter of fact, different experimental conditions may require different computational procedures.[43–47]

3.2.3 Period

So far, we have discussed the computation of three of the six parameters that characterize circadian rhythms: mean level, amplitude, and phase. We shall now discuss the computation of "period" (Figure 3.23). In the precomputer era, inspection of actograms was virtually the only procedure available. Consider Figure 3.24. In the top panel (A), the activity pattern drifts to the left. This tells us that the circadian period is shorter than 24 hours, because the activity onsets are a little earlier each consecutive day. But how much shorter than 24 hours is it? We can answer this question in three different ways. An intuitive way is to notice that the onsets advance

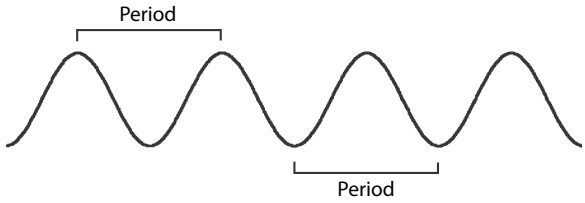


FIGURE 3.23 Period. Period is one of the parameters that characterize a circadian rhythm.

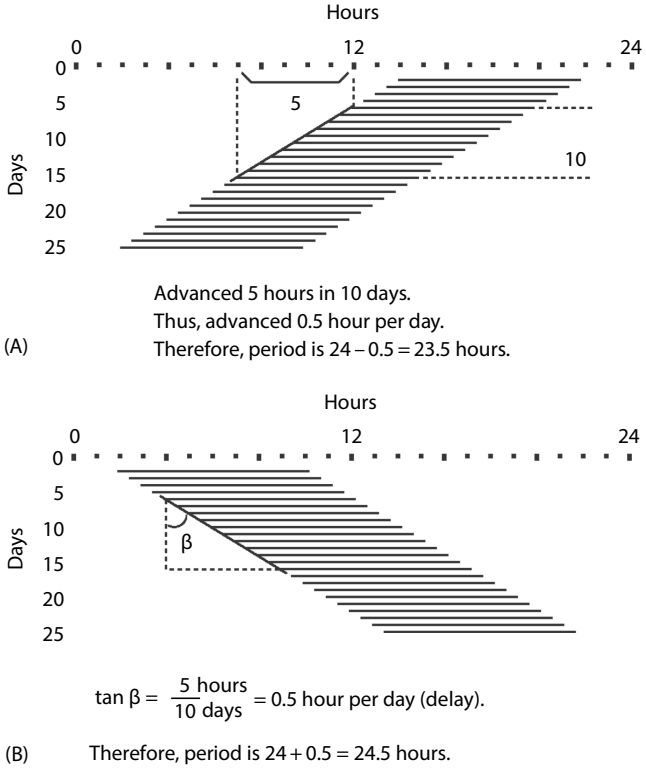


FIGURE 3.24 Calculation of circadian period using actograms. The slope of an imaginary line connecting the daily onsets of activity provides a measure of circadian period. This may be done in an intuitive manner (A) or by the use of basic geometrical principles (B).

5 hours in 10 days. This means that, on average, there is an advance of 0.5 hour each day. Therefore, the period must be 24 – 0.5 = 23.5 hours. We could repeat the procedure for Panel B and find out that, in that case, the period is 24.5 hours. Another way to solve the problem is by using elementary geometry (see Panel B). As you may remember, the slope of a line equals the tangent of its angle to the vertical. As you may also remember, the tangent of an angle in a right triangle equals the length of the opposite side divided by the length of the adjacent side. Thus, we can easily calculate the slope as 0.5 hour per day (and then add it to 24 to obtain the period of 24.5 hours). The third solution requires a background in basic statistics but is also simple: we may use linear regression to find the equation that describes the change in onset times as a function of elapsed days. The slope of the regression line will indicate how much the period deviates from 24 hours (which, for the data in Panel B, should be +0.5). Thus, we find that the period is 24.5 hours. Not surprisingly, the three methods yield the same answer. As a matter of fact, the three methods are just different elaborations of the same basic procedure.

Inspection of actograms can still be used today for the computation of circadian period. However, there are now many other possibilities. One of them, the method of "modulo adjustment," still involves the inspection of actograms but requires the use of a computer so that we can force the actograms to display the data in more convenient ways. In this method, we determine the period of a time series by adjusting the time scale used to construct the actogram. As shown in Figure 3.25, we may start with activity records that are clearly shorter than 24 hours when plotted on a standard 24-hour scale (Panel A). We then shorten the time scale (i.e., reduce the "modulus") until the onsets are vertically aligned. At this point (i.e., Panel D), we have reached the period of the rhythm, which happens to be 22.4 hours. The program **Plot** allows you to use this procedure (see Exercise 3.4).

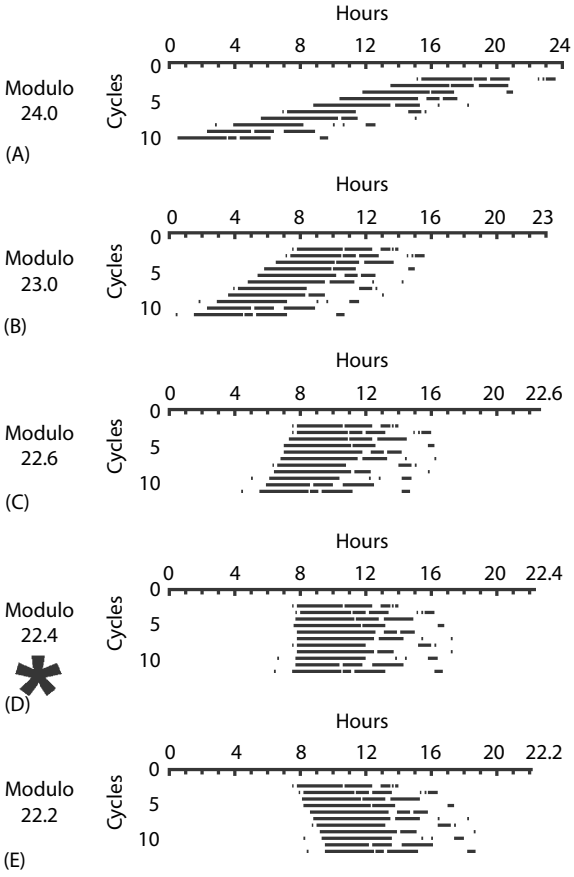


FIGURE 3.25 Calculation of circadian period by adjustment of plot modulo. The period of a rhythm, such as this rhythm of running-wheel activity of a golden hamster, can be determined by successive adjustments of the plot scale (plot modulo, as depicted in Panels A–E). When the correct modulus is reached (in this case, 22.4 hours, as indicated by the asterisk), the activity onsets align on a straight vertical line. (Adapted from Refinetti, R., *Behav. Genet.*, 28, 153, 1998.)

A problem in the use of actograms for the determination of circadian period is that circadian rhythms are rarely as distinct and regular as the activity rhythm depicted in Figure 3.25. If the rhythm is noisy or irregular, data analysis by visual inspection can easily be corrupted by the inspector's subjective biases. Therefore, automated data analysis procedures that are independent of human observers are much more reliable. Computational details of the procedures described in this section will be omitted, but they can be found in a review article that I coauthored with Halberg[41] as well as in the individual bibliographic references provided later.

One automated method for the computation of circadian period is "spectral analysis" (also called "Fourier analysis," after Jean Baptiste Fourier, the nineteenth-century French mathematician who developed it). This method was mentioned briefly at the beginning of the chapter when the concept of "frequency domain" was introduced. Fourier's revolutionary insight was that any time series, regardless of its shape or regularity, can be simulated by a series of sine and cosine waves of various frequencies. For instance, let us say that we want to build a time series that looks like a string of square waves (Figure 3.26). We could start with a single sine wave that has the same period as that of the square wave that we want to build (Panel A). We would then add a sine wave with shorter period (Panel B) to obtain a slightly modified waveform (Panel C). If we continued this procedure through many steps, we would eventually reach a waveform that is essentially square (Panel H). Of course, our goal in the analysis of circadian rhythms is the very opposite: we want to go from the complex wave to the series of simple waves, in the hope that we can identify the components of the rhythm. That's no problem. Fourier and others have worked out the mathematics of the whole process,[48–52] so that we can easily describe complex waves as sums of simple waves. As shown in Figure 3.27, a simple sine wave (A) yields a single peak in the Fourier "periodogram" (B), whereas a square wave (E) yields a series of peaks corresponding to the various rhythmic components (F). Now, look back at Figure 3.1 (the *idealized* body temperature rhythm of a female golden hamster). The periodogram for that data set is shown in Figure 3.28. Notice that the periodogram has a major peak at 24 hours (corresponding to the circadian component), a smaller peak at 8 hours (corresponding to the ultradian component), and a third peak at 96 hours (corresponding to the

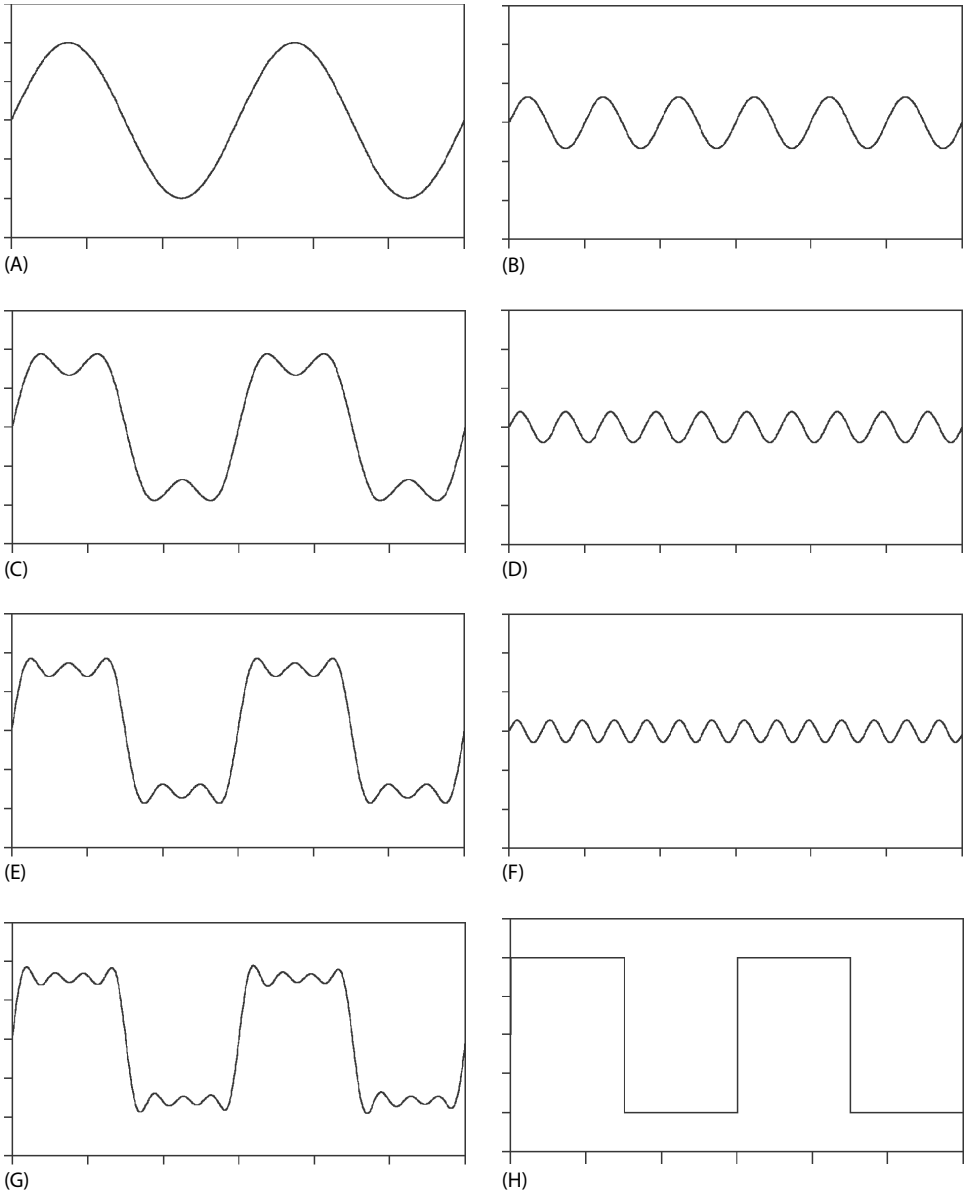


FIGURE 3.26 How Fourier analysis works. This "reverse-engineering" figure shows how a square wave (H) can be built out of the sum of many sine waves (A + B + D + F). Panels C, E, and G are intermediary steps. Fourier analysis actually does the very opposite: it breaks down a complex wave into a set of simple sine waves.

estrous component). The peak at 96 hours is wider because the time resolution of Fourier analysis is lower for longer periods (lower frequencies). The periodogram also shows three other small peaks that are not very meaningful. They represent the periodogram's *interpretation* of the noise component.

The program **Fourier** (see Table 3.1) can be used for the computation of circadian period by Fourier analysis, although other programs are better suited for this purpose. Fourier analysis is an excellent tool for the analysis of ultradian rhythms (see Exercise 4.5), but it is not ideal in the analysis of circadian rhythms. First, as just mentioned, its temporal resolution is low for low frequencies (long periods). Thus, for a typical data set containing 2400 data points collected at 6-minute intervals for 10 consecutive days, Fourier analysis evaluates periods of 2400/1, 2400/2, 2400/3, etc., so that, in the circadian range, only periods of 20.0, 21.8, 24.0, and 26.7 hours (that is, 2400/12, 2400/11, 2400/10, and 2400/9 intervals, respectively) are actually tested.[53] This resolution is unacceptable; one would expect at least a 6-minute (0.1 hour) resolution. Second, Fourier analysis' main asset (namely, its sensitivity to all rhythmic components of the time series) becomes a liability when one has to analyze noisy data sets. If the amount of noise is small, the periodogram is only mildly contaminated (as exemplified by the three small peaks in Figure 3.28); however, if the amount of noise is substantial (not a rare condition in biological rhythms), Fourier analysis loses sensitivity to rhythmic components much faster than do some other analytical methods.[54]

Numerous other methods for the determination of circadian period have been developed or adapted, including

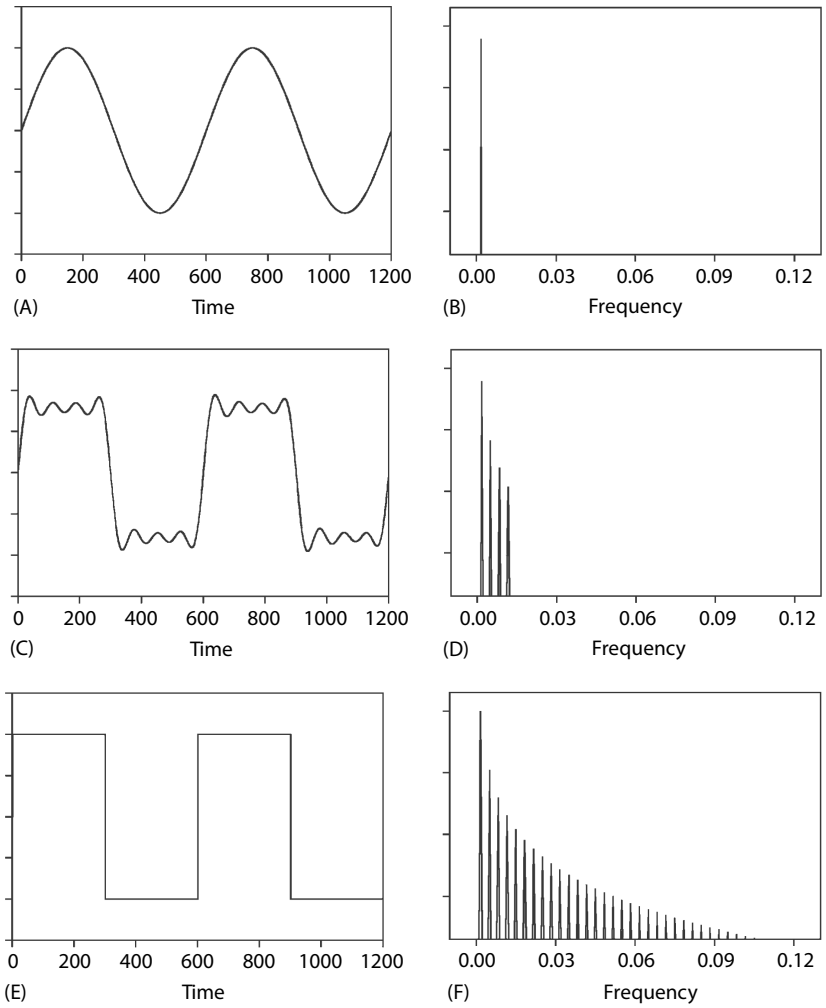


FIGURE 3.27 Actual Fourier analysis. Fourier analysis yields a periodogram that indicates the magnitude of each component of a wave. The wave in (A) is a pure sine wave, so that its periodogram has a single peak (B). The wave in (C) can be decomposed into four sine waves with different frequencies and magnitudes (D). The square wave shown in (E) is complex and can be described only by a large number of sine waves (F). Notice that the wave in Panel C is the same as the wave shown in Figure 3.26 (Panel G).

serial autocorrelation,[55–58] inter-onset averaging,[59–61] iterative harmonics,[62] acrophase counting,[63] singular value decomposition,[64] maximum entropy spectral analysis,[65] and nonlinear multiple components analysis.[23] Some of these methods are more reliable than others. Two methods that have been shown to be particularly suitable for the analysis of circadian period are the Enright periodogram[54] and the Lomb–Scargle periodogram.[66]

The "Enright periodogram," proposed by James Enright,[67] is based on the same principle of temporal alignment used by the method of modulo adjustment discussed earlier. As shown in Figure 3.29, we can determine the period of a rhythmic time series by inspecting the alignment of segments plotted on various time scales. However, instead of relying directly on the alignment of the segments, we use the average waveform of the segments (bottom row in Figure 3.29). If a time scale (modulo) is different from the period of the rhythm, the average waveform tends to be flat, whereas a waveform with a distinct peak is seen when the modulo matches the period of the rhythm. To avoid human subjectivity in the determination of what is and what is not a flat wave, a mathematical index (usually a quotient of variances) is used. Each time scale (and, therefore, each candidate period) is assigned an index. The candidate period associated with the largest index is considered to be the true period. Two distinct but similar implementations of the procedure were published by Dörrscheidt and Beck[68] and by Sokolove and Bushell.[69] The latter, which is referred to as the "chi-square periodogram," has become particularly popular in the analysis of circadian periodicity.[70–86] Both the program **Tau** and the program **Rhythm** (see Table 3.1) can be used for data analysis by the chi-square periodogram procedure. **Tau** is meant for the specific computation of circadian period, whereas **Rhythm** is meant for exploratory analysis of rhythmicity in general.

The "Lomb–Scargle periodogram" is just as good as the Enright periodogram but has the advantage of allowing the analysis of data that were collected at irregular intervals rather than under a rigorous protocol with measurements conducted, say, every 6 minutes or every hour. It also allows the analysis of data sets that were intended to have equally spaced observations but that are missing one or more values because of

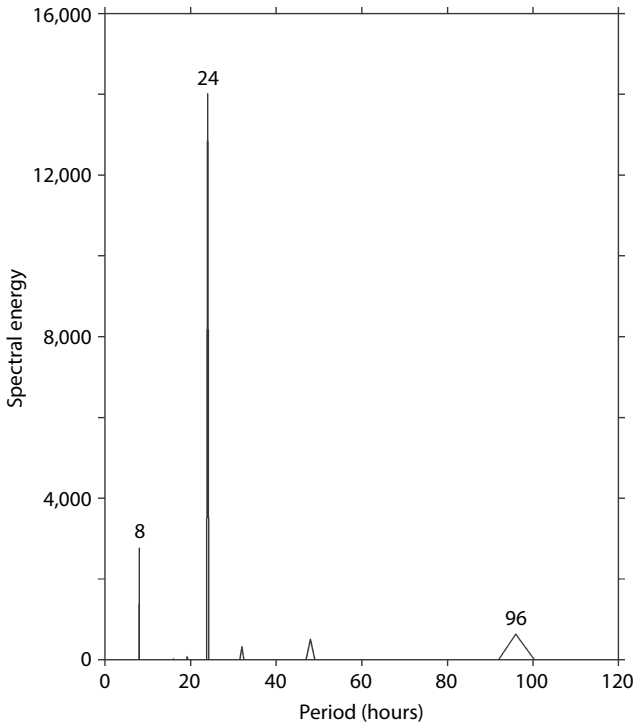


FIGURE 3.28 Fourier analysis in action. This periodogram is the result of Fourier analysis of the idealized body temperature rhythm of a female golden hamster previously shown in Figure 3.1. A large 24-hour component, as well as several smaller components (including 8 and 96 hours), can be seen. The analysis was conducted on a long (92 days) time series with the same parameters as the shorter series shown in Figure 3.1.

equipment failure or other adversity. The procedure is based on N.R. Lomb's adaptation of Fourier analysis to unequally spaced time series.[87] As we will see shortly, the Lomb–Scargle periodogram is susceptible to artifacts,[88,89] but so is the Enright periodogram. The Lomb–Scargle periodogram has been shown to match or surpass Sokolove and Bushell's implementation of the Enright periodogram in the analysis of circadian rhythms,[66] although it has not been widely adopted by circadian physiologists. The program **LSP** (see Table 3.1) can be used for data analysis by the Lomb–Scargle periodogram procedure.

The Enright periodogram (chi-square periodogram) and the Lomb–Scargle periodogram are called "periodograms" because the results of the analyses are routinely plotted in graphs similar to the Fourier periodogram. The term *periodogram* was introduced by Schuster in 1898 for the analysis of meteorological data[90] and used by Halberg in 1956 for the analysis of circadian rhythms.[91] Consider Figure 3.30. Panel A shows 3-day segments of four 10-day-long data sets. The top three data sets were created by computer as, respectively, sine waves with a period of 24.0 hours, sine waves with a period of 12.0 hours, and square waves with a period of 24.0 hours. The fourth data set corresponds to records of running-wheel activity of a golden hamster. Analysis of the hamster data by the method of modulo adjustment revealed a period of 24.0 hours. Now, the Panels B and C in Figure 3.30 show the Enright (chi-square) and Lomb–Scargle periodograms for the corresponding data sets. Notice that both periodogram procedures correctly detected the 24.0-hour period of the sine wave, as indicated by the tall peaks (top row). Both procedures also detected the 12.0-hour period of the other sine wave (second row), but the Enright periodogram incorrectly detected also a 24.0-hour component in the 12.0-hour rhythm. The detection of harmonics that do not actually exist ($12.0 \times 2 = 24.0$ in this case) is a recognized deficiency of the Enright periodogram.[54,68] Thus, when analyzing circadian rhythms, one must make sure that the data set does not contain submultiple oscillations that might be responsible for the appearance of a peak in the circadian range. Filtering out of high-frequency oscillations prior to periodogram analysis is a simple way to accomplish this. Most of the time, this precaution is not necessary because circadian rhythmicity is much more robust than ultradian rhythmicity. If an ultradian peak is smaller than the circadian peak, one can safely interpret both peaks as being legitimate. This is the case in the periodograms for the square wave (third row). Small peaks at 12.0 hours appear alongside with the large peaks at 24.0 hours, and they are legitimate peaks equivalent to the peaks that we saw in the Fourier periodogram for a square wave in Panel F of Figure 3.27, even though they are not *real* peaks in the sense that the square wave does not recur in 12-hour intervals. Notice that the Enright periodogram handled the square wave better than the Lomb–Scargle periodogram did, as demonstrated by the height of the 24.0-hour peaks. Finally, both periodograms identified 24.0-hour rhythmicity in the data set of running-wheel activity (bottom row).

The method of "cosinor rhythmometry" that we discussed for the analysis of phase was originally used for the characterization of phase, mesor, and amplitude of rhythms under the assumption of a 24-hour period,[19,29] but there is no reason why the process cannot be used in various iterations with varying periods within the circadian range, so that a periodogram can be built.[62] The program **Cosinor** (see Table 3.1) uses the iterative cosinor procedure to provide an estimate of circadian period as well as estimates of mesor, amplitude, and acrophase. Because the cosinor procedure is also capable of handling unequally spaced observations as well as equally spaced observations, I consider **Cosinor** to be the best program for the analysis of circadian rhythms. Nonetheless, because scientists are very skeptical people, you are advised to always use at least two separate methods when analyzing your data.[92]

3.2.4 Waveform and Robustness

The fifth parameter of circadian rhythms is "waveform." The four data sets shown in Figure 3.31 have the same mean level, same amplitude, same phase (on average), and same period. Yet, they are clearly different from each other. Their "waveforms" are different: some are regular, some are irregular, some are smooth, and some are rather complex. Unfortunately (or fortunately, if you are getting tired of abstract discussions of data analysis procedures), circadian physiologists have given very little attention to this issue.

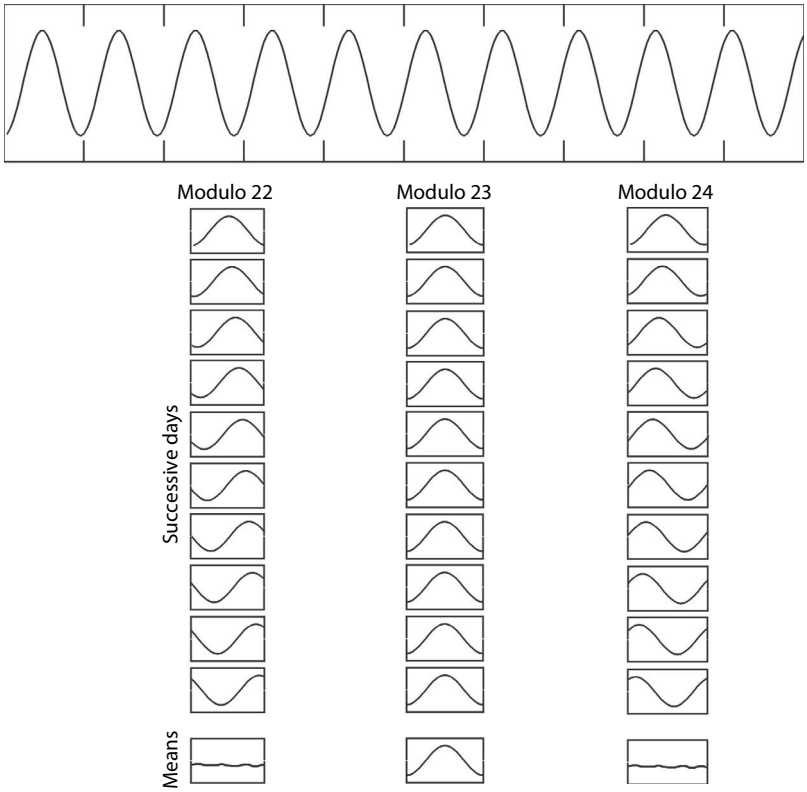


FIGURE 3.29 Calculation of circadian period by the Enright periodogram. The principle of operation of the Enright periodogram procedure is the same as that of the procedure involved in the calculation of circadian period by the method of modulo adjustment of actograms. The difference between the two procedures is that the Enright periodogram procedure provides a numerical value for the strength of each potential period (bottom row).

In principle, one could obtain a quantitative description of waveforms by comparing the various rhythmic components revealed by spectral analysis of the rhythms. An attempt in this direction is made in Figure 3.32 for the body temperature rhythms of four mammalian species maintained under a 24-hour light–dark cycle. To ensure that the waveforms are representative, average values from 7 animals per species were used. The waves for 7 consecutive days were evaluated by Fourier analysis (although only 2 days are shown in Panel A to facilitate visual inspection). To eliminate high-frequency oscillations, all data sets were reduced to 2-hour resolution (i.e., one value each 2 hours). The use of 7 days with 12 values per day ensures that Fourier analysis can detect 24-hour rhythmicity properly. Panel A indicates that the rhythms of different species have different waveforms, and the Panel B is expected to explain the differences. The periodograms of the four species consistently show large peaks at 24 hours and smaller peaks at 12 hours. The other peaks vary from species to species, thus defining spectral profiles that may serve as tools for "rhythm finger-printing." Thus, the horse's rhythm, which resembles to some extent a pure sine wave, yields only very small additional peaks in the periodogram. The laboratory rat's rhythm, which has conspicuous "horns," yields a relatively large 8-hour peak in addition to the 12- and 24-hour peaks. The dog's rhythm, which seems "noisier" than the rhythms of the other species, has many small additional peaks in the high-frequency range (i.e., periods shorter than 12 hours). Whether these interspecies differences in spectral profile are reproducible in other laboratories, and whether they can be extended to other species, remains to be determined.

Finally, the sixth parameter that characterizes circadian rhythms is "robustness." This term has two meanings in statistics. The traditional meaning is that of "tolerance to violations of assumptions." Thus, a statistical test such as the ANOVA is said to be robust because it performs well even if the assumptions about the properties of the data are violated. The other meaning of robustness has to do with the strength of a rhythm. Many circadian physiologists used the term informally to denote the strength of a rhythm (how *clean* a rhythm is)[93–96] before a formal definition of the new meaning was given. The robustness of a rhythm is its strength and regularity. The robustness of a rhythm is distinct from its amplitude, as well as from its other four parameters. Robustness is a measure of the *stationarity* of a rhythm.[97] Look at Figure 3.32 again and compare the waveforms of dogs and horses (Panel A). In horses, the second daily cycle in the oscillation of body temperature is very similar to the first cycle; in dogs, the second cycle differs considerably from the first one. That is, the rhythm of horses is quite consistent (almost *stationary*), whereas the rhythm of dogs is rather variable (not truly stationary).

Because circadian rhythms are not strictly stationary,[97] and because the use of most methods of time series analysis

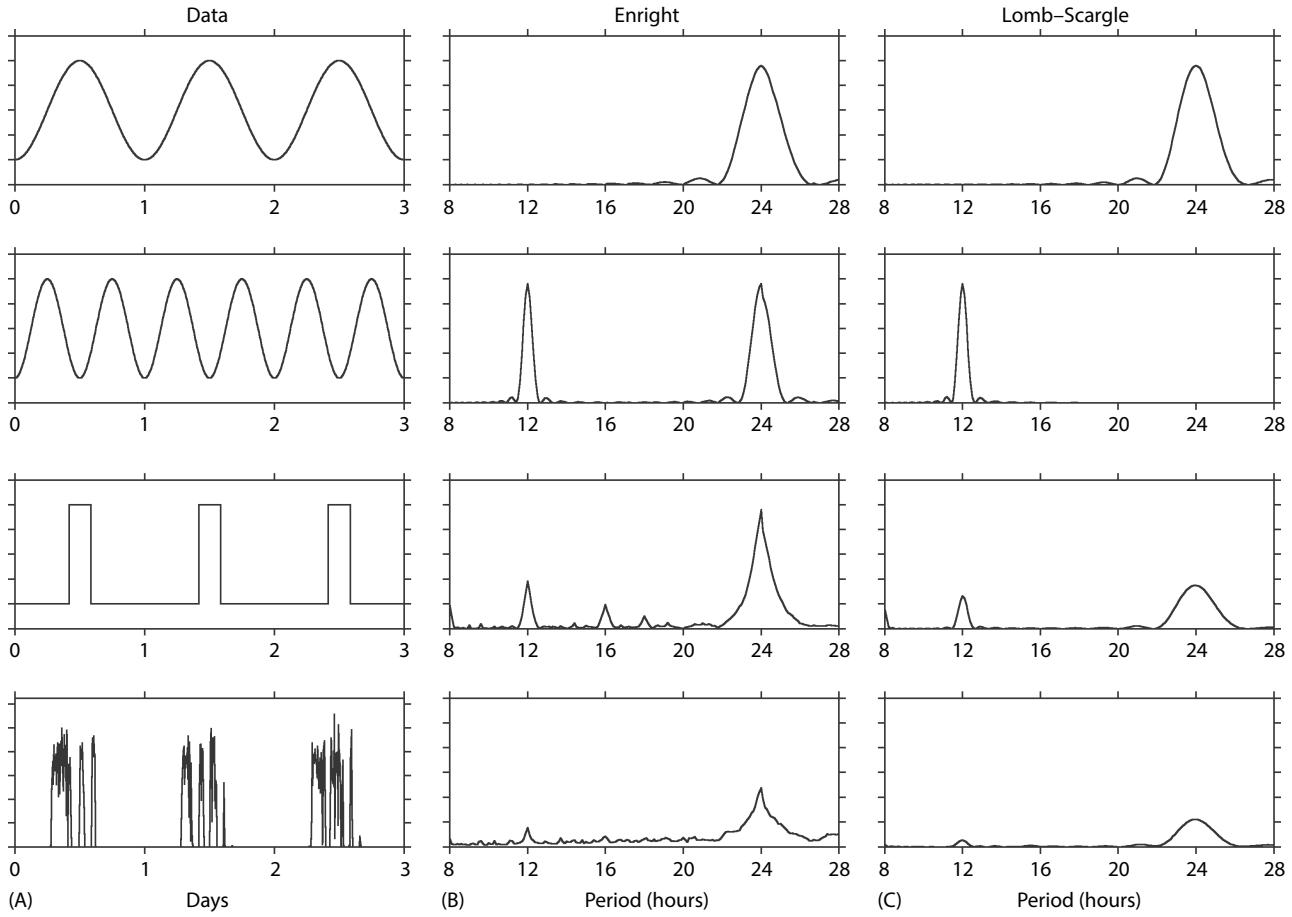


FIGURE 3.30 Enright and Lomb–Scargle periodograms in action. Panel A shows 3-day segments of three artificial data sets and of a data set containing records of running-wheel activity of a golden hamster. Panel B shows Enright (chi-square) periodograms for the corresponding data sets on the left panels. Panel C shows Lomb–Scargle periodograms for the same data sets. All data sets were 10 days long, even though only 3 days are shown. See text for details about interpretation of the periodograms. (Adapted from Refinetti, R., *J. Theor. Biol.*, 227, 571, 2004.)

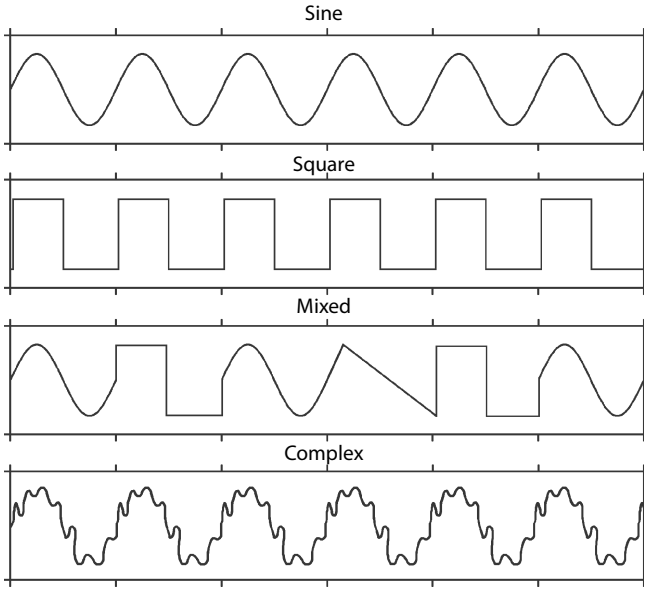


FIGURE 3.31 Waveform. The four graphs show four different rhythms that have clearly distinct waveforms despite having the same period, phase, mean level, and amplitude.

implies the assumption that the oscillatory process under study is stationary, one might argue that no time series analysis of circadian rhythms is reliable. From the viewpoint of mathematics, the argument might be correct. From a physiological perspective, the inaccuracy of the procedures is often no greater than that resulting from standard "biological noise." An alternative procedure for handling the nonstationarity of circadian rhythms is the wavelet transform, which was developed specifically to handle nonstationary time series.[5] While this allows the analysis of a nonstationary *time series*, it is not clear that it provides any advantage in the analysis of *rhythms*. After all, when one talks about rhythms, one is thinking about a process that repeats itself over time. For this reason, one must state the obvious: if the process is so nonstationary as not to repeat itself over time, then one would not call it a rhythm. The process might still be of interest to a mathematician (and some physiologists), but would not be of direct concern to the circadian physiologist. In any event, wavelets can be used for the analysis of circadian rhythms.[98]

The idea of quantifying rhythm robustness by means of the Q_P statistic of the chi-square periodogram was informally introduced more than 30 years ago.[99] The Q_P statistic is

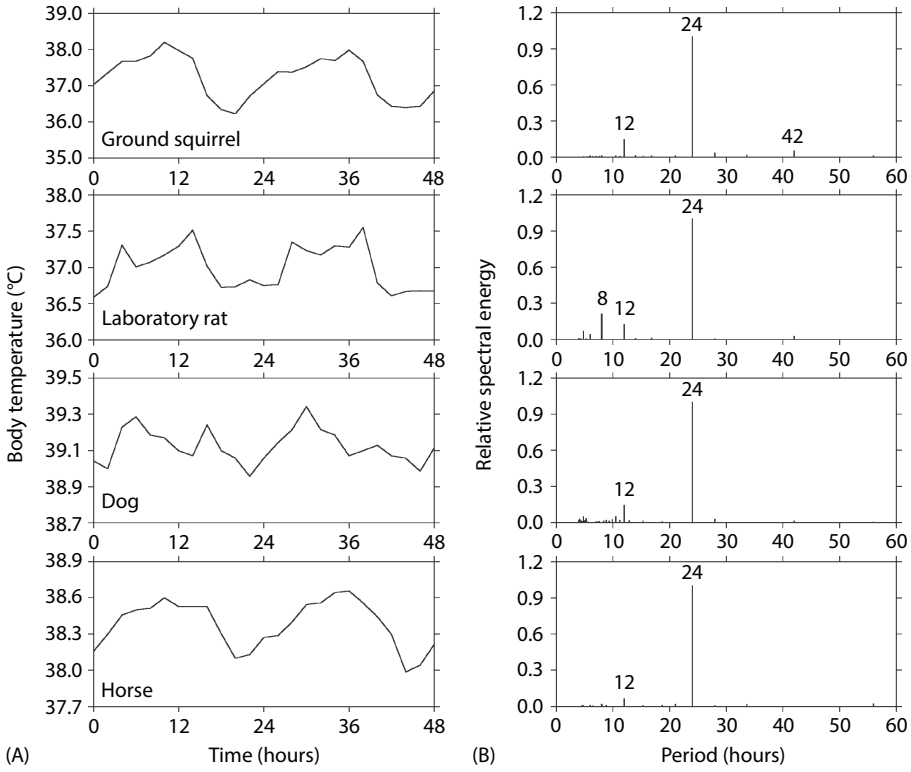


FIGURE 3.32 Quantifying waveform by Fourier analysis. (A) The waveforms of the body temperature rhythms of four mammalian species were quantified by Fourier analysis. The data sets were 7 days long (although only 2 days are shown) and contained mean body temperature values of 7 individuals with 2-hour resolution. A resolution of 2 hours was used to eliminate high-frequency oscillations. (B) The periodograms for all four species show a strong circadian (24-hour) component as well as other smaller components that vary from species to species. (Data from archives of the Refinetti lab. Periodogram analyses previously unpublished.)

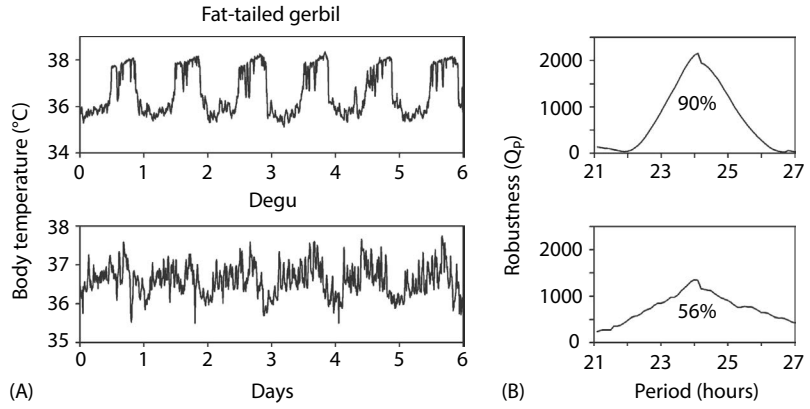


FIGURE 3.33 Calculation of rhythm robustness. (A) Six-day segments of 10-day long records of body temperature (with 6-minute resolution) of a fat-tailed gerbil and of a degu are shown along with (B) the Enright (chi-square) periodograms that describe them. Rhythm robustness is calculated as the peak Q_P value expressed as a percentage of the maximal possible Q_P (which in this case was 2400). The rhythm of the fat-tailed gerbil is much more robust than that of the degu. (Adapted from Refinetti, R., *J. Theor. Biol.*, 227, 571, 2004.)

Sokolove and Bushell's version of the index of rhythmicity of the Enright periodogram, as explained earlier. The relationship between Q_P and the subjective impression of the "neatness" of a rhythm can be easily apprehended in Figure 3.33. The "neat" rhythm of body temperature of the fat-tailed gerbil (a small nocturnal rodent) yields a periodogram with a large 24-hour Q_P (90% of the maximal possible Q_P), whereas the "crummy" rhythm of the degu (a diurnal rodent) yields a periodogram with a much smaller 24-hour Q_P (56% of the maximal possible Q_P). The Q_P value is not a perfect index of stationarity because it is sensitive to noise in the data set, but it becomes a very close approximation of a perfect index if the noise is filtered out prior to analysis,[97] as exemplified in Figure 3.34. Of course, one could always question whether

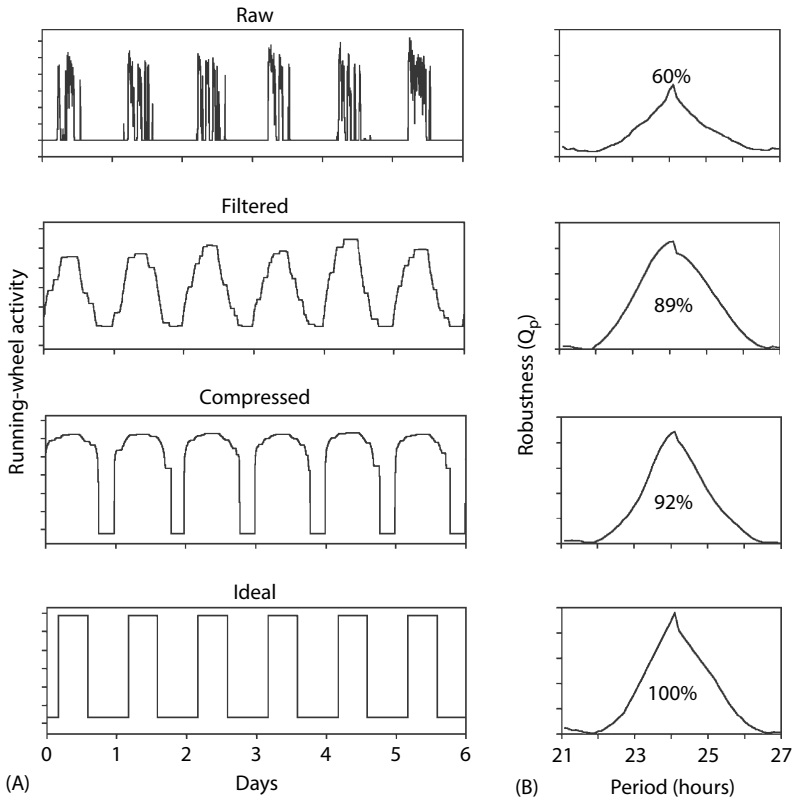


FIGURE 3.34 Exposing rhythm robustness. (A) Filtering, and sometimes compression, of natural data sets (such as the records of running-wheel activity of a golden hamster) helps to expose rhythm robustness (B). A clean square wave is shown as the ideal stationary wave (for these data) with robustness of 100%. (Adapted from Refinetti, R., *J. Theor. Biol.*, 227, 571, 2004.)

biological noise is true noise (i.e., stochastic variation)[100] or some form of deterministic "chaos,"[101,102] but resolution of this issue is not necessary for the analysis of rhythm robustness in the circadian range.

When using the Q_P statistic as an index of rhythm robustness, it is necessary to remember that, when one computes Q_P values for a data set, the more days (or circadian cycles) that one has, the more confident one can be about the nature of the data. This means that Q_P values increase as the number of days used increases. Consequently, comparisons of rhythm robustness between different variables or different individuals must be based on data sets of equal length. However, because the Q_P growth is linear (Figure 3.35), comparisons can be made between data sets of different lengths as long as robustness is expressed not in terms of absolute Q_P values but in terms of percentage of maximum possible values (as was done in Figure 3.34).

There is, of course, no reason why robustness must be quantified by the Q_P statistic. In the cosinor procedure, robustness can be easily calculated as the quotient of the variance associated with the cosine function and the total variance (and then multiplied by 100 to be expressed as percentage of maximal rhythmicity). The **Cosinor** program provides rhythm robustness along with the other rhythmic parameters.

To facilitate the selection of an analytical procedure that is most appropriate for the particular type of data at hand, Figure 3.36 provides a graphical summary of the various procedures described in this chapter. In most cases, the cosinor procedure implemented in the **Cosinor** program (with the periodogram option when needed) is a suitable choice. As previously noted, however, different scientists have their favorite procedures, and it is advisable to analyze the data with more than one procedure whenever possible.

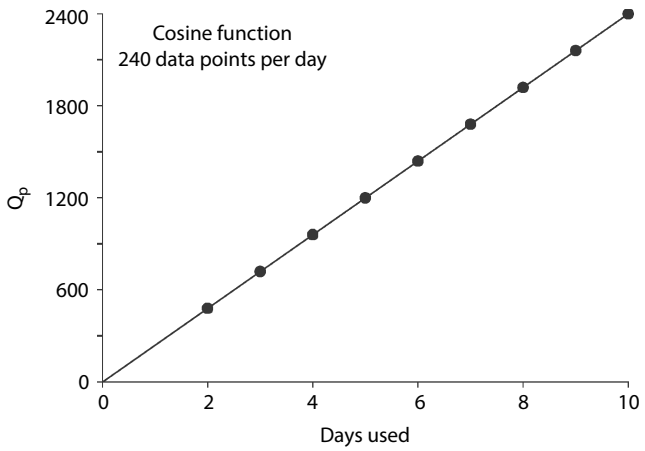


FIGURE 3.35 Maximal Q_P as a function of the length of a data set. The value of peak Q_P obtained by the chi-square periodogram procedure in the analysis of a perfectly rhythmic data set is a linear function of the number of days used in the analysis. For this reason, it is important to express rhythm robustness of an experimental data set as a percentage of the maximal Q_P value.

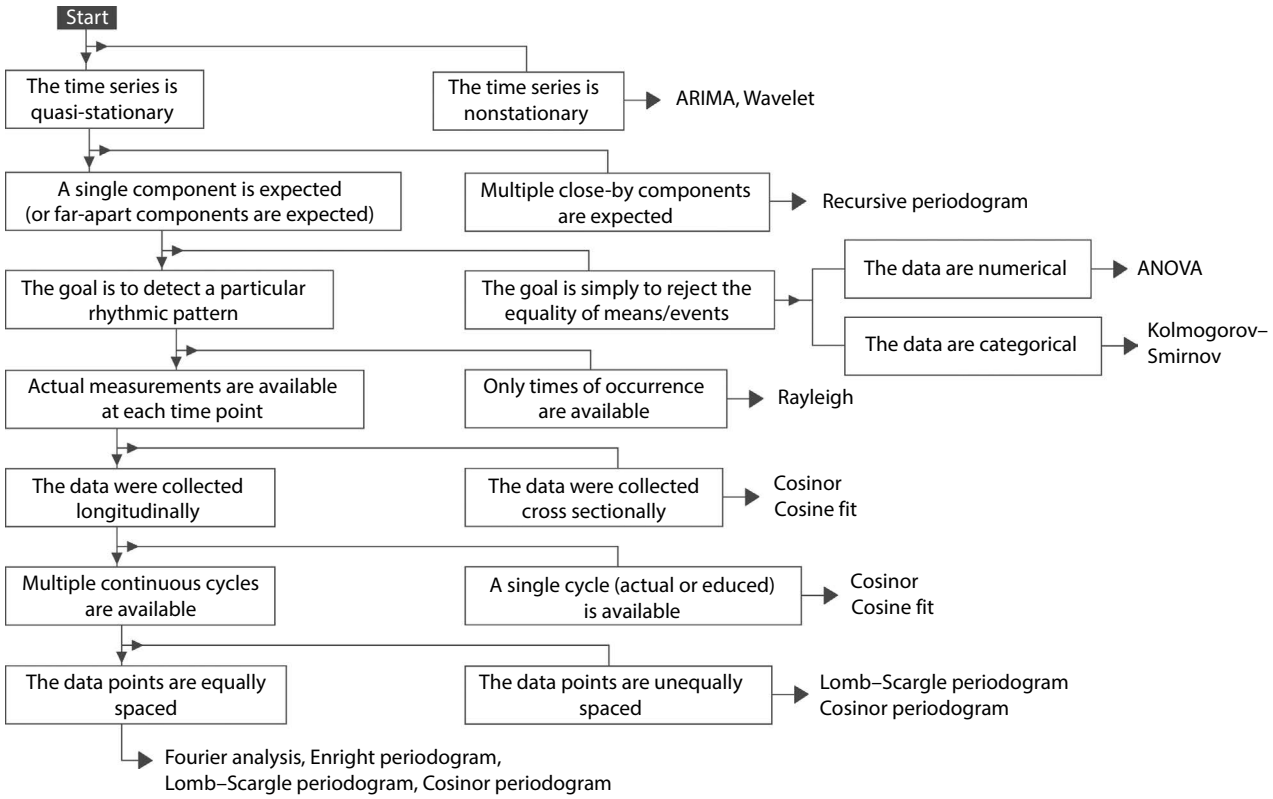


FIGURE 3.36 Guide for choosing a data analysis procedure. This diagram summarizes the various applications of the methods of data analysis described in this chapter.

3.3 STATISTICAL SIGNIFICANCE

The evaluation of statistical significance is an essential element of data analysis. It is an essential element because it allows us to discount, with reasonable confidence, the possibility that we detected rhythmicity in our data by pure luck. Suppose that we have six data points collected over time—every 4 hours for 24 hours (Figure 3.37). If all six readings are identical (Panel A), it would seem reasonable to conclude that there is no daily rhythmicity. But does the pattern in Panel B indicate the presence of rhythmicity? Elementary algebra tells us that three distinct values arranged in groups of 6, with repetition allowed, yield a total of 3^6 combinations. Thus, the sequence of values in Panel B is 1 out of 729 possible sequences—a rather unlikely event. Because it is such an unlikely event, we can be reasonably confident that we did not obtain it by chance. That is, we can be reasonably confident that real rhythmicity caused the particular arrangement of the values over time. Now, what about the other two panels? We might want to say that the arrangement in Panel C is also rhythmic, whereas that in Panel D is not—but, would it be reasonable to do so? When we talk about statistical significance, we are talking precisely about objective criteria for this type of decision. Ronald Fisher, the "father" of the science of statistics (Figure 3.38), believed that this process can provide the basis for "inductive logic."[103]

The issue of "inferential statistics"—which is the name of the general process associated with the computation of statistical significance—has its roots in the limitations of our ability to make scientific observations. Let us say that we want to test if aspirin relieves headaches. We could take 2000 people who are experiencing headaches, give aspirin to 1000 of them and placebo pills to the other 1000—and then check what happens. If every person who received aspirin got better, whereas no one who received placebo got better, it would be very reasonable to conclude that aspirin does indeed relieve headache. Notice, however, that we would be making an "inference," namely, that everyone else in the world will react to the drug the same way that our 1000 "guinea pigs" did—even though we did not, and would not be able to, test them all. As a matter of fact, we would be making the inference that any human being, including those who have not been born yet, will react the same way as our experimental group did. This is a *big* inference that requires more than just a casual hunch. And, of course, the situation is no different in circadian physiology. We cannot observe every circadian rhythm that exists, that has ever existed, and that will ever exist. We must resort to limited *samples* of the larger (virtually infinite) population of circadian rhythms. Suppose that the real phenomenon that we are trying to measure is described by the solid line in Panel A of Figure 3.39. Suppose also that the variations due to normal biological noise are contained between the dashed lines. Let us then sample two data points at random. If we happen to take points *a* and *d*, we will think—correctly—that there is a variation between morning and evening. If we take

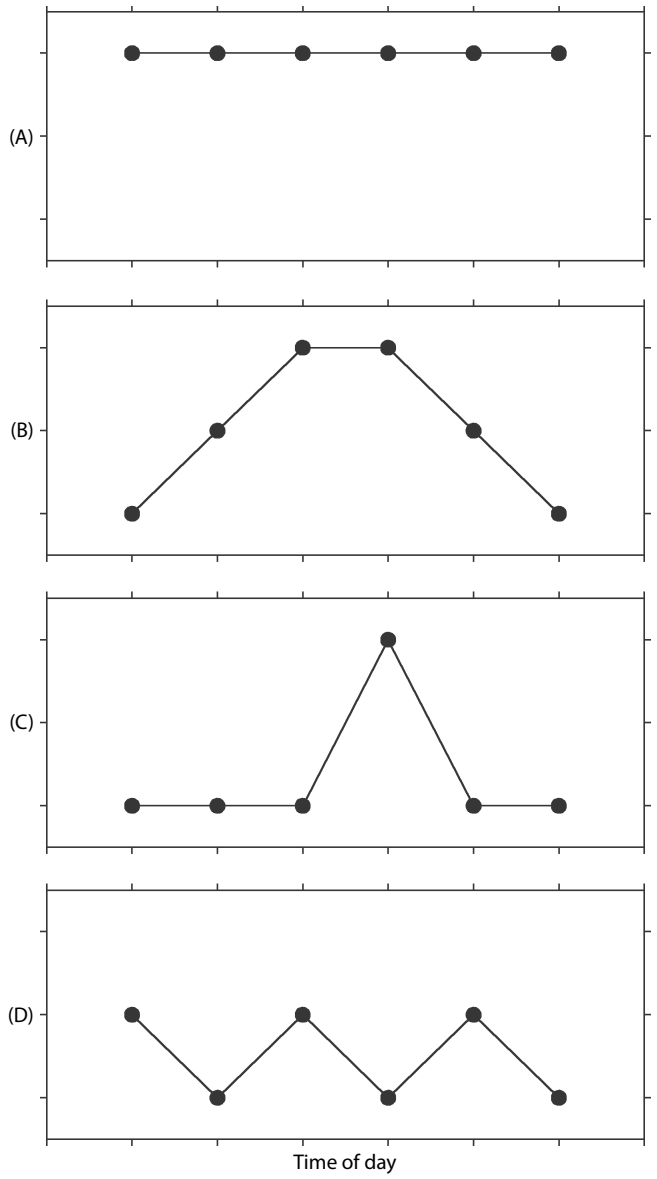


FIGURE 3.37 Importance of statistical testing. This figure shows four hypothetical data sets (A through D), some of which seem to exhibit daily rhythmicity and some of which do not. Which ones are truly rhythmic? Statistical testing is needed to quantify the degree of certainty of our inferences about rhythmicity.

points *a* and *c*, we will be less impressed by the results but will reach the same conclusion. However, if we happen to take points *b* and *c*, we will think that there is no daily variation (or even that there is a very small variation in the opposite direction). The key question is, of course, as follows: what points did we actually get? Since we don't know what the real curve looks like (because we sampled only two points), we cannot know if we got the *correct* points. What can we do? We could take a guess at what the curve looks like and then calculate the probability of getting each pair of points. But again, how can we take a guess? There are millions of possible scenarios! Perhaps we should give up.

All hope is not lost. There is a very sensible solution—if we are willing to lose a battle in order to win the war. The solution

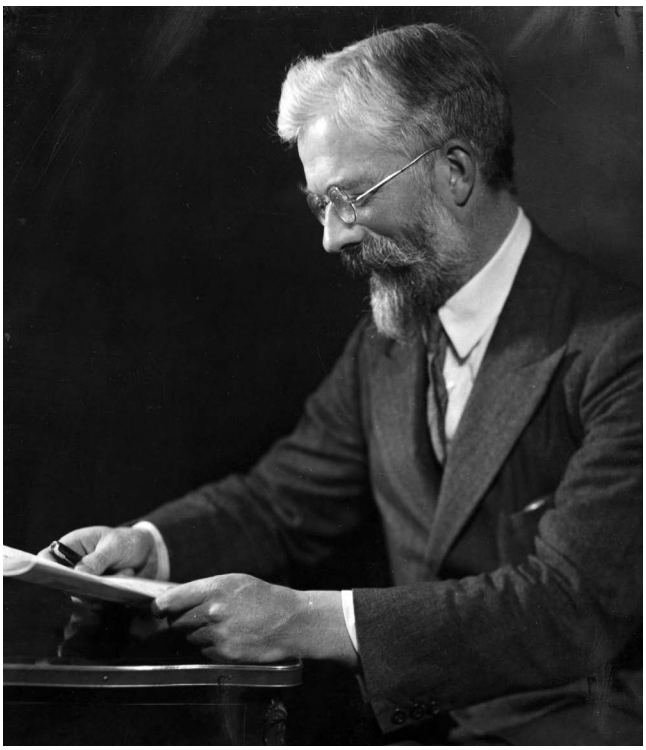


FIGURE 3.38 Sir Ronald Aylmer Fisher (1890–1962). Ronald Fisher is considered the "father" of the science of statistics. (Courtesy of North Carolina State University Libraries, Raleigh, NC.)

is to guess that *there is no real phenomenon*. That is, let us assume that there is no daily rhythmicity, as diagrammed in Panel B of Figure 3.39. We call this the "null hypothesis" (because the hypothesis states that there is no real phenomenon). We can now calculate the probability of obtaining each of the pairs of data points. If the pair that we actually obtained has a very low probability of coming out by chance, then we can assume that it did not come out by chance, and that it came out because our null hypothesis is wrong—that is, we can *reject* the hypothesis. Because the null hypothesis states that there is no rhythmicity, its rejection implies that there is in fact rhythmicity. Victory!

Let us not celebrate too early. The reasoning behind statistical inference is very good, but how do we actually calculate the probabilities? Let us start with a simple case. Suppose that we are simply taking a sample from the population shown in Panel A in Figure 3.40. Let's say that we picked the values marked with an X. This will give us the sample distribution shown in Panel B. The question now is as follows: does the sample distribution look similar enough to the populational distribution? If the sample was randomly drawn, its distribution should look like the populational distribution, and its mean should be similar to the population mean. Of course, we don't know what the population's parameters are—if we knew them, we wouldn't need to draw samples. However, we can use the trick of the null hypothesis again: if we are comparing two means (the famous *t*-test), we assume that the difference between the means is zero, so that the population of differences between means has a mean of zero; if we are

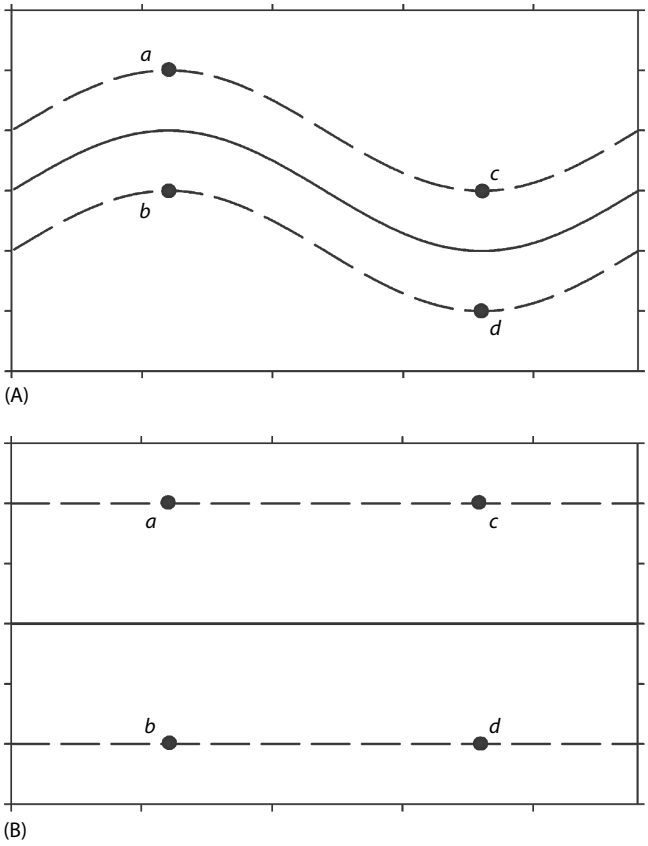


FIGURE 3.39 Problem of inferential statistics. Scientific knowledge is attained by observing samples from a much larger population of data. In both panels in this figure, the mean value of a hypothetical population is indicated by the solid line, and the variability around the mean is indicated by the dashed lines. If, for simplicity, we sample only two data points (say, a and d), we may conclude that the population is rhythmic regardless of whether it is actually rhythmic (Panel A) or not (Panel B).

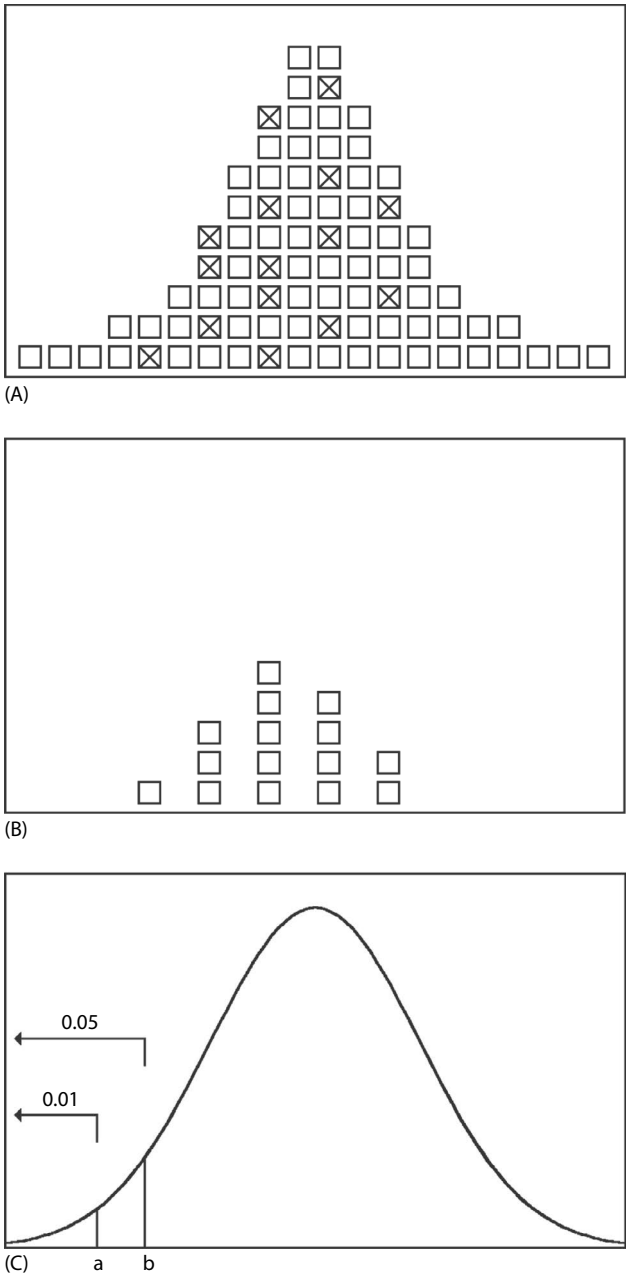


FIGURE 3.40 Rationale of inferential statistics. Because we have to make an inference about a population based on a sample, we need to estimate what other samples would look like. If the population that we are studying looks like the one in Panel A, and we take a sample that looks like the one in Panel B, we need to estimate whether this sample could be reasonably expected to come from the population (if it cannot, then we have something special, something that cannot be explained simply by chance). By drawing many random samples from the population, we can build a "probability density curve" such as that in Panel C. Then we can estimate how likely it is that our experimental results were due to chance alone.

comparing several means (the popular "analysis of variance," or ANOVA), we assume that the quotient of variances is 1, and likewise for other statistical tests.[104–106]

Once we have estimates of the population's parameters, we could draw thousands of random samples from the population, calculate the probabilities associated with all possible sample means, and check where our sample mean falls. Although we can do this for each experiment that we conduct (the so-called bootstrap method),[107–111] the traditional approach is to mathematically derive a generic "probability density curve," such as that shown in Panel C of Figure 3.40. Values below point *a* in the probability density curve occur by chance less than 1% of the time (i.e., point *a* delimits the cutoff for $p < 0.01$). So, if our sample mean is smaller than *a*, we know that our sample is a rare occurrence. But how much rare is rare enough? Commonly, $p < 0.05$ (point *b*) is considered a good breakpoint in the social sciences. This means that the "level of significance" of our decision is 5 in 100 or, in other words, that we are willing to take a 5% chance of rejecting the null hypothesis when the null hypothesis is actually true (the so-called Type I error, symbolized by the letter α). In biological sciences, $p < 0.01$ is often required. In the physical sciences, $p < 0.001$ or lower may be demanded. And why were these breakpoints chosen? This is a question for which I do not have a good answer. Decisions about statistical significance are arbitrary, and politics probably play a major role. As you can

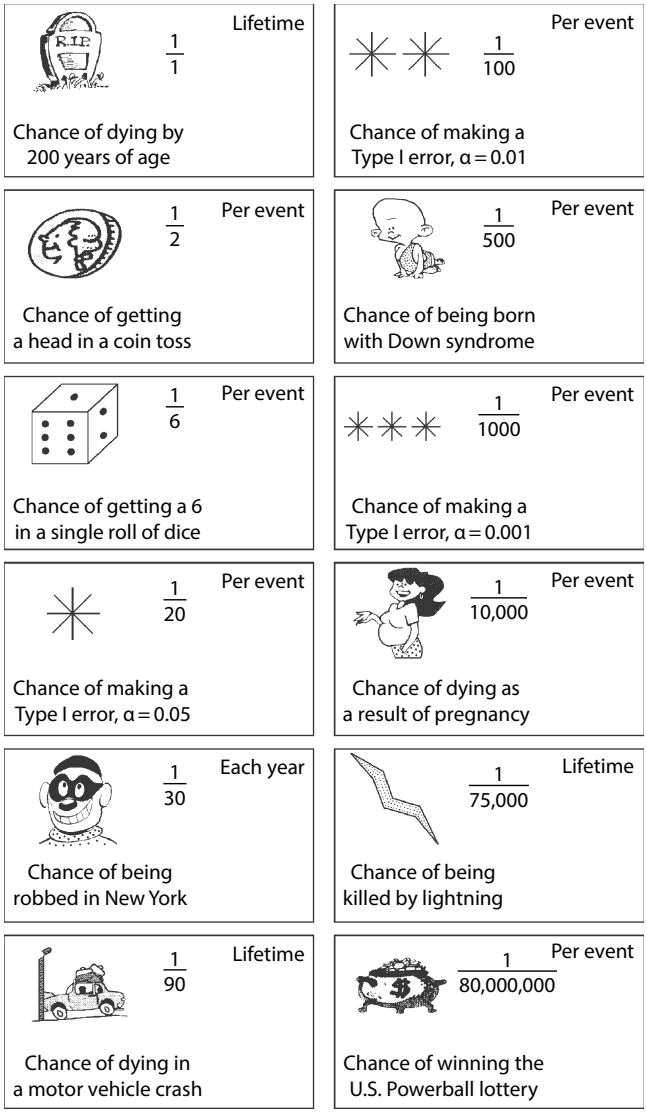


FIGURE 3.41 What are the odds? Although death is a certainty for all mortals (1/1 chance), most events in life take place with a lower probability. In scientific research, a Type I error refers to the situation where we conclude that we found something interesting when our results were actually due to random variation. The probabilities of Type I error usually accepted by researchers ($\alpha = 0.05$, 0.01, and 0.001) span a reasonable range but are not as low as one might think.

see in Figure 3.41, one is a little more likely to commit a Type I error when $\alpha = 0.05$ than to be robbed in New York City. That is, the probability of concluding that we found something interesting when the results are actually due to chance is very high if we choose a significance level of 5%. At the 1% level ($\alpha = 0.01$), one is more likely to make a wrong decision in the test than to be born with Down syndrome. Even the 0.1% level ($\alpha = 0.001$) does not seem to provide adequate protection against Type I errors. This is one reason why serious scientists are skeptical about any single study in a new area. Only after the study has been replicated several times in several laboratories (so that the combined probability of Type I error shrinks to 1 in several millions), do they feel comfortable embracing the findings as scientific truths.

3.3.1 Regular Time Series

Let us now address specifically the statistical significance of procedures used in circadian physiology. For parameters such as mean level, amplitude, and phase, there is usually no need for tests of significance. We often just want to describe them. If we wish to provide an index of variability, we can use standard descriptive statistics. For instance, if we calculated the amplitude of a rhythm by averaging the values (half the range of oscillation) over 10 days, we could compute the SD of this averaged value (as was briefly mentioned in Figure 3.13). The SD is simply the mean of the deviations between the values and the mean—or more accurately: the square root of the mean of the squared deviations. Thus, if we designate each of n values as X_i, the mean as M, and the summation over all values (X_i, from i = 1 to n) as $\Sigma f(X_i)$, then the SD is $\sqrt{\Sigma(X_i - M)^2/n}$. Likewise, if we calculate the average mean level of a rhythm by averaging the mean levels of 10 individuals of a species, we can compute the SD of this averaged value. This would give us an ugly sounding sentence: the SD would be the square root of the mean of the squared deviations of each mean level from the mean of mean levels.

Even when we are just describing the mean level, amplitude, or phase of a rhythm, a small dose of inferential statistics may be desirable. Because the SD refers to the distribution of values in our own sample, we may want to replace it with an index of variability that estimates the variability in anyone's sample. As explained in introductory statistics books,[13–18] our computation of SD should use (n – 1) instead of n as the denominator:

$$SD = \sqrt{\frac{\Sigma(X_i - M)^2}{(n-1)}}$$

Furthermore, if we divide the SD by the square root of the sample size $\left(\sqrt{n}\right)$, we obtain the "standard error" of the mean (SE), a very commonly used statistic. Now, if we can assume that the population of values is normally distributed (a reasonable assumption in many cases), or if we have 30 or more values regardless of the distribution, we can build a "confidence interval" to indicate how confident we are that our sample mean reflects the populational mean. The 95% confidence interval goes from $(M - SE \cdot 1.96)$ to $(M + SE \cdot 1.96)$. The 99% confidence interval goes from $(M - SE \cdot 2.58)$ to $(M + SE \cdot 2.58)$. For instance, when we counted the ducks swimming in a pond (Figure 3.13), we found a mean of 4.5 ducks with an SD of 1.8 ducks. Since we had 10 observations, our n is 10, and our SE is 0.57. Consequently, we can be 95% confident that the real number of ducks in the pond is between 3.4 and 5.6. If we consider only the 5 morning measurements, our 99% confidence interval goes from 4.8 to 7.2.

If we want to compare the mean levels, amplitudes, or phases of two or more groups of observations, we can use standard statistical tests such as *t*-tests and ANOVA.[104–106] However, when determining the acrophase of a rhythm by the fitting of a cosine wave, we need to verify first whether the

fit is a good one. The fitted cosine wave will always have a peak—and we will consider the time of the peak to be the acrophase—but the acrophase will be meaningless if the cosine wave does not fit the data properly. The program **Acro** assesses the goodness of the fit by means of an index computed as the ratio of the sums of squares of the best fit and the worst fit. The smaller the index, the better is the fit. The sampling distribution of this index was previously determined for 30,000 data sets of random numbers, and the probabilities associated with each value were computed. Thus, **Acro** provides the goodness of fit index along with its associated cumulative probability under the assumption of the null hypothesis. Probabilities smaller than the critical value (e.g., $p < 0.01$) indicate that the index is statistically significant. Similarly, to determine whether the cosine fit is meaningful, the program **Cosinor** performs an F test to verify that the amplitude of the fitted wave is significantly larger than zero.[41]

Techniques that are particularly important in circadian physiology have to do with the detection of circadian rhythmicity and the reliability of our estimates of period. Let us start with the Enright (or chi-square) periodogram. The general rationale for significance testing in the Enright periodogram is the same as that for ANOVA. Consider Figure 3.42. If we have a time series like the one shown in Panel A, we can calculate the means for each time of day across the 4 days (the "between-groups" mean, or M_B) as well as the means for each day across the six times of day (the "within-groups" mean, or M_W), as shown in Panel B. The quotient of M_B and M_W is of little use, as it is expected to equal 1 regardless of the actual distribution of the data. On the other hand, the quotient of the deviations from the means (SD_B/SD_W) is a very meaningful ratio. In this case, the quotient is 3.47, which indicates that SD_B is larger than SD_W. In contrast, if there were no consistent rhythmicity in the data (Panel C), the data points would be distributed randomly in the long run, thus leading to an SD_B/SD_W ratio close to 1 (Panel D). To conduct a significance test, we would only need to determine if 3.47 (or 0.80) is significantly different from 1—and we could do it by consulting the appropriate probability density curve (Fisher's F statistic). Figure 3.43 shows the probability density curves for the F

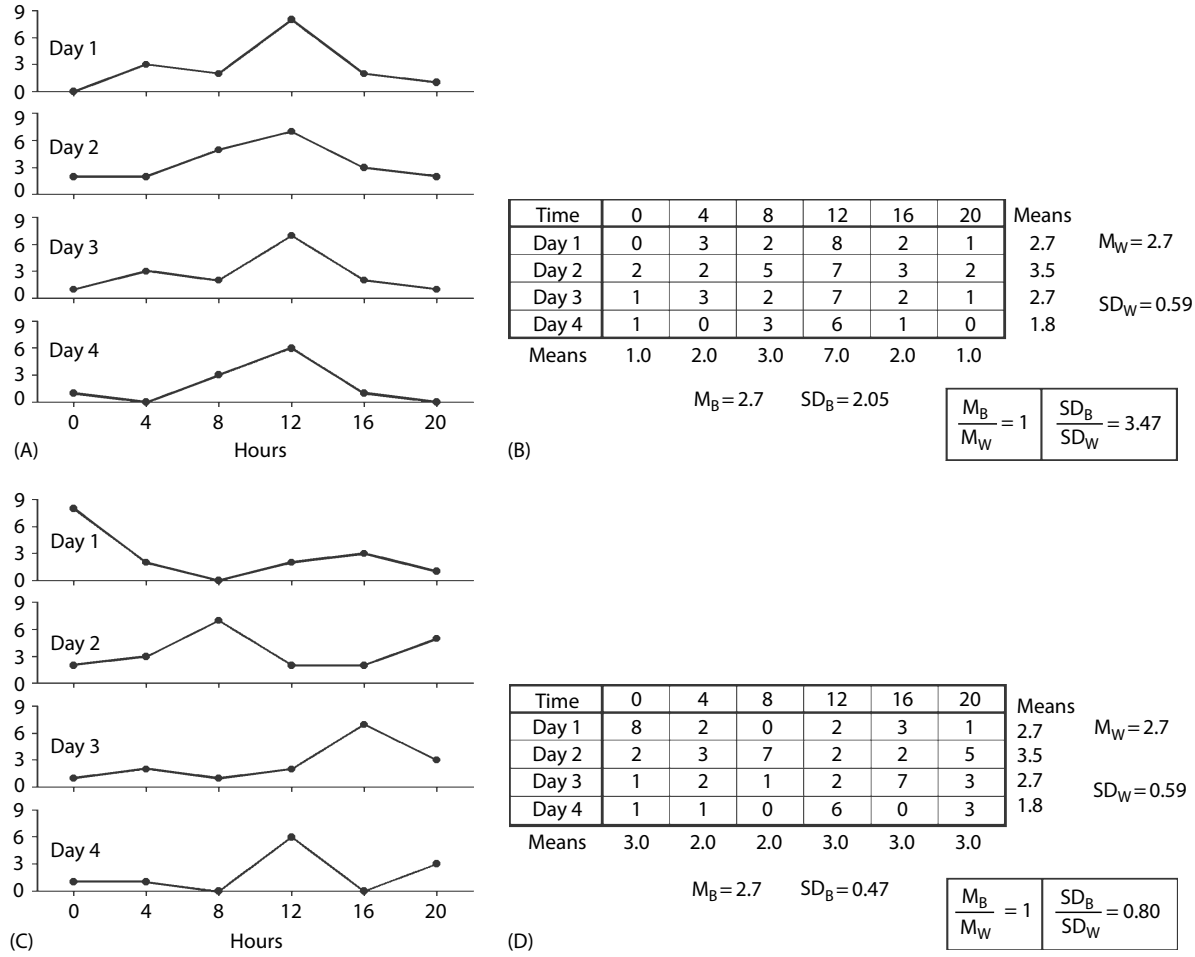


FIGURE 3.42 Developing a statistical test. The rationale behind the widely used analysis of variance (ANOVA), as well as behind the statistical test of the Enright periodogram, is that the variability of the data between groups should be the same as the variability within groups if the data were collected randomly. If the between-group variability substantially exceeds the within-group variability, then random variability cannot account for the results (and, consequently, we have a significant finding at hand). See text for details. *Abbreviations*: M, mean; SD, standard deviation.

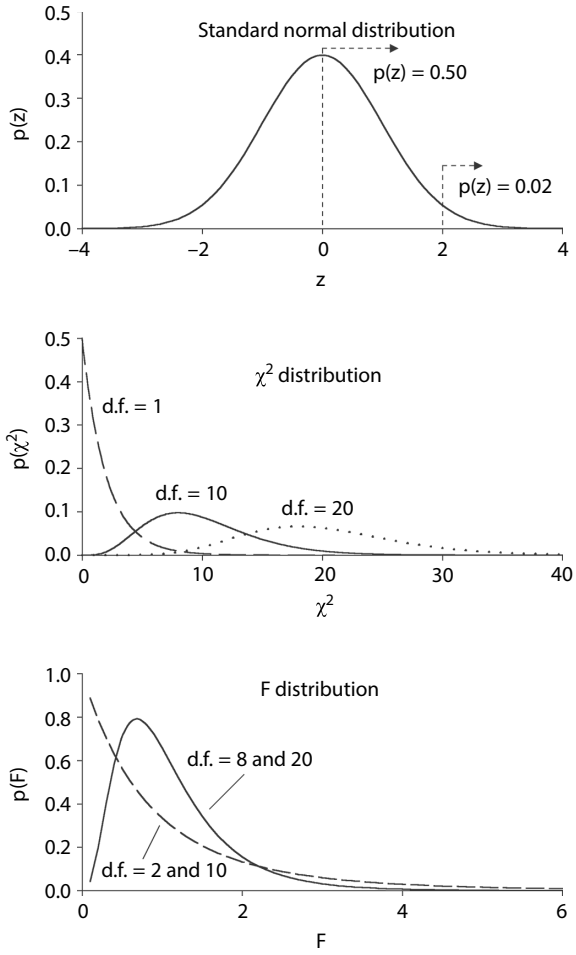


FIGURE 3.43 What do probability density curves look like? The figures show the probability density curves of three commonly used statistics. The χ^2 statistic and the F statistic have multiple probability density curves, and only a few of the curves are shown.

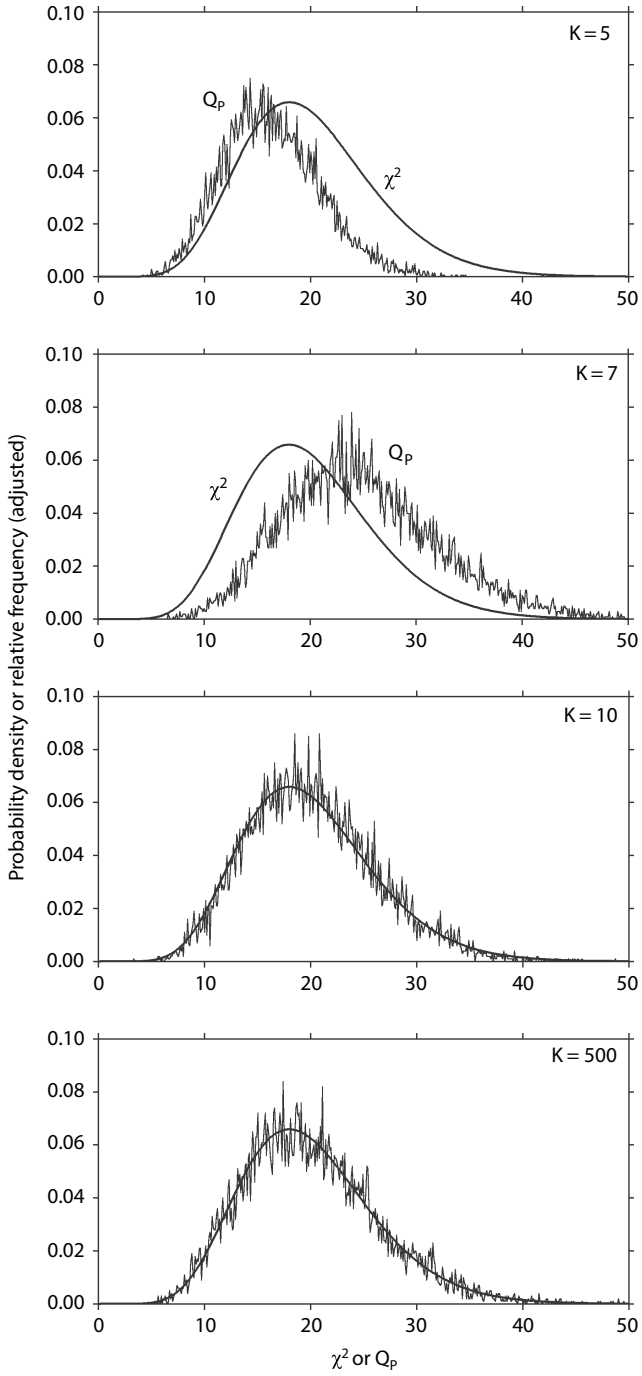


FIGURE 3.44 Just like a χ^2. The Q_P statistic of the chi-square periodogram is distributed as χ^2 when the data set contains 10 or more days ($K \geq 10$). In all four panels, the curve for χ^2 is the probability density curve for 20 degrees of freedom, and the curve for Q_P is the relative frequency curve based on computations for the period of 21 data points on 10,000 sets of random numbers.

statistic and two other commonly used statistics. Notice that, while the probability density curve for the standard normal distribution is relatively simple, both the χ^2 and the F distributions are actually families of functions. The χ^2 distribution depends on the "degrees of freedom" (d.f.) involved. The F distribution depends on two degrees of freedom: one for the numerator and one for the denominator of the F ratio. These days, statistics software packages automatically consult the required probability density curve, so that most users are not even aware that they exist.

The ANOVA is a very powerful tool widely used in significance testing. Unfortunately, the ANOVA can only tell us if the distribution of our data is different from a flat distribution. It does not care whether there is a single daily peak (as is the case for the data set in Panel C of Figure 3.37) or many daily peaks (as in Panel D). With an ANOVA, we cannot determine whether the rhythmicity is circadian or ultradian. The Enright periodogram solves this problem by essentially conducting an ANOVA for each data "fold," as previously illustrated in Figure 3.29. The highest value among the significant values (the peak of the periodogram) is then considered to correspond to the true period of the rhythm. As in the ANOVA, the test of significance for each period in the Enright periodogram could use the F statistic.[68] Sokolove and Bushell used the χ^2 statistic instead,[69] and this is why their implementation is called the chi-square periodogram (chi-square is the spelled-out form of χ^2). It should be pointed out, however, that Sokolove and

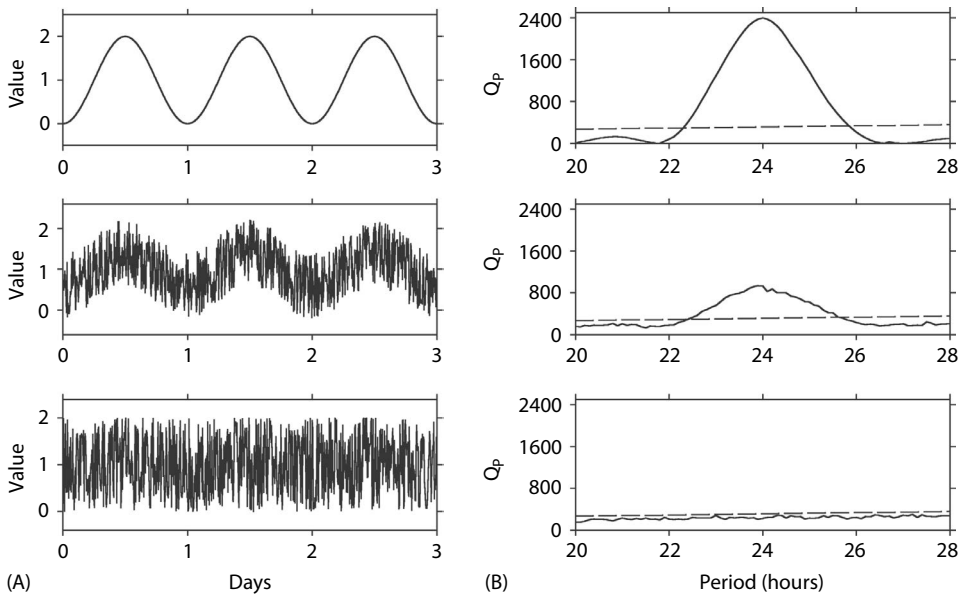


FIGURE 3.45 Determining the statistical significance of peaks in the chi-square periodogram. Panel A shows data sets that are rhythmic but contain variable amounts of noise (random variability). Panel B shows the corresponding chi-square periodograms. The dashed lines in the periodograms indicate the level of significance ($\alpha = 0.001$) without correction for multiple testing. For the data set containing a pure sine wave (top row), the periodogram shows a clear peak at the correct period (24.0 hours), which is well above the significance line. For the data set containing 100% noise (bottom row), the periodogram shows no peak above the significance line.

Bushell's Q_P is distributed as χ^2 only if the time series contains 10 or more days of data, as shown in Figure 3.44. This does not mean that the computed period is incorrect if fewer than 10 days are used, but it does mean that the statistical significance cannot be reliably determined. For this reason, the program **Tau** gives you a warning if you try to use fewer than 10 days, and the program **Rhythm** makes an approximate correction using the relationship shown in Figure 3.35.

I mentioned earlier that the chi-square periodogram is more tolerant of noisy data than Fourier analysis is. This does not mean, however, that it is insensitive to noise. As a matter of fact, we do not want it to be insensitive to noise because the proportion of noise is a relevant property of a rhythm. Figure 3.45 shows periodograms for a sine wave containing different proportions of noise. You can see that the presence of noise reduces the peak of the periodogram and that, as one would expect, there is no peak above the significance line when the time series contains 100% noise (bottom panels). If you are a keen observer, however, you may have noticed that, even for a pure sine wave (top panels), the chi-square periodogram does not contain a sharp peak like the one seen in the Fourier periodogram (for instance, compare this periodogram with that in the top panel of Figure 3.27). There is much more "leakage" of spectral energy in the chi-square periodogram than in the Fourier periodogram. If one has a *clean* time series, and if one is willing to repeat the analysis many times with slightly different segments of the time series, one can obtain much finer temporal resolution with the Fourier periodogram than with the chi-square periodogram. For test of significance in the Fourier periodogram, Fisher developed the necessary statistic many years ago,[112] and an adaptation for the detection of multiple peaks was developed later.[113] Both methods are used in the program **Fourier** (see Table 3.1).

Tests of significance for periodograms raise a problem that many researchers fail to recognize. Because a periodogram typically evaluates 20 or more periods at the same time (say, from 23.0 to 25.0 hours in steps of 0.1 hour), the computation of the desired level of significance of the test is not straightforward. To understand the nature of the problem, consider a racehorse with five horses (Figure 3.46). Let us say that you are placing bets on the winner of the race, and that you want to win the bet. Assuming that all five horses are equally likely to win (admittedly, not a fair assumption in real horse races), your probability of winning a bet in any single race is 1 in 5 (1/5). Thus, your "level of significance" is 20%. Of course, your odds will remain 1/5 no matter how many races you bet on; however, if you keep betting for five races, your odds of picking the winner in "at least one race" are $5 \times 1/5$, or 1. Thus, your "level of significance" becomes 100%. Evidently, winning the bet in at least 1 out of 5 races is no special feat. Similarly, finding a peak in the periodogram may be no special feat. If you choose a significance level of 5% (odds of 1/20) but you have 20 periods in the periodogram, your actual significance level is 100%. Obviously, this is unacceptable. Look at Figure 3.47 now. The curves on the left are sine waves (i.e., they have a rhythmic component), and the curves on the right are straight lines (thus, no rhythmicity). The two curves at the top row are clearly different, but the difference gradually disappears as noise is added to them. At least to my eyes, 90% noise totally obscures the difference between the two curves. Yet, I know that the left curve still has a rhythmic component. Is the rhythmicity biologically relevant or not?

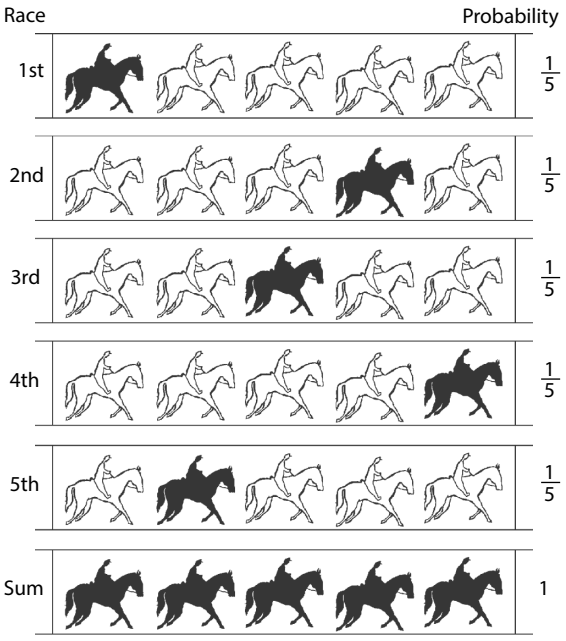


FIGURE 3.46 Inflating the level of significance. The probability of a Type I error in a statistical test is calculated under the assumption that the test will be performed only once. If multiple tests are performed, the computed probability must be corrected. Consider this figure. Assuming that all horses are equally likely to win a race, your probability of picking the winner in any individual race is 1/5. However, if you place bets on 5 races, your probability of picking the winner in at least one race is 1.

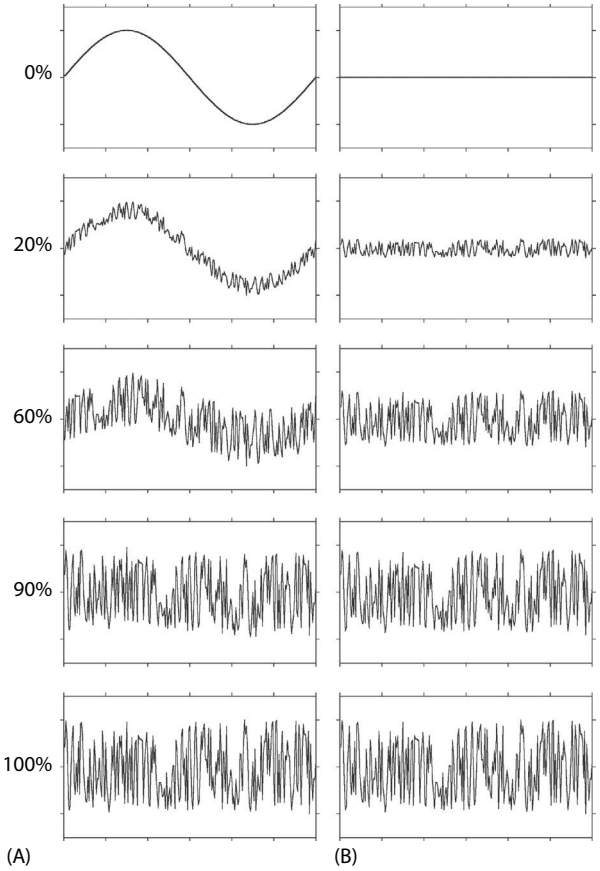


FIGURE 3.47 Statistical significance and statistical power. (A) The graphs show artificial data sets containing sine waves contaminated with variable amounts of noise. (B) The graphs show data sets containing only noise. For 0%, 20%, and 60% noise, the pairs of data sets are clearly distinct. For 90% noise, the two sets look identical, even though the one on the left has 10% signal. Ours eyes do not have the power to see the difference.

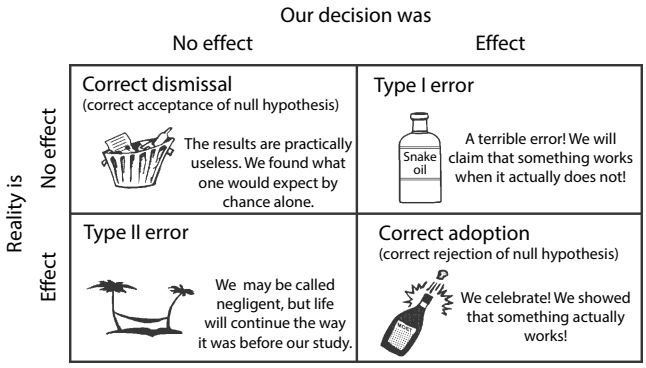


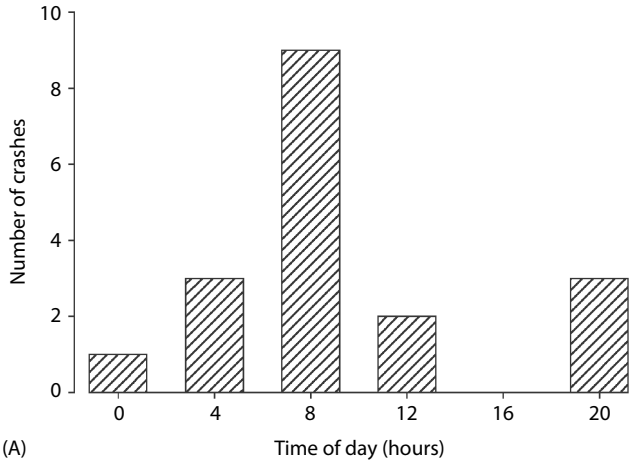
FIGURE 3.48 You can't win! One cannot increase power indefinitely (i.e., eliminate the chances of making a Type II error) without increasing the chances of making a Type I error. In scientific research, Type I errors are considered to be more abominable than Type II errors.

Decisions about the breakpoint between significance and nonsignificance are subjective, as was pointed out earlier (see Figure 3.41). The situation is complicated because hypothesis testing is a "Catch-22" situation. As diagrammed in Figure 3.48, four outcomes are possible when we make a decision to adopt or dismiss an experimental hypothesis. Two of the outcomes present no problem: it is fine to adopt a good hypothesis (i.e., to reject the null hypothesis when the null hypothesis is false), and it is fine to dismiss a bad hypothesis (i.e., to accept the null hypothesis when the null hypothesis is true). However, the other two outcomes constitute errors. We commit a Type I error when we adopt a bad hypothesis (i.e., reject the null hypothesis when the null hypothesis is true), and we commit a Type II error when we discard a good hypothesis (i.e., accept the null hypothesis when the null hypothesis is false). Scientists are usually much more worried about Type I errors than about Type II errors because they do not want to ever be perceived as charlatans trying to sell snake oil. Clinical practitioners, on the other hand, worry quite a bit about Type II errors because they do not want to be blamed for being negligent in the treatment of a patient.

To fully appreciate the relevance of the notions of Type I and Type II error, suppose that you agree to take your neighbor's young child to an amusement park on a sunny Sunday. Suppose also that, after a few hours, the two of you get separated. You start searching for the child frantically. As you look at each child who resembles your missing child, you would like, of course, to succeed as soon as possible, but it will not be tragic if you fail to identify the child right away—as you may keep looking for him or her for a while. It will be tragic, however, if you pick the wrong child and bring him or her to your neighbor's home. The moral of the story is that, if you must

err, it is better to temporarily ignore a true hypothesis than to accept a false hypothesis. A Type II error is more tolerable than a Type I error. To be fair to a clinician's viewpoint, however, I must present you a different anecdote. Suppose that you have just found out that you have a terminal illness. It is possible that you will not live long enough to come back to a cure that you previously dismissed. In this case, you would willingly take the chance of accepting a false hypothesis to ensure that you will not ignore a true hypothesis. This is the type of reasoning that led to the creation of the U.S. National Center for Complementary and Integrative Health (www.nccih.nih.gov). As I will emphasize in Chapter 16, the scientist and the clinician often see things from very different perspectives.

Unfortunately, it is impossible to reduce the probabilities of both Type I and Type II errors. If we decrease the probability of a Type I error (α), we often simultaneously increase the probability of a Type II error (β), and vice versa. There are procedures to increase the "power" of a test (1 – β) without greatly affecting α, but the possibility of an error can never be eliminated. Nonetheless, anyone would agree that an α of 1.00 is useless—if something is sure to happen by chance, it makes no sense to work for it. Thus, we must prevent the level of significance from escalating. A simple solution for the periodogram is to apply a Bonferroni correction[114] to the level of significance. If we wish to keep the *familywise* probability of a Type I error at a level λ as we perform n tests, we must set α at a lower level, so that $\lambda = 1 - (1 - \alpha)^n$. If one is seeking only one peak in the periodogram (which is usually the case when one is attempting to determine the presence of circadian rhythmicity), correction for multiple testing may be considered unnecessary. If, however, one is seeking any possible rhythmicity in a long time series, one must make corrections for multiple testing. After all, when one sets α at 0.05, one is expecting that 1 out of 20 tests will turn out positive even if the data are randomly distributed.



(A)

Time	0	4	8	12	16	20	Total
Observed	1	3	9	2	0	3	18
Expected	3	3	3	3	3	3	18
Difference (+)	2	0	6	1	3	0	12

(B)

Time	0	4	8	12	16	20	Total
Observed	3	3	3	3	3	3	18
Expected	3	3	3	3	3	3	18
Difference (+)	0	0	0	0	0	0	0

(C)

FIGURE 3.49 Rationale of the Kolmogorov–Smirnov test. The data in Panel A suggest the presence of a daily pattern in the temporal distribution of plane crashes. In Panel B, the distribution of the data is compared with a flat distribution (which is what we would expect if there were no daily rhythmicity). The sum of the absolute differences between observed and expected values is a relatively large number (12). In contrast, Panel C shows that, for a data set that is definitely not rhythmic, the sum of the absolute differences is zero. *Note*: the data shown here are fictitious.

3.3.2 Educed Time Series

In some types of studies, the nature of the data is such that quantitative time series cannot be constructed. For example, if we are interested in an individual's daily distribution of the occurrence of headaches, our time series may consist of many days with no events and a few days with isolated single events. In situations such as this one, it is sensible to give up on a thorough longitudinal analysis and, instead, try to analyze the time series as a combined circular process. The combined data set—which is often called an "educed time series" (or educed rhythm) because it is drawn out of the original series—will have the duration of a single day and will, for instance, show 1 headache at midnight, 3 headaches at 1 a.m., 2 headaches at 2 a.m., and so on. The question then will be whether there is daily rhythmicity in the educed rhythm—or, more precisely, whether the distribution of events along the day differs significantly from a flat distribution. A simple way to answer this question is to use the Kolmogorov–Smirnov test.[115] The test is simple and makes no assumptions about the nature of the data. Consider Figure 3.49. The question to be addressed is whether there is a daily pattern in the occurrence of airplane crashes. There are not enough plane crashes each day to allow the construction of a longitudinal time series. However, if observations of the time of day of plane crashes are made over several years, we can obtain an accumulated 1-day cycle as shown in Panel A of Figure 3.49. The graph suggests that more crashes happen around 8 o'clock in the morning than at other times of the day. Is the difference due to chance alone? We can compare the observed distribution of crashes with a flat distribution (which is what we would expect if there were no daily pattern). Panel B shows the observed values plotted in Panel A and the expected values (all 3s in this case). The sum of absolute differences between observed and expected values in this case is 12. In contrast, the sum is 0 if there is no temporal pattern (Panel C). So, if we had a probability density curve for the sum of absolute differences between observed and expected values, we could easily determine whether our score of 12 is very unlikely to occur by chance alone. In actuality, the Kolmogorov–Smirnov test uses a slightly different statistic (namely, the largest difference for accumulated frequencies),[115]

but its logic is the same. The program **Onecycle** (see Table 3.1) performs the Kolmogorov–Smirnov test and provides the probability associated with the computed statistic.

It is important to emphasize that the Kolmogorov–Smirnov test determines only whether the distribution of values differs from a flat distribution. As previously discussed regarding the ANOVA, a distribution that is not flat need not have a single daily peak. It might very well have five or six daily peaks and, therefore, not be any proof of circadian rhythmicity. A better alternative to the Kolmogorov–Smirnov test, if we expect the rhythmicity to have a sinusoidal form, is the Rayleigh test.[116] The Rayleigh test provides a correlation vector, nR^2, which is distributed as χ^2 and, consequently, can be easily tested for significance. The program **Rayleigh** (see Table 3.1) performs the Rayleigh test. Modifications of the test have been proposed to allow the evaluation of specific phases[117] and to construct confidence intervals.[118] A third possibility is to use the **Cosinor** program previously discussed. If the fitted cosine wave has an amplitude significantly different from zero, then the data set can be considered *not* to have a flat distribution—and, of course, if it is not flat, then it may be rhythmic. However, as pointed out by Enright,[119] one must keep in mind that the period of the rhythm is not a free parameter in the cosinor method, so that a finding of significant periodicity does not imply that the period is indeed 24.0 hours or even close to it.

An important consideration when one-cycle data sets are analyzed is whether the cycle is a one-time event or a true rhythm. Suppose that you go to a high school reunion held in a remote tropical island rarely visited by tourists. While enjoying the place, you go to the beach and write down the number of people that you see there. You find a nice daily rhythm with a peak at noon (Panel A in Figure 3.50). This is your one-cycle data set. Now, can you conclude that there is a daily rhythm of number of people at this beach? No, you cannot. Remember: this is a remote island rarely visited by tourists. Most likely, your graph would show a single event if you collected data for a whole year (Panel B). The daily cycle that you observed on that single day is an aberration and not a representative sample of a rhythmic phenomenon. This may sound obvious, but many scientific articles have been published in which the authors talk about annual rhythms of biological function after having collected data for only 1 year, or about weekly rhythms after having collected data for only 1 week, or even about daily rhythms after having collected data for only 1 day. In some cases, it may be reasonable to assume that other cycles would follow the one that was actually observed, but very often the assumption is not justified.

Short time series are also very common in molecular studies that involve the DNA microarray methodology. Usually, gene expression is tracked for a single 24-hour cycle, during which a few subjects are killed for sample collection at intervals of several hours.[120] This raises the issue of the representativeness of any rhythmicity detected in the study, but there is an even greater problem to be dealt with. The great asset of microarrays—namely, their ability to track thousands of genes (or proteins) simultaneously—is also a major liability. When one wishes to test for the presence of daily rhythmicity in 10,000 genes or more in a single study, it is virtually impossible to set a reasonable level of significance for the test of each gene. If we used the Bonferroni correction, we would need to set α at about 0.000005 in order to keep λ at 0.05, which is impractical. In one study in fruit flies, the authors evaluated 14,000 genes and found that 447 of them exhibited circadian rhythmicity. When they calculated how many genes could show rhythmic expression by chance alone, they found

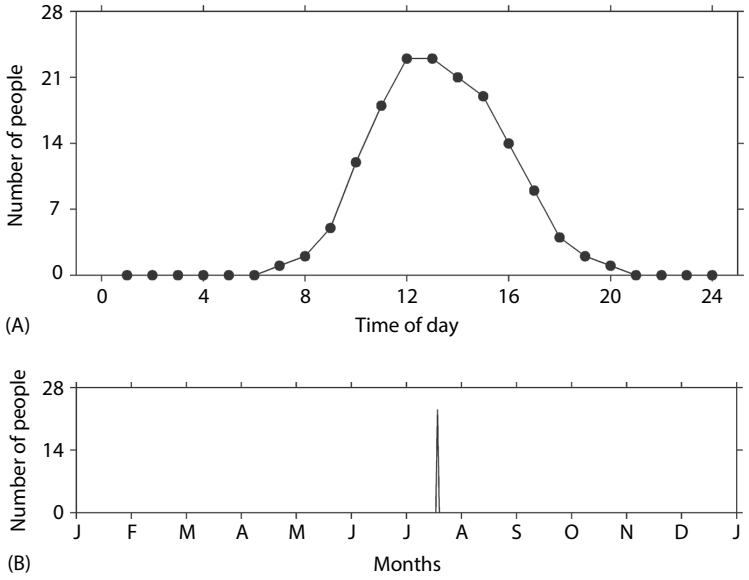


FIGURE 3.50 The issue of representativeness. The data in Panel A suggest that there is a daily pattern in the number of people that can be found at the beach. Any statistical test applied to this data set would reveal a significant effect of time of day. However, it is possible that the real pattern in the number of people that can be found at the beach is the one shown in Panel B, which contains daily rhythmicity on only 1 day out of a year. Thus, studies with data sets containing only one cycle are always suspicious.

the figure to be 298.[121] Thus, the detection of rhythmicity was an artifact in more than half (66%) of the genes initially believed to exhibit rhythmicity. Evidently, something had to be done about it. The authors of the cited study chose a more stringent criterion and ended up with 22 "legitimately" rhythmic genes. However, they themselves acknowledged that their final count was probably an underestimation.[121] By lowering α, one causes an elevation in β and, consequently, a reduction in statistical power. A possible solution is to develop a global test to precede individual comparisons, just like an ANOVA precedes the test of differences between particular groups of means. The design of "planned orthogonal contrasts" should help maintain statistical power without requiring a large reduction in the level of significance.[106,122,123]

A novel approach to multiple comparisons was introduced about 20 years ago by Benjamini and Hochberg.[124] The so-called false discovery rate (FDR) approach consists essentially of taking into consideration only those comparisons that correctly reject the null hypothesis (and, thus, reducing the number of comparisons that require Bonferroni correction by ignoring all the comparisons that fail to reject the null hypothesis). Of course, one never knows which null hypotheses are actually true, but Benjamini and Hochberg demonstrated that the FDR can be controlled if a sequence of four simple steps is followed.[41,124] The FDR can be very useful for multiple comparisons in a variety of studies of circadian rhythms. Its application specifically to the analysis of microarray data has been suggested by statisticians,[125–127] and at least one circadian microarray study has utilized it.[128] Interestingly, the authors of this study did not find the FDR to be particularly useful. As a matter of fact, their results suggested that the majority of genes is rhythmic and that statistical tests should focus on identifying the relatively few genes that are *not* rhythmic.[128] This same group proposed a new test for the detection of periodicity in microarray data based on resampling.[129] The concept of the test is interesting, but its properties must be investigated, so that one can find out whether the high detection rate is due to a reduced probability of Type II error or simply to an increased probability of Type I error. At least three other groups also proposed their own algorithms for detection of circadian rhythmicity in microarray data,[130–132] and at this time it is impossible to ascertain which algorithm is better than the others.

3.3.3 New Statistics

Null-hypothesis significance testing (NHST), as described earlier, is the standard way to certify the reliability of research results and, as such, is regularly presented in undergraduate statistics textbooks.[13–18] It is important to mention, however, that NHST has been challenged in two different fronts on and off for many years.

In one front, some researchers have pointed out that NHST is misused very often—so often, they claim, that it should be discarded and should be replaced by the reporting of confidence intervals.[133–136] I share with others the opinion that problems in significance tests derive not from the tests themselves but from a lack of appreciation for the intricacies of the procedure.[137–144] One of the objections against NHST is that it encourages the arbitrary sectioning of a natural continuum. Although sectioning at a significance point (whether we choose $p < 0.05$ or $p < 0.0001$) is indeed arbitrary, one cannot deny that sometimes it can be very helpful. For instance, it is very reasonable to argue that there is a continuum of people that goes from nasty people to nice people. For most of us, however, there is a threshold that really counts. We will not ask someone out on a date if he or she is below the arbitrary threshold that we set for ourselves. Likewise, we set a threshold for tests of significance, even though we know that there is a continuum of probabilities. Literally, only things above (or below) the threshold are *significant* to us. Thus, to accept a threshold for significance is not to make a metaphysical statement but simply to make a practical decision. As a matter of fact, the main problems attributed to NHST are the result of a user's failure to recognize that p values are relatively arbitrary choices regarding the significance level of a test and that the power of the test needs to be evaluated every time that the test fails to reject the null hypothesis.[142–144]

The other front of assault on NHST is that of statisticians who favor Bayesian inference (after Thomas Bayes, an eighteenth-century mathematician). Bayes studied "conditional probabilities": probabilities of events that are affected by other events, such as the probability of finding someone who speaks Greek given that one is in Greece as opposed to the probability of finding someone who speaks Greek given that one is in France. A theorem named after Bayes provides the means to identify the "posterior" probability of an event given its "prior" probability and a new observation. Because the goal of scientific research can be thought of as the refinement of previous knowledge by new evidence, it makes much sense to think of scientific inference in terms of Bayes' theorem.[145] Instead of having to invert our thought process (as we had to do to derive the notion of null hypothesis), Bayesians claim that we can actually associate probabilities with our original hypothesis, so that we can use the research findings to calculate the posterior probability of our theory.

Mathematicians, particularly those educated in the past 20 years or so, tend to prefer the Bayesian approach. In contrast, humans in general tend to be uncomfortable with conditional probabilities and, instead, prefer operations based on frequencies.[146] Thus, the "frequentist" approach to hypothesis testing (as Bayesians like to refer to NHST) seems to be a more "natural" approach for human beings. I believe that the NHST approach is also more consistent with scientific (research-based) reasoning, whereas the Bayesian approach is more consistent with pure mathematical reasoning, and the explanation is directly related to the timing of the inferential process. Both approaches involve exact computations and multiple relatively arbitrary assumptions, but they do it in a different sequence. Bayesian inference starts with an assumption (the necessarily arbitrary choice of a "prior") and then proceeds to an exact computation. In contrast, NHST starts with exact computations and then proceeds to make assumptions about the relevance of the numbers (as a means to avoid

Type I and Type II errors). Mathematicians seem to be comfortable making assumptions at the beginning, but scientists usually find the notion of "priors" to be unsatisfactory. If we are going to use common sense (inexact thinking), we want to do it when we interpret the meaning of a statistical test, not before we collect our data. Making assumptions before we have the empirical evidence feels like a violation of the principles of empirical research. This is probably the main reason why scientists have been reluctant to adopt Bayesian inference. Whereas there have been many proposals to discard NHST and replace it with Bayesian inference,[147–151] many researchers and statisticians have argued that NHST and Bayesian inference are both valuable and should be applied to different situations.[152–157]

With this, we conclude the discussion of procedures for the analysis of circadian rhythmicity. You learned procedures for the analysis of the mean level, amplitude, phase, period, waveform, and robustness of circadian rhythms, as well as the associated methods for statistical evaluation of significance. We can now move on, in Section II of the book, to the description of rhythmic phenomena in living organisms—or the "phenomenology" of biological rhythms.

SUMMARY

1. Data analysis involves both graphical and numerical procedures. In circadian physiology, the goal of data analysis is to characterize one or more of the six parameters of circadian rhythmicity: mean level, amplitude, phase, period, waveform, and robustness.
2. Calculation of the mean level and amplitude of a rhythm can be based directly on the raw data or on a cosine curve fitted to the data. Phase can be determined by inspection of actograms, computation of the peak of the smoothed rhythm, or identification of the acrophase of the fitted cosine curve. The period of a rhythm is most commonly determined by various methods of inspection of actograms, by Fourier analysis, by the Enright (chi-square) periodogram, by the Lomb–Scargle periodogram, or by the reiterated cosinor procedure. There is no standard procedure to quantify the waveform of circadian rhythms. Robustness, which is an index of the degree of stationarity of the rhythm, may be estimated by periodogram analysis of filtered data sets.
3. Tests of statistical significance are essential for the distinction between real rhythms and random oscillations. Generally, one can be more confident about the results of the tests if one has a long time series covering several cycles of the rhythm, but significance tests can be conducted on educed rhythms as well.

EXERCISES

3.1 Parameters of rhythmic processes. This is a simple exercise. You will run the program **Wave**. If you have not done the computer exercises in previous chapters, you should be reminded that you first need to double-click on the **Circadian** icon to open the program banner and then click on **Wave** (the 8th icon from the right) to start it. **Wave** is a short tutorial on the parameters of rhythmic processes (mean level, amplitude, phase, period, and waveform). The tutorial takes only about 6 minutes to complete, includes background music, and shuts itself down at the end. Go ahead!

3.2 Smoothing a data set. In this exercise, we will use the procedure of moving averages to smooth a data set (i.e., to filter out high-frequency oscillations). The rationale for the procedure was explained in Section 3.2.

1. Let us start by looking at a data set that contains high-frequency oscillations. Start the program **Plot,** select the Data subfolder by double-clicking on it in the *Source* panel, and select the sample data file **A07** by single-clicking on it. This file contains values of metabolic heat production (in W) of a fat-tailed gerbil collected in 6-minute intervals for 6 consecutive days.
2. The program's default values are appropriate for this data set, so you can simply click on the Cartesian plot button (the purple button) now. Browse through the whole data set (6 days) and notice that there is a clear daily rhythm with higher values during the first part of each day. However, there is also considerable *noise* (i.e., ultradian oscillations that do not seem to be regular and that obscure the daily oscillation).
3. To filter the data set, start the program **Moving** (the second icon from the left in the Circadian banner). If needed, select the Data subfolder in the *Source File* panel and then choose the file **A07**. If the box "Same as Source" is checked, the program will automatically change the information in the *Destination File* panel. For this exercise, I suggest that you don't use the default file name (**MAV-A07**); instead, use the name **B07** (i.e., delete **MAV-A07.txt** and type **B07.txt**). There is no need to change the default values for *Data Points* or *Prefilters*.
4. Because ultradian oscillations in **A07** are particularly conspicuous in the range of a few minutes, let us set the *Averaging Window* size to 24 minutes. Type in **4** (i.e., 4 bins or 24 minutes) and click on *Execute*.
5. To save a trip between programs, let us create a second filtered file before we go back to **Plot** to look at the data. Type in a new *Destination File* name (e.g., **C07**), change the *Averaging Window* to **60** (i.e., 60 bins or 6 hours), and click on *Execute*.
6. Now let us look at the results. Go back to **Plot** and load the first file that we created (**B07**, if you followed my suggestion). If the list of files in the Source panel does not show the new file, it is because the list has not been refreshed (i.e., updated). To refresh the list, just double-click on the Circadian folder and then on the Data folder again. Then load **B07**.

7. Notice that the temporal pattern is very similar to that of the original file (**A07**) but the very high-frequency oscillations have been filtered out. You may want to alternate between **A07** and **B07** a few times to observe the difference.
8. Next, let us look at the second file that we created (**C07**). Load it now. The first thing you will notice is probably the fact that the wave pattern is phase-advanced by 3 hours in comparison to **A07**. This is an artifact of the moving averages procedure, which you could easily correct by starting the procedure 3 hours later. The important thing to notice, however, is that **C07** is a much smoother data set. It has only one major peak each day. The daily pattern present in **A07** was preserved, but the ultradian oscillations were filtered out.
9. Oh, yes. You may have noticed also that, although **A07** was 6-days long, both **B07** and **C07** are only 5-days long. In actuality, **B07** is 12 minutes shorter than **A07**, whereas **C07** is 3 hours shorter than **A07**. Because **Plot** only plots full days, a whole day seems to be missing in both **B07** and **C07**.
10. Next, you can practice with other source files. Notice that you have the option to tell the program to discard data points at the beginning or end of the file (using the *Data Points* panel). You may also use the *Prefilter* panels to eliminate outliers. As a matter of fact, you can set the *Averaging Window* size to **1** (and avoid the moving averages filter) if all you want to do is to extract a section from a long data set and eliminate outliers.

3.3 Constructing an actogram. As explained in Section 3.2.2, the actogram is a classic graphic in circadian physiology. Originally used only for records of running-wheel activity, it is now used for practically any type of variable that is recorded over an extended period of time. Data sets must have equally spaced data points in order to generate a meaningful actogram. A few missing points are acceptable, but they must be filled in with a null value to preserve the temporal structure of the data set.

1. Start the program **Plot.**
2. In the *Source* panel, select the Data subfolder by double-clicking on it.
3. Select the data file **A03** by clicking on it. This file contains the records of running-wheel activity of a golden hamster maintained in constant darkness for 36 consecutive days. The number of wheel revolutions is accumulated into 6-minute bins (for a total of 8640 data points in the file). Since the data points are equally spaced, there is no need for time tags (and, accordingly, the file contains no time tags).
4. For now, leave the default values in the *Data* panel. Click on the actogram button (the green button) to display the data. You can see why golden hamsters are the preferred rodent for the study of circadian rhythms. The pattern of activity is very *clean*, with wheel-running neatly restricted to a limited portion of each day. You can also notice that the onsets of activity are neatly arranged one under the other in an almost vertical line, which indicates a free-running period very close to 24.0 hours.
5. Now select the data file **A04** by clicking on it. This file contains the records of running-wheel activity of another hamster maintained in constant darkness for 29 days.
6. Click on the actogram button to display the data. The onsets for this animal clearly deviate from a vertical line, indicating a free-running period slightly longer than 24.0 hours. We will see how to determine the exact period in future exercises. Notice that you can switch to black-and-white display (as you would see in a printed book) by clicking on the brush cup (under the display panel). Please do so now.
7. Next, select the data set **A05**. This file contains the records of body temperature of a Long–Evans rat measured by telemetry every 6 minutes for 6 weeks. A light–dark cycle was present for the first 4 weeks.
8. Click on the actogram button. What do you see? If you are in black-and-white mode, you probably see 42 horizontal straight lines. Why? Because body temperature, unlike locomotor activity, does not go down to zero during the inactive phase of the circadian cycle. By plotting every value above zero, we end up plotting every single data point. Thus, in order to have a useful actogram, we need to clip off the lower values. An arbitrary but convenient clipping level is the mean level of the rhythm. We will see later how to calculate the mean level. For now, click on the *Clip* box and type 36.2.
9. Click on the actogram button. What a difference! Now you have a very legible actogram of the rat's body temperature rhythm. You can clearly see that the animal exhibited a period of 24.0 hours during the 2 weeks under a light–dark cycle and that it freeran with a period longer than 24.0 hours when released into constant darkness.
10. Of course, you don't need to adjust the clipping level if you use different colors for different temperature values. Click on the *Clip* box, delete 36.2, and type 0. Then click on the brush cup to revert to color mode (which will cause an automatic replotting of the data). The resulting actogram is not as clear as the black-and-white version with appropriate clipping level, but it is readable.
11. Finally, select the data set **A06**. This file contains the records of locomotor activity of a pill bug (a small terrestrial crustacean) measured with an infrared photocell for 19 days in constant darkness. As usual, the data resolution is 6 minutes, and the file contains only the ordinate values.

12. Click on the actogram button. You can see why pill bugs are *not* the preferred species in circadian physiology. The records are much *noisier* than those of the hamsters or of the rat. Nonetheless, you will notice that the animal freeruns with a period much shorter than 24.0 hours.

3.4 Determining circadian period by modulo changes in an actogram. Several exercises in Chapters 4 and 5 will deal with the numerical computation of the period of a rhythm. In this exercise, a simple graphical procedure will be demonstrated.

1. Start the program **Plot**.
2. In the *Source* panel, select the Data subfolder by double-clicking on it.
3. Select the data file **A06** by clicking on it. As mentioned in the preceding exercise, this file contains the records of locomotor activity of a pill bug measured with an infrared photocell for 19 days in constant darkness. As usual, the data resolution is 6 minutes, and the file contains no time tags.
4. Click on the actogram button (the green button). Although the records are noisy, it is evident that the circadian period is shorter than 24.0 hours. We know that it is shorter than 24.0 hours because the daily onsets do not align along a vertical line. But what if a day were shorter than 24.0 hours? If the day were as short as the circadian period, the onsets would align along a vertical line. Thus, if we can find out the day length that causes the onsets to align along a vertical line, we will know what the circadian period is.
5. Click on the down arrow by the text "Modulo 24 h" several times. As the plot modulo (day length) shortens, the onsets move toward a vertical line. When you reach 23.3 hours, they are almost perfectly aligned. Thus, the free-running period of this animal is 23.3 hours! (*Note*: The *modulo* operator requires two integers. In computer programs, 23.3 hours are internally processed as 233 integer units.)
6. Now, select the data file **A17**. This data set is more of a challenge. It consists of body temperature measurements of a tree shrew (a primitive primate) conducted every 6 minutes over 7 days under constant light. If you maintain the color display mode, the fact that body temperature never goes down to zero will not be a problem. However, the brevity of the data set (only 7 days) may make it difficult to align the daily "onsets."
7. Click on the actogram button. The plot modulo is automatically returned to 24.0 hours, so the actogram should be sloped to the left (i.e., the period is shorter than 24.0 hours). Adjust the modulo to determine the exact period.
8. If you deemed the period to be 23.8 hours, you were correct. Next, select the data file **A08**. This file contains artificial data constructed as a series of cosine waves with a period of 23.5 hours. Click on the *Clip* box, delete **0**, and type **5** (which is the mean level of the rhythm). Then click on the actogram button and observe the smooth actogram.
9. Now, select the data file **A09**. This is also an artificial file, but 60% of the data points in the preceding file were replaced with random noise in the range of oscillation. Click on the actogram button and observe the noisier but still clear rhythm.
10. Next, select the data file **A10**. This file contains 85% noise. Click on the actogram button and observe the unintelligible actogram. The period is still 23.5 hours, but there is so much noise that you cannot see it. You need a better method to calculate circadian period when the data are noisy (which is very common in mammals with partial suprachiasmatic lesions, for instance). Also, of course, it would be helpful to have a method that calculates circadian period without requiring a human observer—not only to make the experimenter's life easier but also to avoid observer's bias. The exercises in Chapters 4 and 5 will deal with this issue.

SUGGESTIONS FOR FURTHER READING

For more detailed information about topics covered in this chapter, the source articles listed in the "References" section may be consulted. For more general further reading, the following sources may be useful:

Hurlburt, R. T. (2012). *Comprehending Behavioral Statistics*, 5th edn. Dubuque, IA: Kendall Hunt.

An excellent introductory statistics textbook covering descriptive statistics and inferential statistics up to standard analysis of variance. Hurlburt takes an intuitive approach that is missing in most other books. Although the book is nominally targeted at behavioral scientists, the contents are actually general enough to apply to any field of research.

Howell, D. C. (2013). *Statistical Methods for Psychology*, 8th edn. Belmont, CA: Wadsworth.

A wonderful upper-level undergraduate statistics textbook covering the basics of inferential statistics in regression and analysis of variance. The book covers all the material covered in introductory books plus a good deal of advanced topics.

Chatfield, C. (2004). *The Analysis of Time Series: An Introduction*, 6th edn. Boca Raton, FL: CRC Press.

A good introduction to the analysis of time series. The book is intended as an undergraduate textbook and, although it does not specifically address the analysis of circadian rhythms, it provides broad coverage of both theory and practice in time series analysis. *Warning*: despite the author's clear style, the book requires considerable background in college-level statistics and calculus.

Shumway, R. H. and Stoffer, D. S. (2011). *Time Series Analysis and Its Applications*, 3rd edn. New York: Springer.

An advanced textbook on time series analysis concentrating on autoregression and spectral analysis. Similar to Chatfield's book, this book does not specifically address the analysis of circadian rhythms but provides a solid background in time series analysis for readers well trained in statistics and calculus. This third edition provides R code for numerical examples.

Box, G. E. P., Jenkins, G. M., and Reinsel, G. C. (2008). *Time Series Analysis: Forecasting and Control*, 4th edn. New York: Wiley, 2008.

This is a classic book, first published in 1970. Box and Jenkins created the autoregressive model that bears their name. As the title indicates, the goal is forecasting and control, not the characterization of rhythmic patterns, but many readers may find it very useful.

Grinnell, F. (1992). *The Scientific Attitude*, 2nd edn. New York: Guilford Press, 1992.

This eye-opening book is not about mathematical data analysis per se but about the politics of scientific research that can affect the collection and interpretation of research results. Short and well written, this book should be required reading for anyone interested in scientific research.

WEBSITES TO EXPLORE

American Statistical Association: http://www.amstat.org.
OpenStat Free Statistics Program: http://statpages.info/miller/OpenStatMain.htm.
SAS Institute Inc.: http://www.sas.com.
SPSS Inc.: http://www.spss.com.
StatSoft Inc.: http://www.statsoft.com/.
Wavelet Tutorial: http://engineering.rowan.edu/~polikar/WAVELETS/WTtutorial.html.

REFERENCES

1. Brockwell, P. J. and Davis, R. A. (2002). *Introduction to Time Series and Forecasting*, 2nd edn. New York: Springer.
2. Chatfield, C. (2004). *The Analysis of Time Series: An Introduction*, 6th edn. Boca Raton, FL: Chapman & Hall.
3. Hamilton, J. D. (1994). *Time Series Analysis*. Princeton, NJ: Princeton University Press.
4. Shumway, R. H. and Stoffer, D. S. (2011). *Time Series Analysis and Its Implications*, 3rd edn. New York: Springer.
5. Percival, D. B. and Walden, A. T. (2000). *Wavelet Methods for Time Series Analysis*. New York: Cambridge University Press.
6. Madsen, H. (2008). *Time Series Analysis*. Boca Raton, FL: Chapman & Hall.
7. Douc, R., Moulines, E., and Stoffer, D. (2014). *Nonlinear Time Series: Theory, Methods and Applications with R Examples*. Boca Raton, FL: CRC Press.
8. Halberg, F. (1992). Glossary and abbreviations. In: Halberg, F. and Watanabe, H. (Eds.), *Chronobiology and Chronomedicine*. Tokyo, Japan: Medical Review, pp. 5–8.
9. Cornélissen, G., Halberg, F., Schwartzkopff, O., Delmore, P., Katinas, G., Hunter, D., Tarquini, B. et al. (1999). Chronomes, time structures, for chronobioengineering for "a full life". *Biomedical Instrumentation and Technology* 33: 152–187.
10. Refinetti, R. (2005). Time for sex: Nycthemeral distribution of human sexual behavior. *Journal of Circadian Rhythms* 3: art. 4.
11. Levine, J. D., Funes, P., Dowse, H. B., and Hall, J. C. (2002). Signal analysis of behavioral and molecular cycles. *BMC Neuroscience* 3: art. 1.
12. Brown-Brandl, T. M., Yanagi, T., Xin, H., Gates, R. S., Bucklin, R. A., and Ross, G. S. (2003). A new telemetry system for measuring core body temperature in livestock and poultry. *Applied Engineering in Agriculture* 19: 583–589.
13. Hurlburt, R. T. (2012). *Comprehending Behavioral Statistics*, 5th edn. Dubuque, IA: Kendall Hunt.
14. Sprinthall, R. C. (2011). *Basic Statistical Analysis*, 9th edn. Boston, MA: Allyn & Bacon.
15. Moore, D. S., Notz, W. I., and Fligner, M. A. (2013). *The Basic Practice of Statistics*, 6th edn. New York: Freeman.
16. Weiss, N. A. (2011). *Elementary Statistics*, 8th edn. Reading, MA: Addison-Wesley.
17. Aron, A., Coups, E. J., and Aron, E. N. (2013). *Statistics for Psychology*, 6th edn. Upper Saddle River, NJ: Pearson.
18. Pagano, R. R. (2013). *Understanding Statistics in the Behavioral Sciences*, 10th edn. Belmont, CA: Wadsworth.
19. Nelson, W., Tong, Y. L., Lee, J. K., and Halberg, F. (1979). Methods for cosinor rhythmometry. *Chronobiologia* 6: 305–323.
20. Wang, Y. and Brown, M. B. (1996). A flexible model for human circadian rhythms. *Biometrics* 52: 588–596.
21. Ruf, T. (1996). The baseline cosinus function: A periodic regression model for biological rhythms. *Biological Rhythm Research* 27: 153–165.
22. Almirall, H. (1997). Modeling the body temperature throughout the day with a two-term function. *Behavior Research Methods, Instruments, and Computers* 29: 595–599.
23. Alonso, I. and Fernández, J. R. (2001). Nonlinear estimation and statistical testing of periods in nonsinusoidal longitudinal time series with unequidistant observations. *Chronobiology International* 18: 285–308.
24. Wang, Y., Ke, C., and Brown, M. B. (2003). Shape-invariant modeling of circadian rhythms with random effects and smoothing spline ANOVA decompositions. *Biometrics* 59: 804–812.
25. Albert, P. S. and Hunsberger, S. (2005). On analyzing circadian rhythms data using nonlinear mixed models with harmonic terms. *Biometrics* 61: 1115–1122.
26. Akaike, H. (1974). A new look at the statistical model identification. *IEEE Transactions on Automatic Control* AC-19: 716–723.
27. Halberg, F., Cornélissen, G., Katinas, G., Syutkina, E. V., Sothern, R. B., Zaslavskaya, R., Halberg, F. et al. (2003). Transdisciplinary unifying implications of circadian findings in the 1950s. *Journal of Circadian Rhythms* 1: art. 2.
28. Refinetti, R. (1992). Analysis of the circadian rhythm of body temperature. *Behavior Research Methods, Instruments, and Computers* 24: 28–36.
29. Bingham, C., Arbogast, B., Guillaume, G. C., Lee, J. K., and Halberg, F. (1982). Inferential statistical methods for estimating and comparing cosinor parameters. *Chronobiologia* 9: 397–439.
30. Johnson, M. S. (1926). Activity and distribution of certain wild mice in relation to biotic communities. *Journal of Mammalogy* 7: 245–277.
31. Schmid, B., Helfrich-Förster, C., and Yoshii, T. (2011). A new ImageJ plug-in "ActogramJ" for chronobiological analyses. *Journal of Biological Rhythms* 26: 464–467.
32. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology* 106: 253–266.

33. Nelson, D. E. and Takahashi, J. S. (1991). Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *Journal of Physiology* 439: 115–145.
34. Grosse, J., Loudon, A. S. I., and Hastings, M. H. (1995). Behavioural and cellular responses to light of the circadian system of *tau* mutant and wild-type Syrian hamsters. *Neuroscience* 65: 587–597.
35. Sharma, V. K. and Chandrashekaran, M. K. (1999). Precision of a mammalian circadian clock. *Naturwissenschaften* 86: 333–335.
36. Elliott, J. A. and Tarmarkin, L. (1994). Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. *Journal of Comparative Physiology A* 174: 469–484.
37. Meijer, J. H. and De Vries, M. J. (1995). Light-induced phase shifts in onset and offset of running-wheel activity in the Syrian hamster. *Journal of Biological Rhythms* 10: 4–16.
38. Vansteensel, M. J., Deboer, T., Dahan, A., and Meijer, J. H. (2003). Differential responses of circadian activity onset and offset following GABA-ergic and opioid receptor activation. *Journal of Biological Rhythms* 18: 297–306.
39. Refinetti, R. (1992). Non-parametric procedures for the determination of phase markers of circadian rhythms. *International Journal of Biomedical Computing* 30: 49–56.
40. Rosi, F., Greppi, G., Corino, C., Schoen, F., and Solca, F. (1981). An introduction to the study of biological rhythms. *Rivista di Biologia* 74: 155–190.
41. Refinetti, R., Cornélissen, G., and Halberg, F. (2007). Procedures for numerical analysis of circadian rhythms. *Biological Rhythm Research* 38: 275–325.
42. Akashi, M., Soma, H., Yamamoto, T., Tsugitomi, A., Yamashita, S., Yamamoto, T., Nishida, E., Yasuda, A., Liao, J. K., and Node, K. (2010). Noninvasive method for assessing the human circadian clock using hair follicle cells. *Proceedings of the National Academy of Sciences of the United States of America* 107: 15643–15648.
43. Tong, Y. L., Nelson, W. L., Sothern, R. B., and Halberg, F. (1977). Estimation of the orthophase (timing of high values) on a non-sinusoidal rhythm. In: Halberg, F. (Ed.), *Proceedings of the XII International Conference of the International Society for Chronobiology*. Milan, Italy: Il Ponte, pp. 765–769.
44. Klerman, E. B., Lee, Y., Czeisler, C. A., and Kronauer, R. E. (1999). Linear demasking techniques are unreliable for estimating the circadian phase of ambulatory temperature data. *Journal of Biological Rhythms* 14: 260–274.
45. Klerman, E. B., Gershengorn, H. B., Duffy, J. F., and Kronauer, R. E. (2002). Comparisons of the variability of three markers of the human circadian pacemaker. *Journal of Biological Rhythms* 17: 181–193.
46. Daan, S. and Oklejewicz, M. (2003). The precision of circadian clocks: Assessment and analysis in Syrian hamsters. *Chronobiology International* 20: 209–221.
47. Kolodyazhniy, V., Späti, J., Frey, S., Götz, T., Wirz-Justice, A., Kräuchi, K., Cajochen, C., and Wilhelm, F. H. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. *Journal of Biological Rhythms* 26: 55–67.
48. Walker, J. S. (1988). *Fourier Analysis*. New York: Oxford University Press.
49. Körner, T. W. (1988). *Fourier Analysis*. New York: Cambridge University Press.
50. Walker, J. S. (1996). *Fast Fourier Transforms*, 2nd edn. Boca Raton, FL: CRC Press.
51. Bloomfield, P. (2000). *Fourier Analysis of Time Series: An Introduction*, 2nd edn. New York: Wiley.
52. Stade, E. (2005). *Fourier Analysis*. New York: Wiley.
53. Enright, J. T. (1981). Data analysis. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 21–39.
54. Refinetti, R. (1993). Comparison of six methods for the determination of the period of circadian rhythms. *Physiology and Behavior* 54: 869–875.
55. Eijzenbach, V., Sneek, J. H., and Borst, C. (1986). Arterial pressure and heart period in the conscious rabbit: Diurnal rhythm and influence of activity. *Clinical and Experimental Pharmacology and Physiology* 13: 585–592.
56. Lumineau, S., Guyomarc'h, C., Boswell, T., Richard, J. P., and Leray, D. (1998). Induction of circadian rhythm of feeding activity by testosterone implantations in arrhythmic Japanese quail males. *Journal of Biological Rhythms* 13: 278–287.
57. Mormont, M. C., Waterhouse, J., Bleuzen, P., Giacchetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset, J. L., Touitou, Y., and Lévi, F. (2000). Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clinical Cancer Research* 6: 3038–3045.
58. Hassnaoui, M., Pupier, R., and Rehailia, M. (2000). A concordance method for analyzing categorical time series: An application for the search of periodicities. *Biological Rhythm Research* 31: 177–201.
59. Albers, H. E., Gerall, A. A., and Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
60. Rao, A. V. and Sharma, V. K. (2002). A simple approach for the computation of multiple periodicities in biological time series. *Biological Rhythm Research* 33: 487–502.
61. Diambra, L., Lopes, J. R., Menna-Barreto, L., and Rigolino, R. (2002). Ciclograma: A tool for detection of rhythmicities in sleep/wake cycles. *Chronobiology International* 19: 793–803.
62. Klemfuss, H. and Clopton, P. L. (1993). Seeking tau: A comparison of six methods. *Journal of Interdisciplinary Cycle Research* 24: 1–16.
63. Refinetti, R. (1991). An extremely simple procedure for the analysis of circadian and estrous periodicity. *Physiology and Behavior* 50: 655–659.
64. Kanjilal, P. P., Bhattacharya, J., and Saha, G. (1999). Robust method for periodicity detection and characterization of irregular cyclical series in terms of embedded periodic components. *Physical Review E* 59: 4013–4025.
65. Dowse, H. B. (2013). Maximum entropy spectral analysis for circadian rhythms: Theory, history and practice. *Journal of Circadian Rhythms* 11: art. 6.
66. Ruf, T. (1999). The Lomb-Scargle periodogram in biological rhythm research: Analysis of incomplete and unequally spaced time-series. *Biological Rhythm Research* 30: 178–201.
67. Enright, J. T. (1965). The search for rhythmicity in biological time-series. *Journal of Theoretical Biology* 8: 426–468.
68. Dörrscheidt, G. L. and Beck, L. (1975). Advanced methods for evaluating characteristic parameters of circadian rhythms. *Journal of Mathematical Biology* 2: 107–121.
69. Sokolove, P. G. and Bushell, W. N. (1978). The chi square periodogram: Its utility for analysis of circadian rhythms. *Journal of Theoretical Biology* 72: 131–160.
70. Vogelbaum, M. A. and Menaker, M. (1992). Temporal chimeras produced by hypothalamic transplants. *Journal of Neuroscience* 12: 3619–3627.
71. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.

72. Lovegrove, B. G., Heldmaier, G., and Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
73. Wollnik, F. and Schmidt, B. (1995). Seasonal and daily rhythms of body temperature in the European hamster (*Cricetus cricetus*) under semi-natural conditions. *Journal of Comparative Physiology B* 165: 171–182.
74. Sollars, P. J., Kimble, D. P., and Pickard, G. E. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *Journal of Neuroscience* 15: 2109–2122.
75. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
76. Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. *Journal of Comparative Physiology A* 179: 135–142.
77. Hut, R. A., Mrosovsky, N., and Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
78. Earnest, D. J., Liang, F. Q., Ratcliff, M., and Cassone, V. M. (1999). Immortal time: Circadian clock properties of rat suprachiasmatic cell lines. *Science* 283: 693–695.
79. Bertolucci, C., Sovrano, V. A., Magnone, M. C., and Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
80. Shimizu, I., Kawai, Y., Taniguchi, M., and Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
81. Canal-Corretger, M. M., Vilaplana, J., Cambras, T., and Díez-Noguera, A. (2001). Functioning of the rat circadian system is modified by light applied in critical postnatal days. *American Journal of Physiology* 280: R1023–R1030.
82. Chen, W. M. and Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
83. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
84. Wee, R., Castrucci, A. M., Provencio, I., Gan, L., and Van Gelder, R. N. (2002). Loss of photic entrainment and altered free-running circadian rhythms in *math5*$^{-/-}$ mice. *Journal of Neuroscience* 22: 10427–10433.
85. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., and Tomioka, K. (2004). *Drosophila cryb* mutation reveals two circadian clocks that drive locomotor rhythm and have different responsiveness to light. *Journal of Insect Physiology* 50: 479–488.
86. Granados-Fuentes, D., Prolo, L. M., Abraham, U., and Herzog, E. D. (2004). The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *Journal of Neuroscience* 24: 615–619.
87. Lomb, N. R. (1976). Least-squares frequency analysis of unequally spaced data. *Astrophysics and Space Science* 39: 447–462.
88. Schimmel, M. (2001). Emphasizing difficulties in the detection of rhythms with Lomb-Scargle periodograms. *Biological Rhythm Research* 32: 341–345.
89. Van Dongen, H. P. A., Olofsen, E., Van Hartevelt, J. H., and Kruyt, E. W. (1999). Searching for biological rhythms: Peak detection in the periodogram of unequally spaced data. *Journal of Biological Rhythms* 14: 617–620.
90. Schuster, A. (1898). On the investigation of hidden periodicities with application to a supposed 26 day period of metereological phenomena. *Terrestrial Magnetism* 3: 13–41.
91. Koehler, F., Okano, F. K., Elveback, L. R., Halberg, F., and Bittner, J. J. (1956). Periodograms for the study of physiologic daily periodicity in mice and man. *Environmental Medicine and Surgery* 14: 5–30.
92. De Prins, J. and Waldura, J. (1993). Sightseeing around the single cosinor. *Chronobiology International* 10: 395–400.
93. Refinetti, R., Kaufman, C. M., and Menaker, M. (1994). Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters. *Journal of Comparative Physiology A* 175: 223–232.
94. Weinert, D., Fritzche, P., and Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
95. Cheng, P., Yang, Y., and Liu, Y. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 98: 7408–7413.
96. Gonze, D., Halloy, J., and Gldbeter, A. (2002). Robustness of circadian rhythms with respect to molecular noise. *Proceedings of the National Academy of Sciences of the United States of America* 99: 673–678.
97. Refinetti, R. (2004). Non-stationary time series and the robustness of circadian rhythms. *Journal of Theoretical Biology* 227: 571–581.
98. Leise, T. L. and Harrington, M. E. (2011). Wavelet-based time series analysis of circadian rhythms. *Journal of Biological Rhythms* 26: 454–463.
99. Eastman, C. and Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
100. Raser, J. M. and O'Shea, E. K. (2005). Noise in gene expression: Origins, consequences, and control. *Science* 309: 2010–2013.
101. Casdagli, M. (1991). Chaos and deterministic *versus* stochastic non-linear modelling. *Journal of the Royal Statistical Society B* 54: 303–328.
102. Stark, J. and Hardy, K. (2003). Chaos: Useful at last? *Science* 301: 1192–1193.
103. Fisher, R. (1955). Statistical methods and scientific induction. *Journal of the Royal Statistical Society B* 17: 69–78.
104. McPherson, G. (1990). *Statistics in Scientific Investigation*. New York: Springer.
105. Kanji, G. K. (2006). *100 Statistical Tests*, 3rd edn. London, U.K.: Sage.
106. Howell, D. C. (2013). *Statistical Methods for Psychology*, 8th edn. Belmont, CA: Wadsworth.
107. Efron, B. and Tibshirani, R. (1991). Statistical data analysis in the computer age. *Science* 253: 390–395.
108. Hall, P. (1992). *The Bootstrap and Edgeworth Expansion*. New York: Springer.
109. Efron, B. and Tibshirani, R. J. (1993). *An Introduction to the Bootstrap*. Boca Raton, FL: Chapman & Hall.
110. Simon, J. L. (1995). *Resampling: The New Statistics*. Arlington, VA: Resampling Stats.

111. Refinetti, R. (1996). Demonstrating the consequences of violations of assumptions in between-subjects analysis of variance. *Teaching of Psychology* 23: 51–54.
112. Fisher, R. A. (1929). Tests of significance in harmonic analysis. *Proceedings of the Royal Society of London A* 125: 54–59.
113. Siegel, A. F. (1980). Testing for periodicity in a time series. *Journal of the American Statistical Association* 75: 345–348.
114. Krauth, J. (1988). *Distribution-Free Statistics: An Application-Oriented Approach*. New York: Elsevier.
115. Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.
116. Mardia, K. V. (1972). *Statistics of Directional Data*. New York: Academic Press.
117. Proschan, M. A. and Follmann, D. A. (1997). A restricted test of circadian rhythm. *Journal of the American Statistical Association* 92: 717–724.
118. Brazier, K. T. S. (1994). Confidence intervals from the Rayleigh test. *Monthly Notices of the Royal Astronomical Society* 268: 709–712.
119. Enright, J. T. (1989). The parallactic view, statistical testing, and circular reasoning. *Journal of Biological Rhythms* 4: 295–304.
120. Langmead, C. J., Yan, A. K., McClung, C. R., and Donald, B. R. (2003). Phase-independent rhythmic analysis of genome-wide expression patterns. *Journal of Computational Biology* 10: 521–536.
121. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N., and Taghert, P. H. (2002). Influence of the *period*-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* 99: 9562–9567.
122. Kirk, R. E. (1995). *Experimental Design: Procedures for the Behavioral Sciences*, 3rd edn. Pacific Grove, CA: Brooks/Cole.
123. Maxwell, S. E. and Delaney, H. D. (2004). *Designing Experiments and Analyzing Data*, 2nd edn. Mahwah, NJ: Lawrence Erlbaum Associates.
124. Benjamini, Y. and Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B* 57: 289–300.
125. Reiner, A., Yekutieli, D., and Benjamini, Y. (2003). Identifying differentially expressed genes using false discovery rate controlling procedures. *Bioinformatics* 19: 368–375.
126. Storey, J. D. and Tibshirani, R. (2003). Statistical significance for genomewide studies. *Proceedings of the National Academy of Sciences of the United States of America* 100: 9440–9445.
127. Taylor, J., Tibshirani, R., and Efron, B. (2005). The 'miss rate' for the analysis of gene expression data. *Biostatistics* 6: 111–117.
128. Ptitsyn, A. A., Zvonic, S., Conrad, S. A., Scott, L. K., Mynatt, R. L., and Gimble, J. M. (2006). Circadian clocks are resounding in peripheral tissues. *PLoS Computational Biology* 2(3): e16.
129. Ptitsyn, A. A., Zvonic, S., and Gimble, J. M. (2006). Permutation test for periodicity in short time series data. *BMC Bioinformatics* 7: art. S10.
130. Chudova, D., Ihler, A., Lin, K. K., Andersen, B., and Smyth, P. (2009). Bayesian detection of non-sinusoidal periodic patterns in circadian expression data. *Bioinformatics* 25: 3114–3120.
131. Hughes, M. E., Hogenesch, J. B., and Kornacker, K. (2010). JTK_CYCLE: An efficient nonparametric algorithm for detecting rhythmic components in genome-scale data sets. *Journal of Biological Rhythms* 25: 372–380.
132. Thaben, P. F. and Westermark, P. O. (2014). Detecting rhythms in time series with RAIN. *Journal of Biological Rhythms* 29: 391–400.
133. Bakan, D. (1966). The test of significance in psychological research. *Psychological Bulletin* 66: 423–437.
134. Fidler, F., Thomason, N., Cumming, G., Finch, S., and Leeman, J. (2004). Editors can lead researchers to confidence intervals, but can't make them think. *Psychological Science* 15: 119–126.
135. Cumming, G. (2008). Replication and *p* intervals: *p* values predict the future only vaguely, but confidence intervals do much better. *Perspectives on Psychological Science* 3: 286–300.
136. Halsey, L. G., Curran-Everett, D., Vowler, S. L., and Drummond, G. B. (2015). The fickle *P* value generates irreproducible results. *Nature Methods* 12: 179–185.
137. Rosnow, R. L. and Rosenthal, R. (1989). Statistical procedures and the justification of knowledge in psychological science. *American Psychologist* 44: 1276–1284.
138. Zuckerman, M., Hodgins, H. S., Zuckerman, A., and Rosenthal, R. (1993). Contemporary issues in the analysis of data: A survey of 551 psychologists. *Psychological Science* 4: 49–53.
139. Estes, W. K. (1997). Significance testing in psychological research: Some persisting issues. *Psychological Science* 8: 18–20.
140. Altman, D. G. (2002). Poor-quality medical research: What can journals do? *Journal of the American Medical Association* 287: 2765–2767.
141. Hurlbert, S. H. and Lombardi, C. M. (2009). Final collapse of the Neyman-Pearson decision theoretic framework and the rise of the neoFisherian. *Annales Zoologici Fennici* 46: 311–349.
142. Baker, L. F. and Mudge, J. F. (2012). Making statistical significance more significant. *Significance* 9: 29–30.
143. Morey, R. D., Rouder, J. N., Verhagen, J., and Wagenmakers, E. J. (2014). Why hypothesis tests are essential for psychological science: A comment on Cumming (2014). *Psychological Science* 25: 1289–1290.
144. Lakens, D. and Evers, E. R. K. (2014). Sailing from the seas of chaos into the corridor of stability: Practical recommendations to increase the informational value of studies. *Perspectives on Psychological Science* 9: 278–292.
145. Lee, P. M. (2004). *Bayesian Statistics: An Introduction*, 3rd edn. London, U.K.: Wiley.
146. Sedlmeier, P. and Gigerenzer, G. (2001). Teaching Bayesian reasoning in less than two hours. *Journal of Experimental Psychology: General* 130: 380–400.
147. Goodman, S. N. (2001). Of p-values and Bayes: A modest proposal. *Epidemiology* 12: 295–297.
148. Greenland, S. (2008). Bayesian interpretation and analysis of research results. *Seminars in Hematology* 45: 141–149.
149. Dienes, Z. (2011). Bayesian versus orthodox statistics: Which side are you on? *Perspectives on Psychological Science* 6: 274–290.
150. Wetzels, R., Matzke, D., Lee, M. D., Rouder, J. N., Iverson, G. J., and Wagenmakers, E. J. (2011). Statistical evidence in experimental psychology: An empirical comparison using 855 *t* tests. *Perspectives on Psychological Science* 6: 291–298.
151. Kruschke, J. K. (2011). Bayesian assessment of null values via parameter estimation and model comparison. *Perspectives on Psychological Science* 6: 299–312.

152. Bland, J. M. and Altman, D. G. (1998). Bayesians and frequentists. *BMJ* 317: 1151.
153. Bayarri, M. J. and Berger, J. O. (2004). The interplay of Bayesian and frequentist analysis. *Statistical Science* 19: 58–80.
154. Norton, J. D. (2010). There are no universal rules for induction. *Philosophy of Science* 77: 765–777.
155. Refinetti, R. (2011). Bayesian mistake. *Significance* 8: 188.
156. Fraser, D. A. S. (2013). Bayes' confidence. *Science* 341: 1452.
157. Efron, B. (2013). Bayes' theorem in the 21st century. *Science* 340: 1177–1178.

Section II

Phenomenology



A herd of St. Croix sheep going about its daily business.
(Image courtesy of the Agricultural Research Service
of the U.S. Department of Agriculture, Washington, DC.)

This page intentionally left blank

4 Ultradian and Infradian Rhythms

4.1 ENVIRONMENTAL RHYTHMS

Although most of us are not aware of them, environmental rhythms abound on Earth. As exemplified in Table 4.1, there are rhythmic oscillations in our environment that range in period from a few femtoseconds (10^{-15} seconds) to tens of thousands of years. Some of the rhythms are man made, such as 50 or 60 Hz alternated electric current and the 7-day week, whereas others are created by the Earth, the Moon, and the Sun. For instance, ocean tides are caused primarily by the Moon's gravitational attraction and secondarily by the Sun's gravitational attraction.[1] With a few exceptions, the interval between tides corresponds to half a "lunar day"—or 12 hours and 25 minutes (Figure 4.1).

The longest cycles listed in Table 4.1 refer to variations in Earth's orbital parameters.[2] The path of the Earth' revolution around the Sun changes from circular to elliptical over thousands of years, and this variation has exhibited a period of approximately 96,000 years during the last 5 million years (the *orbital eccentricity* rhythm). Also, the Earth's axis of rotation is tilted, and the angle of inclination oscillates with a period of 41,000 years (the *obliquity* rhythm). Furthermore, regardless of the long-term angle of inclination, there is a wobble in the axis of rotation that completes a cycle every 22,000 years (the *precession of the equinoxes* rhythm). These three orbital rhythms have effects on Earth's climate, as suggested in the 1940s by the Croatian mathematician Milutin Milankovich.[3–5] An example of the effect of the orbital eccentricity rhythm on sea surface temperature (SST) can be seen in Figure 4.2. During the last 340,000 years, mean SST of the Pacific Ocean has oscillated between 6°C and 16°C (between 43°F and 61°F) with veiled but noticeable peaks at intervals of approximately 96,000 years (indicated by the vertical arrows). Although not evident by visual inspection, obliquity and precession components can also be identified by spectral analysis in these[6] and other records.[7–9]

If you observed Figure 4.2 carefully, you may have been surprised to notice that ocean temperature has been falling for the past 10,000 years. The surprise was probably due to the fact that you have heard that *global warming* may become a major threat to humanity in the twenty-first century.[10] Is the Earth getting warmer or colder? The answer depends on what temporal perspective you take. In the perspective of the eccentricity rhythm, global temperature is going down, and should fall some 6°C in the next 40,000 years. What is referred to as *global warming* can only be seen in a very narrow temporal window: in the last half-century, the Earth's temperature has risen approximately 0.8°C above the expected level, most likely because of *anthropogenic forcing* (i.e., because of man-made alterations in the atmosphere, such as the increase in carbon dioxide emissions, which create a "greenhouse" effect).[11–19] Although a rise of 0.8°C is not very impressive in comparison to the expected fall of 6°C, the speeds of the two changes are dramatically different. At a rate of 0.8°C per 50 years, a rise of 640°C would be expected in 40,000 years. All life forms on Earth would be dead much before that temperature is reached! So, global temperature has gone down some 4°C in the last 10,000 years, and the 0.8°C rise in the last 50 years does not change the fact that the Earth is following a long-term cooling trend. However, if the short-term rising trend continues, it can easily revert the long-term cooling trend, and life on Earth can indeed be threatened by global warming before the end of the twenty-first century.[10] Migratory patterns of several species of birds have apparently been already affected by global warming,[20,21] and much greater effects on all life forms can be predicted if the warming trend continues.[22] Scientists have been discussing how animals could cope with global warming.[23–25]

Besides the long climatic rhythms caused by variations in orbital parameters, environmental rhythms have been described with periods of a few thousand years,[26,27] a few decades,[28,29] or just a few years. Among the environmental cycles in the latter group are the El Niño Southern Oscillation, which recurs in mild form each year and more strongly in intervals of 2–10 years,[9,30,31] and the 11-year cycle of magnetic fields in sunspots.[32] An 11-year oscillation in the number of sunspots has been known for centuries. Records for the past three centuries are shown in Figure 4.3.

A few years before his death, Halberg claimed to have detected a 7-day cycle in geomagnetic disturbances,[33] although the data presented in the report were anything but convincing. If such a cycle does exist, and if it is not man made, it will provide the first evidence of a putative physical correlate for the week. Unlike the day (derived from the Earth's rotation), the month (derived from the Moon's revolution around the Earth), and the year (derived from the Earth's revolution around the Sun), the week is considered to be an arbitrary human convention without a physical correlate.[34]

Most, if not all, of the environmental rhythms mentioned earlier can affect living organisms and, consequently, can create "biological rhythms" of some sort. However, they cannot possibly induce or reinforce biological rhythmicity in individual organisms whose lives are much shorter than the period of the environmental rhythm. Of all the environmental rhythms on Earth, only those in four temporal domains have been shown to have specific effects on endogenous rhythms of individual organisms (Figure 4.4). As will be discussed

TABLE 4.1
Some Environmental Cycles on Earth[a]

Period	Phenomenon
2×10^{-15} seconds	Oscillation of electromagnetic waves in visible light
2×10^{-2} seconds	Voltage oscillation in alternated current (home electricity)
60 minutes	Sound of bells ringing from the clock tower (day ÷ 24)
12.4 hours	Tides (attractive forces of Sun and Moon)
24 hours	Days (Earth's rotation)
1 week	Work–rest cycle in most of civilized world (day × 7)
30 days	Months (Moon's revolution around the Earth)
365 days	Years (Earth's revolution around the Sun)
11 years	Sunspot cycle (on the Sun, observable from Earth)
22,000 years	Precession of the equinoxes
41,000 years	Variation in Earth's obliquity (axial tilt)
96,000 years	Variation in Earth's orbital eccentricity

[a] This is an illustrative, not exhaustive, list. Some of the environmental cycles, such as those of alternated current, bell rings, and the week, are man made.

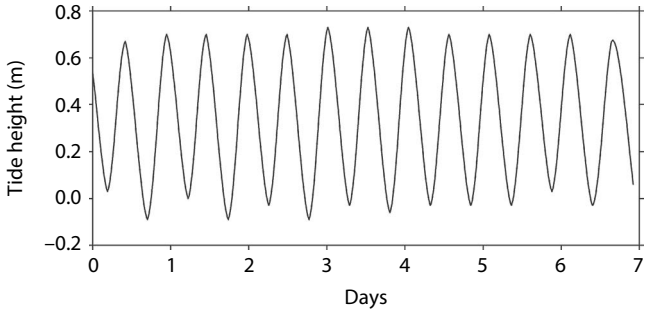


FIGURE 4.1 Going with the tide. The graph shows the variations of tide height at Biscayne Bay (Miami, Florida) during the week of August 8, 2002. The mean time difference between successive high tides is 12.4 hours (12 hours and 25 minutes). (Data courtesy of Tide High and Low, Inc., www.saltwatertides.com.)

later in this chapter, *tidal* cycles (with period of 12.4 hours) are capable of synchronizing *circatidal* rhythms, *daily* cycles (with period of 24 hours) are capable of synchronizing *circadian* rhythms, *lunar* cycles (with period of 29.5 days) are capable of synchronizing *circalunar* rhythms, and *annual* cycles (with period of 1 year) are capable of synchronizing *circannual* rhythms.[35]

As mentioned earlier, *tidal cycles* are caused primarily by regular variations in the Moon's gravitational attraction.[1] As the Earth rotates on its axis each day, a high-water bulge is created under the Moon and another bulge on the opposite side of Earth. This should yield two high tides each day, except that the *lunar day* (the time of the rotation of the Earth with respect to the Moon) is actually 24.8 hours long, so that the interval between two consecutive high tides is 12.4 hours. The Sun also attracts the Earth, so that, when the lunar and solar forces coincide (about twice a month), the tidal variation is greater, thus yielding the so-called *spring tides*. Spring tides

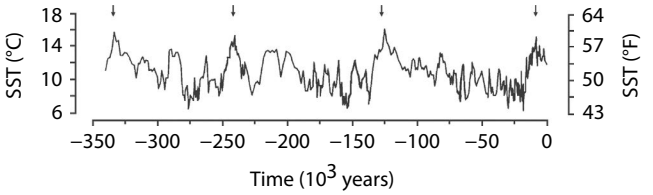


FIGURE 4.2 A long temperature record. The graph shows the SST of the southwest Pacific Ocean during the last 340,000 years, as estimated by the magnesium–calcium ratio in foraminiferal shells. Celsius scale is on the left axis; Fahrenheit scale, on the right axis. Arrows point to temperature peaks with a period of approximately 96,000 years. (Data from Pahnke, S. et al., *Science*, 301, 948, 2003.)

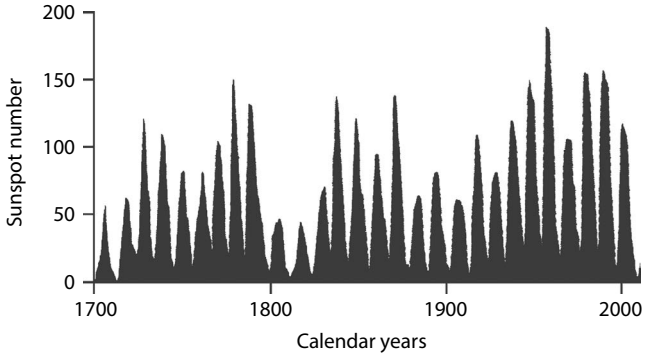


FIGURE 4.3 Sunspot cycle. The number of spots on the Sun oscillates in an 11-year cycle. (Data from Solanki, S.K. and Krivova, N.A., *Science*, 334, 916, 2011.)

are one manifestation of *lunar cycles*, which refer to events related to the revolution of the Moon around the Earth (which takes 29.5 days). Another lunar cycle is the oscillation of nighttime luminosity associated with the phases of the Moon—that is, from almost invisible starlight (0.001 lux) at new moon to maximal nighttime light at full moon (0.1 lux).[36] *Daily cycles* will be discussed in Chapter 5, and the next three paragraphs will deal with *annual cycles*.

Except for people living in the Equator, the experience of annual environmental cycles is a normal part of life. The difference between summer and winter, for example, is as clear as the difference between a green lawn and a snow blanket (Figure 4.5). In the northern hemisphere, the average daily temperature is low in the beginning of the year, rises for 6 months, and then falls again by the end of the year—and this cycle is repeated year after year (Figure 4.6). The alternation of the seasons is due to the Earth's revolution around the Sun (which takes 365.2 days), but the revolution itself is not the sole explanation. The primary cause of the seasons is the tilt of Earth's rotational axis (*obliquity*).[37–39] As the Earth goes around its orbit, it sometimes points toward the Sun and sometimes away from the Sun (Figure 4.7). In the northern hemisphere, summer is experienced when the Earth points toward the Sun, so that the Sun rises higher in the sky and is above the horizon longer; at the same time of the year, the southern hemisphere experiences winter. When the Earth

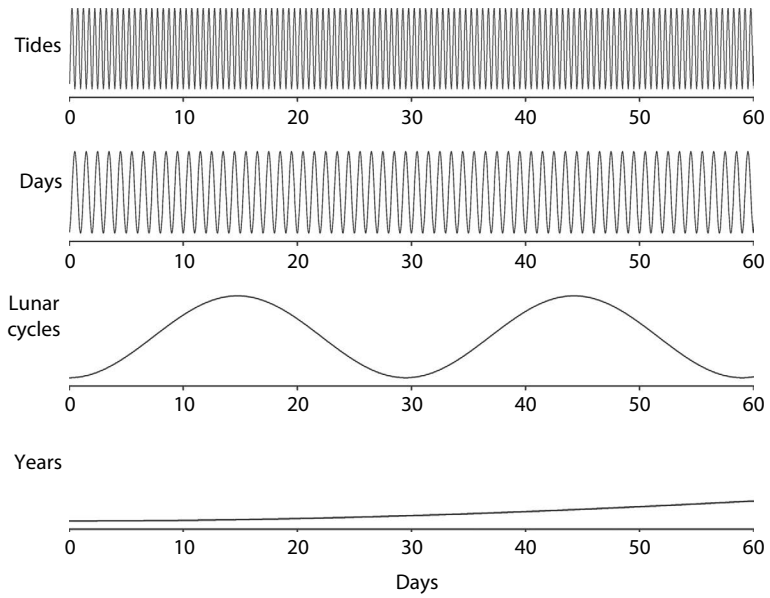


FIGURE 4.4 Clocks that time us. These diagrams provide a visual comparison of the four temporal domains of environmental cycles known to affect the internal clocks of living beings.



FIGURE 4.5 A house in Williamsburg, Virginia. Photographs of the same house taken in the summer and in the winter readily convey the significance of seasonal weather changes. (Photo by R. Refinetti.)

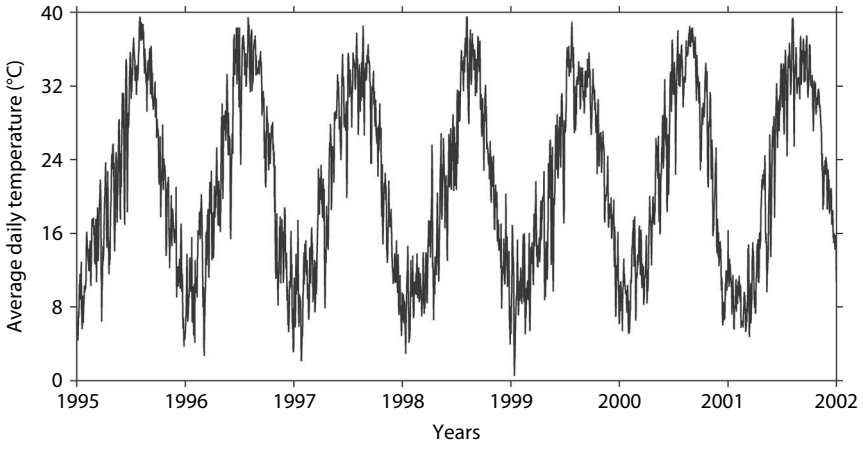


FIGURE 4.6 Hot and cold in Las Vegas. The graph shows the average daily temperature in Las Vegas, Nevada, for 7 consecutive years. Annual rhythmicity is evident. (Data courtesy of Average Daily Temperature Archive, University of Dayton, Dayton, OH.)

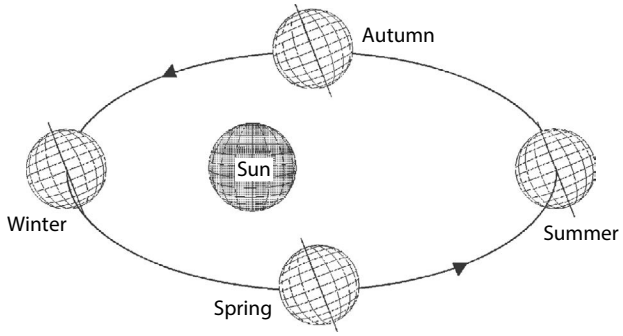


FIGURE 4.7 The Earth, the Sun, and the seasons. The primary cause of the seasons is the tilting of the Earth's rotational axis, which affects the flow of solar energy to each hemisphere as the Earth revolves around the Sun. The designations of summer and winter in this figure apply to the northern hemisphere. When it is summer in the northern hemisphere, it is winter in the southern hemisphere, and vice versa.

points away from the Sun, the northern hemisphere experiences winter and the southern hemisphere experiences summer. The fact that the Sun stays above the horizon longer in the summer explains why the days are longer. For example, at 40° North, the days have 15 hours of sunlight in the summer but only 9 hours of sunlight in the winter. The explanation of the seasonal variation in temperature is a little more complicated, as illustrated in Figure 4.8. Because of the tilt of Earth's axis, the same quantity of sunlight (solar radiation) is spread out over a wider area in the winter ((a) in Panel A) then during the summer ((b) in Panel B). Consequently, less heat per unit area is received in the winter than in the summer and, therefore, the temperatures are lower. Of course, regions close to the Equator are not affected by the Earth's tilt, and this is why there is very little seasonal variation in tropical zones.

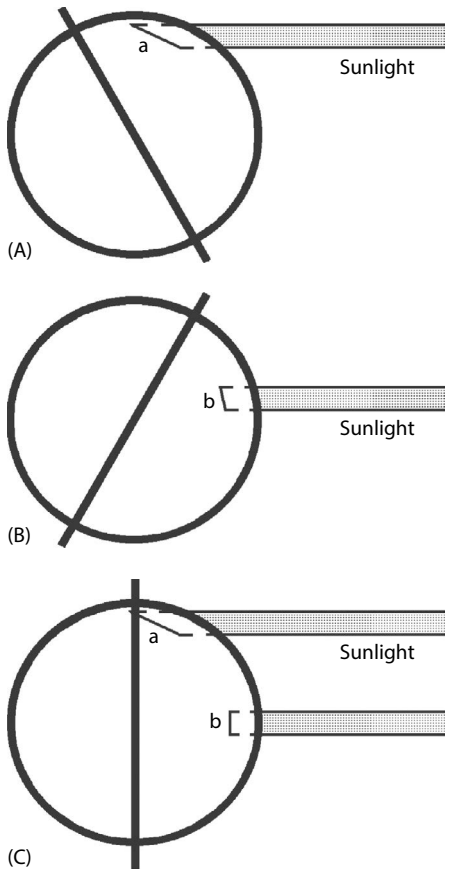


FIGURE 4.8 The deal about obliquity. Because the Earth's rotational axis is tilted (by 23.4°), the flux of solar energy is spread out over a wider surface during the winter (A) than during the summer (B). Latitudinal differences in irradiance, on the other hand, are due to Earth's sphericity and would exist regardless of obliquity (C).

I should note that there is a strong effect of latitude on climate, but that this is a result of Earth's spherical shape, not of its tilt. As shown in Panel C of Figure 4.8, the northern and southern regions would receive less radiation per unit area than the equatorial region even if there were no tilt. This is why the North Pole is cold even in the middle of the summer. I should also note that the Earth's tilt causes the northern hemisphere to be slightly farther from the Sun in the winter and slightly closer to the Sun in the summer. However, this variation in distance from the Sun is so small as to be negligible. Actually, even the much greater variation in distance from the Sun caused by the Earth's elliptic orbit has little effect on climate. This is necessarily true if you think that, when it is winter in the northern hemisphere, it is summer in the southern hemisphere. Obviously, half of the world cannot be far away from the Sun while the other half is close to it. As a matter of fact, look again at Figure 4.7; notice that, in the northern hemisphere, the Earth is closest to the Sun during the winter and farthest during the summer. This may seem counterintuitive, but keep in mind that the average distance between the Earth and the Sun is 150 million km (93 million miles), so that variations of even several thousand kilometers are negligible.

Although most of us characterize the seasons mainly on the basis of temperature—that is, winter is cold and summer is hot—day length and precipitation also follow an annual cycle. The wave form of the precipitation cycle may be somewhat irregular, but day length and temperature have sinusoidal wave forms when averaged over many years (Figure 4.9). It should be noticed, however, that the day-length curve is smoother than the temperature curve. As a matter of fact, the variation in day length progresses smoothly even on a day-to-day basis. Temperature, on the other hand, may show considerable day-to-day variation. For instance, Figure 4.10 shows the curves for average highs and record highs in New York City. Although both curves rise in the summer, the record highs are obvious deviations from the average highs. Whereas the average high in January is 3°C (i.e., barely above freezing), the record high is 20°C (i.e., a typical spring day in the middle of the winter). Therefore, day length is more reliable than temperature as an indicator of the time of year, even though temperature may have a stronger impact on the lives of organisms. Accordingly, we will see later on in this chapter that many organisms use day length, not temperature, as a seasonal clock to help them time their biological rhythms.

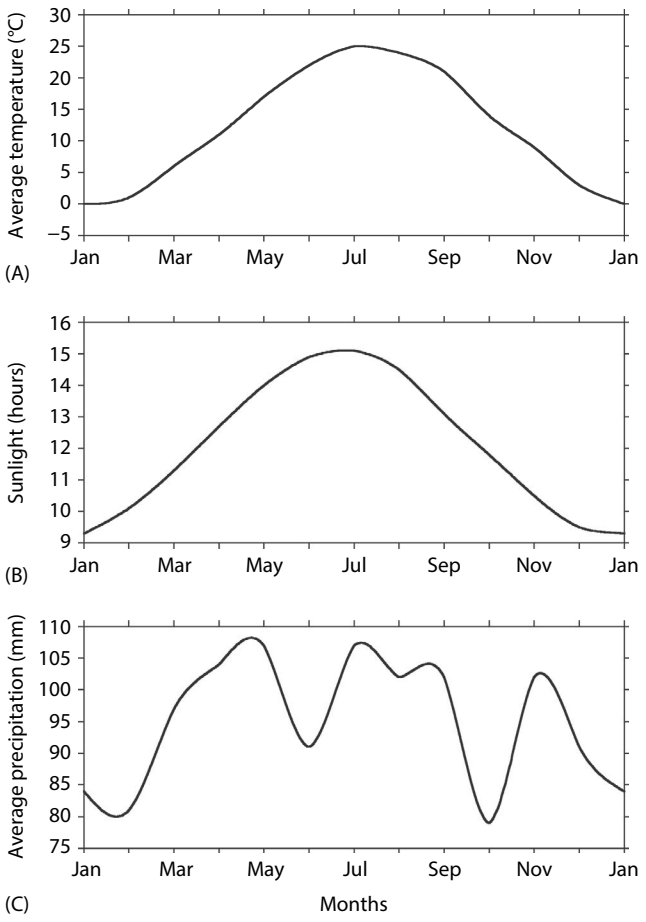


FIGURE 4.9 The weather in New York. The graphs show the seasonal variations in (A) ambient temperature, (B) day length, and (C) precipitation in New York City averaged over the last 30 years. Temperature and day length exhibit a sine wave–like oscillatory pattern, whereas the oscillatory pattern of precipitation is more complex. (Data courtesy of The Weather Channel, www.weatherchannel.com; Tide High and Low, Inc., www.saltwatertides.com.)

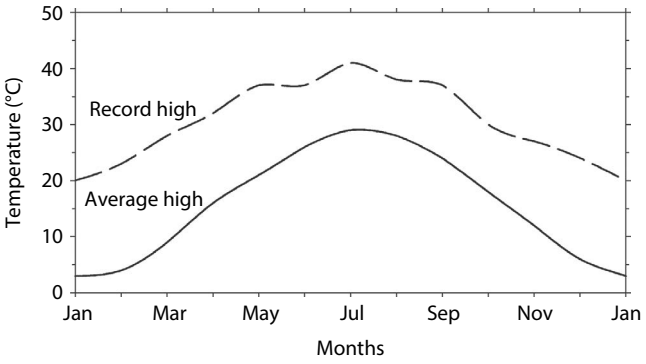


FIGURE 4.10 How hot is New York? Although the average high temperatures in New York City (averaged over 30 years) display a smooth seasonal pattern of oscillation, there is considerable day-to-day variation. For instance, although the average high in January is 3°C (i.e., barely above freezing), the record high was 20°C (in 1967). (Data courtesy of The Weather Channel, www.weatherchannel.com.)

4.2 ULTRADIAN RHYTHMS

Perhaps not surprisingly, circadian physiologists think of biological rhythms either as *circadian rhythms* or as *everything else*. In terms of frequency of oscillation, all rhythms below circadian rhythms are called *infradian* rhythms, whereas all rhythms above circadian rhythms are called *ultradian* rhythms (Figure 4.11). Although there are no strictly defined boundaries, the designation *circadian* is usually reserved for biological oscillations between 10 and 14 μHz (i.e., between 10×10^{-6} and 14×10^{-6} cycles per second). Of course, most biological rhythms are not usually described in terms of frequency of oscillation but in terms of its reciprocal, *period*. Thus, the designation *circadian* is usually reserved for biological rhythms with *periods* between 19 and 28 hours. Notice, however, that the prefixes *infra* and *ultra* become counterintuitive when period is used: *infradian* rhythms have periods *longer* than circadian rhythms, whereas *ultradian* rhythms have periods *shorter* than circadian rhythms. In this section of the chapter, we will discuss *ultradian* rhythms (i.e., high-frequency biological oscillations). Infradian rhythms will be discussed in Sections 4.3 and 4.4, and circadian rhythms in Chapter 5. Although ultradian and infradian rhythms are not, by definition, circadian rhythms (and, therefore, are not strictly in the domain of circadian physiology), it is important to discuss them because they are an integral part of the temporal organization of physiological function.[40,41]

4.2.1 Cardiac and Respiratory Rhythms

Many life forms have vascular systems that facilitate the transport of solutes and cells from one part of the body to another, but only *animals* have elaborate circulatory systems composed of blood vessels and a pumping heart.[42] In vertebrate animals, which have a well-defined heart and a closed circulatory system, the frequency of heart beating (or *heart rate*) is inversely proportional to body size,[43,44] so that a rat's heart at rest beats more than 300 times per minute, whereas a horse's heart beats fewer than 50 times per minute (Figure 4.12). The wave form of the cardiac rhythm depends

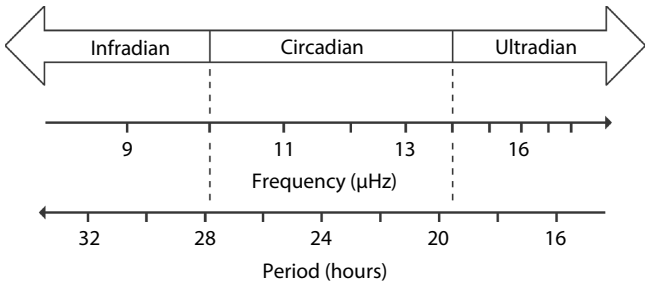


FIGURE 4.11 A self-centered worldview. For circadian physiologists, a biological rhythm is either circadian or "everything else." Oscillatory processes with frequencies below the circadian range are called *infradian*; those above the circadian range are called *ultradian*. Notice that period is the inverse of frequency, so that infradian rhythms have periods longer than circadian rhythms, and ultradian rhythms have periods shorter than circadian rhythms.

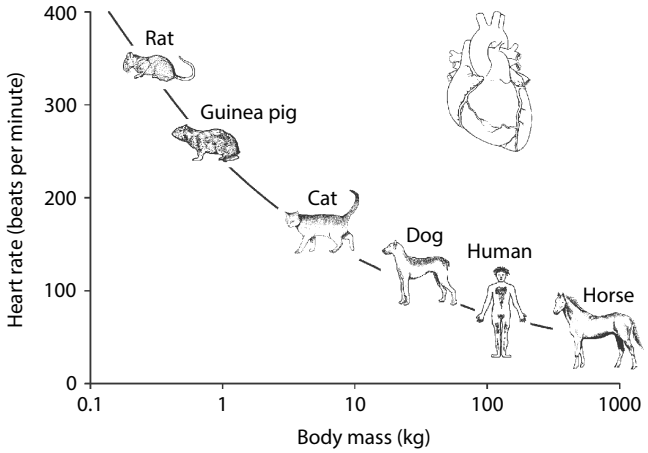


FIGURE 4.12 Heart rate and body size. In mammals, heart rate is a power function of body size. Smaller animals have higher heart rates than larger animals. (Data from Stahl, W.R., *J. Appl. Physiol.*, 22, 453, 1967.)

on the species studied and on the arrangement of electrodes used to record the muscular activity, but a diagram of a typical human *electrocardiogram* is shown in Figure 4.13. A pattern of positive and negative deflections (called the P, Q, R, S, T, and U waves) can be seen.[45] The pattern repeats itself a little more often than once per second, thus yielding a typical human heart rate of 70 beats per minute.

It has been known for many decades that the periodicity of heart beating is driven by a pacemaker located in the *sinoatrial node* of the heart and modulated by the sympathetic and parasympathetic nervous systems.[46–48] Figure 4.14 indicates the location of the sinoatrial node. Individual cells in the sinoatrial node possess pacemaker function, and much has been learned about their operation in recent years.[49,50] Although modulation of heart rate by the autonomic nervous system is ordinarily involuntary, it can be altered by operant conditioning (the so-called *biofeedback* procedure in behavioral medicine).[51–53]

Whereas respiration in most life forms relies on passive diffusion of gases through the integument or through a system of internal air-filled tubes (such as the "tracheal" system of insects), vertebrate animals use gills or lungs to actively extract oxygen from the environment and to excrete carbon dioxide.[44] In vertebrates, the breathing rate—like the heart rate—is inversely proportional to the body size,[43,44] so that a rat at rest breathes more than 70 times per minute, whereas a horse breathes fewer than 10 times per minute (Figure 4.15). In the subgroup of mammals, the flow of air through the lungs is achieved through the concerted action of many muscles, including the diaphragm, which acts like a pump moving up and down within the rib cage, several accessory muscles in the chest and abdomen, and upper-airway muscles in the larynx and pharynx.[54] Contraction of these muscles is coordinated at both the central and peripheral levels, but the rhythmic pattern seems to be generated by conglomerates of respiratory premotor neurons in the lower brainstem (Figure 4.16). The pre-Bötzinger complex (pre-BötC) seems to be the site of the respiratory pacemaker,[55,56] but various groups of cells in the ventral medulla that exhibit preinspiratory activity (pre-I) may also play an essential role.[57] Although individual pre-BötC cells may have pacemaker properties—as individual sinoatrial cardiac cells do—this issue is still under debate.[58] The alternative is that rhythmicity emerges from the interaction of many cells that are not individually rhythmic.

4.2.2 Neuroendocrine Rhythms

The fact that nerve cells in the central nervous system exhibit "spontaneous activity" has been known since the first electrophysiological recordings of single-cell activity in the cerebral cortex.[59,60] Nerve cells in the central nervous system usually have a resting firing rate of a few impulses per second, which means that they exhibit rhythmic behavior with a period of a few tenths of a second.[61] Even before the activity of individual nerve cells could be measured, a rhythmic pattern of brain activity had already been observed. In 1929, Hans Berger published the results of his pioneering work on the human *electroencephalogram* (EEG), a measure of brain activity obtained through electrodes placed on the skull.[62] The brain waves measured in the EEG exhibit a rhythmic pattern with frequencies between about 0.5 and 30 Hz depending on the subject's state of awareness.[63,64] Later studies on changes in brain waves during sleep revealed yet another dimension of ultradian oscillation, as shown in Figure 4.17. Notice the alternation of sleep stages of a typical human adult over the course of a night. Nocturnal sleep is not a constant state of low brain activity. Instead, stages of light and deep sleep alternate in cycles of about 90 minutes throughout the night.

Endocrine glands also exhibit ultradian rhythmicity. Hormones involved in reproduction (such as luteinizing hormone and follicle-stimulating hormone), as well as other hormones such as cortisol and insulin, are secreted rhythmically in intervals of

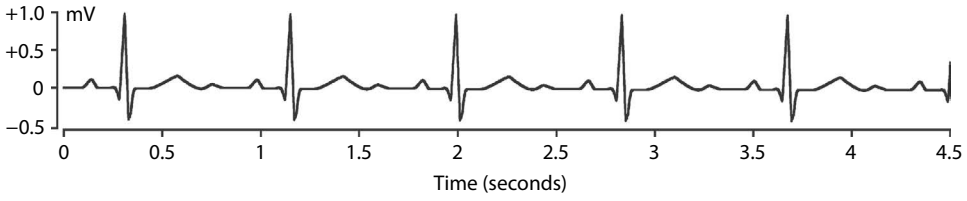


FIGURE 4.13 Cardiac rhythm. Shown is a diagram of a typical human electrocardiogram. The same pattern repeats itself approximately every second (70 beats per minute at rest). The exact configuration of the waves depends on the electrode locations used for the recording. (Adapted from Ganong, W.F., *Review of Medical Physiology*, 20th edn., Lange Medical, New York, 2001.)

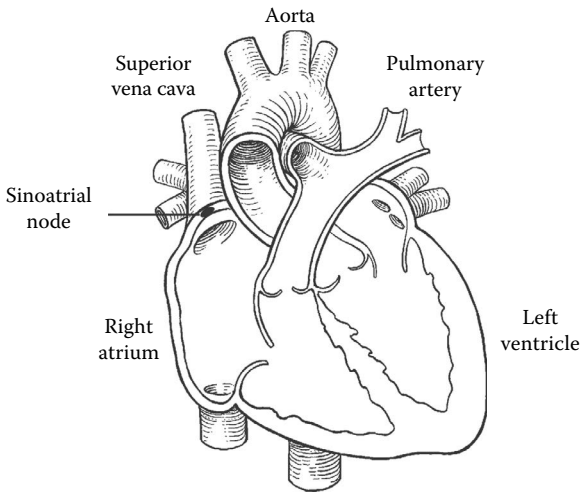


FIGURE 4.14 The cardiac clock. This diagram shows the location of the cardiac pacemaker in the sinoatrial node of the heart. (Adapted from *Medical Illustration Library*, Williams & Wilkins, Baltimore, MD, 1994.)

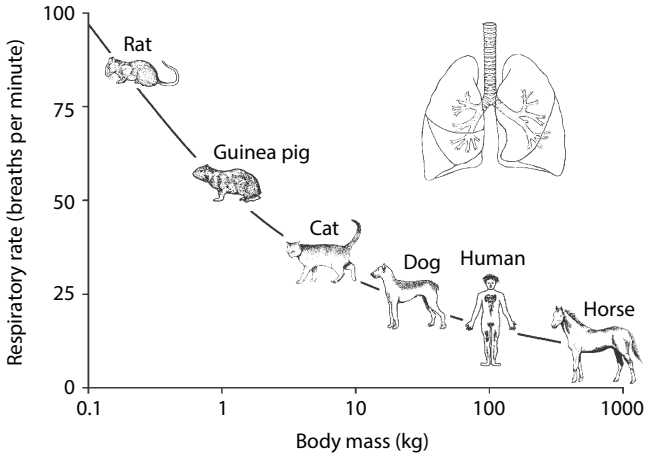


FIGURE 4.15 Respiratory rate and body size. In mammals, the respiratory rate is a power function of body size. Smaller animals breathe more frequently than larger animals. Humans breathe approximately 18 times per minute at rest. (Data from Stahl, W.R., *J. Appl. Physiol.*, 22, 453, 1967.)

approximately one hour.[65–69] Because many endocrine glands are part of a loop that involves also the pituitary gland and the hypothalamus, it is often difficult to determine where the signal for pulsatile secretion is initiated. In the particular case of the reproductive system, gonadotropin-releasing hormone (GnRH, also called luteinizing-hormone-releasing hormone or LHRH) is secreted by the hypothalamus and stimulates the anterior pituitary gland to secrete luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which on their turn stimulate the gonads to secrete estrogens, progestins, and androgens.[45] Hypothalamic neurons that secrete GnRH have been studied *in vitro* and have been shown to possess the intrinsic ability to generate pulsatile secretion, although the pacemaker mechanism may not reside in the secretory neurons themselves.[70]

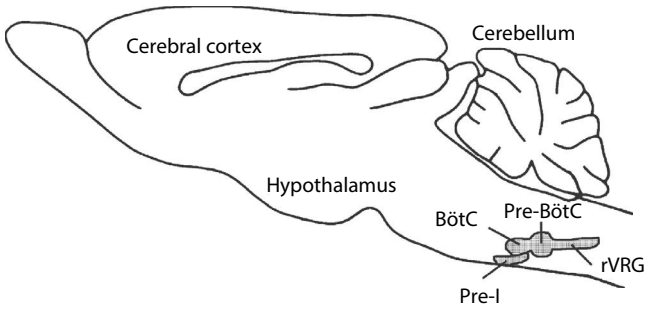


FIGURE 4.16 The respiratory clock. This diagram of the rodent brain shows the location of conglomerates of respiratory bulbospinal premotor neurons (i.e., neurons that control nerves responsible for breathing movements). *Abbreviations*: BötC, Bötzinger complex; Pre-BötC, pre-Bötzinger complex; Pre-I, neurons with preinspiratory discharge patterns; rVRG, rostral ventral respiratory group. (Adapted from Feldman, J.L. et al., *Annu. Rev. Neurosci.*, 26, 239, 2003.)

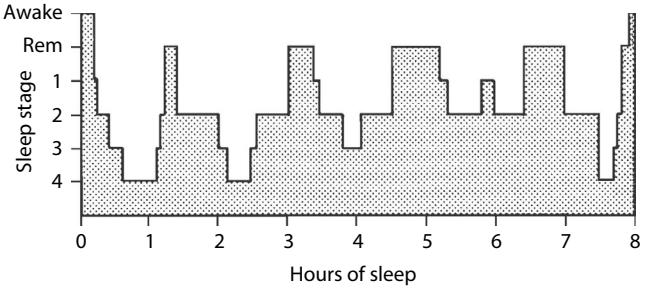


FIGURE 4.17 The cycles of sleep. Shown is the variation of sleep stages (as determined by the pattern of brain waves in the EEG) of a typical human adult over the course of a night. Stages of light and deep sleep alternate with a period of approximately 90 minutes. (Adapted from Shepherd, G.M., *Neurobiology*, 3rd edn., Oxford University Press, New York, 1994.)

4.2.3 Tidal Rhythms

Whereas most life forms inhabit either the ocean or the land, many organisms live in the interface between ocean and land and, therefore, are affected by the cycle of tides (Figure 4.18). Because tides have existed for millions of years, some species have evolved endogenous circatidal rhythmicity as an adaptive mechanism to react in advance to the regular environmental changes produced by the tides. There is still debate about whether there is a specific circatidal clock distinct from the circadian clock (or whether a single clock serves both functions), but it is well established that many intertidal organisms have the ability to generate circatidal rhythms endogenously.[71] Two recent studies on crickets and marine crustaceans have provided very strong evidence in support of the existence of a circatidal clock that is separate from the circadian clock.[72,73]

Figure 4.19 shows the number of fiddler crabs (*Uca uruguayensis*) found on the sand surface of a beach during an interval of slightly more than a day. Two and a half high-tides occurred during this time, and it can be clearly seen that the crabs came out from their burrows to the surface only during



FIGURE 4.18 Living at the beach. Many organisms live by the sea and are affected by the alternation of the tides. (Copyright ArtToday, Tucson, AZ.)

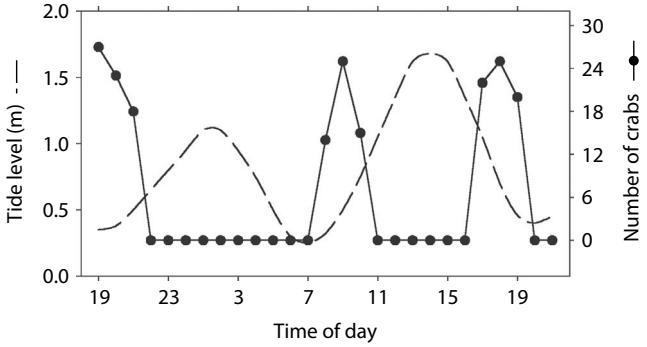


FIGURE 4.19 Showing up at low tide. The graph shows tide level and the number of fiddler crabs (*Uca uruguayensis*) found on the sand surface (i.e., not in burrows) during a 24-hour interval. The crabs clearly come to the surface only at low tide. (Data from de la Iglesia, H.O. et al., *Physiol. Behav.*, 55, 913, 1994.)

low tides. That is, the crabs exhibited a tidal rhythm of burrowing. Not shown in the figure is the fact that the crabs came to the surface only if the low tides occurred during daytime; at nighttime, they stayed in the burrows regardless of the tide level.[74] This means that the tidal rhythm is *gated* by the daily rhythm of light and darkness. Laboratory tests in constant environmental conditions indicated that this particular species of crabs does *not* have an endogenous circatidal clock and simply responds to the ebb and flow of tidal waters.[74]

Gating of tidal rhythms by the daily cycle of light and darkness has been shown in other intertidal organisms,[75–79] and lack of endogenous rhythmicity was observed in a different crab species.[80] More importantly, the existence of endogenous circatidal rhythms has been demonstrated in a variety of organisms, including algae, crustaceans, fishes, and reptiles.[73,75,77,79,81–85] A very elegant laboratory study by Tadashi Akiyama (in Japan) demonstrated the synchronization of free-running circatidal rhythms by 12.5-hour cycles of hydrostatic pressure in zooplankton.[78] Synchronization of endogenous rhythms by environmental cycles must occur in order for an organism to use its internal clock to anticipate changes in the environment. An endogenous clock that cannot be reset by environmental stimuli will freerun indefinitely and will be of very little use for an organism living in a natural environment.

It has been claimed that the mustard plant (*Arabidopsis thaliana*) exhibits tidal rhythmicity in root growth,[86] but the claim is unjustified. The authors monitored root growth in plants maintained under dim constant light and found a mean period of 24.8 hours. Because 24.8 equals 12.4 × 2, the authors inferred that the plants were drawn by the tidal acceleration generated by the orbital motion of the Moon.[86] As will be discussed in Chapters 5 and 6, however, 24.8 hours is well within the circadian range, so that the 24.8-hour rhythm of root growth was much more likely the expression of the well-known circadian rhythmicity of the mustard plant[87] and had nothing to do with the lunar cycle.

4.2.4 Other Ultradian Rhythms

Rhythmic contractions of the smooth muscles of the gastrointestinal tract (stomach and intestines), which occur at the rate of 3–10 cycles per minute, are well known.[88] Several studies have identified ultradian oscillations in various other organs and processes, although replications in multiple laboratories are needed to confirm the findings. One curious finding was that human subjects locked alone in a small room for 5 hours in the afternoon seem to exhibit a rhythmic pattern of impatience that is reflected in bursts of locomotor activity (Figure 4.20). The period of this ultradian rhythm ranges from 0.5 to 2.5 hours in different subjects but seems to be fairly consistent for each individual.[89] An ultradian pattern of activity with a period of 1–2 hours was also noticed in the mobility records of individuals moving between different rooms in their homes.[90] In fiddler crabs (*Uca pugilator*) foraging at the beach, a 21-minute cycle of feeding and retreating to the burrow was observed.[91] Short cycles of feeding (lasting about 30 minutes) were also observed in quail (*Coturnix coturnix*), golden hamsters (*Mesocricetus auratus*), and rats (*Rattus norvegicus*).[92–94]

Other isolated observations of ultradian rhythmicity include those of a 5- to 10-minute oscillation in body contraction of the tiny hydrozoans of the genus *Hydra*,[95] a 10- to 30-minute oscillation in human skin temperature,[96] a 100-minute oscillation in the delta and theta frequency bands of the EEG of rats,[97] a 1- to 4-hour oscillation in the core body temperature of sheep,[98] a 2- to 4-hour oscillation in the level of locomotor activity of voles (genus *Microtus*) and domestic mice (*Mus musculus*),[99–101] a 4- to 5-hour oscillation in the body temperature of lemmings,[102] and a 1.1- to 1.4-hour oscillation in carbon dioxide production of mice, rats, guinea pigs, monkeys, chickens, and quail.[103] The expression "nasal cycle" is sometimes used to describe the alternation of airflow through the nasal passages (with air flowing more easily through the left or right nasal passage in intervals of about 6 hours), although there is little evidence of regular periodicity.[104]

Many investigators conducting studies of circadian rhythms have noticed the presence of high-frequency oscillations that

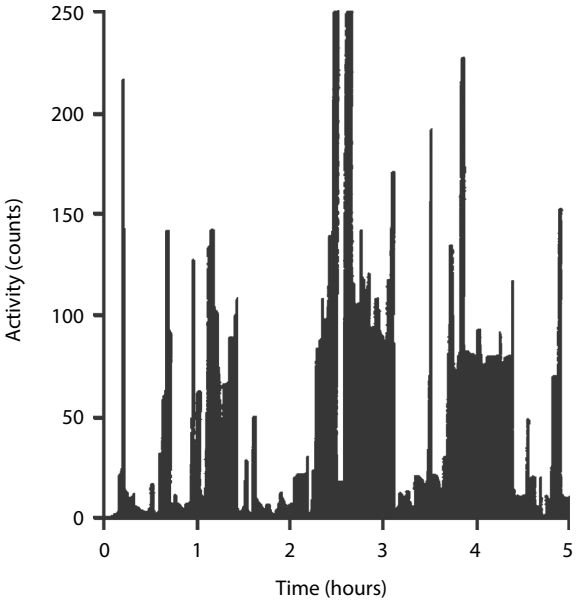


FIGURE 4.20 Killing time. The motor activity records of a young man locked in a small room for 5 hours show an ultradian pattern with a period of approximately 90 minutes. (Data from Grau, C. et al., *Physiol. Behav.*, 57, 411, 1995.)

might constitute ultradian rhythms. Consider the 24-hour record of the body temperature of a golden hamster shown in Figure 4.21. Besides a large daily variation in temperature (which will be discussed in later chapters), one can see rapid oscillations with an average duration of an hour or less. These oscillations may merely reflect biological noise, but they may also be the expression of an ultradian oscillatory process, or even the result of a complex nonlinear (*chaotic*) process.[105–107] In an attempt to characterize ultradian oscillations, spectral analyses of time series were conducted for many variables in various species. In addition to the expected 24-hour component, the analyses revealed one or more ultradian components of 12, 8, and 6 hours.[108–121] Figure 4.22 shows the results of spectral analysis of the body temperature rhythm of the golden hamster. The graphs indicate the percentage of animals whose data exhibited each of the spectral components. When the animals were maintained under a light–dark cycle with 14 hours of light and 10 hours of darkness per day (LD 14:10, Panel A), the 8-hour component was the most common ultradian component. In the absence of a light–dark cycle (LL, constant light, DD, constant darkness, Panel B), the 12-hour component was the most common ultradian component.

As discussed in Chapter 3, spectral analysis describes a complex time series in terms of the sum of multiple simple sine waves. Therefore, the resulting power spectrum may *either* suggest the presence of multiple sources of rhythmicity *or* merely describe the wave form of a single pacemaker that does not produce an ideal sinusoidal signal. At least two sets of experimental observations suggest that the 12-, 8-, and 6-hour components found in previous analyses are *not* true

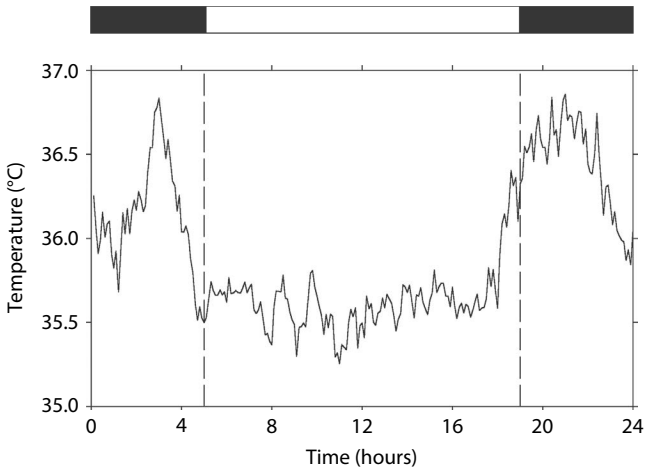


FIGURE 4.21 Small and large oscillations. This 24-hour record of the intra-abdominal temperature of a golden hamster shows not only a large circadian oscillation (to be discussed later on) but also many smaller high-frequency oscillations that may reflect ultradian rhythmicity. The horizontal white and dark bars at the top of the figure indicate the duration of the light and dark phases of the prevailing light–dark cycle. (Data from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999.)

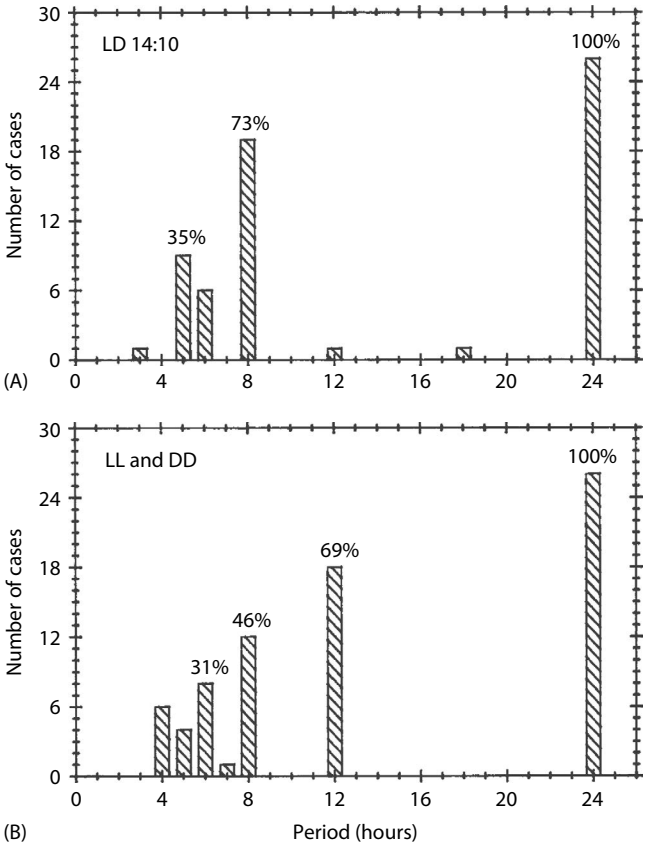


FIGURE 4.22 Relative consistency of ultradian rhythmicity. Time series of the body temperature of 52 golden hamsters were subjected to spectral analysis. Significant 24-hour periodicity was detected in all animals, but detection of ultradian periodicity was less consistent and depended on whether the animals were maintained under a light–dark cycle (LD 14:10, n = 26) (A) or under constant conditions (constant light [LL] or constant darkness [DD], n = 26) (B). (Data from Refinetti, R., *J. Therm. Biol.*, 19, 269–275, 1994.)

ultradian oscillations. One reason to suspect that the power spectra are misleading is that the high-frequency components are harmonics of the fundamental (circadian) frequency. When the same analysis is conducted in mutant hamsters that have a 20-hour circadian period instead of the usual 24-hour period, the "ultradian" components are found to be 10, 6.7, and 5 hours rather than 12, 8, and 6 hours.[122] It would be too much of a coincidence if the periods of the hypothetical ultradian oscillators shrank exactly in proportion to the shrinking of circadian period in the mutant animals. The second reason to question the physiological significance of the high-frequency components is that they disappear (and are replaced by oscillations with periods of 2–5 hours, if at all) in animals whose master circadian clock is surgically destroyed.[114,123–125] The persistence of ultradian oscillations in animals that do not exhibit circadian rhythmicity implies the existence of some form of ultradian timing mechanism, but the period of this mechanism must be shorter than 5 hours (and, therefore, not responsible for the 12-, 8-, and 6-hour oscillations). Further research is necessary to determine whether there is a specific ultradian pacemaker responsible for the oscillations shorter than 5 hours. A possible alternative is that the oscillations are generated by frequency modulation of other ultradian oscillators, such as the one responsible for GnRH pulses. An additional possibility is that the ultradian oscillations result from the operation of an hourglass mechanism associated with the time constant of the sleep homeostat,[126] as will be discussed in Chapter 10.

TABLE 4.2
Period of the Estrous Cycle in Some Vertebrate Species[a]

Period (days)	Species	Sources
1	Chicken	[187,193,194]
1	Quail	[189,190,213]
1	Turkey	[160,564]
4	Golden hamster	[136,141,215]
4	Rat	[172,175,181]
15	Owl monkey	[185]
15	Saki monkey	[154]
16	Antelope	[161]
16	Guinea pig	[195]
17	Sheep	[159,162,176]
20	Deer	[150,163]
21	Cattle	[149,197,199]
21	Degu	[184]
23	Horse	[148,156,178]
28	Human	[130,202,278]
28	Marmoset monkey	[153]
30	Rhesus monkey	[168]
31	Orangutan	[155]
40	Wombat	[164,169]
46	Killer whale	[152]
110	Elephant	[165]
220	Dog	[157,173,174]

[a] Periods shown are average values for each species. Some species exhibit great interindividual variability, including humans (range, 23–36 days) and, most dramatically, dogs (range, 140–380 days).

4.3 INFRADIAN RHYTHMS

In contrast to ultradian rhythms, infradian rhythms are biological processes that cycle with a frequency *lower* than that of circadian rhythms—which means that their period is *longer* than that of circadian rhythms. Infradian rhythms include the reproductive cycles of female animals (*estrous cycle*), the organization of human activities into weeks (*weekly rhythms*), and the monthly alteration of physiological processes associated with the lunar cycle (*lunar rhythms*). Infradian rhythms also include *annual rhythms*, which will be discussed separately in Section 4.4.

4.3.1 Estrous Cycle

Animal reproduction generally requires the joining of male sperm with a female egg. Most female animals do not ovulate on demand, so that reproduction is possible only during the appropriate phase of the ovulatory cycle.[127] As you probably know, many young humans take advantage of this fact and indulge in sexual activity during most of the ovulatory cycle of the woman without having to worry about contraception.[128,129] A woman's estrous cycle—as the estrous cycle of other female primates—is called a *menstrual cycle* because the cycle lasts about a month (*mense* means *month* in Latin). However, there is nothing special about the month. As shown in Table 4.2, estrous cycles vary from 1 day in domestic fowl to 220 days in dogs. The duration of the estrous cycle bears virtually no relationship with infradian environmental cycles or with the body size of the species. In Section 4.4, we will see how reproduction is affected by annual rhythms, and in Chapter 9, we will discuss how estrous cycles interact with circadian rhythms.

The typical oscillation of plasma concentration of sexual hormones during the human menstrual cycle is shown in Figure 4.23. Because the average duration of the menstrual cycle may vary from 23 to 36 days in different women, and because there is variation of a few days from cycle to cycle in most women, all data have been standardized to an ideal 28-day cycle. It is evident that plasma levels of luteinizing hormone are low except at the time of ovulation. Estradiol has a more complex wave form with a peak prior to ovulation, and progesterone peaks much later in the cycle.[127] Notice that also sexual behavior has a rhythmic pattern. Women who do not use birth-control pills (which interfere with the hormonal cycles) initiate sexual activity with a partner more often right after the end of menstruation and at the time of ovulation than at any other time in the cycle.[130] Presumably, the peak after menstruation is due to sanitary considerations, whereas the peak at the time of ovulation is an inherited evolutionary adaptation that favors fertilization and subsequent pregnancy. As a matter of fact, it has been shown that, during the fertile phase of the menstrual cycle, women show preference for male body odor[131] and masculine facial features[132,133]—which,

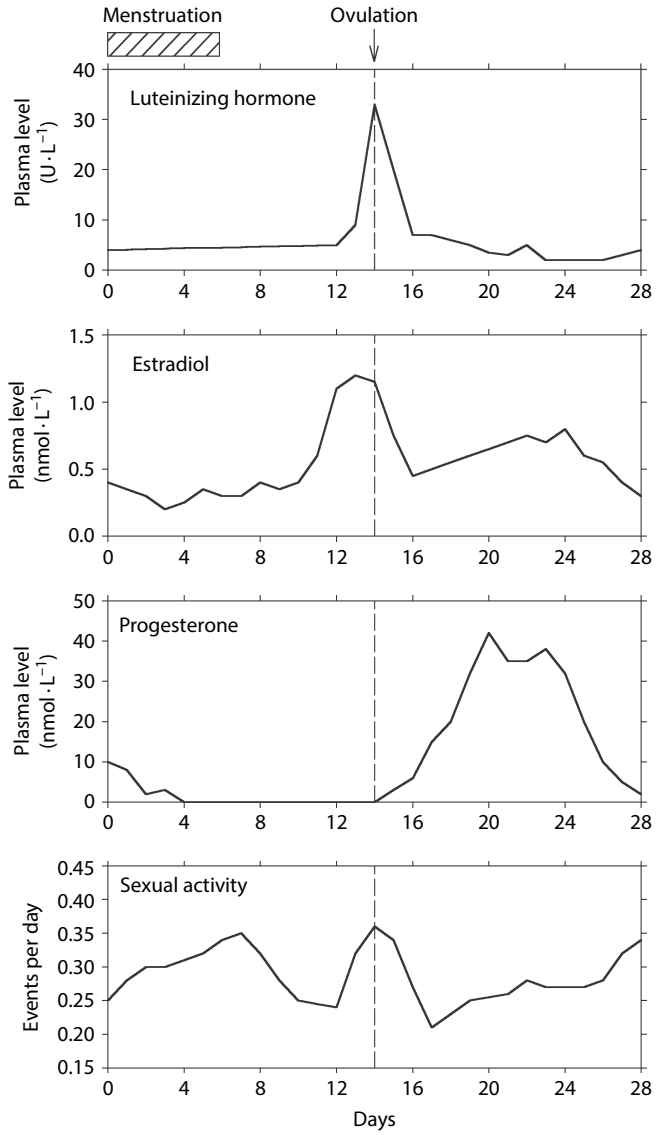


FIGURE 4.23 The menstrual cycle. The menstrual cycle involves not only the cyclic release of an egg (ovulation) throughout a woman's reproductive life but also rhythmic variations in hormone secretion and sexual activity. In women who do not take birth-control pills, self-initiated sexual activity with a partner peaks at the time of ovulation as well as right after menstruation. (Data from Ganong, W.F., *Review of Medical Physiology*, 20th edn., Lange Medical, New York, 2001; Adams, D.B. et al., *N. Engl. J. Med.*, 299, 1145, 1978.)

again, may favor sexual contact and subsequent pregnancy. Approximately one out of six women of reproductive age exhibits "premenstrual syndrome," a condition that most often involves depressed mood and distress prior to and during menstruation. The depressed mood is associated with at least six physical symptoms: headaches, skin changes, gastrointestinal problems, breast changes, and coagulation and heaviness of menstrual bleeding.[134]

Although women are more likely to engage in sexual activity at certain times of the cycle, they can—and do—mate on any day of the month.[135] Females of most other species, however, are sexually receptive only around the time of ovulation



FIGURE 4.24 Wild sex. In many animals, including the lion, the frequency of sexual intercourse is modulated by the female's estrous cycle. (Photo by Michael Wain, Copyright 2003.)

(Figure 4.24). As a matter of fact, many variables besides hormonal secretion exhibit estrous rhythmicity. The female golden hamster, for example, shows 4-day rhythmicity in behavioral sexual receptivity,[136–138] in the pattern of vaginal discharges,[139–141] and in the amount and temporal organization of locomotor activity.[142–146] The estrous modulation of the daily amount of activity of female hamsters is evident in Figure 4.25. Whereas male hamsters exhibit only small variations in activity from day to day, females rhythmically vary their activity levels several fold. A closer look at the estrous variation in the activity pattern of a female hamster is possible in Figure 4.26. The animal was maintained in constant darkness, and, at first inspection, you may perceive only a "messy" free-running circadian rhythm. Notice, however, the consistent alternation of 2 days with much activity and 2 days with little activity. The days with much activity are the day of ovulation and the

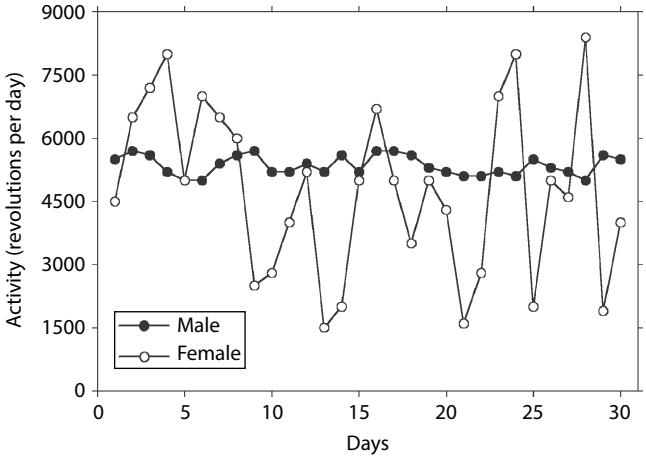


FIGURE 4.25 Run for sex. As exemplified by a pair of golden hamsters housed in separate cages, male hamsters exhibit moderate day-to-day variation in the amount of running-wheel activity, but female hamsters exhibit great variation associated with the 4-day estrous cycle. (Data from Refinetti, R. and Menaker, M., *Behav. Neural Biol.*, 58, 27, 1992.)

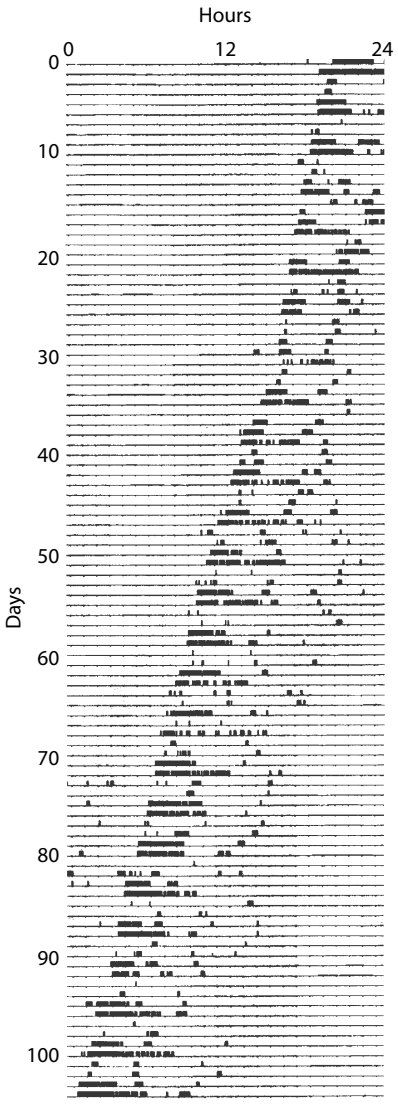


FIGURE 4.26 Estrous signature. The estrous cycle of a female golden hamster is evident in the actogram of her running-wheel activity records under constant environmental conditions (constant temperature and constant darkness). With occasional disruptions, a pattern of 2 days with heavy activity followed by 2 days with sparse activity is evident. (If you are not familiar with actograms, look back at Figure 3.19.) (Data from the archives of the Refinetti lab.)

day immediately preceding it (the days of *estrus* and *proestrus*, respectively), whereas the days with little activity are the remaining days of the estrous cycle (*diestrus* days).

Of course, many species besides the golden hamster exhibit estrous rhythmicity in hormonal secretions,[147–168] vaginal discharges,[157,162,164,169–174] behavioral sexual receptivity,[150,158,159,166,173,175–178] and locomotor activity.[110,147,149,169,170,172,179–185] In birds, egg laying is a very convenient marker of estrous rhythmicity.[186–190] In addition, many species of mammals and birds exhibit estrous rhythmicity in body temperature.[141,151,155,160,169,172,180,181,183–185,189,191–199] Figure 4.27 shows the estrous rhythmicity of rectal temperature of three domestic cows (*Bos taurus*). A sharp rise in temperature (measured daily at dawn) can be seen on the day

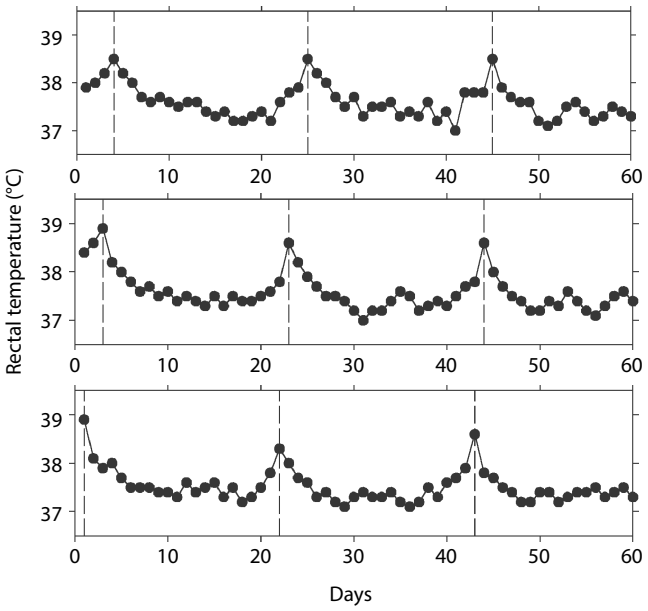


FIGURE 4.27 Bovine estrous cycle. These records of rectal temperature of three cows (*Bos taurus*) were obtained daily at dawn for 2 consecutive months. A sharp rise in temperature can be seen on the day of estrus (vertical dashed lines) every 21 days. Estrus was determined by observation of vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull. (Data from Piccione, G. et al., *BMC Physiol.*, 3, art. 7, 2003.)

of estrus (vertical dashed lines), as determined by vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull. Women also experience an elevation of body temperature immediately after ovulation, so that temperature measurements made during the luteal phase of the menstrual cycle are 0.3°C–0.6°C (~1°F) higher than during the follicular phase.[200–209] The relationship between ovulation and body temperature is consistent enough to be used as a tool for contraception or for its very opposite, the treatment of infertility.[210–212] The postovulatory elevation in body temperature is presumably due to the thermogenic (heat-producing) action of progesterone, but not in all animals. In small rodents, the body temperature rise associated with the estrous cycle is due to the increase in locomotor activity and presumably masks a smaller temperature rise associated directly with progesterone secretion.[141,181]

When animals (or humans) are maintained in an environment devoid of temporal cues, their estrous cycles freerun with periods shorter or longer than those observed under natural conditions.[136,137,185,187,213,214] An example of a free-running estrous cycle is shown in Figure 4.28. A domestic hen (*Gallus domesticus*) was chosen for the illustration because its estrous cycle has a period conveniently short (~28 hours) to be displayed in a standard actogram. One can easily observe the drift of about 4 hours per day in the time of oviposition (and of the peak of the body temperature rhythm). The fact that estrous cycles can freerun under constant environmental conditions indicates that they are not imposed by the environment but, instead, are endogenously generated. As a matter of fact, the endogenous

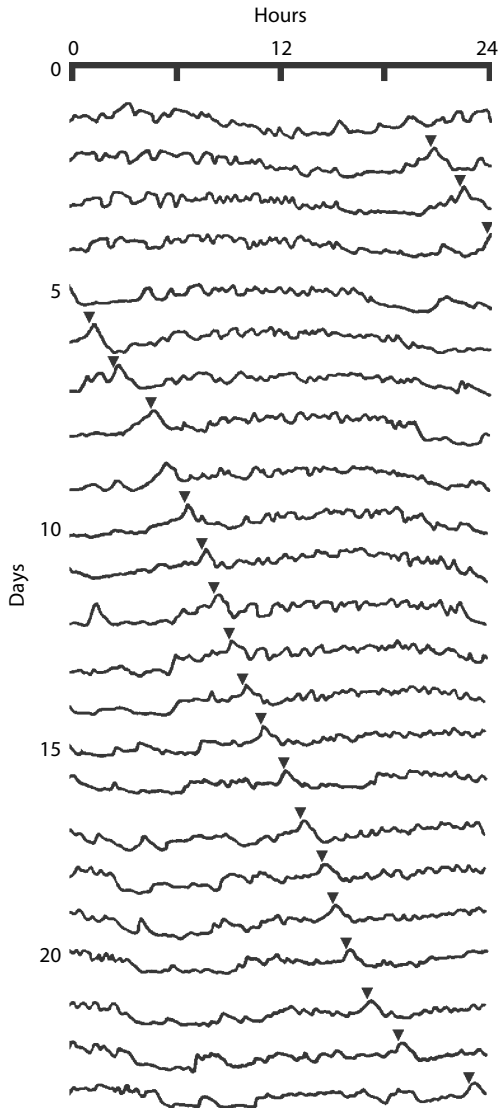


FIGURE 4.28 Laying eggs. The ovulatory cycle of the domestic chicken (*Gallus domesticus*) is so short that it can be plotted in actogram format just like a circadian rhythm. In this figure, the lines indicate body temperature (ranging from 39.5°C to 41.5°C) and the small inverted triangles indicate the time of oviposition (egg laying). Notice that body temperature rises daily at the time of oviposition. (If you are not familiar with actograms, look back at Figure 3.19.) (Data from Kadono, H. and Besch, E.L., Influence of laying cycle on body temperature rhythm in the domestic hen, in: Tanabe, Y., ed., *Biological Rhythms in Birds: Neural and Endocrine Aspects*, Springer, Berlin, Germany, 1980, pp. 91–99.)

nature of estrous rhythmicity could be inferred from the fact that the period of the estrous cycle varies greatly from species to species (see Table 4.2) even though all animals live in the same world. The follicle maturation time of each species is probably the major determinant of the duration of the estrous cycle. As will be discussed in Chapter 9, the circadian system seems to play a role in the synchronization of estrous cycles to the environment. Synchronization is important because, at least in some species, free-running estrous cycles disintegrate after a while, leading to a condition of persistent estrus.[175,215–217]

4.3.2 Weekly Rhythms

The grouping of 7 days into a unit called a *week* is common in most of the world.[34] It is also common for people to organize the week into 5 or 6 days of work and 1 or 2 days of rest. This weekly scheduling of activities results in some obvious biological rhythms, such as the rhythm of churchgoing of people with religious habits (Figure 4.29). Not as obvious is the fact that people drive their cars farther, and are more likely to drive while inebriated, on weekends than on weekdays. As a consequence of this, more traffic accidents take place on Fridays and Saturdays than on other days of the week (Figure 4.30). Whereas the number of presumably unintentional deaths due to traffic accidents also peaks on Fridays and Saturdays, the number of deaths due to suicide peaks on Mondays,[218,219] as shown in Figure 4.31.

Also not surprising is the fact that people's mobility pattern varies depending on the day of the week: during weekdays, people travel to and from work at predictable times, whereas on weekends they go to other places at variable times.[220] They also make more car trips during weekdays than during weekends.[221] The quantity of physical activity, however, does not vary reliably along the week: it seems that some individuals are more active on weekends, whereas others are more active

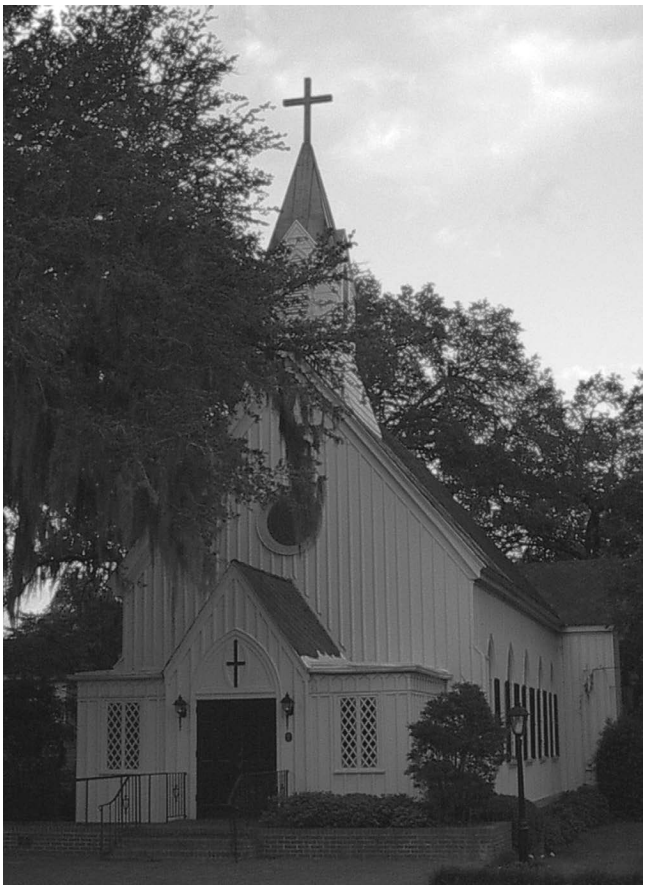


FIGURE 4.29 A small church in Walterboro, South Carolina. Going to church is something that many people do on a weekly basis. Thus, they exhibit a weekly rhythm of churchgoing. (Photo by R. Refinetti.)

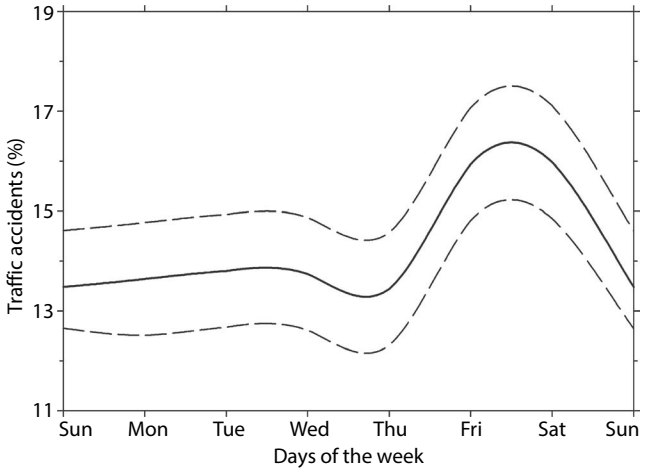


FIGURE 4.30 Watch the road! Highway traffic accidents in the United States show a weekly rhythm. Accidents are more common on Fridays and Saturdays than on other days of the week. The solid line shows the percentage of weekly accidents by day of the week as averaged for the years 1995–1999. The dashed lines indicate the 95% confidence intervals for the means. (Data courtesy of National Center for Statistics and Analysis, United States Department of Transportation.)

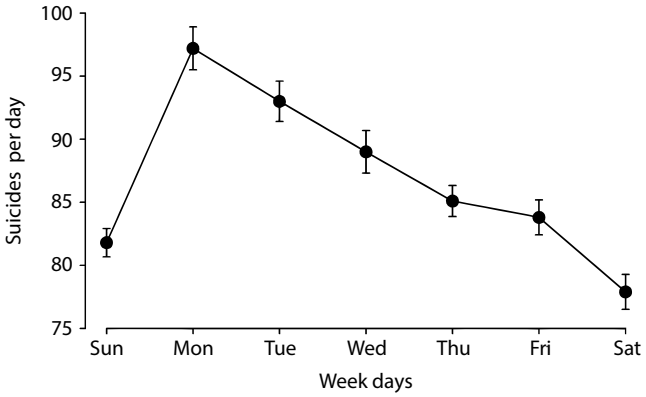


FIGURE 4.31 A day to die. The number of suicides per day in the United States depends on the day of the week. The 31,699 suicides recorded in 2002 were averaged over the 52 weeks of the year, and the error bars indicate the standard errors of the means. (Data courtesy of National Center for Health Statistics, United States Department of Health and Human Services.)

during weekdays, and this distribution varies from country to country.[222] People editing articles on Wikipedia (the free online encyclopedia) do it more often on weekdays or on weekends, depending on location around the world,[223] and scientists refereeing journal manuscripts tend to do it early in the week, starting on Sunday.[224]

People tend to sleep one to three hours longer on Friday and Saturday nights than on weekday nights.[225–227] People also tend to eat more on weekends (2000 kcal·day^{-1}) than during the work week (1800 kcal·day^{-1}).[228] Young married couples have sex almost twice as often on Sundays as on weekdays[229]; as a consequence, testosterone secretion in men is elevated on weekends.[230] In contrast, the frequency of human births is *lower* on weekends than on weekdays,[231–233] possibly because obstetricians consciously or unconsciously want to maintain *their* weekly schedule of work and rest. Patients with fibromyalgia (widespread muscle pain) report more discomfort and pain on Sundays and Mondays than on other days of the week.[234] Blood pressure tends to be higher on Mondays than on other days of the week.[235–237] The frequency of heart attacks (myocardial infarctions and cardiac arrests) in the general population also shows weekly rhythmicity, with most (though not all) studies reporting a peak on Monday.[238–242]

Because the cardiac rhythm, the breathing rhythm, and several circatidal rhythms are endogenously generated, it is natural to wonder whether weekly rhythms are endogenously generated as well. Halberg, who—as discussed in Chapter 1—was the foremost advocate of the discipline of chronobiology, originally pointed out that the week has no environmental counterpart that could guide evolution of endogenous rhythmicity.[243] Later on, however, he coined the adjective *circaseptan* to refer to hypothetical endogenous rhythms with a period of approximately 7 days. Unfortunately, the experimental evidence in support of the existence of circaseptan rhythms is far from compelling. Even if we ignore the issue of endogenesis for a while, the existence of weekly rhythms in bodily functions of individuals is very poorly documented. In an article published in 2003, Halberg and colleagues reported 7-day rhythms of blood pressure and heart rate in human subjects,[244] but the presented data were unconvincing at best. The data shown in Figure 4.32 exemplify the results obtained by Halberg and his colleagues. High-frequency oscillations (apparently of random origin) are so large that not even daily rhythmicity can be easily seen.

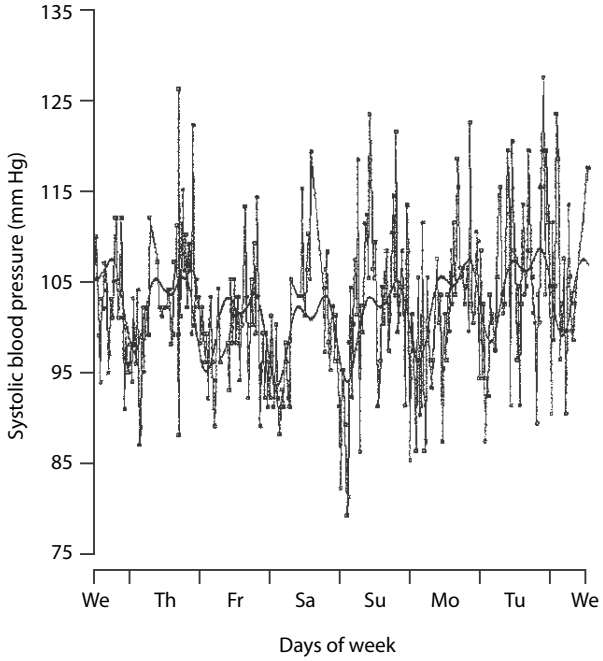


FIGURE 4.32 Weekly or weakly rhythm? It has been suggested that these records of systolic blood pressure of a human subject display weekly rhythmicity, but visual inspection reveals only daily rhythmicity, if any rhythmicity at all. (Data from Lee, M.S. et al., *Biomed. Pharmacother.*, 57, 39s, 2003.)

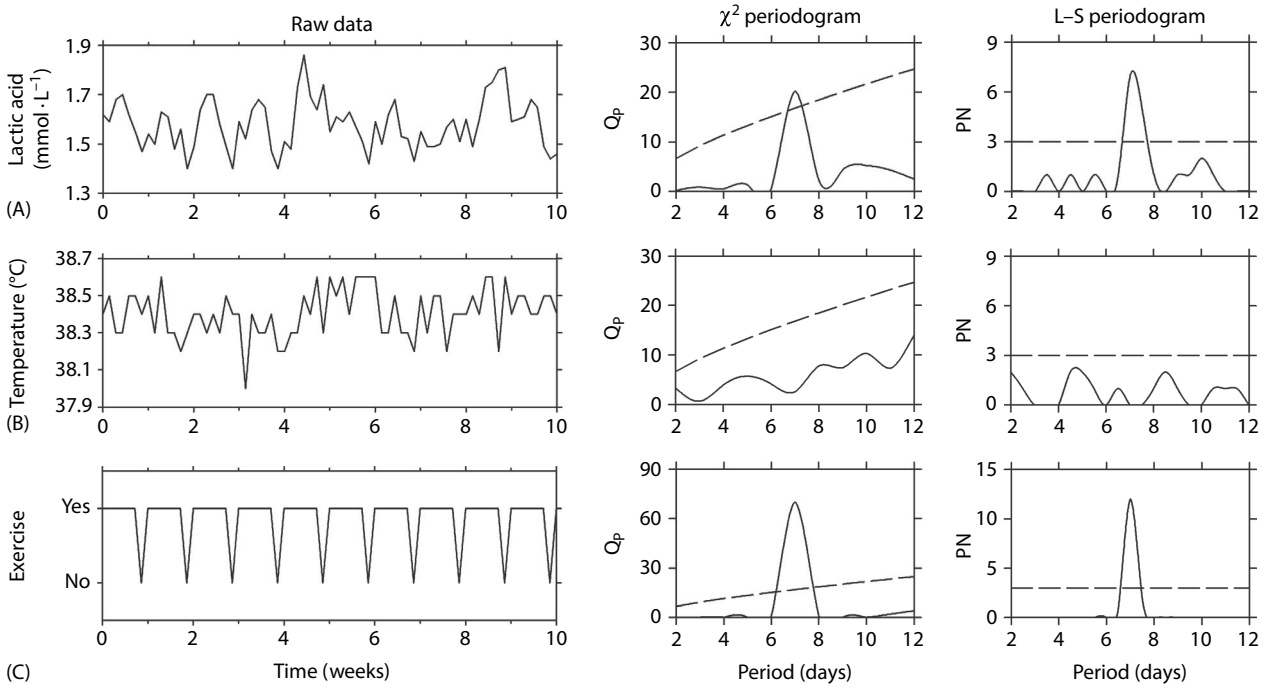


FIGURE 4.33 Feeble weekly rhythmicity in the horse. Raw data and their analyses by the chi square periodogram and Lomb–Scargle periodogram procedures are shown for plasma concentration of lactic acid (A), rectal temperature (B), and schedule of exercise (C) of a horse (*Equus caballus*). There is significant but weak 7-day periodicity in the rhythm of lactic acid concentration and no significant periodicity in the rhythm of rectal temperature. In contrast, the experimenter-imposed schedule of exercise shows robust 7-day periodicity. (If you don't know how to interpret periodograms, look back at Section 3.3.) (Adapted from Piccione, G. et al., *Chronobiol. Int.*, 21, 571, 2004.)

Since data are shown for only one week, any evidence of weekly rhythmicity could only be suggestive, but we need not worry about it because there is no visible evidence of a weekly pattern at all. Cosinor analysis conducted by the authors revealed marginally significant 7-day rhythmicity in some data sets, but—in view of the figures presented—one can only wonder how reliable this "microscopic" statistical analysis is. In another study, Halberg's team claimed to have identified circaseptan rhythms of blood pressure in a set of 11 pairs of newborn twins.[33] Their "evidence," with a probability of Type I error of 0.08, was that there was a smaller within-twins variability than inter-twins variability in the data, which presumably indicates that there is a genetic basis for 7-day rhythmicity. No evidence of a real rhythm was presented.

In one of very few rigorous studies of weekly rhythmicity in individual subjects, 12 horses were studied for 70 consecutive days, so that 10 full 7-day cycles could be monitored.[245] Measurements of plasma concentration of lactic acid, blood pressure, and rectal temperature were made each day in the morning and evening in six athletic horses (subjected to a weekly schedule of fitness training) and six sedentary horses (not subjected to the weekly schedule). Data from one of the athletic horses are shown in Figure 4.33. Plasma concentration of lactic acid showed feeble 7-day rhythmicity (Panel A), which was barely significant as determined by chi-square periodogram analysis (Panel B) and Lomb–Scargle periodogram analysis (Panel C), and rectal temperature showed no 7-day rhythmicity at all (middle row). In contrast, the investigator-imposed schedule of fitness training was clearly rhythmic (bottom row). For the whole group of athletic horses, 7-day rhythmicity was very weak and was present in only one of the parameters (lactic acid concentration). Also, the rhythms did not exhibit a consistent phase relationship with the calendar week. In the sedentary horses (which were not subjected to a weekly schedule of fitness training), the weekly temporal patterns were not significantly rhythmic in any of the variables measured. This suggests that the feeble weekly rhythm observed in athletic horses, if real, was imposed by the weekly exercise schedule and was not generated by an endogenous pacemaker.

4.3.3 Lunar Rhythms

The 29.5-day lunar cycle is the astronomical process that inspired the concept of the *month*, which today is defined independently of the lunar cycle.[246] Like the week, the month has effects on human behavior (Figure 4.34). Also, the menstrual cycle of human females has an average duration very similar to that of a month (28 days) and could be considered a monthly rhythm. It will not be discussed here because it was previously covered in the subsection about estrous cycles. Curiously, there may be a monthly cycle in testosterone secretion of *male* humans that results from the modulation of male sexual activity by the menstrual cycle of their female partners.[230]

More interestingly, the lunar cycle itself can affect organisms through the variation in nighttime luminosity and



FIGURE 4.34 A building under construction. Many construction workers, like many other workers, get paid once every 2 weeks or once a month, which imposes on them a biweekly or monthly rhythm of financial earnings. (Photo by R. Refinetti.)

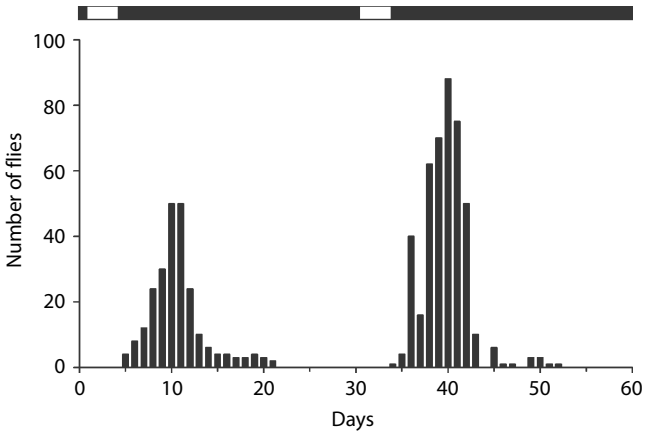


FIGURE 4.35 Lunar rhythm. The graph shows a lunar rhythm in the emergence of *Clunio marinus*, a small fly that inhabits tidal waters. Colonies were maintained under a daily light–dark cycle (12 hours of light and 12 hours of darkness) supplemented with a lunar cycle (artificial moonlight for 4 nights every 30 days, as shown by the horizontal bars at the top of the graph). (Data from Neumann, D., *J. Biol. Rhythms*, 4, 285, 1989.)

tide levels. This variation is modest and has modest effects on organisms, which probably explains why the literature on biological rhythms contains very few studies on lunar rhythms as compared to tidal, daily, and annual rhythms. Although nighttime luminosity can vary from 0.001 lux at the time of a new moon to 0.1 lux at the time of a full moon (a variation of 10^2 lux), daytime luminosity is usually higher than 10,000 lux, which means that the variation between day and night is much stronger (about 10^6 lux).[36] As for the tides, most of the variation is due to Earth's revolution, and the lunar cycle accounts for only about 20% of the variation in water level.[1] The lunar cycle also has a very small effect on Earth's temperature: a variation of 0.02°C from new moon to full moon.[247]

The fact that the lunar oscillation in nighttime luminosity is modest does not mean that nocturnal species cannot take advantage of it. For example, South American owl monkeys (of the genus *Aotus*) are a rare example of nocturnal primates. Argentinean owl monkeys (*Aotus azarai*) exhibit a peculiar behavioral activity pattern: they are active primarily during dawn and dusk when there is a new moon but are fully nocturnal when there is a full moon.[248] Secretion of the hormone melatonin in seagrass rabbitfish (*Siganus canaliculatus*) exhibits lunar rhythmicity in response to the lunar oscillation in nighttime luminosity.[249] Another species of fish, the mummichog (*Fundulus heteroclitus*), spawns rhythmically, with greatest readiness at new moon and full moon.[250]

The results of a well-conducted laboratory study are shown in Figure 4.35. The number of sea flies (*Clunio marinus*) completing metamorphosis from the larval stage was recorded in the laboratory for 2 months, while the larvae were kept under an artificial lunar cycle with four nights of *moonlight* each month (as denoted by the horizontal bar at the top of the figure).[251] Notice that eclosion takes place immediately after the "full moon." Lunar rhythms (and semilunar rhythms associated with spring tides) have been shown to freerun in the absence of environmental lunar cycles[85,252,253] and, therefore, must be endogenously generated. Since they can be synchronized to environmental lunar cycles, it is appropriate to call them *circalunar* rhythms.

An influence of the lunar cycle on human behavior has long been suspected, as demonstrated by the expression "lunatic" applied to mentally ill individuals. In times past, before artificial lighting became a common feature of human adobes, it is possible that night light caused by the full moon was a significant source of sleep disruption, which is known to affect the mental state of psychiatric patients.[254] There have been isolated reports of lunar rhythmicity in human functioning, such as the timing of births,[233] meal size,[255] and sleep parameters.[256,257] The majority of researchers who investigated potential lunar rhythmicity in human physiology and behavior, however, found no evidence of it.[218,222,229,258–267] It is very likely that the influence of the Moon on humans is equivalent to that of other celestial bodies in the solar system—that is, it is meaningful to superstitious people who believe in horoscopes but is nonexistent for the rest of us. Similarly, there is a folkloric belief that the sex of a newborn horse is related to the phase of the moon when it was conceived, but analysis of over 65,000 horse gestations revealed no statistically significant association between moon phase and the proportion of males and females.[268]

4.3.4 Other Infradian Rhythms

A curious infradian rhythm that does not fit into any of the categories discussed earlier is one that involves the Canadian lynx (*Lynx canadensis*) and the Arctic hare (*Lepus arcticus*), both shown in Figure 4.36. The lynx is hunted commercially for its fur, and good records of fur returns have been kept since the early 1800s. As shown in Figure 4.37, fur returns exhibit a remarkably regular



FIGURE 4.36 Predator and prey. (A) The lynx (*Lynx canadensis*) and (B) the Arctic hare (*Lepus arcticus*) are closely connected as predator and prey in the tundra zone of Canada. (Courtesy of National Image Library, United States Fish and Wildlife Service.)

10-year rhythm. The cause of the rhythm is not fully known, but the fur-returns rhythm is definitely due to a rhythm in the size of the lynx population, which is dependent on variations in the population of the lynx's main prey, the hare.[269] A 10-year rhythm in population size has also been documented for various species of voles (genus *Microtus*) in the wild in Europe.[270] Because 10 years is also the approximate period of the cycle of sunspots, it is possible that the phenomena are causally related. The cycle of sunspots has been suggested as the cause of a 10-year cycle of moth population in Norway.[271] Low sunspot activity leads to a thinner ozone layer and, consequently, to higher ultraviolet radiation on the Earth's surface. The higher ultraviolet radiation presumably reduces plant resistance to herbivores, thus favoring an increase in the moth population.[271] Rhythms with approximately 10-year period have also been described for population density of gerbils,[272] abundance of oceanic phytoplankton,[273] tree growth,[274] and urinary excretion of 17-ketosteroid in humans.[275] Unusual infradian rhythms with periods ranging from a few months to several years have been described in dormice,[276] lemmings,[277] and humans.[278] A 10-day rhythm in human dental enamel growth has also been described.[279]

One problem in the study of infradian rhythms is that the possibilities are endless. If one takes almost any time series covering a number of years, one is likely to find at least one temporal pattern that might constitute a rhythm. Deciding whether the temporal pattern truly describes a rhythmic phenomenon is seldom easy. Consider Figure 4.38. The graphs show data about civilian passenger aircraft crashes from the year 1960 to the year 2001. The graph in Panel A shows the number of crashes, whereas the graph in Panel B shows

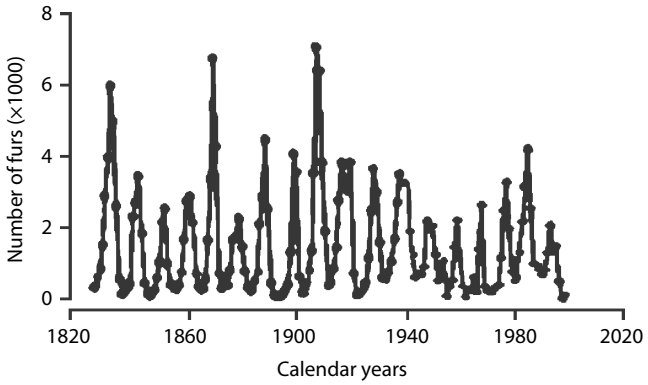


FIGURE 4.37 If you need a fur coat. The number of lynx furs procured each year in an area of approximately 270,000 km^2 in northwest Canada shows a 10-year cycle that results from the cycling of the lynx population. (Data from Stenseth, N.C. et al., *Proc. Natl. Acad. Sci. USA*, 95, 15430, 1998.)

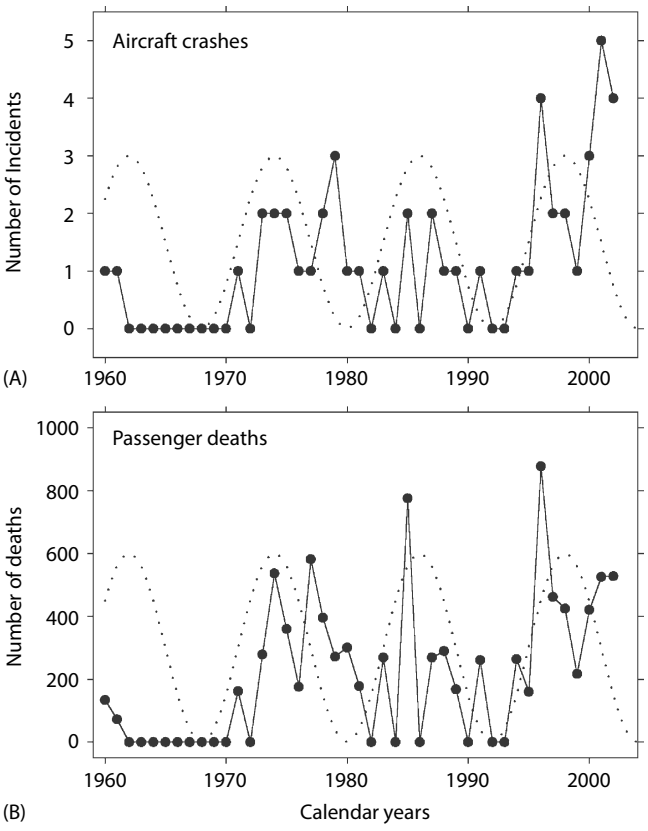


FIGURE 4.38 Is there a 12-year cycle of airplane accidents? The graph in Panel A shows the yearly number of civilian passenger aircraft crashes with more than 150 fatalities per incident over the course of four decades. The graph in Panel B shows the total number of passenger deaths resulting from the crashes. In both graphs, there seems to be a 12-year rhythmic pattern, as suggested by the dotted lines. However, numerical analysis of the data produces inconclusive results. (Data from *TIME Almanac*, TIME Books, Des Moines, IA, 2003.)

the number of passenger deaths resulting from the crashes. In both cases, there seems to be a 12-year rhythm, as suggested by the dotted lines. Are the rhythms real? Kolmogorov–Smirnov tests indicate that the distributions deviate significantly from flat distributions. Chi-square periodogram and Fourier analysis identify significant peaks between 10 and 20 years, but not consistently. So, the rhythms may or may not be real. Until more data are available, no definite decision can be made.

Very common in the popular literature is the notion of an infradian rhythm of human reproduction much longer than the menstrual cycle. When a single woman—or a married woman who has not had children—enters her third or fourth decade of life and starts having the urge to become a mother, it is not uncommon to say that "her biological clock is ticking." The meaning of the expression is that there is some sort of clock in the woman's body that starts ticking louder and louder as menopause approaches. Supposedly, the woman's life will not be complete unless she becomes pregnant and has a child. Several books about late motherhood have the expression *biological clock* in their titles.[280–283] Is this just a metaphor, or is there really some sort of *pregnancy predestination*?

In a world where every life form has a limited life span, it is evident that without reproduction life would soon vanish. In this very general sense, it would be appropriate to speak of pregnancy predestination. If no women had children, and scientists did not develop human cloning right away, human life would soon vanish. But then, natural selection took care of making sex such a pleasurable activity that, for tens of thousands of years, humans did not have to fret about the necessity of reproduction—it just came along as a natural consequence of having sex. Therefore, to the extent that one has a "predestination" to enjoy sex, one could speak of pregnancy predestination. And, of course, anyone who has experienced the strong emotions of parenthood can testify that missing on parenthood would be missing on one of the most significant parts of life. But, metaphors aside, is there a biological clock that sets the time for reproduction? Yes and no. The "yes" refers to a seasonal cycle of reproduction—which is much stronger in other animals than in humans—and to the menstrual cycle. The "no" refers to a clock that would tell women to have children early in life. No evidence has been collected that such a clock might exist.

So, if there is no biological clock that tells women to have children early in life, is it appropriate for mature women to have children? There is no straightforward answer to this question. Although there is no mechanism responsible for the urge to reproduce early in life, people do get older as time goes by, and aging is known to impair various physiological processes. Challenges associated with late motherhood include fertility problems, high risk of chromosomal defects, high rates of miscarriage, and labor complications. On top of this, of course, there are physical and social tensions. Is it all worth it? This is a decision that the future mother must make—together with the father and the physician. While postponing motherhood to the late thirties and forties is not advisable, the idea becomes much more attractive once one has reached that age without having had experienced the joys of motherhood. Figure 4.39 shows the number of live births in the United States as a function of the mother's age. By comparing the 1940 data with the 2000 data, it can be seen not only that there were more births in 2000 than in 1940 (because the population grew in that interval), but also that there was an increase in the proportion of women giving birth at older ages. In the year 2000, women 35–39 years old gave birth to almost half a million babies, and almost 100,000 babies were delivered by women 40 years old or older.

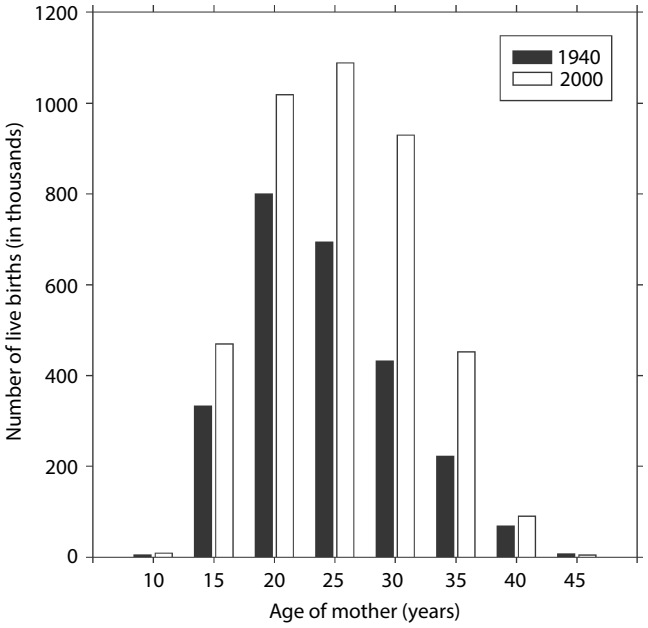


FIGURE 4.39 Are you ready for a baby? The graph shows the number of live births in the United States as a function of the mother's age in 1940 and 2000. Notice not only that there were more births overall in 2000 than in 1940 (primarily because of population growth) but also that there was a substantial increase in the proportion of women giving birth in the 25–35-year-old brackets. (Data courtesy of National Center for Health Statistics, United States Centers for Disease Control and Prevention.)

After having talked about the joys of parenthood, it will be fitting to finish this section with a brief curiosity about mice. Adult mice are normally not fond of preweaning infants. As a matter of fact, they usually kill a pup when they see it. However, in order not to hurt their own offspring, they seem to have some sort of infradian clock that reduces their infanticidal instinct at 18–20 days after mating, which is when the dam gives birth to the pups.[284]

4.4 ANNUAL RHYTHMS

Annual rhythms are rhythms that have a period of 1 year. They are, therefore, a subclass of infradian rhythms. However, they are so ubiquitous that they deserve a separate section in this chapter. In most organisms, annual rhythms are related to the alternation of the seasons, but in humans, the calendar year itself can modulate behavior regardless of changes in temperature and day length (Figure 4.40).

Many physiological and behavioral processes exhibit annual rhythmicity. We will first cover annual rhythms studied



FIGURE 4.40 Christmas in South Carolina. Those who celebrate Christmas do it once a year in late December, which institutes a robust annual rhythm of Christmas celebration. (Photo by R. Refinetti.)

under natural or seminatural conditions in the presence of annual environmental cycles. We will call them *seasonal rhythms* because they cycle with the seasons. Afterward, we will cover *circannual rhythms*, which are endogenously generated rhythms that normally cycle with the seasons but can persist in the absence of environmental cycles. Later on in the book (Chapter 9), we will discuss how annual rhythms interact with circadian rhythms.

4.4.1 Seasonal Rhythms

Some seasonal rhythms are rather obvious. For example, it is evident that a muskox (*Ovibos moschatus*) living in the Alaskan tundra must change its eating habits in the winter, when the ground is frozen and covered with snow (Figure 4.41). Equally not surprising is the fact that small animals (both vertebrates and invertebrates) show seasonal variation in burrowing and nest-building behavior.[285–289] If you live in a temperate country and pay your heating bill, you have probably noticed that your heat usage oscillates yearly, similarly to the records shown in Figure 4.42.

Other seasonal rhythms are not obvious at all. For example, under the frozen Arctic tundra lives a large community of microbes (mostly fungi) that reaches maximal biomass in the late winter and dwindles in the summer.[290] Phytoplankton populations in oceans also exhibit seasonal variation, with



FIGURE 4.41 Muskox bull and colleagues on frozen tundra. Not seen in this picture of the frozen Alaskan tundra is the large community of fungi that thrives under the snow cover. (Courtesy of National Image Library, United States Fish and Wildlife Service.)

different species being more abundant at different times of the year.[291] Likewise, it is not *a priori* evident that people diagnosed as schizophrenics are more often born in the winter than in the other seasons[292,293] or that human sensory processes exhibit seasonal variation.[294,295] Other curious seasonal rhythms include the lowering of the body temperature of prairie dogs (which are not hibernators) by 3°C in the winter,[296] a sixfold increase in the number of deer-vehicle crashes in the late autumn,[297] a doubling of the duration of water dives by elephant seals in the winter,[298] and a 30% rise in the occurrence of human spouse battering in the summer.[299] Particularly bizarre for us humans who are active all year-round is the fact that spadefoot toads (genuses *Scaphiopus* and *Spea*, Figure 4.43) spend almost the whole year buried underground in a dormant state and are active for only a few weeks each year when torrential summer rains provide a suitable surface-level environment for this terrestrial amphibian living in North American deserts.[300,301]

If you usually feel "blue" in the winter and jovial in the summer, you will be glad to know that a seasonal variation in mood is normal in humans, especially in women.[302] In Chapter 16, we will discuss the more unusual case of individuals who become severely depressed in the winter (*seasonal affective disorder*). The winter is also a bad time of the year for people with cardiovascular disorders, as the frequency of heart attacks (myocardial infarction, ventricular fibrillation, cardiac arrest, etc.) varies seasonally by about 3% and peaks in the winter.[238–240,303–305] Other causes of death besides heart attacks also exhibit seasonal variation, so that the total number of deaths per day shows a clear seasonal rhythm that peaks in the winter (Figure 4.44). At least in Finland, however, the summer is the season with the highest number of deaths by suicide.[306] In the Netherlands, women are twice as likely to be infected with the sexually transmitted human papilloma virus in the summer than in the winter.[307] Scientists submitting their research articles for publication in the journal *Psychological Science* do so much more often in the summer than at other times of the year.[224]

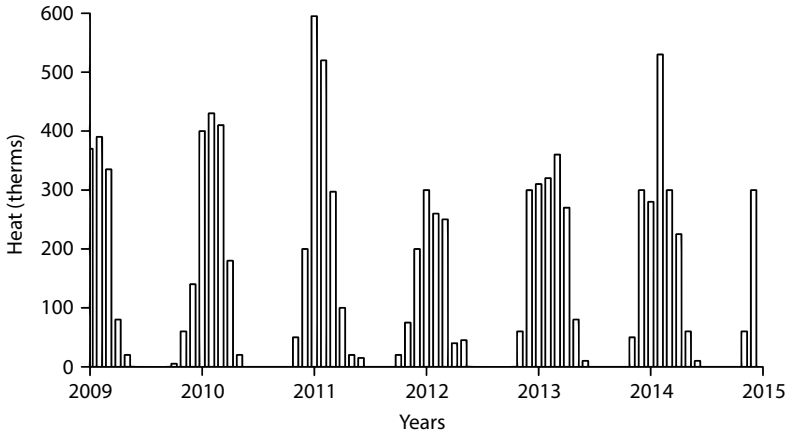


FIGURE 4.42 Heating the house. The graph shows the monthly heat usage (estimated by the volume of gas consumed) of a house in South Carolina over 6 years. A clear yearly oscillation (with peaks in the boreal winter) is visible. (Data courtesy of South Carolina Electric and Gas Company.)



FIGURE 4.43 Spadefoot toad. A spadefoot toad (*Scaphiopus holbrookii*) is up and about only a few weeks each year. (Photo courtesy of Stan Trauth.)

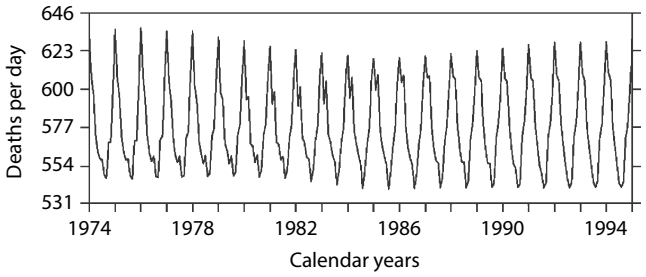


FIGURE 4.44 A season to die. As shown by these data from the Canadian Vital Statistics Database, there is a robust annual rhythm in human deaths. Most deaths occur in January. (Data from Trudeau, R., *Stat. Canada Health Rep.*, 9(1), 43, 1997.)

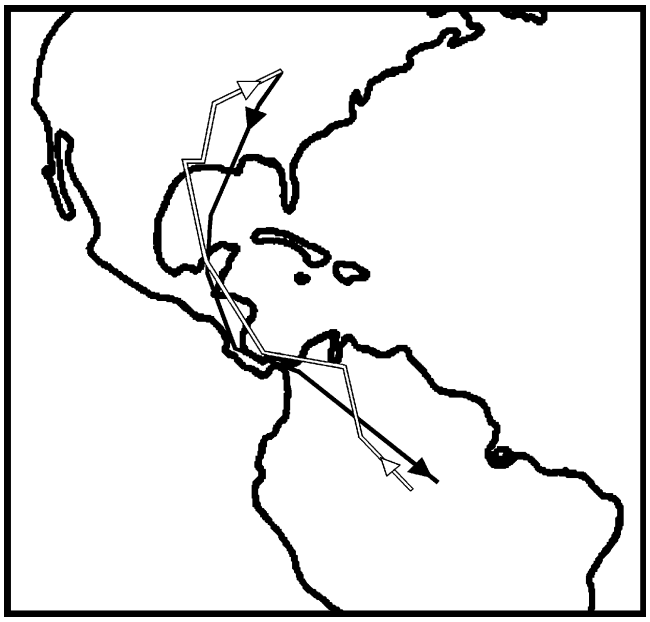


FIGURE 4.45 Long-distance bird flight. The arrows show the flight path of a purple martin (*Progne subis*) that migrated from Pennsylvania to South America in the fall (black arrows) and returned in the spring (white arrows). (Data from Stutchbury, B.J. et al., *Science*, 323, 896, 2009.)

If you spend some time outdoors, you may have observed the phenomenon of *animal migration* around winter time.[308–322] For example, if you live in the northern hemisphere, you may have observed birds flying south in the fall and flying north in the spring. The diagram shown in Figure 4.45 depicts the path flown by a purple martin (*Progne subis*) that bred in Pennsylvania, flew to the Amazon in the fall, and returned to Pennsylvania in the spring—a 15,000 km trip![321] Even more amazing is a leatherback sea turtle (*Dermochelys coriacea*), which was outfitted with a tracking device in Indonesia and swam all the way to California, then to Hawaii, and then back to California, for a total of 20,558 km![323]

Humans engage in seasonal migration too. For instance, Niger (in West Africa) has a predominantly agricultural economy that triggers seasonal relocation of farm workers to low-density agricultural areas during the rainy season and to high-density urban areas during the dry season.[324] In areas less dependent on agriculture, a different type of migration is very common: residents of the United States make 657 million

long-distance summer trips during the 16-week period between Memorial Day weekend and Labor Day weekend each year.[325] Considering that the whole population of the United States is less than 400 million, it is clear that, on average, everyone makes at least one long-distance summer trip each year.

Migration is a complex phenomenon, but its main cause (in evolutionary terms) is likely the depletion of food resources in high-latitude zones in the winter. Animals that do not migrate must deal with the shortage of food and the low environmental temperatures of the winter, and we will now discuss in some detail a number of physiological parameters that exhibit seasonal rhythmicity: body mass, cold-induced thermogenesis, food intake, heterothermy, melatonin secretion, molting, and reproductive capacity.

Figure 4.46 shows the records of average body mass of five European hamsters (*Cricetus cricetus*) housed in an outdoor enclosure in Germany for 2½ years. Notice that the animals regularly gained weight in the summer and lost weight in the winter. Body mass was considerably lower during the first 6 months because the hamsters were juveniles not yet fully grown, but the rhythmic pattern was already evident then. Seasonal changes of body mass in animals maintained under natural or simulated environmental cycles have been recorded in birds,[326–331] rodents,[289,332–352] and other animals.[353–360]

The most obvious feature of the winter is the cold weather, which transforms aqueous precipitation from rain to snow. Although we will not discuss the process of thermoregulation in detail until Chapter 10, it should be obvious that warm-blooded animals (mainly birds and mammals) must be able to produce more body heat during the winter than during the summer, so that they can counteract the environmental cold (Figure 4.47). At any time of the year, birds and mammals increase their metabolic rate when exposed to lower temperatures, but their ability to do so is enhanced by winter *acclimatization*.[361–363] Consider Figure 4.48, which shows the mean metabolic rates of wild rats (*R. norvegicus*) captured in Canada during the summer (mean ambient temperature: 20°C) and the winter (mean ambient temperature: –6°C) and tested at various environmental temperatures. Notice that the metabolic rates of the two groups of rats are very similar when the animals are tested between 0°C and 20°C, but that winter-acclimatized rats have greater thermogenic (i.e., heat-producing) capacity, as demonstrated by the higher metabolic rates at test temperatures of –20°C and below. At –40°C, the metabolic rate of

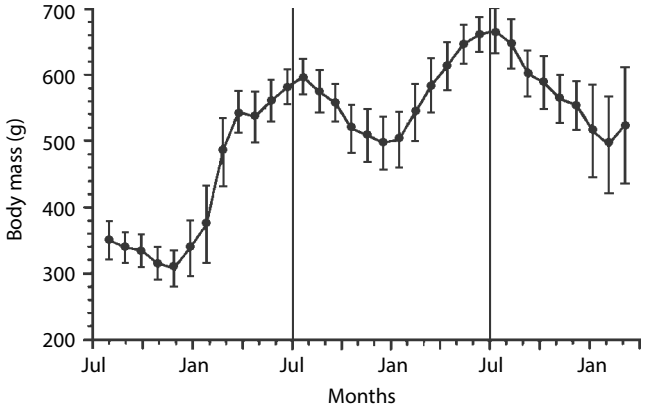


FIGURE 4.46 Gain and lose weight. Many animals gain weight in the summer and lose weight in the winter. This graph shows the mean variation in body mass (± SE) of five European hamsters (*Cricetus cricetus*) maintained in an outdoor enclosure in Germany for 3 years. (Data from Wollnik, F. and Schmidt, B., *J. Comp. Physiol. B*, 165, 171, 1995.)



FIGURE 4.47 A cold wolf. As the temperature of the environment falls in the winter, even well-insulated animals must increase their metabolic rate in order to maintain their body temperature at the normal level. (Courtesy of Yellowstone National Park Wildlife Graphics, United States National Park Service, Washington, DC.)

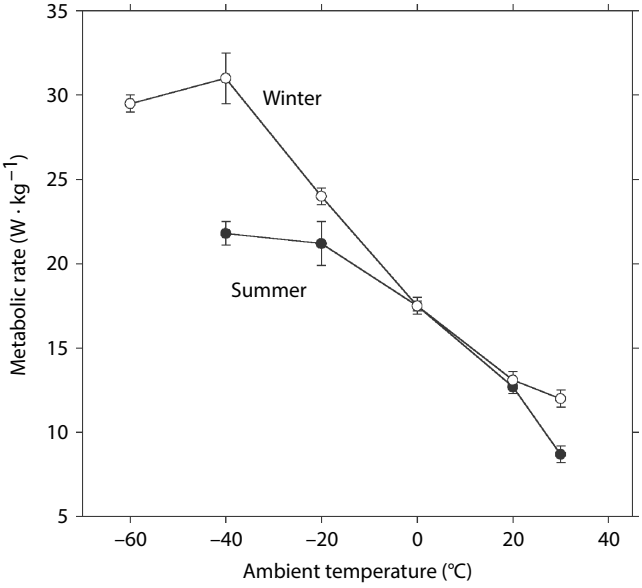


FIGURE 4.48 Compensating for the cold of winter. The graph shows the metabolic rates of wild rats (*Rattus norvegicus*) captured in Canada in the summer and in the winter and tested at different ambient temperatures. Winter-acclimatized rats show greater heat-producing capacity (cold-induced thermogenesis) at ambient temperatures below –10°C (14°F). Each data point corresponds to the mean (± SE) of approximately 8 animals. (Data from Hart, J.S. and Heroux, O., *Canad. J. Zool.*, 41, 711, 1963.)

winter-acclimatized rats is 40% higher than that of summer-acclimatized rats. Thus, the capacity for *cold-induced thermogenesis* oscillates with the seasons. The process of metabolic acclimatization seems to be less effective in birds than in mammals,[364] but it has been reported in both classes.[365–370]

As previously pointed out in Section 4.1, winter is characterized not only by low environmental temperature but also by "short days." That is, the *photoperiod* of winter days is shorter than that of summer days. Therefore, it is natural to wonder whether winter acclimatization is caused by the low temperature or by the short photoperiod. One way to investigate the issue is to keep the animals in the laboratory with a constant photoperiod and to vary only the adaptation temperature (thus producing *acclimation* but not full *acclimatization*). Figure 4.49 shows the mean metabolic rates of domestic mice (*M. musculus*) acclimated to the cold (4°C) or to a neutral environment (26°C) for four weeks before being tested at various temperatures. Both groups show an elevation of metabolic rate at low test temperatures, and the elevation is greater in the cold-adapted group than in the control group. Thus, acclimation alone (i.e., without the effect of photoperiod) can produce an increase in cold-induced thermogenesis. Similar results have been obtained in various studies in rats,[371–376] birds,[377–380] and other animals.[381–385] However, notice in Figure 4.49 that the effect of cold acclimation is rather modest when compared to the effect of test temperature. For instance, the metabolic rate of the cold-acclimated group is only 16% higher than that of the control group at –4°C, whereas metabolic rate is about 300% higher at –4°C than at 26°C in both groups. Thus, the effect of cold acclimation is relatively small and may account for only a fraction of the overall effect of winter acclimatization. To verify this, animals may be kept under a constant ambient temperature and be subjected to varying photoperiods. Although some studies based on this approach revealed an effect of photoperiod alone,[347,368,386,387] others did not.[338,388,389]

To better access the roles of acclimation and photoperiod in the seasonal variation of cold-induced thermogenesis, several investigators compared the effects of photoperiod alone with the effects of photoperiod combined with cold acclimation. The results of a study on Siberian hamsters (*Phodopus sungorus*) are shown in Figure 4.50. The first thing to notice is that these animals have the ability to increase heat production fivefold (from about 10 to 50 $W \cdot kg^{-1}$) without any seasonal adaptation. The next thing to notice is that the effect of seasonal variation in thermogenic capacity (a rise of about 20 $W \cdot kg^{-1}$ from summer to winter) is modest in comparison to the effect of acute exposure to cold (a rise of about 60 $W \cdot kg^{-1}$ from basal metabolic rate). Importantly, it should be noticed that there is a seasonal variation in cold-induced

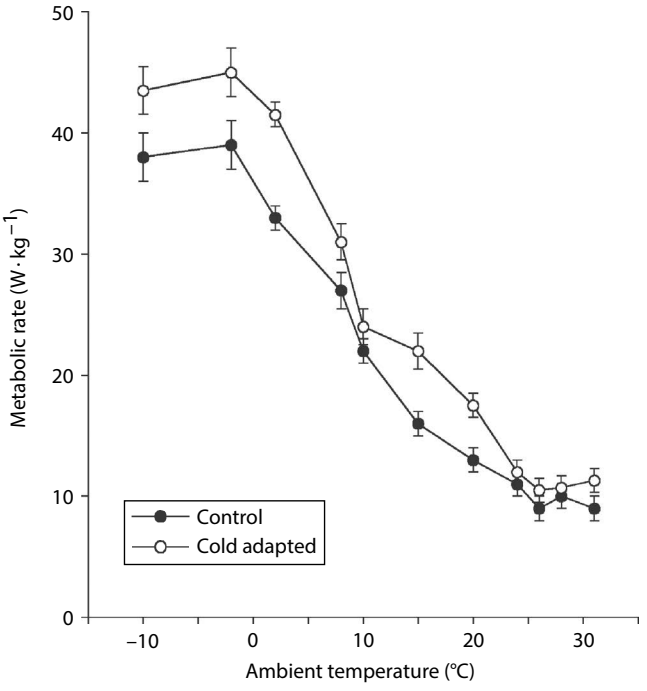


FIGURE 4.49 Isolating the effect of ambient temperature. The graph shows the metabolic rates of domestic mice (*M. musculus*) maintained in the laboratory under cold (4°C) or neutral (26°C) conditions for four weeks and then tested at different ambient temperatures. Although cold-adapted (cold-acclimated) mice exhibit higher metabolic rates than control mice at most test temperatures, the difference is small when compared to the effect of the test temperature itself. Each data point corresponds to the mean (± SE) of approximately 8 animals. (Data from Oufara, S. et al., *Am. J. Physiol*., 253, R39, 1987.)

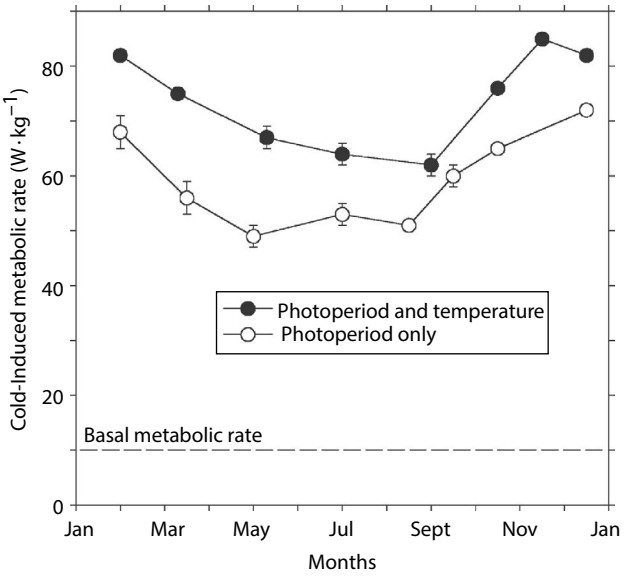


FIGURE 4.50 Isolating the effect of photoperiod. The graph shows the maximal cold-induced metabolic rate (the metabolic rate obtained at the lowest ambient temperature at which the animals could prevent a fall in body temperature) of Siberian hamsters (*Phodopus sungorus*) kept either outdoors in Germany or indoors at thermoneutrality (23°C) under a natural photoperiod matching the outdoor's photoperiod. Notice that, although the two groups exhibit similar seasonal variations in cold-induced thermogenesis, hamsters exposed to variations in both photoperiod and temperature exhibit higher metabolic rates than hamsters exposed only to variations in photoperiod. Each data point corresponds to the mean (± SE) of 9–18 animals. The horizontal dashed line indicates the mean metabolic rate at thermoneutrality. (Data from Heldmaier, G. et al., *J. Comp. Physiol. B*, 149, 1, 1982.)

thermogenesis in animals that were adapted only to photoperiodic variation (open circles), although the absolute values are higher in animals adapted to variation in both photoperiod and ambient temperature (closed circles). Thus, the occurrence of seasonal variation in cold-induced thermogenesis can be explained by the variation in photoperiod alone, but the full effect of acclimatization requires variations in ambient temperature as well. Considering that the range of seasonal oscillation was about 20 $W \cdot kg^{-1}$ in both groups and that the fully acclimatized group was about 15 $W \cdot kg^{-1}$ above the group adapted only to photoperiod, one might conclude that temperature adaptation accounts for a slightly smaller proportion of the full seasonal variation in thermogenic capacity than photoperiod does. Although the exact proportion of the two environmental factors varied in other studies conducted on Siberian hamsters,[390–392] rats,[393] deer mice,[394] and pouched mice,[395] it is clear that both variables can affect cold-induced thermogenesis. A study on collard lemmings failed to identify an effect of either temperature or photoperiod,[335] which may reflect simply species differences or differences in research methods. Many more studies in these and other species are needed before a solid conclusion can be reached. At this time, the only possible generalization is that, in most animals, acclimatization of cold-induced thermogenesis is attained partially by seasonal fluctuations in ambient temperature and partially by seasonal fluctuations in photoperiod.

Eating is an essential activity for all animals (Figure 4.51). Although food must be ingested all year-round, animals exposed to natural seasonal fluctuations in the environment



FIGURE 4.51 A hungry prairie dog. As the temperature of the environment falls in the winter, homeothermic animals must increase their intake of food in order to maintain the high metabolic rate necessary for the preservation of normal body temperature. (Copyright ArtToday, Tucson, AZ.)

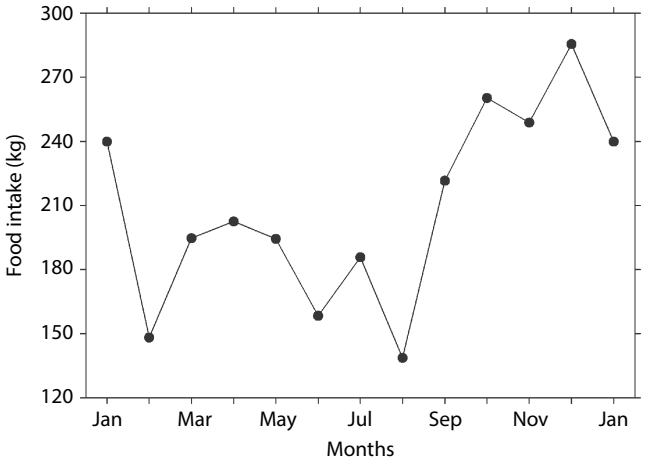


FIGURE 4.52 Burn your food. As shown by these records of food intake of a South African fur seal (*Arctocephalus pusillus*) kept outdoors throughout the year at the Toronto Zoo (in Canada), many animals consume more food during the winter than during the summer. (Data from Shearer, D.S. et al., *Proceedings of the First Annual Conference of the Nutrition Advisory Group of the American Zoo and Aquarium Association*, Vol. 1, pp. 213–217, 1995.)

exhibit seasonal fluctuations in food intake.[341,353,360,396–400] For example, Figure 4.52 shows the annual variation of food intake of a South African fur seal (*Arctocephalus pusillus*) housed outdoors in the Toronto Zoo (in Canada). Although the data show some small oscillations due to normal biological noise, it is clear that the seal ingests more food in the colder months (October through January) than during the warmer months. As was the case for cold-induced thermogenesis, photoperiod and ambient temperature are the two most likely environmental signals affecting food intake. Regarding ambient temperature, we earlier saw that exposure to a cold environment evokes cold-induced thermogenesis in mammals and birds. Thus, more energy is expended—and more food must be ingested—when ambient temperature is low. This specific effect of ambient temperature is illustrated in Figure 4.53. Groups of laboratory rats (*R. norvegicus*) were housed at different ambient temperatures under the same photoperiod, and their daily food intake was measured. The figure clearly shows that more food was consumed when the ambient temperature was lower. Increased food intake at lower ambient temperature has been documented in a large number of studies in rats[401–406] and other species.[407–412]

So, temperature alone might explain the seasonal variation in food intake. However, the situation is more complicated. Studies conducted under natural seasonal conditions or under simulated photoperiods did not agree about when the increase in food intake takes place. In some studies, food intake was greater during the winter (or during the short photoperiod),[359,396] but in most other studies, food intake was greater during the summer (or during the long photoperiod).[333,341,344,345,353,355,360,397,398,413] Of course, ambient temperature can be a stimulus for increased food intake only for animals that increase their food intake in the winter.

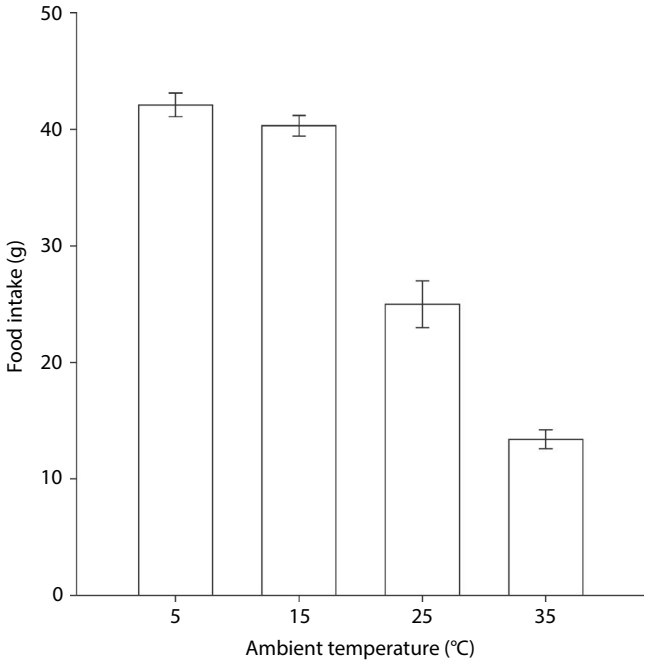


FIGURE 4.53 Isolating the effect of ambient temperature. This graph shows the mean food intake of groups of laboratory rats kept under identical photoperiods but different ambient temperatures. Food intake is greater at lower temperatures. Each bar corresponds to the mean (± SE) of 10 rats. (Data from Witty, R.T. and Long, J.F., *Am. J. Physiol.*, 219, 1359, 1970.)

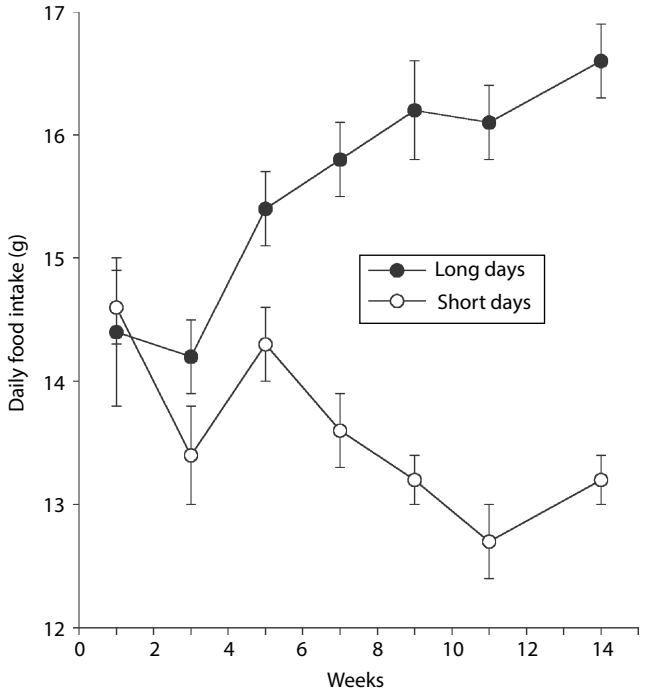


FIGURE 4.54 Isolating the effect of photoperiod. This graph shows the mean daily food intake of two groups of laboratory rats maintained under the same ambient temperature (23°C) but different photoperiods (short days, 8 hours of light per day; long days, 16 hours of light per day) for 14 consecutive weeks. Food intake is greatly affected by photoperiod, even though rats are not particularly seasonal animals. Each data point is the mean (± SE) of 10 or 11 rats. (Data from Shoemaker, M.B. and Heideman, P.D., *BMC Physiol.*, 2, art. 11, 2002.)

For all other animals that show seasonal variation in ingestive behavior, photoperiod must be the predominant stimulus. One might think that only animals that require large energetic reserves for winter migration or hibernation would need to increase food intake during the summer and autumn, but this is not true. As shown in Figure 4.54, for instance, even laboratory rats—which do not migrate or hibernate—ingest more food under a long photoperiod than under a short photoperiod. Many more laboratory studies involving separate and combined manipulation of ambient temperature and photoperiod in a variety of species are necessary for a systematic quantification of the relative roles of the two variables in the control of food intake. Of course, reduction of food intake in the winter could be a simple consequence of seasonal variations in food availability. However, in most studies, seasonal variation in intake was observed even when food was provided in abundance. Thus, the reduction of food intake in the winter must result from elaborate physiological adjustments made in response to the environmental changes (or in response to an endogenous process that anticipates the environmental changes, as will be discussed later). Food availability may be an *ultimate* cause of the variation in food intake (in the sense that the response is an adaptive strategy to deal with the predictable shortage of food in the winter), but it is not necessarily a *proximal* cause of the seasonal variation in food intake.

Another parameter of seasonal rhythmicity that we must discuss is *heterothermy*. The term *heterothermy* refers to the partial blockade of thermoregulatory processes during winter *hibernation* or summer *estivation*.[414] Hibernators include ground squirrels[332,351,415–430] (Figure 4.55), hamsters,[337,431–439] chipmunks,[440–442] dormice,[443,444] bats,[445–447] marmots,[344,448] hedgehogs,[449,450] prairie dogs,[341] some marsupials,[451,452] monotremes,[453] and even primitive primates.[454] Bears experience prolonged sleep episodes in the winter but do not exhibit the drastic reduction in



FIGURE 4.55 Master hibernator. Various animals from temperate regions, such as the ground squirrel, use hibernation as a mechanism to deal with the energetic challenges of the winter. (Courtesy of National Image Library, United States Fish and Wildlife Service, Washington, DC.)

metabolic rate and body temperature of true hibernators.[455–457] Estivation is most common in invertebrates and lower vertebrates but has been described in mammals as well.[443,458]

As will be discussed in Chapter 10, some species of mammals and birds experience brief episodes of low body temperature that last only a few hours each day and that are usually classified as a form of *daily torpor* as opposed to actual hibernation.[459] Arthropods (such as insects, crustaceans, and arachnids) have their own process of seasonal inactivity, although, of course, they are cold blooded all year-round. The period of inactivity in arthropods during which growth stops (called *diapause*) is under the control of both ambient temperature and photoperiod.[460–465] Some plants also exhibit seasonal inactivity, as illustrated by the *Hosta plantaginea* plant shown in Figure 4.56. The seasonal changes in this plant are evident, but, what the figure does not show is that the same pattern can be observed year after year, thus confirming the rhythmicity of the phenomenon. At least one species of fish that lives in Antarctic waters (black rockcod, *Notothenia coriiceps*) exhibits a mechanism of metabolic suppression that resembles hibernation in mammals and birds.[466]

The hibernation pattern of a hedgehog (*Erinaceus europaeus*) is illustrated in Figure 4.57. The animal was housed under natural photoperiod and natural ambient temperature in France. Notice that body temperature, which had been relatively constant at 37°C during the summer, exhibited many prolonged falls during the autumn and winter. In November and December, when ambient temperature fell below 0°C, body temperature went down to just a few degrees above freezing. Also notice that the duration of the hypothermic bouts was longer during the colder months. Except during the interbout intervals of euthermia, the animal remained still, as if sleeping. One aspect of these data that is atypical is the early entry into hibernation (late August) and early arousal (early January). In this respect, the hibernation pattern of a European ground squirrel (*Spermophilus citellus*) shown in Figure 4.58 is more typical. This animal was also housed under natural photoperiod and natural ambient temperature (in the Netherlands) and exhibited many hypothermic bouts, which were longer during the colder months. The first bout occurred in late September, and the last one in late March. Not noticeable in the scales used in this figure is the fact that the body temperature of squirrels of various species often exhibits a slow, modest decline for several days before the first torpor bout of the hibernation season.[429,467,468]

Studies on various species of hibernators have shown that, although low ambient temperature and lack of food may facilitate entry into hibernation, photoperiod is the main environmental factor controlling the timing of hibernation.[469,470] Most investigators agree that an animal entering hibernation is not simply a chilled warm-blooded animal. Instead, it is believed that hibernation is a *regulated* state—that is, a state of intended low metabolism that allows body temperature to fall along with ambient temperature.[469–477] Figure 4.59 shows the records of body temperature and metabolism of a hibernating squirrel during a brief episode of arousal and return to torpor. It can be noticed that, when the animal re-enters torpor, the fall in body temperature lags behind the fall in metabolism, thus confirming the regulatory nature of the process.[478] We will return to this matter in Chapter 10.

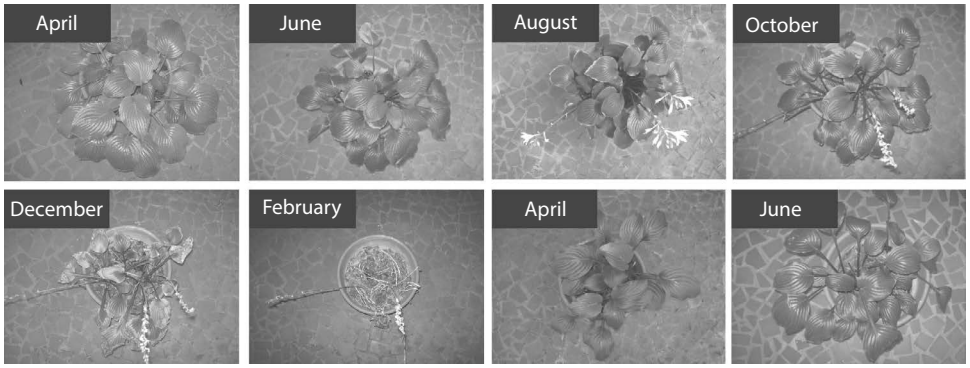


FIGURE 4.56 Seasonal plant. This specimen of *Hosta plantaginea* living at 33°N grows new leaves in April, flowers in August, becomes dormant in December, and restarts the yearly cycle in April. (Photo and montage by R. Refinetti.)

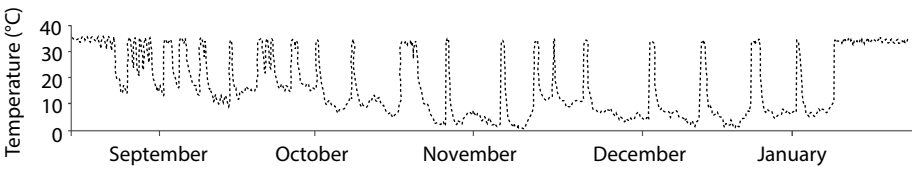


FIGURE 4.57 Hedgehog sleeps through the winter. The records of body temperature of a hedgehog (*Erinaceus europaeus*) maintained under natural conditions of illumination and ambient temperature in France show a clear pattern of hibernation during the fall and early winter. Brief periods of normothermia are interspersed with longer periods of deep hypothermia. (Data from Saboureau, M. et al., *Comp. Rend. Acad. Sci. Paris*, 313(3), 93, 1991.)

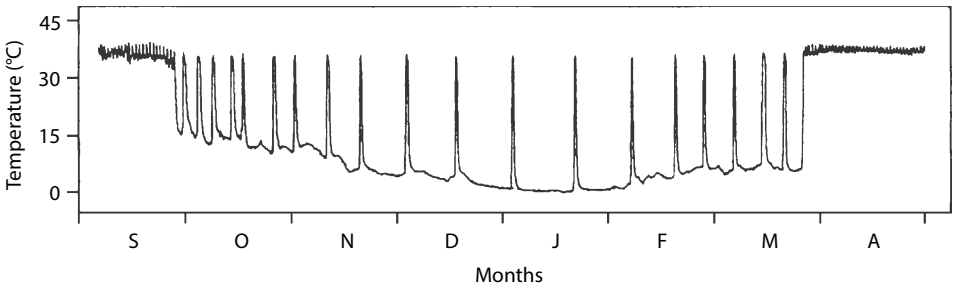


FIGURE 4.58 Squirrel sleeps through the winter. The records of body temperature of a European ground squirrel (*Spermophilus citellus*) maintained under natural conditions of illumination and ambient temperature in Holland show a clear pattern of hibernation throughout the fall and winter. Brief periods of normothermia are interspersed with longer periods of deep hypothermia. (Data from Hut, R.A. et al., *J. Comp. Physiol. B*, 172, 47, 2002.)

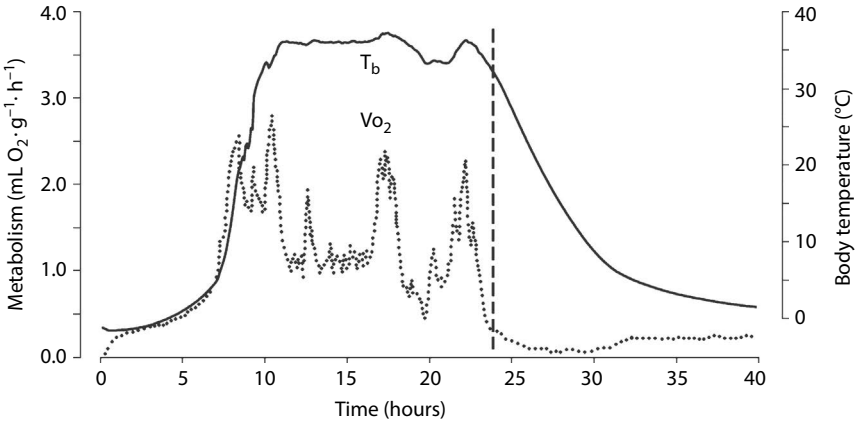


FIGURE 4.59 Back to sleep. The graph shows the records of body temperature and metabolism of a hibernating Arctic ground squirrel during a brief episode of arousal and return to torpor. (Data from Karpovich, S.A. et al., *J. Comp. Physiol. B*, 179, 691, 2009.)

Although all mammals and birds can sense the seasonal variation in photoperiod, most of them cannot hibernate and cannot tolerate the low body temperature associated with hibernation. Thus, scientists have always wondered what special physiological mechanism allows hibernators to hibernate. There are certainly special features developed over evolution, but what is the immediate signal for initiation of torpor bouts? Recently, a research team analyzed 231 metabolites in the blood of ground squirrels at 7 different stages of the circannual cycle and identified a pattern of metabolites that accumulate (or deplete) during torpor and are restored during interbout arousal,[479] which could explain why hibernators undergo several cycles of torpor and arousal instead of remaining in deep torpor throughout the winter. A separate team of researchers isolated and tested a protein complex (which they called *hibernation-specific protein*) that may be the chemical signal for initiation of torpor.[442] A third research team provided suggestive evidence that the sleep-related substance *adenosine* may be the actual chemical signal.[480]

Red deer (*Cervus elaphus*) do not hibernate but do exhibit a seasonal variation in subcutaneous temperature, as illustrated by the combined data for nine deer kept outdoors in the Slovak Republic (Figure 4.60). Subcutaneous temperature is more dependent on ambient temperature than core temperature is, but the seasonal variation in subcutaneous temperature in this case is not a mere reflex of the seasonal variation in ambient temperature because the lowest values of subcutaneous temperature are observed in March, 2 months after the lowest values of ambient temperature.[481] Notice that the return of values of subcutaneous temperature to a higher level takes place at approximately the same time as the return of food availability (as indicated by the energy content of ingested food). Presumably, the fall in subcutaneous temperature is a consequence of a controlled hypometabolic state designed to save energy during the winter.

Hormone secretion is yet another physiological parameter that exhibits seasonal rhythmicity. Although seasonal rhythmicity in the secretion of various hormones has been documented in many species,[344,355,357,397,482–485] the hormone *melatonin* (not to be confused with the skin pigment *melanin*) has received special attention because it seems to be the link between photoperiod and the various seasonal rhythms in the body. Melatonin (5-methoxy-*N*-acetyltryptamine) was first isolated in 1958.[486] Its structural formula is shown in Figure 4.61. In vertebrates, melatonin is synthesized mainly in the pineal gland—but also in the eyes—and is secreted into the general circulation. The secretion shows seasonal rhythmicity,[487–495] as exemplified in Figure 4.62. The data refer to serum melatonin concentration in goats housed indoors under a light–dark cycle matching the outdoor photoperiod in

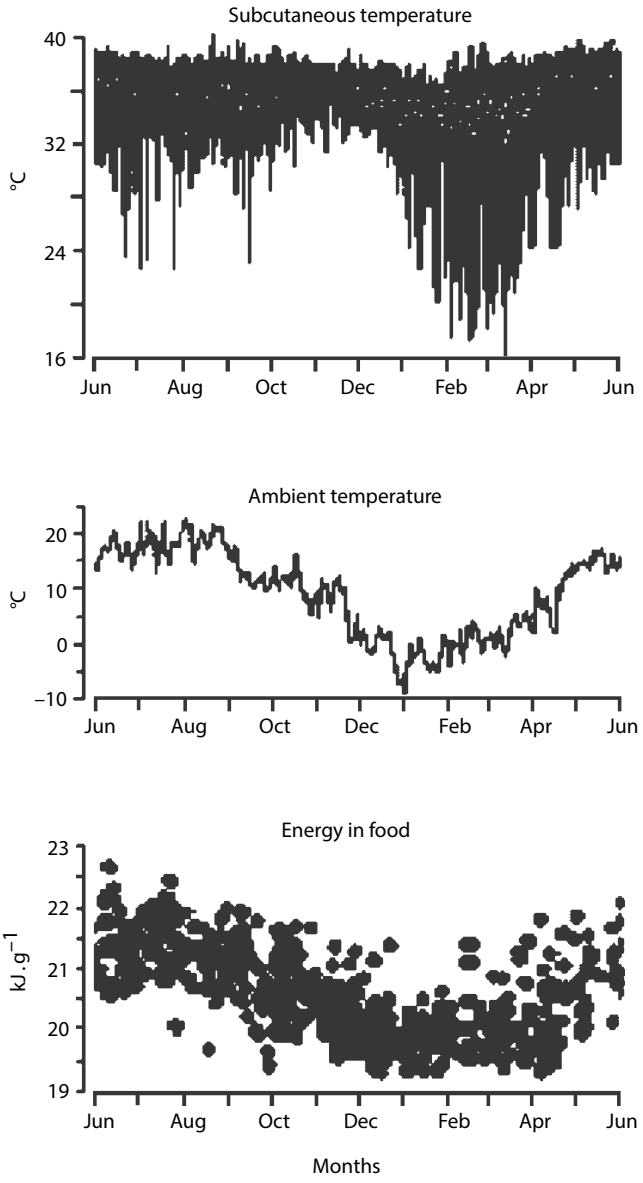


FIGURE 4.60 Deer gets cold in the winter. The red deer (*Cervus elaphus*) does not hibernate, but its subcutaneous temperature is lower during the winter. Notice that the lowest level of subcutaneous temperature is achieved 2 months after the lowest ambient temperature in this animal kept outdoors in the Slovak Republic. Thus, the fall in subcutaneous temperature is not merely a reflection of the fall in ambient temperature but a regulated process due perhaps to the lower energy content of food. (Data from Arnold, W. et al., *Am. J. Physiol.*, 286, R174, 2004.)

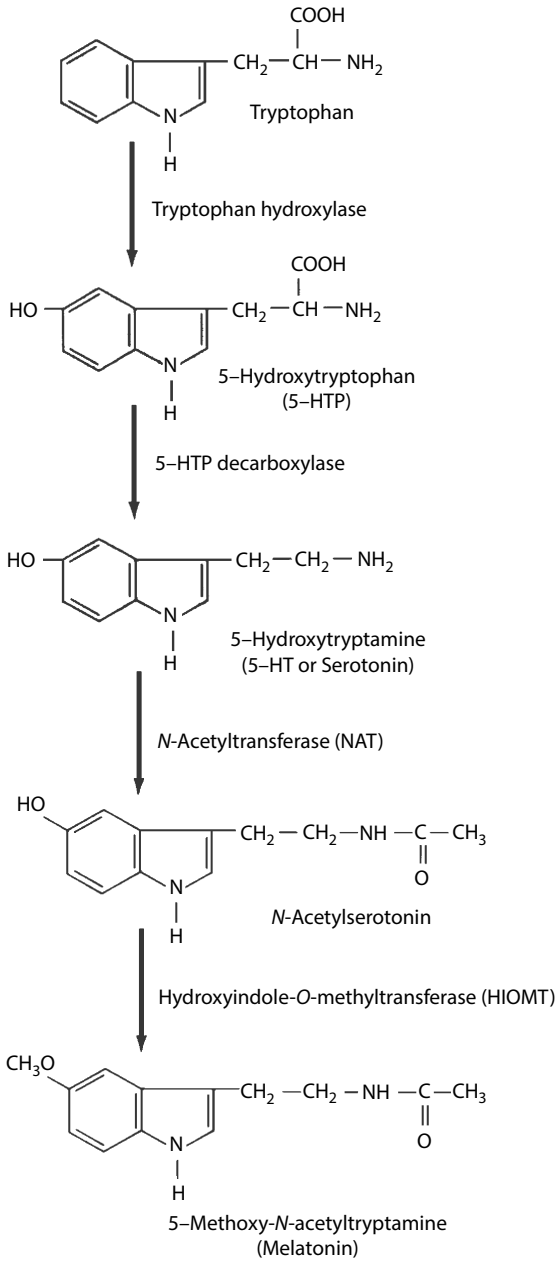


FIGURE 4.61 Making melatonin. Shown are the steps in the synthesis of melatonin from dietary tryptophan. The enzymes involved in the reactions are listed next to the arrows. Some reactions require cofactors that are not shown. (Adapted from Feldman, R.S. and Quenzer, L.F., *Fundamentals of Neuropsychopharmacology*, Sinauer, Sunderland, MA, 1984.)

Finland. Notice that melatonin secretion is less intense in the summer than in the winter.

The phenomenon of *photoperiodism*—that is, the modulation of physiological processes by changes in photoperiod—has been extensively studied in plants since the early twentieth century.[496] In vertebrate animals, melatonin plays a central role in communicating photoperiodic information to the various organs in the body.[497] As will be discussed in Chapter 11, melatonin secretion is inhibited by light (perceived through the eyes in mammals) and has a short half-life, so that melatonin is present in the blood for a shorter interval under long photoperiods (i.e., summer) than under short photoperiods (i.e., winter). The short or long melatonin signal then acts on specialized brain centers or directly on the appropriate organs. This mechanism is actually more complicated because, in many species, melatonin secretion is also under circadian control, and the circadian system itself is responsive to light. We shall return to this topic after we have discussed the properties of the circadian system in future chapters.

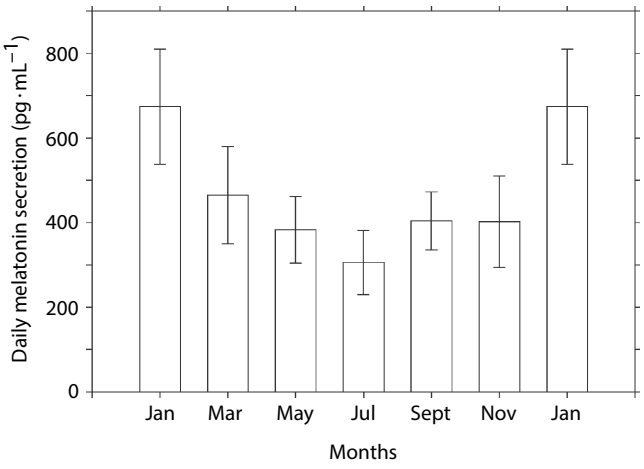


FIGURE 4.62 Finnish goats. The graph shows the mean daily melatonin secretion (measured in the serum) of goats housed indoors with a variable light–dark cycle matching the outdoor photoperiod in Finland throughout the year. An annual rhythm of melatonin secretion is clearly seen. Each bar corresponds to the mean (± SE) of 7 goats. (Data from Alila-Johansson, A. et al., *J. Biol. Rhythms*, 16, 254, 2001.)

Two other physiological parameters that exhibit photoperiodism are *molting* and *reproductive capacity*. Many species undergo a seasonal change in pelage,[328,346,369,498–507] as exemplified by the change in fur color in Siberian hamsters (Figure 4.63). Seasonal changes in reproductive capacity have been particularly well studied, probably because of the fundamental role that reproduction plays in the preservation of species. Some organisms reproduce all year-round (within the constraints of the estrous cycle), whereas others reproduce only under short photoperiods, and still others only under long photoperiods. Photoperiodic reproduction has been described in plants,[508–511] invertebrates,[462,465,512] lower vertebrates,[250,513–516] birds,[328,517–519] and many mammalian species, including Siberian hamsters,[289,504,520–523] Syrian hamsters,[138,140,215,432,524–529] other rodents,[333,334,337,339,342,345,347–349,351,436,530–535] and other mammals.[148,150,161,352,357,483,506,536–542] Deer (Figure 4.64) are short-day breeders, whereas dormice (a species of small nocturnal rodents) are long-day breeders. Figure 4.65 shows that plasma testosterone levels in male dormice are low during much of the year but rise sharply in the summer.

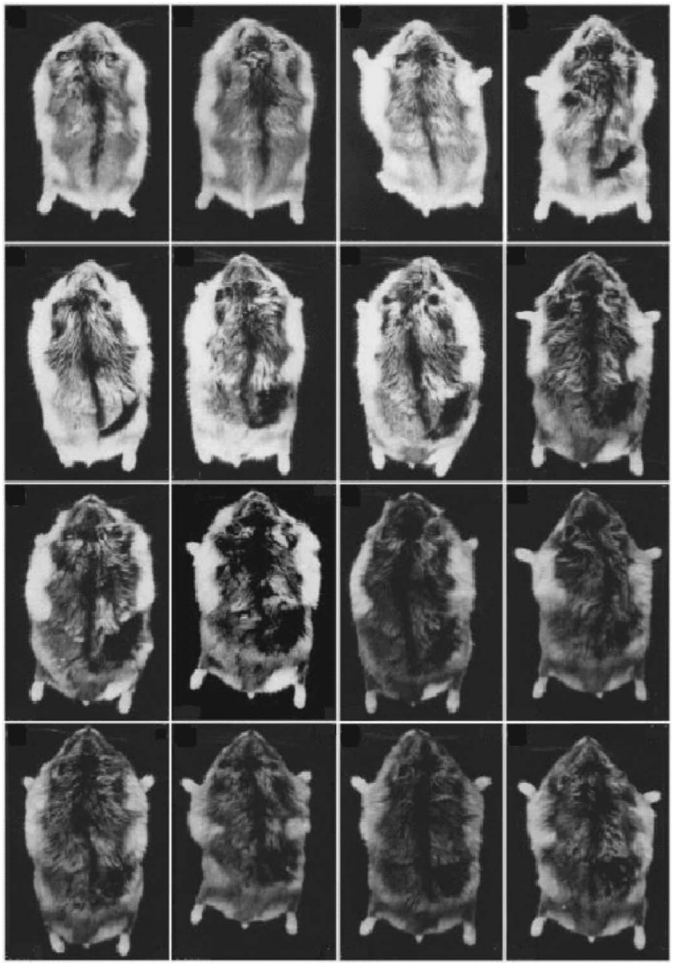


FIGURE 4.63 Winter white. The Siberian hamster (*Phodopus sungorus*) exhibits an annual rhythm of pelage coloration, as shown in this series of photographs. The sequence is left to right and then top to bottom. The pelage is light in the winter (top left) and dark in the summer (bottom right). (From Kuhlmann, M.T. et al.: Molting in the Djungarian hamster (*Phodopus sungorus* Pallas): Seasonal or continuous process? *Journal of Experimental Zoology*. 2003. 295A, 160–171. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reprinted with permission.)



FIGURE 4.64 White-tail deer. Several animal species, including the deer, undergo an annual cycle of reproductive capability. (Photo by Scott Bauer; Agricultural Research Service, United States Department of Agriculture, Washington, DC.)

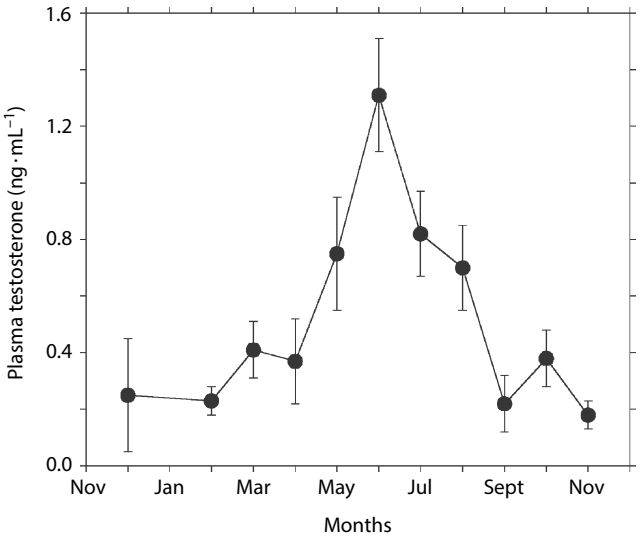


FIGURE 4.65 The season for sex. In males of many species, the production of testosterone varies with the seasons. In fat dormice (*Glis glis*) kept indoors but subjected to the natural variation in photoperiod and ambient temperature in France, testosterone secretion peaks in the early summer. Each data point corresponds to the mean (± SE) of 7 dormice. (Data from Jallageas, M. et al., *J. Biol. Rhythms*, 6, 343, 1991.)

In some cases, the reproductive season is so short, that it is practically impossible to distinguish estrous rhythmicity from annual reproductive rhythmicity. For example, Magellanic penguins (*Spheniscus magellanicus*) lay a single clutch of two eggs per year during the spring.[543] Should we call this a long (1 year) estrous cycle with no seasonal variation or, instead, a brief estrous cycle limited to a short reproductive season? In humans, the reproductive "season" lasts for the whole year (i.e., humans are capable of reproducing all year-round). Nevertheless, the frequency of human conception and birth exhibits a small seasonal variation.[233,544–548] Also, adolescent girls reach menarche (i.e., the first menstrual cycle) more often in January and July than in other months of the year.[549]

4.4.2 Circannual Rhythms

Because seasons have existed for millions of years, some species have evolved endogenous circannual rhythmicity as an adaptive mechanism to react in advance to the regular environmental changes associated with the seasons. Some authors refer to these endogenously generated rhythms as Type II rhythms, in contrast to Type I rhythms that require the presence of seasonal environmental cues.[550] It should be pointed out, however, that both types of rhythms are affected by environmental cycles. Type I rhythms are *evoked* by environmental cycles, whereas Type II rhythms are *synchronized* to environmental cycles. The anatomical location of the circannual pacemaker is yet to be identified,[470,551,552] although the pituitary gland and the hypothalamus are likely candidates.[553,554]

In order to demonstrate that a seasonal rhythm is a circannual rhythm, one must show that the rhythm freeruns under constant conditions—and that it does so with a period at least slightly different from the period of the environmental cycle to which it is usually synchronized. This means that the organism must be studied for many consecutive years until enough data are available for appropriate analysis. Consequently, it is not surprising that very few studies have demonstrated the existence of circannual rhythms. Still, several variables have been adequately studied in various species of birds[331,555–559] and mammals.[148,334,336,342,442,507,534,540,560–563] An example is given in Figure 4.66. A chipmunk (*Tamias striatus*) was maintained in the laboratory for 6 years at a constant environmental temperature and in constant darkness (caused by surgical blinding). A clear annual rhythm of food intake can be observed. Notice that there are 7 cycles in 6 years, which implies that the free-running period is approximately 10 months (and, therefore, is significantly shorter than the 12 months of a calendar year). Since there is no reason to suspect the existence of an undetected environmental cycle with period of 10 months, it is reasonable to assume that the 10-month periodicity is endogenously generated.

Figure 4.67 shows the circannual rhythm of body mass of a golden-mantled ground squirrel (*Spermophilus lateralis*). This squirrel was housed indoors under a simulated natural photoperiod, but the animal did not actually respond to the photoperiod because its pineal gland (the main source of melatonin) was surgically removed prior to the study. A clear rhythm of body mass can be observed. Since almost 5 full cycles are completed in 4 years, the free-running rhythm has a period of 9–10 months. A third example is shown in Figure 4.68. The testicular size (testis width) of a tropical bird (the stonechat, *Saxicola torquata*) was measured for over 7 consecutive years (although only 6 years are shown), while the bird was housed under constant ambient temperature and constant photoperiod with 13 hours of light and 11 hours of darkness per day. Notice the presence of 7 peaks of testicular width in 6 years, which indicates a free-running period of 10 months.

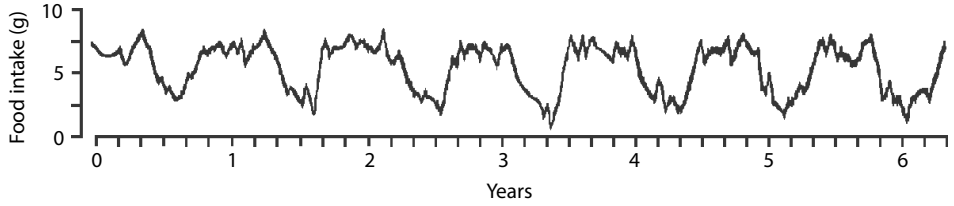


FIGURE 4.66 Circannual rhythm of food intake. In many animals, the annual rhythm of food intake is controlled by an internal clock. This graph shows the variation in food intake of a chipmunk (*Tamias striatus*) maintained in the laboratory for 6 years. Ambient temperature was maintained constant all year-round (24°C), and the animal was blinded to eliminate the influence of photoperiod. Despite the absence of seasonal changes in the environment, a clear rhythm of food intake can be observed. The occurrence of 7 cycles in 6 years implies a free-running period of approximately 10 months. (Data from Richter, C.P., *Proc. Natl. Acad. Sci. USA*, 75, 3517, 1978.)

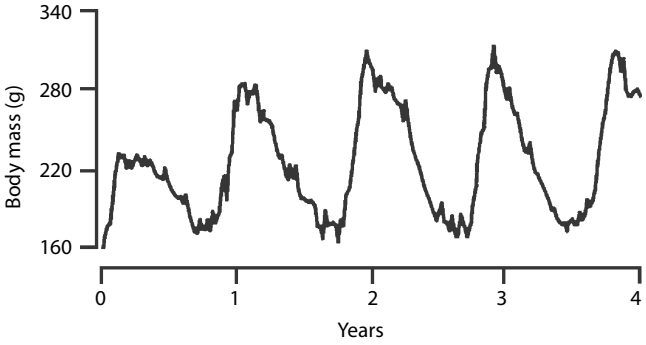


FIGURE 4.67 Circannual rhythm of body weight. In many animals, the annual rhythm of body mass is controlled by an internal clock. This graph shows the variation in body mass of a golden-mantled ground squirrel (*Spermophilus lateralis*) maintained in the laboratory for 4 years. Ambient temperature was maintained constant all year-round (23°C), and the animal was pinealectomized to eliminate the influence of photoperiod. Despite the absence of seasonal cues, a clear rhythm of body mass can be observed. The beginning of Year 0 corresponds to the summer. (Data from Hiebert, S.M. et al., *J. Biol. Rhythms*, 15, 126, 2000.)

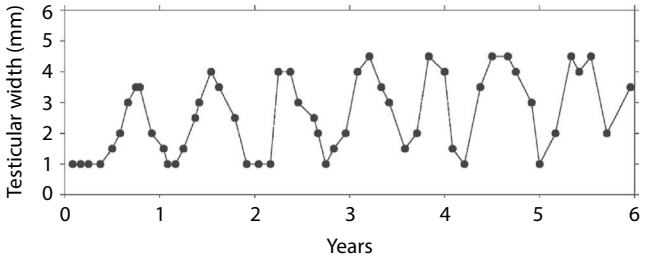


FIGURE 4.68 Circannual rhythm of reproductive capability. In many animals, the annual rhythm of reproductive capability is controlled by an internal clock. This graph shows the variation in testicular size of a tropical bird, the stonechat (*Saxicola torquata axillaris*), maintained in the laboratory for over 6 years. Ambient temperature (20°C) and photoperiod (13 hours of light and 11 hour of darkness per day) were maintained constant throughout the years. Despite the absence of seasonal cues, a clear rhythm of testicular size can be observed. The occurrence of 7 cycles in 6 years implies a free-running period of approximately 10 months. (Data from Gwinner, E. and Dittami, J., *Science*, 249, 906, 1990.)

This concludes our discussion of infradian and ultradian rhythms. Having discussed rhythms with periods longer and shorter than that of circadian rhythms, we are now ready to examine the phenomenology of circadian rhythms in Chapter 5, so that we can later move to Section III of the book, where we will discuss the endogenous and exogenous mechanisms responsible for overt rhythmicity.

SUMMARY

1. Rhythmic oscillations in the environment range in period from a few femtoseconds (10^{-15} seconds) to tens of thousands of years. Of all the environmental rhythms on Earth, only those in four temporal domains have been shown to have specific effects on endogenous rhythms of individual organisms: tidal, daily, lunar, and annual rhythms.
2. Ultradian rhythms are biological rhythms that have periods shorter than circadian rhythms (i.e., shorter than approximately 19 hours). They include cardiac, respiratory, neuroendocrine, gastrointestinal, tidal, and other rhythms. Although many ultradian rhythms are endogenously generated by some sort of pacemaker, only tidal rhythms are regularly synchronized to environmental cycles.
3. Infradian rhythms are biological rhythms that have periods longer than circadian rhythms (i.e., longer than approximately 28 hours). They include estrous, weekly, lunar, annual, and other rhythms. Although many infradian rhythms are endogenously generated by some sort of pacemaker, only lunar and annual rhythms can be fully synchronized to environmental cycles with periods similar to the endogenous periods.
4. Annual rhythms constitute a particularly ubiquitous class of infradian rhythms. Physiological parameters that exhibit annual rhythmicity include body mass, cold-induced thermogenesis, food intake, heterothermy, melatonin secretion, pelage molting, and reproductive capacity. Many, but not all, annual rhythms are endogenously generated and can be synchronized to annual environmental cycles. These rhythms are called *circannual rhythms*.

EXERCISES

4.1 Hamster estrous cycle. The estrous cycle of the female golden hamster is a good example of an infradian rhythm. When maintained under a light–dark cycle with 14 hours of light per day (LD 14:10), the female hamster exhibits a very regular estrous cycle that repeats itself every 4 days. A very convenient variable for monitoring the stages of the cycle is vaginal mucus secretion. For this exercise, you will need one or more female golden hamsters housed individually under LD 14:10. All you need to do is to inspect the vaginal secretion once a day for about 3 weeks. The inspection should be conducted right before lights-off (or during the dark phase under dim red light). Gentle pressure around the vagina may be necessary to cause the secretion to be expelled. The table below will help you identify the stages of the estrous cycle. Don't be discouraged if sometimes you are unable to identify the stage. Remember that you will be conducting inspections every day, so that a few missing data points will not prevent you from seeing the overall pattern. After you have collected data for about three weeks, prepare a simple graph with the stage of the cycle on the ordinate (Y-axis) and days on the abscissa (X-axis). You should be able to observe a 4-day cycle. *Note: Depending on where you live, you may need a permit to conduct this exercise because it involves a vertebrate species (even though no invasive procedures are involved). Check with your local authorities first. If you are a university student, ask your professor about it.*

Stage	Name	Characteristics
1	Estrus	Mucus is relatively thick, opaque, and stringy.
2	Diestrus 1	Mucus hardens into a soft, opaque plug.
3	Diestrus 2	Soft plug becomes hard and waxy but may have fallen off, leaving no conspicuous mucus.
4	Proestrus	Mucus is clear or cloudy, sparse, and not stringy.

4.2 Detecting rhythmicity in a data set. In this exercise, we will use the program **Rhythm** to detect rhythmicity in various data sets. As explained in Sections 3.2 and 3.3, this program uses the chi-square periodogram procedure to evaluate the presence of statistically significant rhythmicity. If you have not done the computer exercises in previous chapters, you should be reminded that you first need to double-click on the **Circadian** icon to open the program banner and then click on **Rhythm** (the fourth icon from the left) to start it.

1. Start the program **Rhythm**.
2. If necessary, switch to the Data subfolder and then select the file **A30** in the *Source File* panel. This file contains the values of mean daily temperature in the city of Chicago during a 4-year interval. You cannot inspect the data using **Plot**, but a graph is provided in Figure 4.69.

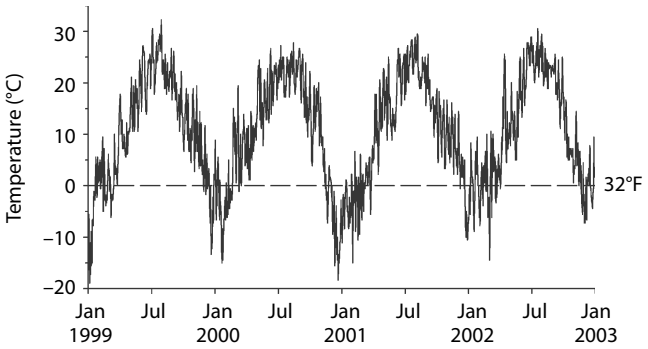


FIGURE 4.69 Ups and downs in Chicago. The graph shows the mean daily temperature in Chicago (IL) from January 1999 to 2003. There is a clear annual rhythm with higher temperatures in the summer and lower temperatures in the winter. (Data courtesy of National Weather Service, United States National Oceanic and Atmospheric Administration.)

3. **Rhythm** requires equally spaced data with no missing points (and no time tags). **A30** complies with these requirements.
4. In the *Data Resolution* panel, set the Bin size to **1** and click on the Days option button (because the file contains data collected once a day).
5. In the *Target Periodicity* panel, set the Period to **12** and click on the Months option button (because inspection of Figure 4.69 clearly suggests the existence of 12-month rhythmicity).
6. Click on *Execute*. The *Results* panel will report that there is a statistically significant 12-month periodicity in the data, as we expected. Details are also reported, including the mean level of the rhythm (10.5°C, or 51°F) and the range of oscillation (51.2°C, or 124°F). Importantly, notice the statement that "no harmonics were detected." Higher-frequency oscillations (such as weekly or monthly) might have been present in the data set even though we only asked the program to look for a yearly oscillation. It is good to know that **Rhythm** is looking after our interests!
7. Checking for lower harmonics is especially important if we ask for the wrong target periodicity. To see this, select the data file **A04** by clicking on it. As we saw in Exercise 3.3, this file contains the records of running-wheel activity of a golden hamster maintained in constant darkness for 29 days. If you don't remember the data set, you may want to open **Plot** and inspect the data in the actogram format before proceeding.
8. Now back to our exercise. You should have already selected the file **A04**. In the *Data Resolution* panel, we need to enter the correct information (i.e., the bin size must be 6 and the unit must be minutes). Just for fun, let us set the *Target Periodicity* to **48** and hours (rather than **24** and hours, or **1** and days, as it would be reasonable to do). Then click on *Execute*.

9. What happened? The program identified significant 48-hour periodicity, but it was smart enough to check also for 24-hour periodicity and to warn us that the significant 48-hour periodicity may be just an artifact. If you think about it, a process that repeats itself every 24 hours is also rhythmic in a 48-hour scale (i.e., it repeats itself exactly twice every 48 hours).
10. So, let us go ahead and change the *Target Periodicity* to **24** hours and click on *Execute* again. This time, the program tells us that there is significant periodicity but that no harmonics were detected. Thus, we can be reasonably confident that the true periodicity is 24 hours (as inspection of the actogram clearly suggested).
11. As a final warning, keep in mind that we looked only for broad periodicity. When **Rhythm** tells us that there is 24-hour periodicity in data set **A04**, it is actually telling us that there is periodicity between about 23 and 25 hours. The exercises in Chapter 5 will deal with programs designed to determine the exact period of a circadian rhythm.

4.3 Detecting rhythmicity in a single cycle. A process that completes a cycle only once is not really a rhythmic process. Thus, determining that a single cycle is indeed cyclic is no assurance of rhythmicity. In the real world, however, it is sometimes the case that we have only one cycle to deal with (and we presumably have a good reason to assume that the cycle would repeat itself if we could observe it). As described in Section 3.3.2, the program **Onecycle** (the third icon from the left in the Circadian banner) provides a simple means to determine the presence of periodicity in a single cycle.

1. Start the program **Onecycle**.
2. The program is very simple and does not even require data files. We can simply type in the values. It is assumed that the data points are equally spaced, but the units of time are irrelevant.
3. Let us say that you measured the growth of a plant once a day for a week and that you noticed that they grew more on Monday and Tuesday than on any other day. Is there a weekly rhythm? Starting on Sunday, you obtained the following measurements of growth (in millimeters): 10, 15, 15, 12, 10, 10, and 10. Type in these numbers and click on *OK*.
4. Perhaps as a surprise to you, the program tells us that the distribution of growth over the days of the week is not significantly different from a random pattern ($D = 0.0627$, $p > 0.05$). Thus, we do not even have to wonder whether the one-cycle periodicity reflects a real periodicity. In this case, there is no statistically significant one-cycle periodicity to start with.
5. Click on *Cancel* to clear the data entry panel and enter another data set. For this data set, you stood by a public monument for a whole day and counted the number of people who were there (excluding yourself). Your unit of time measurement was 2 hours, so that you have 12 data points, as follows: 0, 0, 0, 2, 10, 15, 12, 16, 10, 4, 0, and 0.
6. Inspection of the data suggests the existence of a daily cycle. Nobody was at the monument at midnight, at 2 a.m., or at 4 a.m. Two nerds came at 6 a.m., 10 people came at 8 a.m., and so on. Is this one-cycle periodicity significant? If you have not typed the numbers yet, do it now. Then click on *OK*.
7. In this case, the temporal distribution is significantly different from a random pattern ($D = 0.3043$, $p < 0.01$). Of course, we don't know whether the cycle would occur again if you were desperate enough to stay up for another 24 hours and make additional observations. But at least we can say that the increase in the number of visitors during business hours was real on the day that you were there.
8. *Note:* This program doesn't actually test for rhythmicity. It tests for homogeneity in the distribution of the data points. If the program fails to find a significant difference, it is reasonable to assume that there isn't rhythmicity; however, if the program finds a significant difference, the difference could be between any two (or more) data points. A more reliable test of rhythmicity is the *cosinor* test (see Exercise 5.5).

4.4 Detecting periodicity in a sequence of infrequent events. In this exercise, we will use the program **Rayleigh** (the sixth program from the left in the Circadian banner) to detect periodicity in a sequence of infrequent events. As explained in Section 3.3.2, this program uses the Rayleigh test to determine whether the events are significantly concentrated around a single time of day.

1. Start the program **Rayleigh**.
2. The program is very simple and does not even require data files. We can simply type in the values.
3. Let us say that you live by the beach and spend a lot of time just watching the ocean. You have been doing nothing but drinking beer for a week. However, you were diligent enough to write down the times when you saw a ship sailing by in the horizon. Your records look like this:

Day	Time	Time in Decimal Format
Day 1	12 noon, 4 p.m.	12, 16
Day 2	1:30 p.m.	13.5
Day 3	3 p.m., 5 p.m.	15, 17
Day 4	11 a.m., 2 p.m., 6 p.m.	11, 14, 18
Day 5	1 p.m.	13
Day 6	3 p.m., 4:30 p.m.	15, 16.5
Day 7	12 noon, 5 p.m.	12, 17

4. Now you wonder whether the transit of ships in front of your house shows daily rhythmicity. To find out if there is a sinusoidal pattern in your data, type in the values from the rightmost column of the table. Make sure to type one value per line. After you typed the 13 lines, click on *OK*.

5. If you typed the values correctly, you obtained an nR^2 of 9.537, which is significant at a level below 0.0001. Thus, you can conclude that there is a daily pattern in the transit of ships. It would seem that ships come along mostly in the afternoon. Of course, I assume that you were awake at other times of the day and that you did not see ships because the ships were not there.
6. Let us try another data set. Suppose that you work at the emergency room of a hospital and that you write down the times when patients arrive. Your records for three randomly selected days indicate the following times:

Day	Times of Arrival
May 27	8.3, 8.8. 9.5, 10.1, 11.0, 11.4, 12.1, 12.4
June 3	12.6, 12.9, 13.5, 14.1, 16.2, 18.7
June 8	15.3, 20.2, 21.4, 22.2, 22.8, 23.3, 23.8

7. Click on *Cancel* to start the new data set. Then type in the times of arrival and click on *OK*.
8. This time around we found no evidence of significant rhythmicity. Our nR^2 of 2.601 has a probability greater than 0.05 ($p = 0.074$) under the null hypothesis.

4.5 Analyzing ultradian rhythms. You learned in Section 3.2.3 that Fourier analysis is often a poor choice to evaluate circadian rhythmicity but is a very powerful tool in the analysis of ultradian rhythmicity. In this exercise, we will use the program **Fourier** (the fifth program from the left in the Circadian banner) to evaluate multiple rhythmicity in various data sets.

1. Start the program **Fourier**.
2. If necessary, switch to the Data subfolder and then select the file **A25** in the *Source* panel. This file contains an artificial data set generated by computer. Two cosine waves (with periods of 12 and 24 hours) are combined into a single data string. The resolution is 6 minutes.
3. Before we proceed, you should inspect the data set. Open **Plot**, select **A25**, and click on the Cartesian plot button (the purple button). You should easily see the 12-hour and the 24-hour components. Notice that the time series is perfectly stationary (i.e., the wave form is the same day after day).
4. Now go back to **Fourier** and click on *OK*. The spectral energy associated with each of many periods will be shown on the display panel. Most periods have zero energy because the data set is composed of pure cosine waves. Scroll down to the period of 11.99 (i.e., 12 hours) and notice that its spectral energy is indicated as statistically significant. By scrolling a little further down, you can see that the spectral energy for the period of 23.99 (i.e., 24 hours) is also statistically significant.
5. Next, select the data file **A26**. This file also contains an artificial data set generated by computer, but now there are four waves combined (with periods of 6, 10, 12, and 24 hours). As earlier, you may wish to inspect the data using **Plot**. When you are done, move to the next step.
6. Click on *OK*, wait for the analysis to be completed, and scroll down to see significant peaks at 6, 10, 12 (actually, 11.99), and 24 (actually, 23.99) hours.
7. Too obvious? If you think so, try data file **A27**. The time series in this file is a combination of a cosine wave with a period of 23.5 hours and a cosine wave with a period of 24.5 hours. By using **Plot**, you will notice that the resulting wave form is not quite what you expected. Worse, by using **Fourier**, you will notice that the combination of a 23.5-hour wave with a 24.5-hour wave does not yield two peaks (at 23.5 and 24.5) but a single peak at the mean of the two periods (i.e., 24.0). Go ahead! Gain some humility at the complexity of numerical data analysis!
8. I realize that your real interest must be in the analysis of actual (biological) data. So let us analyze data file **A04**. We have looked at this file in previous exercises. It contains the records of running-wheel activity of a golden hamster maintained in constant darkness for 29 days. Go back to **Plot** and inspect **A04** both as an actogram and in Cartesian mode. In actogram mode, you should notice that the period of the rhythm is longer than 24.0 hours (at least for the first half of the record, it is 24.1 hours). In Cartesian mode, you should notice that the time series is clearly not stationary (i.e., the wave form varies quite a bit from day to day).
9. Now, let us use **Fourier** to analyze **A04**. Select **A04** in the *Source* panel and click on *OK*. We could change "Bins to use" in the *Data* panel in order to analyze the full 29 days (i.e., 6960 bins), but let us leave the default value of 2400 bins to analyze the first 10 days only.
10. Scroll down the display panel and notice that the spectral energy of all periods is greater than zero. This is the norm, not the exception, in actual biological rhythms. Notice also that the period of 24.1 hours is not listed (instead, the main period is listed as 23.99). As discussed in Section 3.2.3, Fourier analysis often lacks resolution in the circadian range. In contrast, notice that the analysis is much more detailed than we care for in the ultradian range. Clearly, Fourier analysis is an excellent tool for the analysis of ultradian rhythms but not as good for the analysis of circadian rhythms.

SUGGESTIONS FOR FURTHER READING

As mentioned in the previous chapter, the source articles listed in the "References" section may be consulted for detailed information about topics covered in this chapter. For more general further reading, the following sources may be useful:

Foster, R. G. and Kreitzman, L. (2009). *Seasons of Life: The Biological Rhythms that Enable Living Things to Thrive and Survive*. New Haven, CT: Yale University Press.

A pleasant and instructive general audience book dealing with seasonal rhythms in plants, animals, and humans.

Aschoff, J. (Ed.) (1981). *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum.

A classic in the biological rhythms literature. Although more than 30 years old, this edited book still provides useful information on circadian, tidal, lunar, and annual rhythms.

Dunlap, J. C., Loros, J. J., and DeCoursey, P. J. (Eds.) (2004). *Chronobiology: Biological Timekeeping*. Sunderland, MA: Sinauer.

A multiauthor textbook on biological rhythms covering circadian and circannual rhythms.

Koukkari, W. L. and Sothern, R. B. (2006). *Introducing Biological Rhythms*. New York: Springer.

Published in the same year as the second edition of *Circadian Physiology*, this book is aimed at a similar audience but has more coverage of rhythms outside the circadian range.

Numata, H. and Helm, B. (Eds.) (2014). *Annual, Lunar, and Tidal Clocks: Patterns and Mechanisms of Nature's Enigmatic Rhythms*. Tokyo, Japan: Springer.

All 16 chapters in this edited book concentrate on tidal, lunar, or seasonal rhythms in a variety of vertebrate and invertebrate species.

Stillman, B. and Stewart, D. (Eds.) (2008). *Clocks and Rhythms* (*Cold Spring Harbor Symposia on Quantitative Biology*, Vol. 72). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

This edited volume is based on the presentations at the 72nd Cold Spring Harbor Symposium on Quantitative Biology in 2007. In addition to circadian rhythms, covered topics include development, aging, and seasonal rhythms.

Saunders, D. S., Steel, C. G. H., Vafopoulou, X., and Lewis, R. D. (2002). *Insect Clocks*, 3rd edn. New York: Elsevier.

Directed at active researchers in the field, this book covers circadian and annual rhythms in insects in great detail.

Lyman, C. P., Willis, J. S., Malan, A., and Wang, L. C. H. (1982). *Hibernation and Torpor in Mammals and Birds*. New York: Academic Press.

Although this book is now over 30 years old, it remains a valuable review of early studies on hibernation and daily torpor in mammals and birds.

WEBSITES TO EXPLORE

Endocrine Society: http://www.endo-society.org.

Lunar Cycle: http://www.moonconnection.com/moon_cycle.phtml.

PubMed (U.S. National Library of Medicine): http://www.ncbi.nlm.nih.gov/pubmed/.

Seasons (University of Tennessee): http://csep10.phys.utk.edu/astr161/lect/time/seasons.html.

Sun and the Seasons: http://www.timeanddate.com/calendar/seasons.html.

Tides and Currents in the United States: http://tidesandcurrents.noaa.gov/.

REFERENCES

1. Thurman, H. V. and Trujillo, A. P. (2005). *Essentials of Oceanography*, 8th edn. Upper Saddle River, NJ: Prentice Hall.
2. Bradley, R. S. (1999). *Paleoclimatology: Reconstructing Climates of the Quaternary*, 2nd edn. San Diego, CA: Academic Press.
3. Kerr, R. A. (1987). Milankovitch climate cycles through the ages. *Science* 235: 973–974.
4. Daintith, J., Mitchell, S., Tootill, E., and Gjertsen, D. (1994). *Biographical Encyclopedia of Scientists*, 2nd edn. Bristol, CT: Institute of Physics.
5. Woodward, F. I. (1987). *Climate and Plant Distribution*. Cambridge, U.K.: Cambridge University Press.
6. Pahnke, K., Zahn, R., Elderfield, H., and Schulz, M. (2003). 340,000-year centennial-scale marine record of Southern Hemisphere climatic oscillation. *Science* 301: 948–952.
7. Jansen, J. H. F., Kuijpers, A., and Troelstra, S. R. (1986). A mid-Brunhes climatic event: Long-term changes in global atmosphere and ocean circulation. *Science* 232: 619–622.
8. Beaufort, L., Garidel-Thoron, T. de, Mix, A. C., and Pisias, N. G. (2001). ENSO-like forcing on oceanic primary production during the late Pleistocene. *Science* 293: 2440–2444.
9. Koutava, A., Lynch-Stieglitz, J., Marchitto, T. M., and Sachs, J. P. (2002). El Niño-like pattern in Ice Age tropical Pacific Sea surface temperature. *Science* 297: 226–230.
10. Karl, T. R. and Trenberth, K. E. (2003). Modern global climate change. *Science* 302: 1719–1723.
11. Schneider, S. H. (1989). The greenhouse effect: Science and policy. *Science* 243: 771–781.
12. Pollack, H. N., Huang, S., and Shen, P. Y. (1998). Climate change record in subsurface temperatures: A global perspective. *Science* 282: 279–281.
13. Crowley, T. J. (2000). Causes of climate change over the past 1000 years. *Science* 289: 270–277.
14. Stott, P. A., Tett, S. F. B., Jones, G. S., Allen, M. R., Mitchell, J. F. B., and Jenkins, G. J. (2000). External control of 20th century temperature by natural and anthropogenic forcing. *Science* 290: 2133–2137.
15. Jones, P. D., Osborn, T. J., and Briffa, K. R. (2001). The evolution of climate over the last millennium. *Science* 292: 662–667.
16. Vinnikov, K. Y. and Grody, N. C. (2003). Global warming trend of mean tropospheric temperature observed by satellites. *Science* 302: 269–272.
17. Karoly, D. J., Braganza, K., Stott, P. A., Arblaster, J. M., Meehl, G. A., Broccoli, A. J., and Dixon, K. W. (2003). Detection of a human influence on North American climate. *Science* 302: 1200–1203.
18. Luterbacher, J., Dietrich, D., Xoplaki, E., Grosjean, M., and Wanner, H. (2004). European seasonal and annual temperature variability, trends, and extremes since 1500. *Science* 303: 1499–1503.
19. Keeling, R. F. (2008). Recording Earth's vital signs. *Science* 319: 1771–1772.
20. Cotton, P. A. (2003). Avian migration phenology and global climate change. *Proceedings of the National Academy of Sciences of the United States of America* 100: 12219–12222.
21. Jonzén, N., Lindén, A., Ergon, T., Knudsen, E., Vik, J. O., Rubolini, D., Piacentini, D. et al. (2006). Rapid advance of spring arrival dates in long-distance migratory birds. *Science* 312: 1959–1961.
22. Roberts, L. (1988). Is there life after climate change? *Science* 242: 1010–1012.

23. Kearney, M., Shine, R., and Porter, W. P. (2009). The potential for behavioral thermoregulation to buffer "cold-blooded" animals against climate warming. *Proceedings of the National Academy of Sciences of the United States of America* 106: 3835–3840.
24. Hofmann, G. E. and Todgham, A. E. (2010). Living in the now: Physiological mechanisms to tolerate a rapidly changing environment. *Annual Review of Physiology* 72: 127–145.
25. Bradshaw, W. E. and Holzapfel, C. M. (2010). Light, time, and the physiology of biotic response to rapid climate change in animals. *Annual Review of Physiology* 72: 147–166.
26. Bond, G., Showers, W., Cheseby, M., Lotti, R., Almasi, P., de Menocal, P., Priore, P., Cullen, H., Hajdas, I., and Bonani, G. (1997). A pervasive millenial-scale cycle in North Atlantic Holocene and Glacial climates. *Science* 278: 1257–1266.
27. Oppo, D. W., McManus, J. F., and Cullen, J. L. (1998). Abrupt climate events 500,000 to 340,000 years ago: Evidence from subpolar North Atlantic sediments. *Science* 279: 1335–1338.
28. Scuderi, L. A. (1993). A 2000-year tree ring record of annual temperatures in the Sierra Nevada mountains. *Science* 259: 1433–1436.
29. Chavez, F. P., Ryan, J., Lluch-Cota, S. E., and Ñiquen, M. (2003). From anchovies to sardines and back: Multidecadal change in the Pacific Ocean. *Science* 299: 217–221.
30. Kerr, R. A. (1990). The climate system as a ticking clock. *Science* 249: 1246–1247.
31. Burdick, A. (1991). El Niño antiguo. *Sciences* 31(3): 8–9.
32. Kondratyev, K. Y. and Nikolsky, G. A. (1970). Solar radiation and solar activity. *Quarterly Journal of the Royal Meteorological Society* 96: 509–522.
33. Cornélissen, G., Engebretson, M., Johnson, D., Otsuka, K., Burioka, N., Posch, J., and Halberg, F. (2001). The week, inherited in neonatal human twins, found also in geomagnetic pulsations in isolated Antarctica. *Biomedicine and Pharmacotherapy* 55: 32–50.
34. Zerubavel, E. (1985). *The Seven Day Circle: The History and Meaning of the Week*. New York: Free Press.
35. Aschoff, J. (1981). A survey on biological rhythms. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 3–10.
36. Zaha, M. A. (November 1972). Shedding some needed light on optical measurements. *Electronics* 6: 91–96.
37. Trewartha, G. T. and Horn, L. H. (1980). *An Introduction to Climate*, 5th edn. New York: McGraw-Hill.
38. Miller, A. and Thompson, J. C. (1983). *Elements of Meteorology*, 4th edn. Columbus, OH: Charles E. Merrill.
39. Danielson, E. W., Levin, J., and Abrams, E. (2003). *Meteorology*, 2nd edn. Boston, MA: McGraw-Hill.
40. Cornélissen, G., Halberg, F., Schwartzkopff, O., Delmore, P., Katinas, G., Hunter, D., Tarquini, B. et al. (1999). Chronomes, time structures, for chronobioengineering for "a full life". *Biomedical Instrumentation and Technology* 33: 152–187.
41. Gillette, M. U. and Sejnowski, T. J. (2005). Biological clocks coordinately keep life on time. *Science* 309: 1196–1198.
42. Barnes, R. S. K. (1998). *The Diversity of Living Organisms*. Malden, MA: Blackwell.
43. Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *Journal of Applied Physiology* 22: 453–460.
44. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th edn. New York: Cambridge University Press.
45. Barrett, K. E., Barman, S. M., Boitano, S., and Brooks, H. L. (2012). *Ganong's Review of Medical Physiology*, 24th edn. New York: McGraw-Hill.
46. Meek, W. J. and Eyster, J. A. E. (1914). Experiments on the origin and propagation of the impulse in the heart. IV. The effect of vagal stimulation and of cooling on the location of the pacemaker within the sino-auricular node. *American Journal of Physiology* 34: 368–383.
47. Bozler, E. (1943). The initiation of impulses in cardiac muscle. *American Journal of Physiology* 138: 273–282.
48. Hutter, O. F. and Trautwein, W. (1956). Vagal and sympathetic effects on the pacemaker fibers in the sinus venosus of the heart. *Journal of General Physiology* 39: 715–733.
49. Accili, E. A., Proenza, C., Baruscotti, M., and DiFrancesco, D. (2002). From funny current to HCN channels: 20 years of excitation. *News in Physiological Sciences* 17: 32–37.
50. Monfredi, O., Maltsev, V. A., and Lakatta, E. G. (2013). Modern concepts concerning the origin of the heartbeat. *Physiology* 28: 74–92.
51. Miller, N. E. (1978). Biofeedback and visceral learning. *Annual Review of Psychology* 29: 373–404.
52. Bindu, P. N. and Desiraju, T. (1988). Success of autonomic operant conditioning of heart rate without involving contractions of somatic skeletal muscles. *Indian Journal of Physiology and Pharmacology* 32: 231–251.
53. Hatch, J. P., Borcherding, S., and Norris, L. K. (1990). Cardiopulmonary adjustments during operant heart rate control. *Psychophysiology* 27: 641–648.
54. Hilaire, G. and Pásaro, R. (2003). Genesis and control of the respiratory rhythm in adult mammals. *News in Physiological Sciences* 18: 23–28.
55. Smith, J. C., Ellenberger, H. H., Ballanyi, K., Richter, D. W., and Feldman, J. L. (1991). Pre-Bötzinger complex: A brainstem region that may generate respiratory rhythm in mammals. *Science* 254: 726–729.
56. Koshiya, N. and Smith, J. C. (1999). Neuronal pacemaker for breathing visualized *in vitro*. *Nature* 400: 360–363.
57. Onimaru, H. and Homma, I. (2003). A novel functional neuron group for respiratory rhythm generation in the ventral medulla. *Journal of Neuroscience* 23: 1478–1486.
58. Feldman, J. L., Mitchell, G. S., and Nattie, E. E. (2003). Breathing: Rhythmicity, plasticity, chemosensitivity. *Annual Review of Neuroscience* 26: 239–266.
59. Adrian, E. D. (1941). Afferent discharges to the cerebral cortex from peripheral sense organs. *Journal of Physiology* 100: 159–191.
60. Mountcastle, V. B. (1957). Modality and topographic properties of single neurons of cat's somatic sensory cortex. *Journal of Neurophysiology* 20: 408–434.
61. Lydic, R. (1989). Central pattern-generating neurons and the search for general principles. *FASEB Journal* 3: 2457–2468.
62. Berger, H. (1929). Über das Elektrenkephalogramm des Menschen. *Archiv für Psychiatrie und Nervenkrankheiten* 87: 527–570.
63. Shepherd, G. M. (1994). *Neurobiology*, 3rd edn. New York: Oxford University Press.
64. Kandel, E. R., Schwartz, J. H., and Jessell, T. M. (2000). *Principles of Neural Science*, 4th edn. New York: McGraw-Hill.
65. Knobil, E. (1987). A hypothalamic pulse generator governs mammalian reproduction. *News in Physiological Sciences* 2: 42–43.
66. Loudon, A. S. I., Wayne, N. L., Krieg, R., Iranmanesh, A., Veldhuis, J. D., and Menaker, M. (1994). Ultradian endocrine rhythms are altered by a circadian mutation in the Syrian hamster. *Endocrinology* 135: 712–718.

67. Weibel, L., Spiegel, K., Follenius, M., Ehrhart, J., and Branderberger, G. (1996). Internal dissociation of the circadian markers of the cortisol rhythm in night workers. *American Journal of Physiology* 270: E608–E613.
68. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L., and Bitman, J. (1999). Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. *Domestic Animal Endocrinology* 16: 41–55.
69. Bergendahl, M., Evans, W. S., and Veldhuis, J. D. (1996). Current concepts on ultradian rhythms of luteinizing hormone secretion in the human. *Human Reproduction Update* 2: 507–518.
70. Matagne, V., Lebrethon, M. C., Gérard, A., and Bourguignon, J. P. (2003). *In vitro* paradigms for the study of GnRH neuron function and estrogen effects. *Annals of the New York Academy of Sciences* 1007: 129–142.
71. Palmer, J. D. (2000). The clocks controlling the tide-associated rhythms of intertidal animals. *BioEssays* 22: 32–37.
72. Takekata, H., Matsuura, Y., Goto, S. G., Satoh, A., and Numata, H. (2012). RNAi of the circadian clock gene *period* disrupts the circadian rhythm but not the circatidal rhythm in the mangrove cricket. *Biology Letters* 8: 488–491.
73. Zhang, L., Hastings, M. H., Green, E. W., Tauber, E., Sladek, M., Webster, S. G., Kyriacou, C. P., and Wilcockson, D. C. (2013). Dissociation of circadian and circatidal timekeeping in the marine crustacean *Eurydice pulchra*. *Current Biology* 23: 1863–1873.
74. de la Iglesia, H. O., Rodríguez, E. M., and Dezi, R. E. (1994). Burrow plugging in the crab *Uca uruguayensis* and its synchronization with photoperiod and tides. *Physiology and Behavior* 55: 913–919.
75. Al-Adhub, A. H. Y. and Naylor, E. (1975). Emergence rhythms and tidal migrations in the brown shrimp *Crangon crangon* (L.). *Journal of the Marine Biological Association of the United Kingdom* 55: 801–810.
76. Barlow, R. B., Powers, M. K., Howard, H., and Kass, L. (1986). Migration of *Limulus* for mating: Relation to lunar phase, tide height, and sunlight. *Biological Bulletin* 171: 310–329.
77. Wikelski, M. and Hau, M. (1995). Is there an endogenous tidal foraging rhythm in marine iguanas? *Journal of Biological Rhythms* 10: 335–350.
78. Akiyama, T. (2004). Entrainment of the circatidal swimming activity rhythm in the cumacean *Dimorphostylis asiatica* (Crustacea) to 12.5-hour hydrostatic pressure cycles. *Zoological Science* 21: 29–38.
79. Chabot, C. C., Skinner, S. J., and Watson, W. H. (2008). Rhythms of locomotion expressed by *Limulus polyphemus*, the American horseshoe crab: I. Synchronization by artificial tides. *Biological Bulletin* 215: 34–45.
80. Honegger, H. W. (1976). Locomotor activity in *Uca crenulata*, and the response to two Zeitgebers, light-dark and tides. In: DeCoursey, P. J. (Ed.), *Biological Rhythms in the Marine Environment*. Columbia, SC: University of South Carolina Press, pp. 93–102.
81. Palmer, J. D. (1967). Daily and tidal components in the persistent rhythmic activity of the crab, *Sesarma*. *Nature* 215: 64–66.
82. Palmer, J. D. and Round, F. A. (1967). Persistent, vertical-migration rhythms in benthic microflora. VI. The tidal and diurnal nature of the rhythm of the diatom *Hantzschia virgata*. *Biological Bulletin* 132: 44–55.
83. Saigusa, M. (2002). Hatching controlled by the circatidal clock, and the role of the medulla terminalis in the optic peduncle of the eyestalk, in an estuarine carb *Sesarma haematocheir*. *Journal of Experimental Biology* 205: 3487–3504.
84. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
85. Cummings, S. M. and Morgan, E. (2001). Time-keeping system of the eel pout, *Zoarces viviparus*. *Chronobiology International* 18: 27–46.
86. Barlow, P. W. and Fisahn, J. (2012). Lunisolar tidal force and the growth of plant roots, and some other of its effects on plant movements. *Annals of Botany* 110: 301–318.
87. McClung, C. R. (2006). Plant circadian rhythms. *Plant Cell* 18: 792–803.
88. Sanders, K. M., Ördög, T., Koh, S. D., and Ward, S. M. (2000). A novel pacemaker mechanism drives gastrointestinal rhythmicity. *News in Physiological Sciences* 15: 291–298.
89. Grau, C., Escera, C., Cilveti, R., García, M., Mojón, A., Fernandez, J. R., and Hermida, R. C. (1995). Ultradian rhythms in gross motor activity of adult humans. *Physiology and Behavior* 57: 411–419.
90. Austin, D., Cross, R. M., Hayes, T., and Kaye, J. (2014). Regularity and predictability of human mobility in personal space. *PLOS ONE* 9: e90256.
91. Wilkens, J. L. and Fingerman, M. (1965). Heat tolerance and temperature relationships of the fiddler crab, *Uca pugilator*, with reference to body coloration. *Biological Bulletin* 128: 133–141.
92. Lumineau, S., Guyomarc'h, C., and Richard, J. P. (2000). Ontogeny of the ultradian rhythm of activity in Japanese quail. *Chronobiology International* 17: 767–776.
93. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A., and Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
94. Zorrilla, E. P., Inoue, K., Fekete, E. M., Tabarin, A., Valdez, G. R., and Koob, G. F. (2005). Measuring meals: Structure of prandial food and water intake of rats. *American Journal of Physiology* 288: R1450–R1467.
95. Passano, L. M. and McCullough, C. B. (1964). Co-ordinating systems and behaviour in *Hydra*. I. Pacemaker system of the periodic contractions. *Journal of Experimental Biology* 41: 643–664.
96. Doust, J. W. L. (1979). Periodic homeostatic fluctuations of skin temperature in the sleeping and waking state. *Neuropsychobiology* 5: 340–347.
97. Grass, K., Prast, H., and Philippu, A. (1995). Ultradian rhythm in the delta and theta frequency bands of the EEG in the posterior hypothalamus of the rat. *Neuroscience Letters* 191: 161–164.
98. Mohr, E. and Krzywanek, H. (1990). Variations of core-temperature rhythms in unrestrained sheep. *Physiology and Behavior* 48: 467–473.
99. Halle, S. (1995). Diel pattern of locomotor activity in populations of root voles, *Microtus oeconomus*. *Journal of Biological Rhythms* 10: 211–224.
100. van der Veen, D. R., Le Minh, N., Gos, P., Arneric, M., Gerkema, M. P., and Schibler, U. (2006). Impact of behavior on central and peripheral circadian clocks in the common vole *Microtus arvalis*, a mammal with ultradian rhythms. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3393–3398.
101. Dowse, H., Umemori, J., and Koide, T. (2010). Ultradian components in the locomotor activity rhythms of the genetically normal mouse, *Mus musculus*. *Journal of Experimental Biology* 213: 1788–1795.

102. Haim, A., Saarela, S., Hohtola, E., and Zisapel, N. (2004). Daily rhythms of oxygen consumption, body temperature, activity and melatonin in the Norwegian lemming *Lemmus lemmus* under northern summer photoperiod. *Journal of Thermal Biology* 29: 629–633.
103. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G., and Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
104. Eccles, R. (1996). A role for the nasal cycle in respiratory defence. *European Respiratory Journal* 9: 371–376.
105. Goldberger, A. L., Rigney, D. R., and West, B. J. (1990). Chaos and fractals in human physiology. *Scientific American* 262(2): 42–49.
106. Goldberger, A. L. (1991). Is the normal heartbeat chaotic or homeostatic? *News in Physiological Sciences* 6: 87–91.
107. Stark, J. and Hardy, K. (2003). Chaos: Useful at last? *Science* 301: 1192–1193.
108. Jilge, B. (1985). The rhythm of food and water ingestion, faeces excretion and locomotor activity in the guinea pig. *Zeitschrift für Versuchstierkunde* 27: 215–225.
109. Stupfel, M., Gourlet, V., Court, L., Mestries, J., Parramon, A., and Mérat, P. (1987). Periodic analysis of ultradian (40 min < tau < 24 h) respiratory variations in laboratory vertebrates of various circadian activities. *Chronobiologia* 14: 365–375.
110. Wollnik, F. and Turek, F. W. (1988). Estrous correlated modulations of circadian and ultradian wheel-running activity rhythms in LEW/Ztm rats. *Physiology and Behavior* 43: 389–396.
111. Kronauer, R. E. and Jewett, M. E. (1992). The relationship between circadian and hemicircadian components of human endogenous temperature rhythms. *Journal of Sleep Research* 1: 88–92.
112. Golombek, D. A., Ortega, G., and Cardinali, D. P. (1993). Wheel running raises body temperature and changes the daily cycle in golden hamsters. *Physiology and Behavior* 53: 1049–1054.
113. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
114. Refinetti, R. (1994). Circadian modulation of ultradian oscillation in the body temperature of the golden hamster. *Journal of Thermal Biology* 19: 269–275.
115. Ortega, G. J., Romanelli, L., and Golombek, D. A. (1994). Statistical and dynamical analysis of circadian rhythms. *Journal of Theoretical Biology* 169: 15–21.
116. Robinson, E. L. and Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
117. Scheibe, K. M., Berher, A., Langbein, J., Streich, W. J., and Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biological Rhythm Research* 30: 216–233.
118. Benstaali, C., Bogdan, A., and Touitou, Y. (2002). Effect of a short photoperiod on circadian rhythms of body temperature and motor activity in old rats. *Pflügers Archiv* 444: 73–79.
119. Hermida, R. C., Ayala, D. E., Fernández, J. R., Mojón, A., Alonso, I., and Calvo, C. (2002). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiology International* 19: 461–481.
120. Hadtstein, C., Wühl, E., Soergel, M., Witte, K., and Schaefer, F. (2004). Normative values for circadian and ultradian cardiovascular rhythms in childhood. *Hypertension* 43: 547–554.
121. Sans-Fuentes, M. A., Díez-Noguera, A., and Cambras, T. (2010). Light responses of the circadian system in leptin deficient mice. *Physiology and Behavior* 99: 487–494.
122. Refinetti, R. (1996). Ultradian rhythms of body temperature and locomotor activity in wild-type and tau-mutant hamsters. *Animal Biology* 5: 111–115.
123. Gerkema, M. P., Groos, G. A., and Daan, S. (1990). Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. *Journal of Biological Rhythms* 5: 81–95.
124. Eastman, C. I., Mistlberger, R. E., and Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology and Behavior* 32: 357–368.
125. Chiesa, J. J., Cambras, T., Carpentieri, A. R., and Díez-Noguera, A. (2010). Arrhythmic rats after SCN lesions and constant light differ in short time scale regulation of locomotor activity. *Journal of Biological Rhythms* 25: 37–46.
126. Phillips, A. J. K., Fulcher, B. D., Robinson, P. A., and Klerman, E. B. (2013). Mammalian test/activity patterns explained by physiologically based modeling. *PLoS Computational Biology* 9: e1003213.
127. Johnson, M. H. and Everitt, B. J. (2000). *Essential Reproduction*, 5th edn. Oxford, U.K.: Blackwell.
128. Allgeier, E. R. and Allgeier, A. R. (2000). *Sexual Interactions*, 5th edn. Boston, MA: Houghton Mifflin.
129. Crooks, R. and Baur, K. (2005). *Our Sexuality*, 9th edn. Pacific Grove, CA: Brooks/Cole.
130. Adams, D. B., Gold, A. R., and Burt, A. D. (1978). Rise in female-initiated sexual activity at ovulation and its suppression by oral contraceptives. *New England Journal of Medicine* 299: 1145–1150.
131. Rikowski, A. and Grammer, K. (1999). Human body odour, symmetry and attractiveness. *Proceedings of the Royal Society of London B* 266: 869–874.
132. Penton-Voak, I. S., Perrett, D. I., Castles, D. L., Kobayashi, T., Burt, D. M., Murray, L. K., and Minamisawa, R. (1999). Menstrual cycle alters face preference. *Nature* 399: 741–742.
133. Macrae, C. N., Alnwick, K. A., Milne, A. B., and Schloerscheidt, A. M. (2002). Person perception across the menstrual cycle: Hormonal influences on social-cognitive functioning. *Psychological Science* 13: 532–536.
134. Kiesner, J. (2009). Physical characteristics of the menstrual cycle and premenstrual depressive symptoms. *Psychological Science* 20: 763–770.
135. Gangestad, S. W. and Thornhill, R. (2008). Human oestrus. *Proceedings of Royal Society B* 275: 991–1000.
136. Alleva, J. J., Waleski, M. V., and Alleva, F. R. (1971). A biological clock controlling the estrous cycle of the hamster. *Endocrinology* 88: 1368–1379.
137. Fitzgerald, K. M. and Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences of the United States of America* 73: 2923–2927.
138. Swann, J. and Turek, F. W. (1982). Cycle of lordosis behavior in female hamsters whose circadian activity rhythm has split into two components. *American Journal of Physiology* 243: R112–R118.
139. Orsini, M. W. (1961). The external vaginal phenomena characterizing the stages of the estrous cycle, pregnancy, pseudo-pregnancy, lactation, and the anestrous hamster, *Mesocricetus auratus*. *Proceedings of the Animal Care Panel* 11: 193–206.

140. Seegal, R. F. and Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. *Biology of Reproduction* 12: 223–231.
141. Refinetti, R. and Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.
142. Richards, M. P. M. (1966). Activity measured by running wheels and observation during the oestrous cycle, pregnancy and pseudopregnancy in the golden hamster. *Animal Behaviour* 14: 450–458.
143. Morin, L. P., Fitzgerald, K. M., and Zucker, I. (1977). Estradiol shortens the period of hamster circadian rhythms. *Science* 196: 305–307.
144. Takahashi, J. S. and Menaker, M. (1980). Interaction of estradiol and progesterone: Effects on circadian locomotor rhythm of female golden hamsters. *American Journal of Physiology* 239: R497–R504.
145. Davis, F. C., Stice, S., and Menaker, M. (1987). Activity and reproductive state in the hamster: Independent control by social stimuli and a circadian pacemaker. *Physiology and Behavior* 40: 583–590.
146. Fritzsche, P., Riek, M., and Gattermann, R. (2000). Effects of social stress on behavior and corpus luteum in female golden hamsters (*Mesocricetus auratus*). *Physiology and Behavior* 68: 625–630.
147. Sridaran, R. and McCormack, C. E. (1980). Parallel effects of light signals on the circadian rhythms of running activity and ovulation in rats. *Journal of Endocrinology* 85: 111–120.
148. Palmer, E., Driancourt, M. A., and Ortavant, R. (1982). Photoperiodic stimulation of the mare during winter anoestrus. *Journal of Reproduction and Fertility* 32(S): 275–282.
149. Lewis, G. S. and Newman, S. K. (1984). Changes throughout estrous cycles of variables that might indicate estrus in dairy cows. *Journal of Dairy Science* 67: 146–152.
150. Curlewis, J. D., Loudon, A. S. I., and Coleman, A. P. M. (1988). Oestrus cycles and the breeding season of the Père David's deer hind (*Elaphurus davidianus*). *Journal of Reproduction and Fertility* 82: 119–126.
151. Rajamahendran, R., Robinson, J., Desbottes, S., and Walton, J. S. (1989). Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels, and ovulation in dairy cows. *Theriogenology* 31: 1173–1182.
152. Walker, L. A., Cornell, L., Dahl, K. D., Czekala, N. M., Dargen, C. M., Joseph, B., Hsueh, A. J. W., and Lasley, B. L. (1988). Urinary concentrations of ovarian steroid hormone metabolites and bioactive follicle-stimulating hormone in killer whales (*Orcinus orchus*) during ovarian cycles and pregnancy. *Biology of Reproduction* 39: 1013–1020.
153. Häster, L. and Erkert, H. G. (1993). Alteration of circadian period length does not influence the ovarian cycle length in common marmosets, *Callithrix j. jacchus* (Primates). *Chronobiology International* 10: 165–175.
154. Shideler, S. E., Savage, A., Ortuño, A. M., Moorman, E. A., and Lasley, B. L. (1994). Monitoring female reproductive function by measurement of fecal estrogen and progesterone metabolites in the white-faced saki (*Pithecia pithecia*). *American Journal of Primatology* 32: 95–108.
155. Asa, C. S., Fischer, F., Carrasco, E., and Puricelli, C. (1994). Correlation between urinary pregnanediol glucuronide and basal body temperature in female orangutans, *Pongo pygmaeus*. *American Journal of Primatology* 34: 275–281.
156. Nagamine, N., Nambo, Y., Nagata, S., Nagaoka, K., Tsunoda, N., Taniyama, H., Tanaka, Y., Tohei, A., Watanabe, G., and Taya, K. (1998). Inhibin secretion in the mare: Localization of inhibin alpha, beta-A, and beta-B subunits in the ovary. *Biology of Reproduction* 59: 1392–1398.
157. Kooistra, H. S., Okkens, A. C., Bevers, M. M., Popp-Snijders, C., van Haaften, B., Dieleman, S. J., and Schoemaker, J. (1999). Concurrent pulsatile secretion of luteinizing hormone and follicle-stimulating hormone during different phases of the estrous cycle and anestrus in Beagle bitches. *Biology of Reproduction* 60: 65–71.
158. McMillan, H. J. and Wynne-Edwards, K. E. (1999). Divergent reproductive endocrinology of the estrous cycle and pregnancy in dwarf hamsters (*Phodopus*). *Comparative Biochemistry and Physiology A* 124: 53–67.
159. Gray, C. A., Bartol, F. F., Taylor, K. M., Wiley, A. A., Ramsey, W. S., Ott, T. L., Bazer, F. W., and Spencer, T. E. (2000). Ovine uterine gland knock-out model: Effects of gland ablation on the estrous cycle. *Biology of Reproduction* 62: 448–456.
160. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W., and Bacon, W. L. (2000). Circadian rhythm of the preovulatory surge of luteinizing hormone and its relationships to rhythms of body temperature and locomotor activity in turkey hens. *Biology of Reproduction* 62: 1452–1458.
161. Skinner, D. C., Richter, T. A., Malpaux, B., and Skinner, J. D. (2001). Annual ovulation cycles in an aseasonal breeder, the springbok (*Antidorcas marsupialis*). *Biology of Reproduction* 64: 1176–1182.
162. Duggavathi, R., Bartlewski, P. M., Barrett, D. M. W., and Rawlings, N. C. (2003). Use of high-resolution transrectal ultrasonography to assess changes in numbers of small ovarian antral follicles and their relationships to the emergence of follicular waves in cyclic ewes. *Theriogenology* 60: 495–510.
163. García, A. J., Landete-Castillejos, T., Gomez-Brunet, A., Garde, J. J., and Gallego, L. (2003). Characteristics of the oestrus cycle of Iberian red deer (*Cervus elaphus hispanicus*) assessed by progesterone profiles. *Journal of Experimental Zoology* 298A: 143–149.
164. West, M., Galloway, D., Shaw, J., Trouson, A., and Paris, M. C. J. (2004). Oestrous cycle of the common wombat, *Vombatus ursinus*, in Victoria, Australia. *Reproduction, Fertility and Development* 16: 339–346.
165. Brown, J. L., Walker, S. L., and Moeller, T. (2004). Comparative endocrinology of cycling and non-cycling Asian (*Elephas maximus*) and African (*Loxodonta africana*) elephants. *General and Comparative Endocrinology* 136: 360–370.
166. Atsalis, S., Margulis, S. W., Bellem, A., and Wielebnowski, N. (2004). Sexual behavior and hormonal estrus cycles in captive aged lowland gorillas (*Gorilla gorilla*). *American Journal of Primatology* 62: 123–132.
167. Cavigelli, S. A., Monfort, S. L., Whitney, T. K., Mechref, Y. S., Novotny, M., and McClintock, M. K. (2005). Frequent serial fecal corticoid measures from rats reflect circadian and ovarian corticosterone rhythms. *Journal of Endocrinology* 184: 153–163.
168. Barger, L. K., Hoban-Higgins, T. M., and Fuller, C. A. (2008). Assessment of circadian rhythms throughout the menstrual cycle of female rhesus monkeys. *American Journal of Primatology* 70: 19–25.
169. Peters, D. G. and Rose, R. W. (1979). The oestrous cycle and basal body temperature in the common wombat (*Vombatus ursinus*). *Journal of Reproduction and Fertility* 57: 453–460.

170. Basterfield, L., Lumley, L. K., and Mathers, J. C. (2009). Wheel running in female C57BL/6J mice: Impact of oestrus and dietary fat and effects on sleep and body mass. *International Journal of Obesity* 33: 212–218.
171. Kopp, C., Ressel, V., Wigger, E., and Tobler, I. (2006). Influence of estrous cycle and ageing on activity patterns in two inbred mouse strains. *Behavioural Brain Research* 167: 165–174.
172. Brobeck, J. R., Wheatland, M., and Strominger, J. L. (1947). Variations in regulation of energy exchange associated with estrus, diestrus and pseudopregnancy in rats. *Endocrinology* 40: 65–72.
173. Bouchard, G., Youngquist, R. S., Vaillancourt, D., Krause, G. F., Guay, P., and Paradis, M. (1991). Seasonality and variability of the interestrous interval in the bitch. *Theriogenology* 36: 41–50.
174. Beijerink, N. J., Dieleman, S. J., Kooistra, H. S., and Okkens, A. C. (2003). Low doses of bromocriptine shorten the interestrous interval in the bitch without lowering plasma prolactin concentration. *Theriogenology* 60: 1379–1386.
175. Hardy, D. F. (1970). The effect of constant light on the estrous cycle and behavior of the female rat. *Physiology and Behavior* 5: 421–425.
176. Johnson, G. A., Stewart, M. D., Gray, C. A., Choi, Y., Burghardt, R. C., Yu-Lee, L. Y., Bazer, F. W., and Spencer, T. E. (2001). Effects of the estrous cycle, pregnancy, and interferon tau on 2′,5′-oligoadenylate synthetase expression in the ovine uterus. *Biology of Reproduction* 64: 1392–1399.
177. Zehr, J. L., Tannenbaum, P. L., Jones, B., and Wallen, K. (2000). Peak occurrence of female sexual initiation predicts day of conception in rhesus monkeys (*Macaca mulatta*). *Reproduction, Fertility and Development* 12: 397–404.
178. Handler, J., Wüstenhagen, A., Schams, D., Kindahl, H., and Aurich, C. (2004). Estrous cycle characteristics, luteal function, secretion of oxytocin and plasma concentrations of 5-keto-13,14-dihydro-PGF2α after administration of low doses of prostaglandin F2α in pony mares. *Theriogenology* 61: 1573–1582.
179. Francis, A. J. P. and Coleman, G. J. (1988). The effect of ambient temperature cycles upon circadian running and drinking activity in male and female laboratory rats. *Physiology and Behavior* 43: 471–477.
180. Yang, Y. and Gordon, C. J. (1996). Ambient temperature limits and stability of temperature regulation in telemetered male and female rats. *Journal of Thermal Biology* 21: 353–363.
181. Kent, S., Hurd, M., and Satinoff, E. (1991). Interactions between body temperature and wheel running over the estrous cycle in rats. *Physiology and Behavior* 49: 1079–1084.
182. Dolatshad, H., Campbell, E. A., O'Hara, L., Maywood, E. S., Hastings, M. H., and Johnson, M. H. (2006). Developmental and reproductive performance in circadian mutant mice. *Human Reproduction* 21: 68–79.
183. Redden, K. D., Kennedy, A. D., Ingalls, J. R., and Gilson, T. L. (1993). Detection of estrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. *Journal of Dairy Science* 76: 713–721.
184. Labyak, S. E. and Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
185. Rauth-Widmann, B., Fuchs, E., and Erkert, H. G. (1996). Infradian alteration of circadian rhythms in owl monkeys (*Aotus lemurinus griseimembra*): An effect of estrous? *Physiology and Behavior* 59: 11–18.
186. Bhatti, B. M. and Morris, T. R. (1978). Entrainment of oviposition in the fowl using light-dark cycles. *British Poultry Science* 19: 333–340.
187. Kadono, H. and Besch, E. L. (1980). Influence of laying cycle on body temperature rhythm in the domestic hen. In: Tanabe, Y. (Ed.), *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin, Germany: Springer, pp. 91–99.
188. Konishi, T. (1980). Circadian rhythm of ovipositional time in Japanese quail. In: Tanabe, Y. (Ed.), *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin, Germany: Springer, pp. 79–90.
189. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
190. Houdelier, C., Guyomarc'h, C., and Lumineau, S. (2002). Daily temporal organization of laying in Japanese quail: Variability and heritability. *Chronobiology International* 19: 377–392.
191. Marrone, B. L., Gentry, R. T., and Wade, G. N. (1976). Gonadal hormones and body temperature in rats: Effects of estrous cycles, castration and steroid replacement. *Physiology and Behavior* 17: 419–425.
192. Krohmer, R. W. and Crews, D. (1987). Temperature activation of courtship behavior in the male red-sided garter snake (*Thamnophis sirtalis parietalis*): Role of the anterior hypothalamus-preoptic area. *Behavioral Neuroscience* 101: 228–236.
193. Bobr, L. W. and Sheldon, B. L. (1977). Analysis of ovulation-oviposition patterns in the domestic fowl by telemetry measurement of deep body temperature. *Australian Journal of Biological Sciences* 30: 243–257.
194. Kadono, H., Besch, E. L., and Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
195. Czaja, J. A. and Butera, P. C. (1986). Body temperature and temperature gradients: Changes during the estrous cycle and in response to ovarian steroids. *Physiology and Behavior* 36: 591–596.
196. Wrenn, T. R., Bitman, J., and Sykes, J. F. (1958). Body temperature variations in dairy cattle during the estrous cycle and pregnancy. *Journal of Dairy Sciences* 41: 1071–1076.
197. Fordham, D. P., Rowlinson, P., and McCarthy, T. T. (1988). Oestrus detection in dairy cows by milk temperature measurement. *Research in Veterinary Science* 44: 366–374.
198. Kyle, B. L., Kennedy, A. D., and Small, J. A. (1998). Measurement of vaginal temperature by radiotelemetry for the prediction of estrus in beef cows. *Theriogenology* 49: 1437–1449.
199. Piccione, G., Caola, G., and Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
200. Harvey, O. L. and Crockett, H. E. (1932). Individual differences in temperature changes of women during the course of the menstrual cycle. *Human Biology* 4: 453–468.
201. Rubenstein, B. B. and Lindsley, D. B. (1937). Relation between human vaginal smears and body temperatures. *Proceedings of the Society for Experimental Biology and Medicine* 35: 618–619.
202. Martin, P. L. (1943). Detection of ovulation by the basal temperature curve with correlating endometrial studies. *American Journal of Obstetrics and Gynecology* 46: 53–62.
203. Kleitman, N. and Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
204. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.

205. Severino, S. K., Wagner, D. R., Moline, M. L., Hurt, S. W., Pollak, C. P., and Zendell, S. (1991). High nocturnal body temperature in premenstrual syndrome and late luteal phase dysphoric disorder. *American Journal of Psychiatry* 148: 1329–1335.
206. Nakayama, K., Yoshimuta, N., Sasaki, Y., Kadokura, M., Hiyama, T., Takeda, A., Sasaki, M., and Ushijima, S. (1992). Diurnal rhythm in body temperature in different phases of the menstrual cycle. *Japanese Journal of Psychiatry and Neurology* 46: 235–237.
207. Kattapong, K. R., Fogg, L. F., and Eastman, C. I. (1995). Effect of sex, menstrual phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International* 12: 257–266.
208. Baker, F. C., Waner, J. I., Vieira, E. F., Taylor, S. R., Driver H. S., and Mitchell, D. (2001). Sleep and 24 hour body temperatures: A comparison in young men, naturally cycling women and women taking hormonal contraceptives. *Journal of Physiology* 530: 565–574.
209. Baker, F. C., Driver, H. S., Paiker, J., Rogers, G. G., and Mitchell, D. (2002). Acetaminophen does not affect 24-h body temperature or sleep in menstrual cycle luteal phase in young women. *Journal of Applied Physiology* 92: 1684–1691.
210. Tompkins, P. (1944). The use of basal temperature graphs in determining the date of ovulation. *Journal of the American Medical Association* 124: 698–700.
211. Moghissi, K. S. (1980). Prediction and detection of ovulation. *Fertility and Sterility* 34: 89–98.
212. Martinez, A. R., van Hooff, M. H. A., Schoute, E., van der Meer, M., Broekmans, F. J. M., and Hompes, P. G. A. (1992). The reliability, acceptability and applications of basal body temperature (BBT) records in the diagnosis and treatment of infertility. *European Journal of Obstetrics and Gynecology and Reproductive Biology* 47: 121–127.
213. Houdelier, C., Guyomarc'h, C., Lumineau, S., and Richard, J. P. (2002). Circadian rhythms of oviposition and feeding activity in Japanese quail: Effects of cyclic administration of melatonin. *Chronobiology International* 19: 1107–1119.
214. Reinberg, A., Halberg, F., Ghata, J., and Siffre, M. (1966). Spectre thermique (rythmes de la température rectale) d'une femme adulte avant, pendant et après son isolement souterrain de trois mois. *Comptes Rendus de l'Académie des Sciences* 262: 782–785.
215. Kent, G. C., Ridgway, P. M., and Strobel, E. F. (1968). Continual light and constant estrus in hamsters. *Endocrinology* 82: 699–703.
216. Johns, M. A., Feder, H. H., and Komisaruk, B. R. (1980). Reflex ovulation in light-induced persistent estrus (LLPE) rats: Role of sensory stimuli and the adrenals. *Hormones and Behavior* 14: 7–19.
217. Albers, H. E., Gerall, A. A., and Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
218. Maldonado, G. and Kraus, J. F. (1991). Variation in suicide occurrence by time of day, day of week, month, and lunar phase. *Suicide and Life-Threatening Behavior* 21: 174–187.
219. Johnson, H., Brock, A., Griffiths, C., and Rooney, C. (2005). Mortality from suicide and drug-related poisoning by day of the week in England and Wales, 1993–2002. *Health Statistics Quarterly* 27: 13–16.
220. Herder, E. and Siehndel, P. (2012). Daily and weekly patterns in human mobility. *CEUR Workshop Proceedings* 872: art. 3.
221. Song, C., Qu, Z., Blumm, N., and Barabási, A. L. (2010). Limits of predictability in human mobility. *Science* 327: 1018–1021.
222. Refinetti, R., Sani, M., Jean-Louis, G., Pandi-Perumal, S. R., Durazo-Arvizu, R. A., Dugas, L. R., Kafensztok, R. et al. (2015). Evidence for daily and weekly rhythmicity but not lunar or seasonal rhythmicity of physical activity in a large cohort of individuals from five different countries. *Annals of Medicine* 47: 530–537.
223. Yasseri, T., Sumi, R., and Kertész, J. (2012). Circadian patterns of Wikipedia editorial activity: A demographic analysis. *PLOS ONE* 7: e30091.
224. Cutting, J. E. (2007). Rhythms of research. *Psychological Science* 18: 1023–1026.
225. Binkley, S., Tome, M. B., Crawford, D., and Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
226. Mauvieux, B., Gouthière, L., Sesboüe, B., and Davenne, D. (2003). Etudes comparées des rythmes circadiens et reflet actimétrique du sommeil de sportifs et de sédentaires en poste régulier de nuit. *Canadian Journal of Applied Physiology* 28: 831–887.
227. Roenneberg, T., Wirz-Justice, A., and Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
228. De Castro, J. M. (1991). Weekly rhythms of spontaneous nutrient intake and meal pattern of humans. *Physiology and Behavior* 50: 729–738.
229. Palmer, J. D., Udry, J. R., and Morris, N. M. (1982). Diurnal and weekly, but no lunar rhythm in human copulation. *Human Biology* 54: 111–121.
230. Hirschenhauser, K., Frigerio, D., Grammer, K., and Magnusson, M. S. (2002). Monthly patterns of testosterone and behavior in prospective fathers. *Hormones and Behavior* 42: 172–181.
231. Ruffieux, C., Marazzi, A., and Paccaud, F. (1992). The circadian rhythm of the perinatal mortality rate in Switzerland. *American Journal of Epidemiology* 135: 936–951.
232. Anderka, M., Declercq, E. R., and Smith, W. (2000). A time to be born. *American Journal of Public Health* 90: 124–126.
233. Mikulecky, M. and Lisboa, H. R. K. (2002). Daily birth numbers in Passo Fundo, South Brazil, 1997–1999: Trends and periodicities. *Brazilian Journal of Medical and Biological Research* 35: 985–990.
234. Bellamy, N., Sothern, R. B., and Campbell, J. (2004). Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. *Journal of Rheumatology* 31: 379–389.
235. Murakami, S., Otsuka, K., Kubo, Y., Shimagawa, M., Yamanaka, T., Ohkawa, S., and Kitaura, Y. (2004). Repeated ambulatory monitoring reveals a Monday morning surge in blood pressure in a community-dwelling population. *American Journal of Hypertension* 17: 1179–1183.
236. Tuomisto, M. T., Terho, T., Korhonen, I., Lappalainen, R., Tuomisto, T., Laippala, P., and Turjanmaa, V. (2006). Diurnal and weekly rhythms of health-related variables in home recordings for two months. *Physiology and Behavior* 87: 650–658.
237. Thomas, C., Wood, G. C., Langer, R. D., and Stewart, W. F. (2008). Elevated blood pressure in primary care varies in relation to circadian and seasonal changes. *Journal of Human Hypertension* 22: 755–760.
238. Cornélissen, G., Breus, T. K., Bingham, C., Zaslavskaya, R., Varshitsky, M., Mirsky, B., Teibloom, M., Tarquini, B., Bakken, E., and Halberg, F. (1993). Beyond circadian chronorisk: Worldwide circaseptan-circasemiseptan patterns of myocardial infarctions, other vascular events, and emergencies. *Chronobiologia* 20: 87–115.

239. Spielberg, C., Falkenhahn, D., Willich, S. N., Wegscheider, K., and Völler, H. (1996). Circadian, day-of-week, and seasonal variability in myocardial infarction: Comparison between working and retired patients. *American Heart Journal* 132: 579–585.
240. Allegra, J. R., Cochrane, D. G., Allegra, E. M., and Cable, G. (2002). Calendar patterns in the occurrence of cardiac arrest. *American Journal of Emergency Medicine* 20: 513–517.
241. Kinjo, K., Sato, H., Shiotani, I., Kurotobi, T., Ohnishi, Y., Hishida, E., Nakatani, D. et al. (2003). Variation during the week in the incidence of acute myocardial infarction: Increased risk for Japanese women on Saturdays. *Heart* 89: 398–403.
242. Savopoulos, C., Ziakas, A., Hatzitolios, A., Delivoria, C., Kounanis, A., Mylonas, S., Tsougas, M., and Psaroulis, D. (2006). Circadian rhythm in sudden cardiac death: A retrospective study of 2,665 cases. *Angiology* 57: 197–204.
243. Kaiser, H., Cornélissen, G., and Halberg, F. (1990). Paleochronobiology: Circadian rhythms, gauges of adaptive Darwinian evolution; about 7-day (circaseptan) rhythms, gauges of integrative internal evolution. *Progress in Clinical and Biological Research* 341B: 755–762.
244. Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K., and Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s-44s.
245. Piccione, G., Caola, G., and Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
246. Couderc, P. (1971). Calendar. *Encyclopedia Americana* 5: 184–190.
247. Balling, R. C. and Cerveny, R. S. (1995). Influence of lunar phase on daily global temperatures. *Science* 267: 1481–1483.
248. Fernandez-Duque, E. and Erkert, H. G. (2006). Cathemerality and lunar periodicity of activity rhythms in owl monkeys of the Argentinian Chaco. *Folia Primatologica* 77: 123–138.
249. Rahman, M. S., Kim, B. H., Takemura, A., Park, C. B., and Lee, Y. D. (2004). Effects of moonlight exposure on plasma melatonin rhythms in the seagrass rabbitfish, *Siganus canaliculatus*. *Journal of Biological Rhythms* 19: 325–334.
250. Taylor, M. H., Leach, G. J., DiMichele, L., Levitan, W. M., and Jacob, W. F. (1979). Lunar spawning cycle in the mummichog, *Fundulus heteroclitus* (Pisces: Cyprinodontidae). *Copeia* 1979(2): 291–297.
251. Neumann, D. (1989). Circadian components of semilunar and lunar timing mechanisms. *Journal of Biological Rhythms* 4: 285–294.
252. Saigusa, M. (1980). Entrainment of a semilunar rhythm by a simulated moonlight cycle in the terrestrial crab, *Sesarma haematocheir*. *Oecologia* 46: 38–44.
253. Zantke, J., Ishikawa-Fujiwara, T., Arboleda, E., Lohs, C., Schipany, K., Hallay, N., Straw, A. D., Todo, T., and Tessmar-Raible, K. (2013). Circadian and circalunar clock interactions in a marine annelid. *Cell Reports* 5: 99–113.
254. Raison, C. L., Klein, H. M., and Steckler, M. (1999). The moon and madness reconsidered. *Journal of Affective Disorders* 53: 99–106.
255. De Castro, J. M. and Pearcey, S. M. (1995). Lunar rhythms of the meal and alcohol intake of humans. *Physiology and Behavior* 57: 439–444.
256. Röösli, M., Jüni, P., Braun-Fahrländer, C., Brinkhof, M. W. G., Low, N., and Egger, M. (2006). Sleepless night, the moon is bright: Longitudinal study of lunar phase and sleep. *Journal of Sleep Research* 15: 149–153.
257. Cajochen, C., Altanay-Ekici, S., Münch, M., Frey, S., Knoblauch, V., and Wirz-Justice, A. (2013). Evidence that the lunar cycle influences human sleep. *Current Biology* 23: 1485–1488.
258. Gutiérrez-García, J. M. and Tusell, F. (1997). Suicides and the lunar cycle. *Psychological Reports* 80: 243–250.
259. Laverty, W. H. and Kelly, I. W. (1998). Cyclical calendar and lunar patterns in automobile property accidents and injury accidents. *Perceptual and Motor Skills* 86: 299–302.
260. Simón, A. (1998). Aggression in a prison setting as a function of lunar phases. *Psychological Reports* 82: 747–752.
261. Chapman, S. and Morrell, S. (2000). Barking mad? Another lunatic hypothesis bites the dust. *British Medical Journal* 321: 1561–1563.
262. Peters-Engl, C., Frank, W., Kerschbaum, F., Denison, U., Medl, M., and Sevelda, P. (2001). Lunar phases and survival of breast cancer patients: A statistical analysis of 3,757 cases. *Breast Cancer Research and Treatment* 70: 131–135.
263. Waldhoer, T., Haidinger, G., and Vutuc, C. (2002). The lunar cycle and the number of deliveries in Austria between 1970 and 1999. *Gynecologic and Obstetric Investigation* 53: 88–89.
264. Núñez, S., Méndez, L. P., and Aguirre-Jaime, A. (2002). Moon cycles and violent behaviours: Myth or fact? *European Journal of Emergency Medicine* 9: 127–130.
265. Biermann, T., Estel, D., Sperling, W., Bleich, S., Kornhuber, J., and Reulbach, U. (2005). Influence of lunar phases on suicide: The end of a myth? A population-based study. *Chronobiology International* 22: 1137–1143.
266. Schuld, J., Slotta, J. E., Schuld, S., Kollmar, O., Schilling, M. K., and Richter, S. (2011). Popular belief meets surgical reality: Impact of lunar phases, Friday the 13th and Zodiac signs on emergency operations and intraoperative blood loss. *World Journal of Surgery* 35: 1945–1949.
267. Cordi, M., Ackermann, S., Bes, F. W., Hartmann, F., Konrad, B. N., Genzel, L., Pawlowski, M. et al. (2014). Lunar cycle effects on sleep and the file drawer problem. *Current Biology* 24: R549–R550.
268. Aguilar, J. J., Cuervo-Arango, J., and Juliana, L. S. (2015). Lunar cycles at mating do not influence sex ratio at birth in horses. *Chronobiology International* 32: 43–47.
269. Stenseth, N. C., Falck, W., Chan, K. S., Bjørnstad, O. N., O'Donoghue, M., Tong, H., Boonstra, R., Boutin, S., Krebs, C. J., and Yoccoz, N. G. (1998). From patterns to processes: Phase and density dependencies in the Canadian lynx cycle. *Proceedings of the National Academy of Sciences of the United States of America* 95: 15430–15435.
270. Cornulier, T., Yoccoz, N. G., Bretagnolle, V., Brommer, J. E., Butet, A., Ecke, F., Elston, D. A. et al. (2013). Europe-wide dampening of population cycles in keystone herbivores. *Science* 340: 63–66.
271. Selås, V., Hogstad, O., Kobro, S., and Rafoss, T. (2004). Can sunspot activity and ultraviolet-B radiation explain cyclic outbreaks of forest moth pest species? *Proceedings of the Royal Society of London B* 271: 1897–1901.
272. Davis, S., Begon, M., De Bruyn, L., Ageyev, V. S., Klassovskiy, N. L., Pole, S. B., Viljugrein, H., Stenseth, N. C., and Leirs, H. (2004). Predictive thresholds for plague in Kazakhstan. *Science* 304: 736–738.
273. Martinez, E., Antoine, D., D'Ortenzio, F., and Gentili, B. (2009). Climate-driven basin-scale decadal oscillations of oceanic phytoplankton. *Science* 326: 1253–1256.

274. Nintcheu-Fata, S., Katinas, G., Halberg, F., Cornélissen, G., Tolstykh, V., Michael, H. N., Otsuka, K., Schwartzkopff, O., and Bakken, E. (2003). Chronomics of tree rings for chrono-astrobiology and beyond. *Biomedicine and Pharmacotherapy* 57: 24s–30s.
275. Halberg, F., Cornélissen, G., Watanabe, Y., Otsuka, K., Fiser, B., Siegelova, J., Mazankova, V. et al. (2001). Near 10-year and longer periods modulate circadians: Intersecting anti-aging and chronoastrobiological research. *Journal of Gerontology* 56A: M304–M324.
276. Mrosovsky, N., Melnyk, R. B., Lang, K., Hallonquist, J. D., Boshes, M., and Joy, J. E. (1980). Infradian cycles in dormice (*Glis glis*). *Journal of Comparative Physiology A* 137: 315–339.
277. Gilg, O., Hanski, I., and Sittler, B. (2003). Cyclic dynamics in a simple vertebrate predator-prey community. *Science* 302: 866–868.
278. Richter, C. P. (1965). *Biological Clocks in Medicine and Psychiatry*. Springfield, IL: Charles C. Thomas.
279. Fitzgerald, C. M. (1998). Do enamel microstructures have regular time dependency? Conclusions from the literature and a large-scale study. *Journal of Human Evolution* 35: 371–386.
280. McKaughan, M. (1987). *The Biological Clock: Reconciling Careers and Motherhood in the 1980's*. New York: Doubleday.
281. Armstrong, P. (1996). *Beating the Biological Clock: The Joys and Challenges of Late Motherhood*. London, U.K.: Headline.
282. Paulson, R. J., and Sachs, J. (2000). *Rewinding Your Biological Clock: Motherhood Late in Life*. San Francisco, CA: W.H. Freeman.
283. Birrittieri, C. (2004). *What Every Woman Should Know About Fertility and Her Biological Clock*. Franklin Lakes, NJ: New Page Books.
284. Perrigo, G., Bryant, W. C., and vom Saal, F. S. (1990). A unique neural timing system prevents male mice from harming their own offspring. *Animal Behaviour* 39: 535–539.
285. Davis, R. C., Hassall, M., and Sutton, S. L. (1977). The vertical distribution of isopods and diplopods in a dune grassland. *Pedobiologia* 17: 320–329.
286. Perron, F. E. (1978). Seasonal burrowing behavior and ecology of *Aporrhais occidentalis* (Gastropoda: Strombacea). *Biological Bulletin* 154: 463–471.
287. Stark, H. E. (1963). Nesting habits of the California vole, *Microtus californicus*, and microclimatic factors affecting its nests. *Ecology* 44: 663–669.
288. Dufour, B. A. (1978). Le terrier d'*Apodemus sylvaticus* L.: sa construction en terrarium et son adaptation a des facteurs externes et internes. *Behavioural Processes* 3: 57–76.
289. Puchalski, W., Bulova, S. J., Lynch, C. B., and Lynch, G. R. (1988). Photoperiod, temperature and melatonin effects on thermoregulatory behavior in Djungarian hamsters. *Physiology and Behavior* 42: 173–177.
290. Schadt, C. W., Martin, A. P., Lipson, D. A., and Schmidt, S. K. (2003). Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* 301: 1359–1361.
291. Giovannoni, S. J. and Vergin, K. L. (2012). Seasonality in ocean microbial communities. *Science* 335: 671–676.
292. Templer, D. I., Ruff, C. F., Halcomb, P. H., Barthlow, V. L., and Ayers, J. L. (1978). Month of conception and birth of schizophrenics as related to the temperature. *Orthomolecular Psychiatry* 7: 231–235.
293. Kendell, R. E. and Adams, W. (1991). Unexplained fluctuations in the risk for schizophrenia by month and year of birth. *British Journal of Psychiatry* 158: 758–763.
294. Bassi, C. J. and Powers, M. K. (1986). Daily fluctuations in the detectability of dim lights by humans. *Physiology and Behavior* 38: 871–877.
295. Pöllmann, L. (1994). Circadian and circannual variations in the evaluation of thermal comfort in a constant climate. *Indoor Environment* 3: 145–148.
296. Bakko, E. B., Porter, W. P., and Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
297. Haikonen, H. and Summala, H. (2001). Deer-vehicle crashes: Extensive peak at 1 hour after sunset. *American Journal of Preventive Medicine* 21: 209–213.
298. Bennett, K. A., McConnell, B. J., and Fedak, M. A. (2001). Diurnal and seasonal variations in the duration and depth of the longest dives in southern elephant seals (*Mirounga leonina*): Possible physiological and behavioral constraints. *Journal of Experimental Biology* 204: 649–662.
299. Michael, R. P. and Zumpe, D. (1986). An annual rhythm in the battering of women. *American Journal of Psychiatry* 143: 637–640.
300. Dimmitt, M. A. and Ruibal, R. (1980). Environmental correlates of emergence in spadefoot toads (*Scaphiopus*). *Journal of Herpetology* 14: 21–29.
301. Ruibal, R., Tevis, L., and Roig, V. (1969). The terrestrial ecology of the spadefoot toad *Scaphiopus hammondii*. *Copeia* 1969: 571–584.
302. Harmatz, M. G., Well, A. D., Overtree, C. E., Kawamura, K. Y., Rosal, M., and Ockene, I. S. (2000). Seasonal variation of depression and other moods: A longitudinal approach. *Journal of Biological Rhythms* 15: 344–350.
303. Trudeau, R. (1997). Monthly and daily patterns of death. *Statistics Canada Health Reports* 9(1): 43–50.
304. Müller, D., Lampe, F., Wegscheider, K., Schultheiss, H. P., and Behrens, S. (2003). Annual distribution of ventricular tachycardias and ventricular fibrillation. *American Heart Journal* 146: 1061–1065.
305. Reavey, M., Saner, H., Paccaud, F., and Marques-Vidal, P. (2013). Exploring the periodicity of cardiovascular events in Switzerland: Variation in deaths and hospitalizations across seasons, day of the week and hour of the day. *International Journal of Cardiology* 168: 2195–2200.
306. Hiltunen, L., Suominen, K., Lönnqvist, J., and Partonen, T. (2011). Relationship between daylength and suicide in Finland. *Journal of Circadian Rhythms* 9: art. 10.
307. Hrushesky, W. J. M., Sothern, R. B., Rietveld, W. J., Du-Quiton, J., and Boon, M. E. (2006). Sun exposure, sexual behavior and uterine cervical papilloma virus. *International Journal of Biometeorology* 50: 167–173.
308. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
309. Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A. et al. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293: 1310–1314.
310. Hernandez, J., Bonnedahl, J., Waldenström, J., Palmgren, H., and Olsen, B. (2003). *Salmonella* in birds migrating through Sweden. *Emerging Infectious Diseases* 9: 753–755.
311. Paar, J., Oldroyd, B. P., and Kastberger, G. (2000). Giant honeybees return to their nest sites. *Nature* 406: 475.
312. Neumann, P., Koeniger, N., Koeniger, G., Tingek, S., Kryger, P., and Moritz, R. F. A. (2000). Home-site fidelity in migratory honeybees. *Nature* 406: 474–475.

313. Mouritsen, H. and Frost, B. J. (2002). Virtual migration in tethered flying monarch butterflies reveals their orientation mechanisms. *Proceedings of the National Academy of Sciences of the United States of America* 99: 10162–10166.
314. Froy, O., Gotter, A. L., Casselman, A. L., and Reppert, S. M. (2003). Illuminating the circadian clock in monarch butterfly migration. *Science* 300: 1303–1305.
315. Merlin, C., Gegear, R. J., and Reppert, S. M. (2009). Antennal circadian clocks coordinate sun compass orientation in migratory monarch butterflies. *Science* 325: 1700–1704.
316. Radtke, R., Svenning, M., Malone, D., Klementsen, A., Ruzicka, J., and Fey, D. (1996). Migrations in an extreme northern population of Arctic charr *Salvelinus alpinus*: Insights from otolith microchemistry. *Marine Ecology Progress Series* 136: 13–23.
317. Hunter, E., Metcalfe, J. D., and Reynolds, J. D. (2003). Migration route and spawning area fidelity by North Sea plaice. *Proceedings of the Royal Society of London B* 270: 2097–2103.
318. Aarestrup, K., Okland, F., Hansen, M. M., Righton, D., Gargan, P., Castonguay, M., Bernatchez, L. et al. (2009). Oceanic spawning migration of the European eel (*Anguilla anguilla*). *Science* 325: 1660.
319. Rattenborg, N. C., Mandt, B. H., Obermeyer, W. H., Winsauer, P. J., Huber, R., Wikelski, M., and Benca, R. M. (2004). Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLoS Biology* 2: 924–936.
320. Croxall, J. P., Silk, J. R. D., Phillips, R. A., Afanasyev, V., and Briggs, D. R. (2005). Global circumnavigations: Tracking year-round ranges of nonbreeding albatrosses. *Science* 307: 249–250.
321. Stutchbury, B. J., Tarof, S. A., Done, T., Gow, E., Kramer, P. M., Tautin, J., Fox, J. W., and Afanasyev, V. (2009). Tracking long-distance songbird migration by using geolocators. *Science* 323: 896.
322. Altizer, S., Bartel, R., and Han, B. A. (2011). Animal migration and infectious disease risk. *Science* 331: 296–302.
323. Burton, K. W. (2008). An epic journey. *Science* 319: 881.
324. Bharti, N., Tatem, A. J., Ferrari, M. J., Grais, R. F., Djibo, A., and Grenfell, B. T. (2011). Explaining seasonal fluctuations of measles in Niger using nighttime lights imagery. *Science* 334: 1424–1427.
325. Bureau of Transportation Statistics (2003). *National Household Travel Survey Highlights Report*. Washington, DC: U.S. Department of Transportation.
326. Totzke, U., Hubinger, A., and Bairlein, F. (1997). A role for pancreatic hormones in the regulation of autumnal fat deposition of the garden warbler (*Sylvia borin*)? *General and Comparative Endocrinology* 107: 166–171.
327. Piersma, T. and Jukema, J. (2002). Contrast in adaptive mass gains: Eurasian golden plovers store fat before midwinter and protein before prebreeding flight. *Proceedings of the Royal Society of London B* 269: 1101–1105.
328. Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M., and Morton, M. L. (2003). Effects of temperature on photoperiodically induced reproductive development, circulating luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*. *General and Comparative Endocrinology* 131: 143–158.
329. Totzke, U., Hübinger, A., Dittami, J., and Bairlein, F. (2000). The autumnal fattening of the long-distance migratory garden warbler (*Sylvia borin*) is stimulated by intermittent fasting. *Journal of Comparative Physiology B* 170: 627–631.
330. Guglielmo, C. G. and Williams, T. D. (2003). Phenotypic flexibility of body composition in relation to migratory state, age, and sex in the western sandpiper (*Calidris mauri*). *Physiological and Biochemical Zoology* 76: 84–98.
331. Maggini, I. and Bairlein, F. (2010). Endogenous rhythms of seasonal migratory body mass changes and nocturnal restlessness in different populations of Northern Wheatear *Oenanthe oenanthe*. *Journal of Biological Rhythms* 25: 268–276.
332. Pengelley, E. T. and Fisher, K. C. (1966). Locomotor activity patterns and their relation to hibernation in the golden-mantled ground squirrel. *Journal of Mammalogy* 47: 63–73.
333. Dark, J., Zucker, I., and Wade, G. N. (1983). Photoperiodic regulation of body mass, food intake, and reproduction in meadow voles. *American Journal of Physiology* 245: R334–R338.
334. Pohl, H. (1987). Control of annual rhythms of reproduction and hibernation by photoperiod and temperature in the Turkish hamster. *Journal of Thermal Biology* 12: 119–123.
335. Maier, H. A. and Feist, D. D. (1991). Thermoregulation, growth, and reproduction in Alaskan collared lemmings: Role of short day and cold. *American Journal of Physiology* 261: R522–R530.
336. Lee, T. M. and Zucker, I. (1991). Suprachiasmatic nucleus and photic entrainment of circannual rhythms in ground squirrels. *Journal of Biological Rhythms* 6: 315–330.
337. Wollnik, F. and Schmidt, B. (1995). Seasonal and daily rhythms of body temperature in the European hamster (*Cricetus cricetus*) under semi-natural conditions. *Journal of Comparative Physiology B* 165: 171–182.
338. Haim, A., McDevitt, R. M., and Speakman, J. R. (1995). Thermoregulatory responses to manipulations of photoperiod in wood mice *Apodemus sylvaticus* from high latitudes. *Journal of Thermal Biology* 20: 437–443.
339. Mrosovsky, N. (1990). Circannual cycles in golden-mantled ground squirrels: Fall and spring cold pulses. *Journal of Comparative Physiology A* 167: 683–689.
340. Bartness, T. J. (1995). Short day-induced depletion of lipid stores is fat pad- and gender-specific in Siberian hamsters. *Physiology and Behavior* 58: 539–550.
341. Harlow, H. J. (1997). Winter body fat, food consumption and nonshivering thermogenesis of representative spontaneous and facultative hibernators: The white-tailed prairie dog and black-tailed prairie dog. *Journal of Thermal Biology* 22: 21–30.
342. Hiebert, S. M., Thomas, E. M., Lee, T. M., Pelz, K. M., Yellon, S. M., and Zucker, I. (2000). Photic entrainment of circannual rhythms in golden-mantled ground squirrels: Role of the pineal gland. *Journal of Biological Rhythms* 15: 126–134.
343. Chevillard, L., Portet, R., and Cadot, M. (1963). Growth rate of rats born and reared at 5 and 30°C. *Federation Proceedings* 22: 699–702.
344. Concannon, P., Levac, K., Rawson, R., Tennant, B., and Bensadoun, A. (2001). Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. *American Journal of Physiology* 281: R951–R959.
345. Shoemaker, M. B. and Heideman, P. D. (2002). Reduced body mass, food intake, and testis size in response to short photoperiod in adult F344 rats. *BMC Physiology* 2: art. 11.
346. Kuhlmann, M. T., Clemen, G., and Schlatt, S. (2003). Molting in the Djungarian hamster (*Phodopus sungorus* Pallas): Seasonal or continuous process? *Journal of Experimental Zoology* 295A: 160–171.

347. Bartness, T. J. and Wade, G. N. (1984). Photoperiodic control of body weight and energy metabolism in Syrian hamsters (*Mesocricetus auratus*): Role of pineal gland, melatonin, gonads, and diet. *Endocrinology* 114: 492–498.
348. Larkin, J. E., Jones, J., and Zucker, I. (2002). Temperature dependence of gonadal regression in Syrian hamsters exposed to short day lengths. *American Journal of Physiology* 282: R744–R752.
349. Pitrosky, B., Delagrange, P., Rettori, M. C., and Pévet, P. (2003). S22153, a melatonin antagonist, dissociates different aspects of photoperiodic responses in Syrian hamsters. *Behavioural Brain Research* 138: 145–152.
350. Peacock, W. L., Król, E., Moar, K. M., McLaren, J. S., Mercer, J. G., and Speakman, J. R. (2004). Photoperiodic effects on body mass, energy balance and hypothalamic gene expression in the bank vole. *Journal of Experimental Biology* 207: 165–177.
351. Gür, H. and Gür, M. K. (2005). Annual cycle of activity, reproduction, and body mass of Anatolian ground squirrels (*Spermophilus xanthoprymnus*) in Turkey. *Journal of Mammalogy* 86: 7–14.
352. Monecke, S. and Wollnik, F. (2005). Seasonal variations in circadian rhythms coincide with a phase of sensitivity to short photoperiods in the European hamster. *Journal of Comparative Physiology B* 175: 167–183.
353. Fuglei, E. and Øritsland, N. A. (1999). Seasonal trends in body mass, food intake and resting metabolic rate, and induction of metabolic depression in arctic foxes (*Alopex lagopus*) at Svalbard. *Journal of Comparative Physiology B* 169: 361–369.
354. Körtner, G. and Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiology and Behavior* 58: 31–36.
355. Lincoln, G. A., Rhind, S. M., Pompolo, S., and Clarke, I. J. (2001). Hypothalamic control of photoperiod-induced cycles in food intake, body weight, and metabolic hormones in rams. *American Journal of Physiology* 281: R76–R90.
356. Nieminen, P., Mustonen, A. M., Asikainen, J., and Hyvärinen, H. (2002). Seasonal weight regulation of the raccoon dog (*Nyctereutes procyonoides*): Interactions between melatonin, leptin, ghrelin, and growth hormone. *Journal of Biological Rhythms* 17: 155–163.
357. Fitzgerald, B. P. and McManus, C. J. (2000). Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. *Biology of Reproduction* 63: 335–340.
358. Kowalczyk, R., Jedrzejewska, B., and Zalewski, A. (2003). Annual and circadian activity patterns of badgers (*Meles meles*) in Bialowieza Primeval Forest (eastern Poland) compared with other Palaearctic populations. *Journal of Biogeography* 30: 463–472.
359. Génin, F., Schilling, A., and Claustrat, B. (2003). Melatonin and methimazole mimic short-day-induced fattening in gray mouse lemurs. *Physiology and Behavior* 79: 553–559.
360. Peltier, T. C., Barboza, P. S., and Blake, J. E. (2003). Seasonal hyperphagia does not reduce digestive efficiency in an Arctic grazer. *Physiological and Biochemical Zoology* 76: 471–483.
361. Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. London, U.K.: Chapman & Hall.
362. Gordon, C. J. (1993). *Temperature Regulation in Laboratory Rodents*. New York: Cambridge University Press.
363. Roberts, J. C. (1996). Thermogenic responses to prolonged cold exposure: Birds and mammals. In: Fregly, M. J. and Blatteis, C. M. (Eds.), *Handbook of Physiology, Section 4: Environmental Physiology*. New York: Oxford University Press.
364. Dawson, W. R. and Marsh, R. L. (1989). Metabolic acclimatization to cold and season in birds. In: Bech, C. and Reinertsen, R. E. (Eds.), *Physiology of Cold Adaptation in Birds*. New York: Plenum.
365. Hildwein, G. (1970). Capacités thermorégulatrices d'un mammifère insectivore primitif, le tenrec: Leurs variations saisonnières. *Archives des Sciences Physiologiques* 24: 55–71.
366. Veghte, J. H. (1965). Thermal and metabolic responses of the gray jay to cold stress. *Physiological Zoology* 37: 316–328.
367. Hart, J. S. and Heroux, O. (1963). Seasonal acclimatization in wild rats (*Rattus norvegicus*). *Canadian Journal of Zoology* 41: 711–716.
368. Kronfeld-Schor, N., Haim, A., Dayan, T., Zisapel, N., Klingenspor, M., and Heldmaier, G. (2000). Seasonal thermogenic acclimation of diurnally and nocturnally active desert spiny mice. *Physiological and Biochemical Zoology* 73: 37–44.
369. Cooper, S. J. (2002). Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. *Physiological and Biochemical Zoology* 75: 386–395.
370. Jefimow, M., Wojciechowski, M., and Tegowska, E. (2004). Seasonal and daily changes in the capacity for nonshivering thermogenesis in golden hamsters housed under semi-natural conditions. *Comparative Biochemistry and Physiology A* 137: 297–309.
371. Heroux, O., Hart, J. S., and Depocas, F. (1956). Metabolism and muscle activity of anesthetized warm and cold acclimated rats on exposure to cold. *Journal of Applied Physiology* 9: 399–403.
372. Griggio, M. A. (1982). The participation of shivering and nonshivering thermogenesis in warm and cold-acclimated rats. *Comparative Biochemistry and Physiology A* 73: 481–484.
373. Allard, M. and LeBlanc, J. (1988). Effects of cold acclimation, cold exposure, and palatability on postprandial thermogenesis in rats. *International Journal of Obesity* 12: 169–178.
374. Schmidek, W. R., Zachariassen, K. E., and Hammel, H. T. (1983). Total calorimetric measurements in the rat: Influences of the sleep-wakefulness cycle and of environmental temperature. *Brain Research* 288: 261–271.
375. Gwosdow, A. R. and Besch, E. L. (1985). Effect of thermal history on the rat's response to varying environmental temperature. *Journal of Applied Physiology* 59: 413–419.
376. Griggio, M. A. and Tarasantchi, J. (1984). The effect of acclimation temperature on energy metabolism as a function of body weight in rats. *Journal of Thermal Biology* 9: 295–298.
377. Duchamp, C., Barré, H., Delage, D., Rouanet, J. L., Cohen-Adad, F., and Minaire, Y. (1989). Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. *American Journal of Physiology* 257: R744–R751.
378. Hohtola, E. and Stevens, E. D. (1986). The relationship of muscle electrical activity, tremor and heat production to shivering thermogenesis in Japanese quail. *Journal of Experimental Biology* 125: 119–135.
379. Barré, H., Cohen-Adad, F., Duchamp, C., and Rouanet, J. L. (1986). Multilocular adipocytes from muscovy ducklings differentiated in response to cold acclimation. *Journal of Physiology* 375: 27–38.
380. Marjoniemi, K. and Hohtola, E. (2000). Does cold acclimation induce nonshivering thermogenesis in juvenile birds? Experiments with Pekin ducklings and Japanese quail chicks. *Journal of Comparative Physiology B* 170: 537–543.
381. Chevillard, L., Guntz, M. C., Petrovic, V., and Giono, H. (1960). Réactions thermorégulatrices du cobaye adapté au froid et à 30°C. *Comptes Rendus de Séances de la Société de Biologie de Paris* 154: 2017–2020.

382. Pohl, H. (1965). Temperature regulation and cold acclimation in the golden hamster. *Journal of Applied Physiology* 20: 405–410.
383. Oufara, S., Barré, H., Rouanet, J. L., and Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *American Journal of Physiology* 253: R39–R45.
384. Smith, B. K. and Dawson, T. J. (1984). Changes in the thermal balance of a marsupial (*Dasyuroides byrnei*) during cold and warm acclimation. *Journal of Thermal Biology* 9: 199–204.
385. Pohl, H. and Hart, J. S. (1965). Thermoregulation and cold acclimation in a hibernator, *Citellus tridecemlineatus*. *Journal of Applied Physiology* 20: 398–404.
386. Haim, A. (1996). Food and energy intake, non-shivering thermogenesis and daily rhythm of body temperature in the bushy-tailed gerbil *Sekeetamys calurus*: The role of photoperiod manipulations. *Journal of Thermal Biology* 21: 37–42.
387. Saarela, S. and Heldmaier, G. (1987). Effect of photoperiod and melatonin on cold resistance, thermoregulation and shivering/nonshivering thermogenesis in Japanese quail. *Journal of Comparative Physiology B* 157: 625–633.
388. Haim, A. and Martinez, J. J. (1992). Seasonal acclimatization in the migratory hamster *Cricetulus migratorius*: The role of photoperiod. *Journal of Thermal Biology* 17: 347–351.
389. McDevitt, R. M. and Andrews, J. F. (1994). The importance of nest utilization as a behavioural thermoregulatory strategy in *Sorex minutus*, the pygmy shrew. *Journal of Thermal Biology* 19: 97–102.
390. Heldmaier, G., Steinlechner, S., and Rafael, J. (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *Journal of Comparative Physiology* 149: 1–9.
391. Heldmaier, G. and Jablonka, B. (1985). Seasonal differences in thermogenic adaptation evoked by daily injections of noradrenaline. *Journal of Thermal Biology* 10: 97–99.
392. Rafael, J. and Vsiansky, P. (1985). Photoperiodic control of the thermogenic capacity in brown adipose tissue of the Djungarian hamster. *Journal of Thermal Biology* 10: 167–170.
393. Hagelstein, K. A. and Folk, G. E. (1979). Effects of photoperiod, cold acclimation and melatonin on the white rat. *Comparative Biochemistry and Physiology C* 62: 225–229.
394. Blank, J. L. and Ruf, T. (1992). Effect of reproductive function on cold tolerance in deer mice. *American Journal of Physiology* 263: R820–R826.
395. Ellison, G. T. H., Skinner, J. D., and Haim, A. (1992). The relative importance of photoperiod and temperature as cues for seasonal acclimation of thermoregulation in pouched mice (*Saccostomus campestris*: Cricetidae) from southern Africa. *Journal of Comparative Physiology B* 162: 740–746.
396. Shearer, D. S., Valdes, E. V., Qyarzun, S. E., Steinsky, L., and Atkinson, J. L. (1995). Food intake patterns in captive South African fur seals (*Arctocephalus pusillus pusillus*). *Proceedings of the First Annual Conference of the Nutrition Advisory Group of the American Zoo and Aquarium Association*, Toronto, Ontario, Vol. 1, pp. 213–217.
397. Rhind, S. M., McMillen, S. R., Pekas, J. C., and Duff, E. (2001). The role of cholecystokinin in the expression of seasonal variation in the feed intake and eating pattern of red deer (*Cervus elaphus*). *Physiology and Behavior* 73: 211–216.
398. Storeheier, P. V., Sehested, J., Diernaes, L., Sundset, M. A., and Mathiesen, S. D. (2003). Effects of seasonal changes in food quality and food intake on the transport of sodium and butyrate across ruminal epithelium of reindeer. *Journal of Comparative Physiology B* 173: 391–399.
399. De Castro, J. M. (1991). Seasonal rhythms of human nutrient intake and meal pattern. *Physiology and Behavior* 50: 243–248.
400. Moon, H. K. and Shim, J. E. (2004). Seasonal variation of food consumption patterns in Korea. *Asia Pacific Journal of Clinical Nutrition* 13: S167.
401. Brobeck, J. R. (1948). Food intake as a mechanism of temperature regulation. *Yale Journal of Biology and Medicine* 20: 545–549.
402. Witty, R. T. and Long, J. F. (1970). Effect of ambient temperature on gastric secretion and food intake in the rat. *American Journal of Physiology* 219: 1359–1363.
403. Yamauchi, C., Fujita, S., Obara, T., and Ueda, T. (1981). Effects of room temperature on reproduction, body and organ weights, food and water intake, and hematology in rats. *Laboratory Animal Science* 31: 251–258.
404. Cormarèche-Leydier, M. (1981). The effect of ambient temperature on rectal temperature, food intake and short term body weight in the capsaicin desensitized rat. *Pflügers Archiv* 389: 171–174.
405. Armitage, G., Harris, R. B. S., Hervey, G. R., and Tobin, G. (1984). The relationship between energy expenditure and environmental temperature in congenitally obese and non-obese Zucker rats. *Journal of Physiology* 350: 197–207.
406. Refinetti, R. (1988). Effects of food temperature and ambient temperature during a meal on food intake in the rat. *Physiology and Behavior* 43: 245–247.
407. Gasnier, A. and Mayer, A. (1939). Recherches sur la régulation de la nutrition. III. Mécanismes régulateurs de la nutrition et intensité du métabolisme. *Annales de Physiologie Physicochimique et Biologique* 15: 186–194.
408. Iampietro, P. F., Bass, D. E., and Buskirk, E. R. (1958). Caloric intake during prolonged cold exposure. *Metabolism* 7: 149–153.
409. Savory, C. J. (1986). Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowls. *Physiology and Behavior* 38: 353–357.
410. Manning, J. M. and Bronson, F. H. (1990). The effects of low temperature and food intake on ovulation in domestic mice. *Physiological Zoology* 63: 938–948.
411. Henderson, D., Fort, M. M., Rashotte, M. E., and Henderson, R. P. (1992). Ingestive behavior and body temperature of pigeons during long-term cold exposure. *Physiology and Behavior* 52: 455–469.
412. Agetsuma, N. (2000). Influence of temperature on energy intake and food selection by macaques. *International Journal of Primatology* 21: 103–111.
413. Kauffman, A. S., Cabrera, A., and Zucker, I. (2001). Energy intake and fur in summer- and winter-acclimated Siberian hamsters (*Phodopus sungorus*). *American Journal of Physiology* 281: R519–R527.
414. IUPS Thermal Commission (2001). Glossary of terms for thermal physiology. *Japanese Journal of Physiology* 51: 245–280.
415. Lyman, C. P. (1954). Activity, food consumption and hoarding in hibernators. *Journal of Mammalogy* 35: 545–552.
416. Dawe, A. R. and Spurrier, W. A. (1969). Hibernation induced in ground squirrels by blood transfusion. *Science* 163: 298–299.
417. Michener, G. R. (1983). Spring emergence schedules and vernal behavior of Richardson's ground squirrels: Why do males emerge from hibernation before females? *Behavioral Ecology and Sociobiology* 14: 29–38.
418. Ruit, K. A., Bruce, D. S., Chiang, P. P., Oeltgen, P. R., Welborn, J. R., and Nilekani, S. P. (1987). Summer hibernation in ground squirrels (*Citellus tridecemlineatus*) induced by injection of whole or fractionated plasma from hibernating black bears (*Ursus americanus*). *Journal of Thermal Biology* 12: 135–138.

419. Frerichs, K. U., Kennedy, C., Sokoloff, L., and Hallenbeck, J. M. (1994). Local cerebral blood flow during hibernation, a model of natural tolerance to "cerebral ischemia". *Journal of Cerebral Blood Flow and Metabolism* 14: 193–205.
420. Buck, M. J., Squire, T. L., and Andrews, M. T. (2002). Coordinate expression of the PDK4 gene: A means of regulating fuel selection in a hibernating mammal. *Physiological Genomics* 8: 5–13.
421. Twente, J. W. and Twente, J. A. (1965). Effects of core temperature upon the duration of hibernation of *Citellus lateralis*. *Journal of Applied Physiology* 20: 411–416.
422. Larkin, J. E., Franken, P., and Heller, H. C. (2002). Loss of circadian organization of sleep and wakefulness during hibernation. *American Journal of Physiology* 282: R1086–R1095.
423. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
424. Tattersall, G. J. and Milsom, W. K. (2003). Transient peripheral warming accompanies the hypoxic metabolic response in the golden-mantled ground squirrel. *Journal of Experimental Biology* 206: 33–42.
425. Kauffman, A. S., Paul, M. J., and Zucker, I. (2004). Increased heat loss affects hibernation in golden-mantled ground squirrels. *American Journal of Physiology* 287: R167–R173.
426. Millesi, E., Prossinger, H., Dittami, J. P., and Fieder, M. (2001). Hibernation effects on memory in European ground squirrels (*Spermophilus citellus*). *Journal of Biological Rhythms* 16: 264–271.
427. Hut, R. A., Barnes, B. M., and Daan, S. (2002). Body temperature patterns before, during, and after semi-natural hibernation in the European ground squirrel. *Journal of Comparative Physiology B* 172: 47–58.
428. Barnes, B. M. (1989). Freeze avoidance in a mammal: Body temperatures below 0°C in an Arctic hibernator. *Science* 244: 1593–1595.
429. Gür, M. K., Refinetti, R., and Gür, H. (2009). Daily rhythmicity and hibernation in the Anatolian ground squirrel under natural and laboratory conditions. *Journal of Comparative Physiology B* 179: 155–164.
430. Williams, C. T., Sheriff, M. J., Schmutz, J. A., Kohl, F., Øivind, T., Buck, C. L., and Barnes, B. M. (2011). Data logging of body temperatures provides precise information on phenology of reproductive events in a free-ling Arctic hibernator. *Journal of Comparative Physiology B* 181: 1101–1109.
431. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
432. Jansky, L., Haddad, G., Kahlerová, Z., and Nedoma, J. (1984). Effect of external factors on hibernation of golden hamsters. *Journal of Comparative Physiology B* 154: 427–433.
433. Oklejewicz, M., Daan, S., and Strijkstra, A. M. (2001). Temporal organisation of hibernation in wild-type and *tau* mutant Syrian hamsters. *Journal of Comparative Physiology B* 171: 431–439.
434. Lyman, C. P., O'Brien, R. C., Greene, G. C., and Papafrangos, E. D. (1981). Hibernation and longevity in the Turkish hamster *Mesocricetus brandti*. *Science* 212: 668–670.
435. Lyman, C. P., O'Brien, R. C., and Bossert, W. H. (1983). Differences in tendency to hibernate among groups of Turkish hamsters (*Mesocricetus brandti*). *Journal of Thermal Biology* 8: 255–257.
436. Jagiello, G. M., Fang, J. S., Sung, W. K., Hembree, W. C., and Ducayen, M. B. (1992). Genetic characteristics of spermatogenesis in the Turkish hamster (*Mesocricetus brandti*) subjected to reduced temperature or light. *Journal of Thermal Biology* 17: 175–184.
437. Waßmer, T. and Wollnik, F. (1997). Timing of torpor bouts during hibernation in European hamsters (*Cricetus cricetus* L.). *Journal of Comparative Physiology B* 167: 270–279.
438. Wassmer, T. (2004). Body temperature and above-ground patterns during hibernation in European hamsters (*Cricetus cricetus* L.). *Journal of Zoology* 262: 281–288.
439. Siutz, C., Pluch, M., Ruf, T., and Millesi, E. (2012). Sex differences in foraging behaviour, body fat and hibernation patterns of free-ranging common hamsters. In: Ruf, T., Bieber, C., Arnold, W., and Millesi, E. (Eds.), *Living in a Seasonal World: Thermoregulatory and Metabolic Adaptations*. Berlin, Germany: Springer, pp. 155–165.
440. Pivorun, E. B. (1976). A biotelemetry study of the thermoregulatory patterns of *Tamias striatus* and *Eutamias minimus* during hibernation. *Comparative Biochemistry and Physiology A* 53: 265–271.
441. French, A. R. (2000). Interdependency of stored food and changes in body temperature during hibernation of the eastern chipmunk, *Tamias striatus*. *Journal of Mammalogy* 81: 979–985.
442. Kondo, N., Sekijima, T., Kondo, J., Takamatsu, N., Tohya, K., and Ohtsu, T. (2006). Circannual control of hibernation by HP complex in the brain. *Cell* 125: 161–172.
443. Wilz, M. and Heldmaier, G. (2000). Comparison of hibernation, estivation and daily torpor in the edible dormouse, *Glis glis*. *Journal of Comparative Physiology B* 170: 511–521.
444. Gür, M. K., Bulut, S., Gür, H., and Refinetti, R. (2014). Body temperature patterns and use of torpor in an alpine glirid species, the woolly dormouse. *Acta Theriologica* 59: 299–309.
445. Menaker, M. (1959). Endogenous rhythms of body temperature in hibernating bats. *Nature* 184: 1251–1252.
446. Menaker, M. (1964). Frequency of spontaneous arousal from hibernation in bats. *Nature* 203: 540–541.
447. Stawski, C., Turbill, C., and Geiser, F. (2009). Hibernation by a free-ranging subtropical bat (*Nyctophilus bifax*). *Journal of Comparative Physiology B* 179: 433–441.
448. Arnold, W., Heldmaier, G., Ortmann, S., Pohl, H., Ruf, T., and Steinlechner, S. (1991). Ambient temperatures in hibernacula and their energetic consequences for Alpine marmots (*Marmota marmota*). *Journal of Thermal Biology* 16: 223–226.
449. Fowler, P. A. and Racey, P. A. (1990). Daily and seasonal cycles of body temperature and aspects of heterothermy in the hedgehog *Erinaceus europaeus*. *Journal of Comparative Physiology B* 160: 299–307.
450. Saboureau, M., Vignault, M. P., and Ducamp, J. J. (1991). L'hibernation chez le Hérisson (*Erinaceus europaeus* L.) dans son environnement naturel: Étude par biotélémétrie des variations de la température corporelle. *Comptes Rendus de l'Académie des Sciences* 313: 93–100.
451. Körtner, G., Song, X., and Geiser, F. (1998). Rhythmicity of torpor in a marsupial hibernator, the mountain pygmy-possum (*Burramys parvus*), under natural and laboratory conditions. *Journal of Comparative Physiology B* 168: 631–638.
452. Geiser, F. (2007). Yearlong hibernation in a marsupial mammal. *Naturwissenschaften* 94: 941–944.
453. Nicol, S. C. and Andersen, N. A. (2007). Cooling rates and body temperature regulation of hibernating echidnas (*Tachyglossus aculeatus*). *Journal of Experimental Biology* 210: 586–592.
454. Blanco, M. B., Dausmann, K. H., Ranaivoarisoa, J. F., and Yoder, A. D. (2013). Underground hibernation in a primate. *Scientific Reports* 3: art. 1768.
455. Nelson, R. A., Wahner, H. W., Jones, J. D., Ellefson, R. D., and Zollman, P. E. (1973). Metabolism of bears before, during, and after winter sleep. *American Journal of Physiology* 224: 491–496.

456. Watts, P. D. (1989). Whole body thermal conductance of denning ursids. *Journal of Thermal Biology* 14: 67–70.
457. Øivind, T., Blake, J., Edgar, D. M., Grahn, D. A., Heller, H. C., and Barnes, B. M. (2011). Hibernation in black bears: Independence of metabolic suppression from body temperature. *Science* 331: 906–909.
458. MacMillen, R. E. (1965). Aestivation in the cactus mouse, *Peromyscus eremicus*. *Comparative Biochemistry and Physiology* 16: 227–248.
459. Cooper, C. E. and Geiser, F. (2008). The "minimal boundary curve for endothermy" as a predictor of heterothermy in mammals and birds: A review. *Journal of Comparative Physiology B* 178: 1–8.
460. Stross, R. G. and Hill, J. C. (1965). Diapause induction in Daphnia requires two stimuli. *Science* 150: 1462–1464.
461. Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemistry and Physiology A* 77: 513–517.
462. Lair, K. P., Bradshaw, W. E., and Holzapfel, C. M. (1997). Evolutionary divergence of the genetic architecture underlying photoperiodism in the pitcher-plant mosquito, *Wyeomyia smithii*. *Genetics* 147: 1873–1883.
463. Wang, X., Ge, F., Xue, F., and You, L. (2004). Diapause induction and clock mechanism in the cabbage beetle, *Colaphellus bowringi* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology* 50: 373–381.
464. Ogden, N. H., Lindsay, L. R., Beauchamp, G., Charron, D., Maarouf, A., O'Callaghan, C. J., Waltner-Toews, D., and Barker, I. K. (2004). Investigation of relationships between temperature and developmental rates of tick *Ixodes scapularis* (Acari: Ixodidae) in the laboratory and field. *Journal of Medical Entomology* 41: 622–633.
465. Tachibana, S. I. and Numata, H. (2004). Effects of temperature and photoperiod on the termination of larval diapause in *Lucilia sericata* (Diptera: Calliphoridae). *Zoological Science* 21: 197–202.
466. Campbell, H. A., Fraser, K. P. P., Bishop, C. M., Peck, L. S., and Egginton, S. (2008). Hibernation in an Antarctic fish: On ice for winter. *PLOS ONE* 3: e1743.
467. Scott, G. W., Fisher, K. C., and Love, J. A. (1974). A telemetric study of the abdominal temperature of a hibernator, *Spermophilus richardsonii*, maintained under constant conditions of temperature and light during the active season. *Canadian Journal of Zoology* 52: 653–658.
468. Sheriff, M. J., Williams, C. T., Kenagy, G. J., Buck, C. L., and Barnes, B. M. (2012). Thermoregulatory changes anticipate hibernation onset by 45 days: Data from free-living Arctic ground squirrels. *Journal of Comparative Physiology B* 182: 841–847.
469. Hudson, J. W. (1973). Torpidity in mammals. In: Whittow, G. C. (Ed.), *Comparative Physiology of Thermoregulation*, Vol. 3. New York: Academic Press, pp. 97–165.
470. Wang, L. C. H. and Lee, T. F. (1996). Torpor and hibernation in mammals: Metabolic, physiological, and biochemical adaptations. In: Fregly, M. J. and Blatteis, C. M. (Eds.), *Handbook of Physiology, Section 4: Environmental Physiology*, Vol. 1. New York: Oxford University Press, pp. 507–532.
471. Davis, D. E. (1976). Hibernation and circannual rhythms of food consumption in marmots and ground squirrels. *Quarterly Review of Biology* 51: 477–514.
472. Lyman, C. P. (1982). Entering hibernation. In: Lyman, C. P., Willis, J. S., Malan, A., and Wang, L. C. H. (Eds.), *Hibernation and Torpor in Mammals and Birds*. New York: Academic Press, pp. 37–53.
473. French, A. R. (1988). The patterns of mammalian hibernation. *American Scientist* 76: 568–575.
474. Snyder, G. K. and Nestler, J. R. (1990). Relationships between body temperature, thermal conductance, Q_{10} and energy metabolism during daily torpor and hibernation in rodents. *Journal of Comparative Physiology B* 159: 667–675.
475. Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology* 66: 239–274.
476. Heldmaier, G., Ortmann, S., and Elvert, R. (2004). Natural hypometabolism during hibernation and daily torpor in mammals. *Respiratory Physiology & Neurobiology* 141: 317–329.
477. Heldmaier, G. (2011). Life on low flame in hibernation. *Science* 331: 866–867.
478. Karpovich, S. A., Tøien, Ø., Buck, C. L., and Barnes, B. M. (2009). Energetics of arousal episodes in hibernating Arctic ground squirrels. *Journal of Comparative Physiology B* 179: 691–700.
479. Epperson, L. E., Karimpour-Fard, A., Hunter, L. E., and Martin, S. L. (2011). Metabolic cycles in a circannual hibernator. *Physiological Genomics* 43: 799–807.
480. Jinka, T. R., Tøien, Ø., and Drew, K. L. (2011). Season primes the brain in an Arctic hibernator to facilitate entrance into torpor mediated by adenosine A1 receptors. *Journal of Neuroscience* 31: 10752–10758.
481. Arnold, W., Ruf, T., Reimoser, S., Tataruch, F., Onderscheka, K., and Schober, F. (2004). Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). *American Journal of Physiology* 286: R174–R181.
482. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2003). Serum cortisol levels in goats exhibit seasonal but not daily rhythmicity. *Chronobiology International* 20: 65–79.
483. Guerin, M. V. and Matthews, C. D. (1998). Alterations of estrous activity in the ewe by circadian-based manipulation of the endogenous pacemaker. *Journal of Biological Rhythms* 13: 60–69.
484. Souza, M. I. L., Bicudo, S. D., Uribe-Velásquez, L. F., and Ramos, A. A. (2002). Circadian and circannual rhythms of T3 and T4 secretions in Polwarth-Ideal rams. *Small Ruminant Research* 46: 1–5.
485. Gay, F., Valiante, S., Sciarrillo, R., de Falco, M., Laforgia, V., and Capaldo, A. (2010). Annual and daily serum aldosterone and catecholamine patterns in males of the Italian crested newt, *Triturus carnifex* (Amphibia, Urodela). *Italian Journal of Zoology* 77: 384–390.
486. Lerner, A. B., Case, J. D., Takahashi, Y., Lee, T. H., and Mori, W. (1958). Isolation of melatonin, the pineal gland factor that lightens melanocytes. *Journal of the American Chemical Society* 80: 2587.
487. Thrun, L. A., Moenter, S. M., O'Callaghan, D., Woodfill, C. J. I., and Karsch, F. J. (1995). Circannual alterations in the circadian rhythm of melatonin secretion. *Journal of Biological Rhythms* 10: 42–54.
488. Guerin, M. V., Deed, J. R., Kennaway, D. J., and Matthews, C. D. (1995). Plasma melatonin in the horse: Measurements in natural photoperiod and in acutely extended darkness throughout the year. *Journal of Pineal Research* 19: 7–15.
489. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2001). Seasonal variation in endogenous serum melatonin profiles in goats: A difference between spring and fall? *Journal of Biological Rhythms* 16: 254–263.
490. Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K., and Tabata, M. (2003). Variations in plasma melatonin levels of the rainbow trout (*Oncorhynchus mykiss*) under various light and temperature conditions. *Zoological Science* 20: 1011–1016.

491. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J., and Simonneaux, V. (2003). Mechanisms regulating the marked seasonal variation in melatonin synthesis in the European hamster pineal gland. *American Journal of Physiology* 284: R1043–R1052.
492. Bertolucci, C., Foà, A., and Van't Hof, T. J. (2002). Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. *Hormones and Behavior* 41: 414–419.
493. García, A., Landete-Castillejos, T., Zarazaga, L., Garde, J., and Gallego, L. (2003). Seasonal changes in melatonin concentrations in female Iberian red deer (*Cervus elaphus hispanicus*). *Journal of Pineal Research* 34: 161–166.
494. Piccione, G., Caola, G., and Refinetti, R. (2007). Annual rhythmicity and maturation of physiological parameters in goats. *Research in Veterinary Science* 83: 239–243.
495. Haritou, S. J. A., Zylstra, R., Ralli, C., Turner, S., and Tortonese, D. J. (2008). Seasonal changes in circadian peripheral plasma concentrations of melatonin, serotonin, dopamine and cortisol in aged horses with Cushing's disease under natural photoperiod. *Journal of Neuroendocrinology* 20: 988–996.
496. Bünning, E. (1960). Circadian rhythms and the time measurement in photoperiodism. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 249–256.
497. Gorman, M. R., Borman, B. D., and Zucker, I. (2001). Mammalian photoperiodism. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 481–508.
498. Rust, C. C., Shackelford, R. M., and Meyer, R. K. (1965). Hormonal control of pelage cycles in the mink. *Journal of Mammalogy* 46: 549–565.
499. Rust, C. C. (1962). Temperature as a modifying factor in the spring pelage change of sort-tailed weasels. *Journal of Mammalogy* 43: 323–328.
500. Harris, G. D., Huppi, H. D., and Gessaman, J. A. (1985). The thermal conductance of winter and summer pelage of *Lepus californicus*. *Journal of Thermal Biology* 10: 79–81.
501. Walsberg, G. E. (1991). Thermal effects of seasonal coat change in three subarctic mammals. *Journal of Thermal Biology* 16: 291–296.
502. Al-Hilli, F. and Wright, E. A. (1988). The effects of environmental temperature on the hair coat of the mouse. *Journal of Thermal Biology* 13: 21–24.
503. Duncan, M. J. and Goldman, B. D. (1984). Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, *Phodopus sungorus*. I. Role of the gonads and the pituitary. *Journal of Experimental Zoology* 230: 89–95.
504. Goldman, S. L., Dhandapani, K., and Goldman, B. D. (2000). Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 417–428.
505. Palchykova, S., Deboer, T., and Tobler, I. (2003). Seasonal aspects of sleep in the Djungarian hamster. *BMC Neuroscience* 4: art. 9.
506. Burkhardt, J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *Journal of Agricultural Science* 37: 64–68.
507. Lincoln, G. A., Andersson, H., and Hazlerigg, D. (2003). Clock genes and the long-term regulation of prolactin secretion: Evidence for a photoperiodic/circannual timer in the pars tuberalis. *Journal of Neuroendocrinology* 15: 390–397.
508. Oda, Y. (1969). The action of skeleton photoperiods on flowering in *Lemna perpusilla*. *Plant Cell Physiology* 10: 399–409.
509. Park, D. H., Somers, D. E., Kim, Y. S., Choy, Y. H., Lim, H. K., Soh, M. S., Kim, H. J., Kay, S. A., and Nam, H. G. (1999). Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285: 1579–1582.
510. Roden, L. C., Song, H. R., Jackson, S., Morris, K., and Carre, I. A. (2002). Floral responses to photoperiod are correlated with the timing of rhythmic expression relative to dawn and dusk in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the United States of America* 99: 13313–13318.
511. Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A., and Coupland, G. (2004). Photoreceptor regulation of CONTANS protein in photoperiodic flowering. *Science* 303: 1003–1006.
512. Madhavan, K. and Shribbs, J. M. (1981). Role of photoperiod and low temperature in the control of ovigerous molt in the terrestrial isopod, *Armadillidium vulgare* (Latreille, 1804). *Crustaceana* 41: 263–270.
513. Razani, H., Hanyu, I., and Aida, K. (1987). Critical daylength and temperature level for photoperiodism in gonadal maturation of goldfish. *Experimental Biology* 47: 89–94.
514. Okuzawa, K., Furukawa, K., Aida, K., and Hanyu, I. (1989). Effects of photoperiod and temperature on gonadal maturation and plasma steroid and gonadotropin levels in a cyprinid fish, the honmoroko *Gnathopogon caerulescens*. *General and Comparative Endocrinology* 75: 139–147.
515. Chang, C. F., Hu, H. J., and Sun, L. T. (1993). Effects of temperature and photoperiod on ovarian development in female ayu, *Plecoglossus altivelis*. *Journal of Thermal Biology* 18: 197–201.
516. Park, Y. J., Takemura, A., and Lee, Y. D. (2006). Lunar-synchronized reproductive activity in the pencil-streaked rabbitfish *Siganus doliatus* in the Chuuk Lagoon, Micronesia. *Ichthyological Research* 53: 179–181.
517. King, V. M., Bentley, G. E., and Follett, B. K. (1997). A direct comparison of photoperiodic time measurement and the circadian system in European starlings and Japanese quail. *Journal of Biological Rhythms* 12: 431–442.
518. Zivkovic, B. D., Underwood, H., Steele, C. T., and Edmonds, K. (1999). Formal properties of the circadian and photoperiodic systems of Japanese quail: Phase response curve and effects of T-cycles. *Journal of Biological Rhythms* 14: 378–390.
519. Bentley, G. E., Spar, B. D., MacDougall-Shackleton, S. A., Hahn, T. P., and Ball, G. F. (2000). Photoperiod regulation of the reproductive axis in male zebra finches, *Taeniopygia guttata*. *General and Comparative Endocrinology* 117: 449–455.
520. Puchalski, W. and Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: Effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.
521. Anchordoquy, H. C. and Lynch, G. R. (2000). Timing of testicular recrudescence in Siberian hamsters is unaffected by pinealectomy or long-day photoperiod after 9 weeks in short days. *Journal of Biological Rhythms* 15: 406–416.
522. Larkin, J. E., Freeman, D. A., and Zucker, I. (2001). Low ambient temperature accelerates short-day responses in Siberian hamsters by altering responsiveness to melatonin. *Journal of Biological Rhythms* 16: 76–86.
523. Zhou, S., Cagampang, F. R. A., Stirland, J. A., Loudon, A. S. I., and Hopkins, S. J. (2002). Different photoperiods affect proliferation of lymphocytes but not expression of cellular, humoral, or innate immunity in hamsters. *Journal of Biological Rhythms* 17: 392–405.
524. Gaston, S. and Menaker, M. (1967). Photoperiodic control of hamster testis. *Science* 158: 925–928.

525. Pratt, B. L. and Goldman, B. D. (1986). Activity rhythms and photoperiodism of Syrian hamsters in a simulated burrow system. *Physiology and Behavior* 36: 83–89.
526. Maywood, E. S., Buttery, R. C., Vance, G. H. S., Herbert, J., and Hastings, M. H. (1990). Gonadal responses of the male Syrian hamster to programmed infusions of melatonin are sensitive to signal duration and frequency but not to signal phase nor to lesions of the suprachiasmatic nuclei. *Biology of Reproduction* 43: 174–182.
527. Heideman, P. D. and Bronson, F. H. (1993). Sensitivity of Syrian hamsters (*Mesocricetus auratus*) to amplitudes and rates of photoperiodic change typical of the tropics. *Journal of Biological Rhythms* 8: 325–337.
528. Shimomura, K., Nelson, D. E., Ihara, N. L., and Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
529. Loudon, A. S. I., Ihara, N., and Menaker, M. (1998). Effects of a circadian mutation on seasonality in Syrian hamsters (*Mesocricetus auratus*). *Proceedings of the Royal Society of London B* 265: 517–521.
530. Jallageas, M., Mas, N., and Nouguier-Soulé, J. (1991). Control of annual endocrine rhythms in the edible dormouse: Nonprimary effect of photoperiod. *Journal of Biological Rhythms* 6: 343–352.
531. Edmonds, K. E. and Stetson, M. H. (2001). Effects of age and photoperiod on reproduction and the spleen in the marsh rice rat (*Oryzomys palustris*). *American Journal of Physiology* 280: R1249–R1255.
532. Edmonds, K. E., Riggs, L., and Stetson, M. H. (2003). Food availability and photoperiod affect reproductive development and maintenance in the marsh rice rat (*Oryzomys palustris*). *Physiology and Behavior* 78: 41–49.
533. Sicard, B., Fuminier, F., Maurel, D., and Boissin, J. (1993). Temperature and water conditions mediate the effects of day length on the breeding cycle of a Sahelian rodent, *Arvicanthis niloticus*. *Biology of Reproduction* 49: 716–722.
534. Concannon, P. W., Parks, J. E., Roberts, P. J., and Tennant, B. C. (1992). Persistent free-running circannual reproductive cycles during prolonged exposure to a constant 12L:12D photoperiod in laboratory woodchucks (*Marmota monax*). *Laboratory Animal Science* 42: 382–391.
535. Concannon, P. W., Castracane, V. D., Rawson, R. E., and Tennant, B. C. (1999). Circannual changes in free thyroxine, prolactin, testes, and relative food intake in woodchucks, *Marmota monax*. *American Journal of Physiology* 277: R1401–R1409.
536. Holloway, J. C. and Geiser, F. (1996). Reproductive status and torpor of the marsupial *Sminthopsis crassicaudata*: Effect of photoperiod. *Journal of Thermal Biology* 21: 373–380.
537. Génin, F. and Perret, M. (2003). Daily hypothermia in captive grey mouse lemurs (*Microcebus murinus*): Effects of photoperiod and food restriction. *Comparative Biochemistry and Physiology B* 136: 71–81.
538. García, A. J., Landete-Castillejos, T., Garde, J. J., and Gallego, L. (2002). Reproductive seasonality in female Iberian red deer (*Cervus elaphus hispanicus*). *Theriogenology* 58: 1553–1562.
539. Ebling, F. J. P., Lincoln, G. A., Wollnik, F., and Anderson, N. (1988). Effects of constant darkness and constant light on circadian organization and reproductive responses in the ram. *Journal of Biological Rhythms* 3: 365–384.
540. Karsch, F. J., Robinson, J. E., Woodfill, C. J., and Brown, M. B. (1989). Circannual cycles of luteinizing hormone and prolactin secretion in ewes during prolonged exposure to a fixed photoperiod: Evidence for an endogenous reproductive rhythm. *Biology of Reproduction* 41: 1034–1046.
541. Chemineau, P., Daveau, A., Cognié, Y., Aumont, G., and Chesneau, C. (2004). Seasonal ovulatory activity exists in tropical Creole female goats and Black Belly ewes subjected to a temperate photoperiod. *BMC Physiology* 4: art. 12.
542. Ishikawa, A., Sakamoto, H., Katagiri, S., and Takahashi, Y. (2003). Changes in sexual behavior and fecal steroid hormone concentrations during the breeding season in female Hokkaido brown bears (*Ursus arctos yesoensis*) under captive conditions. *Journal of Veterinary Medical Science* 65: 99–102.
543. Fowler, G. S., Wingfield, J. C., Boersma, P. D., and Sosa, R. A. (1994). Reproductive endocrinology and weight change in relation to reproductive success in the Magellanic penguin (*Spheniscus magellanicus*). *General and Comparative Endocrinology* 94: 305–315.
544. Roenneberg, T. and Aschoff, J. (1990). Annual rhythm of human reproduction: I. Biology, sociology, or both? *Journal of Biological Rhythms* 5: 195–216.
545. Randall, W. (1990). The solar wind and human birth rate: A possible relationship due to magnetic disturbances. *International Journal of Biometeorology* 34: 42–48.
546. Randall, W. (1995). The annual temporal pattern of human births in the USA. *Biological Rhythm Research* 26: 505–520.
547. Chandwani, K. D., Cech, I., Smolensky, M. H., Burau, K., and Hermida, R. C. (2004). Annual pattern of human conception in the state of Texas. *Chronobiology International* 21: 73–93.
548. Mongrain, V., Paquet, J., and Dumont, M. (2006). Contribution of the photoperiod at birth to the association between season of birth and diurnal preference. *Neuroscience Letters* 406: 113–116.
549. Matchock, R. L., Susman, E. J., and Brown, F. M. (2004). Seasonal rhythms of menarche in the United States: Correlates to menarcheal age, birth age, and birth month. *Women's Health Issues* 14: 184–192.
550. Zucker, I., Lee, T. M., and Dark, J. (1991). The suprachiasmatic nucleus and annual rhythms of mammals. In: Klein, D. C., Moore, R. Y., and Reppert, S. M. (Eds.), *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press, pp. 246–259.
551. Zucker, I. (2001). Circannual rhythms: Mammals. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 509–528.
552. Ruby, N. F. (2003). Hibernation: When good clocks go cold. *Journal of Biological Rhythms* 18: 275–286.
553. Lincoln, G. A., Andersson, H., and Loudon, A. (2003). Clock genes in calendar cells as the basis of annual timekeeping in mammals: A unifying hypothesis. *Journal of Endocrinology* 179: 1–13.
554. Lincoln, G. A., Clarke, I. J., Hut, R. A., and Hazlerigg, D. G. (2006). Characterizing a mammalian circannual pacemaker. *Science* 314: 1941–1944.
555. Gwinner, E. (1977). Photoperiodic synchronization of circannual rhythms in the European starling (*Sturnus vulgaris*). *Naturwissenschaften* 64: 44.
556. Gwinner, E. and Dittami, J. (1990). Endogenous reproductive rhythms in a tropical bird. *Science* 249: 906–908.
557. Piersma, T. (2002). When a year takes 18 months: Evidence for a strong circannual clock in a shorebird. *Naturwissenschaften* 89: 278–279.
558. Lumineau, S. and Guyomarc'h, C. (2000). Circadian rhythm of activity during the annual phases in the European quail, *Coturnix coturnix*. *Comptes Rendus de l'Académie des Sciences* 323: 793–799.

559. Scheuerlein, A. and Gwinner, E. (2002). Is food availability a circannual zeitgeber in tropical birds? A field experiment on stonechats in tropical Africa. *Journal of Biological Rhythms* 17: 171–180.
560. Mrosovsky, N. (1975). The amplitude and period of circannual cycles of body weight in golden-mantled ground squirrels with medial hypothalamic lesions. *Brain Research* 99: 97–116.
561. Richter, C. P. (1978). Evidence for existence of a yearly clock in surgically and self-blinded chipmunks. *Proceedings of the National Academy of Sciences of the United States of America* 75: 3517–3521.
562. Zucker, I., Boshes, M., and Dark, J. (1983). Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. *American Journal of Physiology* 244: R472–R480.
563. Anchordoquy, H. C. and Lynch, G. R. (2000). Evidence of an annual rhythm in a small proportion of Siberian hamsters exposed to chronic short days. *Journal of Biological Rhythms* 15: 122–125.
564. Liu, H. K., Nestor, K. E., Long, D. W., and Bacon, W. L. (2001). Frequency of luteinizing hormone surges and egg production rate in turkey hens. *Biology of Reproduction* 64: 1769–1775.

5 Daily and Circadian Rhythms

5.1 ENVIRONMENTAL AND POPULATIONAL RHYTHMS

If you paid attention to the title of this chapter, you may be wondering why I called it "daily and circadian rhythms." Are not daily rhythms the same thing as circadian rhythms? Yes and no. According to the *American Heritage Dictionary*, the adjective *daily* applies to something that happens or is done every day or once a day.[1] A similar description is given for the adjective *circadian* (Figure 5.1). To understand the difference between the two words, we need to look at their histories.

Daily derives from the Old English *dæglic* and dates back to the twelfth century.[1] *Circadian* was created by Halberg in the 1950s by the combination of the Latin terms *circa* (about) and *dies* (day).[2] Thus, *circadian* literally means "approximately daily." In the 1950s and most of the 1960s, biologists were still arguing about the existence of truly endogenous biological rhythms in the circadian range.[3] Halberg—whose historical significance was discussed in Chapter 1—was certain that circadian rhythms were endogenously generated and felt the need for a term that could emphasize the endogenous nature of the rhythms. He felt that the designation "approximately daily" (*circadian*) would convey the idea of endogenesis, because a process that can have a period different from that of environmental cycles must be produced endogenously. In conversations with me, he recollected that the term *circadian* was created in 1950 or 1951, although he did not formally introduce it to the scientific community until 1959.[2] The new word became popular instantly. As a matter of record, the U.S. National Library of Medicine's PubMed database lists 16 journal articles published in the year 1960 alone that have the word *circadian* in the title. The entries for over 1500 articles published by the end of 1969 can be retrieved by the term *circadian* in the title, abstract, or indexing field. The count surpasses 38,000 for entries through the end of the year 1999.

Curiously, Halberg himself contributed to confusion in the use of the new word by suggesting that *circadian* could be used to designate both free-running rhythms and rhythms observed under a 24-hour environmental cycle.[2] His reasoning was that an endogenous rhythm does not stop being endogenous when it is synchronized to an environmental cycle, which is true. Yet, a rhythm observed under a 24-hour environmental cycle need not be an endogenous rhythm. This subtle distinction eluded many researchers who were not specialists in circadian physiology. It was Aschoff—the second member of the triad of forefathers of circadian physiology discussed in Chapter 1—who formalized three requirements for the appropriate use of the term *circadian*.[4] A biological rhythm is said to be *circadian* if (1) it is endogenously generated, (2) it has a free-running period close to 24 hours, and (3) it can be modified (synchronized) by environmental cycles with 24-hour periods. Strictly speaking, all three requirements must be met to justify the use of the term *circadian*. (As mentioned in Chapter 4, a period "close to 24 hours" means a period between approximately 19 and 28 hours.)

The term *daily*—used to denote rhythms with a period of 24 hours whose endogenous nature has not been ascertained or has been disproved by experimental research—is not as unique as the term *circadian*. Many synonyms are currently in use. *Daily* is certainly preferable to *diurnal*, which has been used in this sense by many authors[5–12] but should be reserved for the meaning of "during the *daylight* segment of a day" (i.e., "during the *photophase*") as an antonym for *nocturnal*. *Daily* is also preferable to the unnecessary neologism *diel*, which has been used for many years mostly by researchers with an ecological background.[13–20] Another alternative is the term *nycthemeral* (from the Greek *nychthémeron*, which means "the duration of a night and a day"). *Nycthemeral* has been in use since at least 1884[21] and has been adopted by many authors, particularly in Europe.[22–28] Although I personally prefer *daily* to *nycthemeral*, the latter is probably a better technical term—because it does not have the double meaning of "every day" and "once a day" that *daily* has, and because it follows the traditional derivation of scientific terms from the Greek or Latin. A much less elegant alternative is the use of the noun *24 hour* as an adjective, such as in "24-hour rhythm."[29–34]

In this section, we will examine *environmental rhythms* with 24-hour periods as well as some human *populational rhythms* (i.e., rhythms that can be detected at the level of groups of people but not necessarily in single individuals). Daily and circadian rhythms in individual behavioral (voluntary) functions will be examined in Section 5.2, and rhythms in autonomic (involuntary) functions will be examined in Section 5.3.

5.1.1 Environmental Rhythms

On Earth, the Sun rises and sets every 24 hours (Figure 5.2). The alternation of day and night is determined by the rotation of the Earth on its axis.[35–37] During each rotation, the side of the Earth exposed to the Sun experiences daylight, whereas the opposite side experiences the darkness of the night (Figure 5.3). Unfortunately, the English language—like many other languages—uses the same word to designate both the interval between two sunrises (*day*) and the interval between sunrise and sunset (*day*), which can be confusing at times. The interval between sunset and sunrise is uniquely designated as *night*.

Cir–ca–di–an (sər–kā′ dē–ən) *adj. Biol.*
Of or exhibiting approximately 24-hour periodicity.
[<Lat. *circā*, around + *diēs*, day.]

FIGURE 5.1 What does circadian mean? This is the definition of *circadian* given by a popular dictionary. (From *The American Heritage Dictionary of the English Language*, Houghton Mifflin, New York, 1994.)



FIGURE 5.2 Sunrise over Pelican Island National Wildlife Refuge, Florida. The alternation of day and night constitutes a robust environmental daily rhythm. (Courtesy of National Image Library, United States Fish and Wildlife Service.)

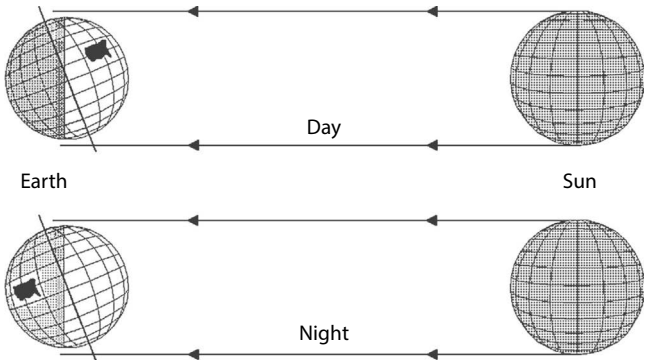


FIGURE 5.3 Day and night. The alternation of day and night results from the Earth's rotation around its axis. The side of the Earth exposed to the Sun experiences the day, whereas the opposite side experiences the night.

A day—in the sense of *nychthémeron*—corresponds to the interval of time required for a full rotation of the Earth. This depends on how we define a full rotation, however. The time required for a full rotation with respect to the stars is called a *sidereal day* and is about 4 minutes shorter than the *solar day*, which corresponds to the time required for a full cycle of the apparent motion of the Sun in the sky.[38,39] The apparent motion of the Sun is actually not uniform, so that a *civil day* is defined as the *mean* solar day (24.0 hours). A civil day has two *civil twilights*: dawn and dusk. The durations of the light segment of the day (the dawn-to-dusk interval) and the dark segment of the day (the dusk-to-dawn interval) depend on latitude and season, as previously discussed in Chapter 4 (Section 4.1). For instance, at 50°N, the dawn-to-dusk interval lasts 16 hours in July but only 8 hours in December. Although days with long dawn-to-dusk intervals are often called "long days" and days with short dawn-to-dusk intervals are called "short days," the actual duration of the day (dawn-to-dawn) is, of course, always 24 hours. Nonetheless, because civil clock times vary not only with latitude and season but also with geopolitical factors, different locations on Earth have very different days (in the sense of the dawn-to-dusk interval) at a given time of the year. Figure 5.4 shows the timing of the photophase of the natural light–dark cycle in six different cities on January 29. Particularly noteworthy is the fact that noon is *not* truly "midday" even in Quito, which lies on the Equator. In Puerto Williams (Chile), the afternoon accounts for two thirds of the day.

The most conspicuous difference between day and night is the change in illumination. On a clear day, outdoor illuminance is in the order of 10^4 lux, whereas on a clear night, with a full moon, illuminance is in the order of 10^{-1} lux.[40] Although not as dramatic, and not as regular, there is also a daily variation in ambient temperature and relative humidity.[35–37] Figure 5.5 shows records of illuminance and air temperature for a typical summer day in the little town of Walterboro, South Carolina. On this overcast day, outdoor illuminance rose to 5000 lux within 2 hours after sunrise, stayed up throughout the day, and fell in the evening. For comparison, a typical record of indoor illuminance (such as that of a vivarium room) is also shown. Although the lights come on instantly at the flip of a switch (or under the control of a timer), the night–day transition is not much different from the outdoors one. What is clearly different is the much lower level attained after the transition (200–300 lux). Outdoors, the change in illumination that takes place at dawn and dusk is not limited to light intensity. There is also a change in spectral composition (color), with less green light (and relatively more blue and red light) when the Sun is below the horizon.[41]

The lower panel in Figure 5.5 shows the records of outdoor and indoor temperature. Depending on the quality of the air-conditioning system, indoor temperature may oscillate as little as 1°C or less throughout the day, while outdoor temperature oscillates as much as 7°C or more. The difference between day and night temperatures varies depending on latitude, season, and other factors—including short-term weather changes, as is evident in the 4-day records of air temperature for the city of Miami shown in Figure 5.6. Day–night differences in temperature seem to be undergoing a small reduction of approximately 1°C per 100 years in most of the world.[42]

Many organisms inhabit *microhabitats* that are not exposed to the full range of daily variation in illumination and temperature. In these cases, the influence of environmental cycles is diminished. For instance, aquatic organisms generally experience a much smaller daily variation in environmental temperature than

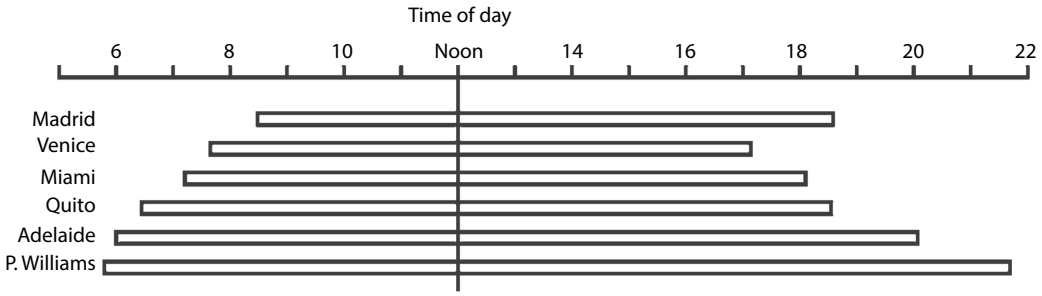


FIGURE 5.4 Varying day lengths. For each of the six cities, the horizontal white bars indicate the hours of sunlight on January 29. The cities are Madrid (Spain), Venice (Italy), Miami (Florida), Quito (Equador), Adelaide (Australia), and Puerto Williams (Chile). (Data from Time and Date, www.timeanddate.com.)

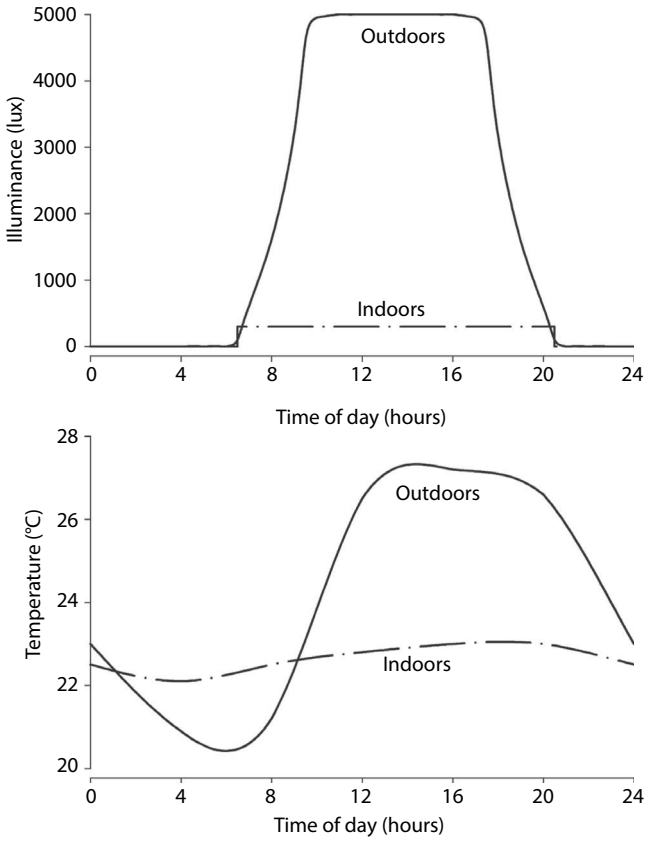


FIGURE 5.5 A typical mild summer day in Walterboro, South Carolina. The graphs show the daily variation of illuminance (luminosity) and ambient temperature during a mild summer day, as measured by the author. The indoor readings refer to an air-conditioned room with artificial lighting controlled by a timer.

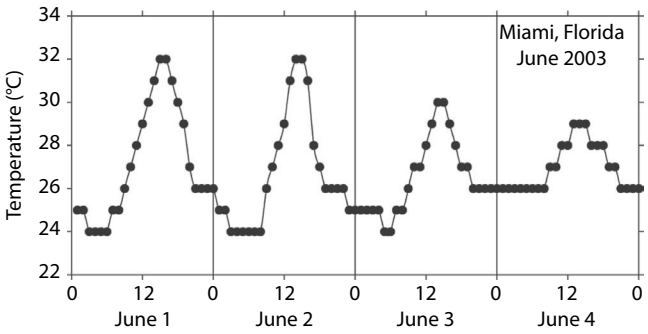


FIGURE 5.6 Late spring in Miami, Florida. The graph shows hourly measurements of ambient temperature in Miami during the first 4 days of June 2003. Although the temperature rose and fell each day, the range of oscillation was much greater during the first 2 days than during the last 2 days. (Data from The Weather Channel, www.weatherchannel.com.)

do terrestrial animals, because rivers and oceans warm up and cool off more slowly than does the lower atmosphere. Organisms that live in subterranean environments are exposed to smaller fluctuations in both illumination and temperature. Animals that spend the day in burrows or shelters but come out at night generally experience smaller variation in environmental illumination even when traveling long distances on the surface. As will be discussed in Chapter 9, however, evolutionary adaptations of morphological and functional traits have *not* been accompanied by major alterations in the properties of the circadian system.

5.1.2 Populational Rhythms

Many important events in one's life occur too seldom to characterize a rhythmic process. Each one of us is born only once, gets married only a few times, and occasionally has a major motor-vehicle accident or a life-threatening health problem. However, if these events are considered at a populational level, they may very well exhibit daily rhythmicity. Let us take the example of *sexual activity*. The average American in his or her early adult years has sex 2–3 times a week.[43] Therefore, it makes no sense to look for a daily rhythm of sexual activity at the individual level. However, different individuals have sex at different times of the day on different occasions, so that a populational daily rhythm (an *educed rhythm*) may exist. As a matter of fact, Figure 5.7 shows the results from a study of 78 young married couples living in North Carolina who kept detailed records of their sexual activity for 3 months.[44] It is evident that although young married couples have sex almost any time of the day, most episodes take place around 11 p.m. We discussed similar results from South Carolina (with most episodes of sexual activity occurring at bedtime) in Chapter 3,[45] and a third study in Poland obtained comparable results.[46] The findings in all three studies are in agreement with those obtained in a survey about the time of day of the first intercourse of young women, in which it was found that over 85% of the ladies had lost their virginity at night.[47]

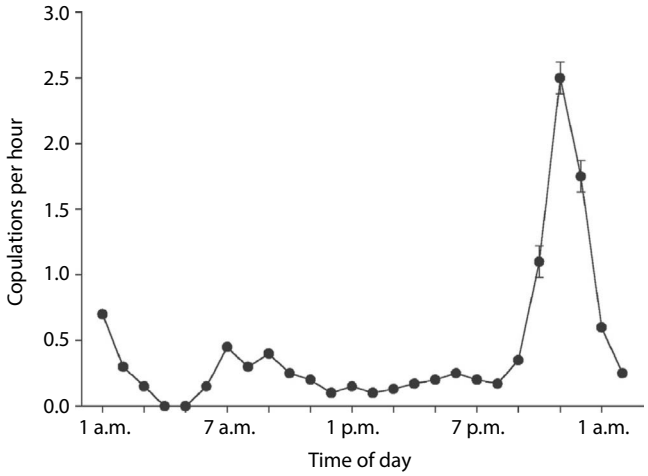


FIGURE 5.7 Time for sex. The sexual encounters of 78 young married couples were recorded for 3 consecutive months. Shown are the mean copulation rates (±SE) across the day. There is a clear populational rhythm with a pronounced peak around 11 o'clock at night. (Data from Palmer, J.D. et al., *Hum. Biol.*, 54, 111, 1982.)



FIGURE 5.8 A newborn baby. There is a daily rhythm in the birth of human babies. (Copyright ArtToday, Tucson, AZ.)

Although sexual activity is the route to reproduction, it is unlikely that the time of day of intercourse bears any relationship with the time of day of parturition (i.e., the act of giving birth) that takes place 9 months later. Nonetheless, the time of day of parturition does exhibit a populational rhythm (Figure 5.8). Several studies, based on thousands of births recorded over many years, have consistently found a daily rhythm of parturition that peaks around noon.[48–51] As an example, the hourly distribution of deliveries at the Rambam Medical Center, in Israel, is shown in Figure 5.9. The top graph shows the distribution for 41,626 deliveries over an 8-year interval, whereas the lower graph shows the distribution for only 5,289 deliveries that required *urgent* cesarean sections. There is a clear daily pattern of total deliveries, with high numbers between 8 a.m. and 4 p.m. Because the interval between 8 a.m. and 4 p.m. corresponds to the usual day-shift of hospital personnel, it is not unreasonable to wonder whether physicians' convenience dictates the time of delivery.

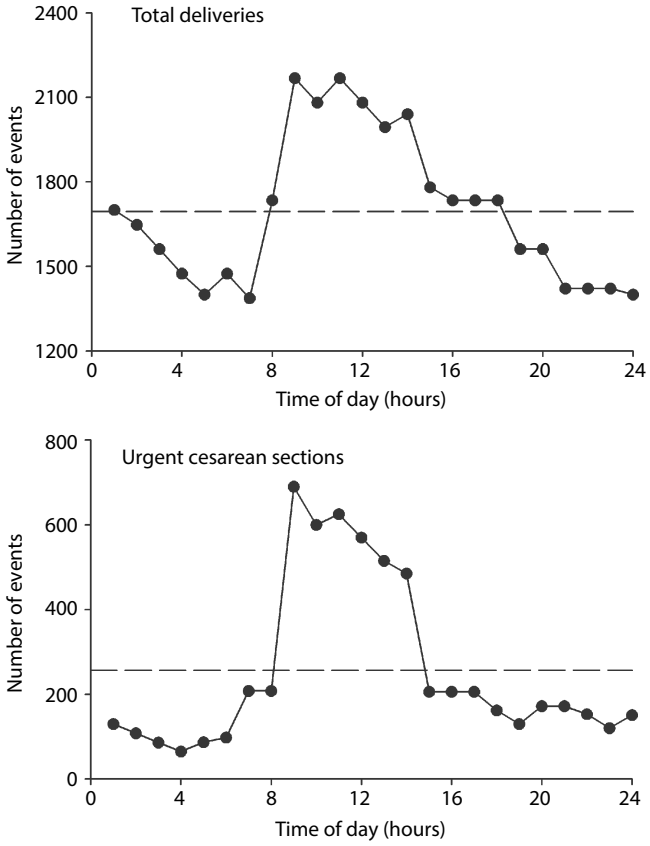


FIGURE 5.9 The time to be born. The graphs show the daily distributions of deliveries (total deliveries and urgent cesarean deliveries) over an 8-year interval in an Israeli maternity hospital (Rambam Medical Center). The distributions clearly deviate from a flat pattern corresponding to the 24-hour mean (dashed line). Most deliveries occur between 8 a.m. and 4 p.m. (Data from Goldstick, O. et al., *Israel Med. Assoc. J.*, 5, 564, 2003.)

The authors of this study reasoned that, if the daily pattern of deliveries is due to physicians' convenience rather than to a biological rhythm, then the pattern should not be present in deliveries involving urgent cesarean sections (that is, deliveries for which an ethical physician would not permit mere convenience to play an important role). As shown by the bottom graph, the daily distribution of urgent deliveries is very similar to that of total deliveries, which suggests that the daily pattern of total deliveries is a *bona fide* biological rhythm. The *bona fide* nature of the rhythm is supported by an unrelated study in Canada, in which it was found that the onset of labor (which takes place in the expectant mother's home and should not be affected by physicians' convenience) also exhibits daily rhythmicity, with a peak between 8 p.m. and 2 a.m.[48]

Road traffic is another populational phenomenon that exhibits daily rhythmicity. *Rush hour* traffic (Figure 5.10) is very common in large cities at the beginning and end of the workday. A study on highway accidents in Sweden, involving 12,535 accidents with personal injury over an interval of 5 years, found sharp peaks at rush hours (8 a.m. and 5 p.m.).[52] Although this is an interesting statistic in its own right, the authors of the study also computed the hourly distribution of



FIGURE 5.10 Stuck in traffic. Congested traffic is a common occurrence in large cities. It follows a daily rhythm with greater congestion at the beginning and end of the business day. (Copyright ArtToday, Tucson, AZ.)

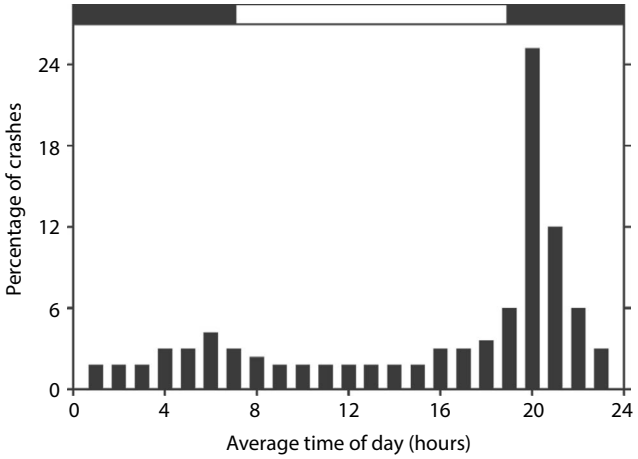


FIGURE 5.11 Road kill. The graph shows the daily distribution of vehicle crashes with moose (*Alces alces*) in Finnish highways from 1989 to 1997. The horizontal dark and white bars at the top indicate the duration of darkness and sunlight, respectively. Because the times of sunrise and sunset vary during the year, the percentage of crashes is plotted not as a function of actual time of day but as a function of time before and after sunset and sunrise. It is evident that most collisions occur shortly after sunset. (Data from Haikonen, H. and Summala, H., *Am. J. Prevent. Med.*, 21, 209, 2001.)

accidents after correction for the daily variation in regular traffic volume. That is, more accidents are expected to occur when there are more vehicles on the road—but is there a daily variation in the occurrence of accidents when the number of accidents is expressed as a percentage of the volume of traffic? The answer is *yes*. Accidents are five times more likely to occur at 4 a.m. than at other times of the day.[52] Presumably, drivers are sleepier at 4 o'clock in the morning and are more prone to fall asleep while driving or simply not to concentrate on the driving task. Poor visibility at night might also be a causative agent—but, if so, one would expect a high frequency of accidents also early in the night, which is not the case. Poor driver visibility is also unlikely to be responsible for an increase in animal–vehicle crashes shortly after sunset. As shown in Figure 5.11—which is based on the records of 13,379 vehicle crashes with wild moose in Finland over a 9-year interval—there are many more crashes shortly after sunset than shortly before sunrise.[53] Here, the temporal pattern is probably not related to a human rhythm of attention either, as 8 p.m. is not a particularly low time for human performance. Instead, the increase in the number of crashes at 8 p.m. is probably due to a greater movement of moose at this time. The daily organization of moose behavior evidently can have significant effects on humans.

Several million people around the world voluntarily edit articles in Wikipedia, the open-access encyclopedia. A study of the time of day when edits are made, with adjustment for local time, revealed a global pattern with a minimum of activity at 6 a.m. followed by a rapid rise, a high plateau from noon to 10 p.m., and a fall to the next trough at 6 a.m.[54] Strong daily rhythmicity is also found in the use of online social networks.[55]

Many other populational rhythms have been described. For instance, suicides tend to occur during light hours (that is, mostly in the morning and afternoon, and not at night).[32,56–60] The morning is the most common time for heart attacks[61–68] and stroke.[69–71] Unexpected postoperative deaths are more common late at night,[72,73] whereas newborn perinatal mortality is higher in the evening.[49] Migraine attacks in children are much more common during the day (especially the afternoon) than at night.[74] Aggressive behavior of psychiatric patients is more common in the early afternoon,[75] whereas accidents in children are more common in the early evening.[76–78] Medical students and residents are more likely to be accidentally exposed to pathogens during the day, when the hospital is busiest; however, if correction is made for the number of people on duty, accidental exposures are much more common at night, with a peak at 11 p.m.[79]

The physiological processes responsible for populational rhythms are not known. However, the fact that populational rhythms can be detected implies that the individuals that make up the population are synchronized. If they were not synchronized, their individual processes would be scattered all over the day, and no consistent populational pattern would emerge. Therefore, populational rhythms cannot truly free-run and, except under special circumstances, cannot be considered to be *circadian* rhythms. We can only say that they are *daily* rhythms. Of course, these daily rhythms could be

endogenously generated and be only modulated by environmental factors (rather than fully caused by environmental factors). In order to determine the likelihood of the endogenous nature of the rhythms, one must first conduct studies on rhythms that can be measured at the level of individuals. Such studies will be reviewed in the next two sections.

5.2 BEHAVIORAL RHYTHMS

Before we start looking at particular daily and circadian rhythms of behavior (this section) and of autonomic functions (Section 5.3), it is important to briefly discuss the functional significance of circadian rhythmicity. Evolutionary and ecological considerations will be addressed in Chapter 9.

Although you may have never thought about it, there is much more in a day than just the alternation of sunlight and darkness. It is true that your house does not move from one part of town to another—and back—during the course of a day, and the number of molecules in one mole of nitrogen does not change from daytime to nighttime, but many things oscillate daily. If you are a typical human being, you most likely walk around and do things during the day and sleep at night—you are a *diurnal* organism. If you have mice in your attic, you probably know that they are active during the night and rest during the day—they are *nocturnal* organisms. Regular, daily oscillations have been recorded under controlled conditions in numerous species for a variety of physiological variables, including locomotor activity, eating and drinking, excretion, learning capability, heart rate, blood pressure, body temperature, hormone secretion, and many others. Although daily rhythmicity is more robust in some organisms than in others, and more robust in some physiological variables than in others, the *effect size* of daily rhythmicity compares very favorably with that of other important phenomena in the physical and biological worlds. By "effect size," I mean the magnitude and consistency of the oscillation, as compared to random and nonrandom variations caused by other factors. Mathematically, the effect size can be estimated as the quotient of the standard deviation of the mean difference between time points and the standard deviation of the whole data set (that is, σ_M/σ). Look at Table 5.1. The effect size of the daily rhythm of body temperature averages 0.82 (in a possible range of 0–1) for several mammalian species. This effect size is smaller than that of a geophysical process such as the annual cycle of air temperature in New York City, but it is also much larger than that of many well-respected processes, such as the gain in body weight of growing rats, the academic advantage of smart university students over their less fortunate colleagues, or the day-to-day variability in the number of wheel revolutions performed by male laboratory mice. Thus, the daily body temperature rhythm—as a representative

TABLE 5.1
Comparison of the *Effect Size* of Circadian Rhythmicity with That of Other Biological and Physical Phenomena[a]

Phenomenon	Effect Size	Comments
Change in an adult person's height (effect size calculated for 12 consecutive measurements of the height of six people)	0.00	The height of a person is always the same (and different from that of the others). Thus, repeated measurements result in no variability.
Difference in the body weights of growing rats (effect size calculated for the body weight of 12 rats measured 6 times at weekly intervals starting at 1 month of age)	0.12	Different rats have different body weights, but rats gain weight very rapidly during the first months of life, so that the effect of individual differences becomes almost negligible.
Difference is the performance of students in a class (effect size calculated for the performance of 12 students in six graded activities in a psychology class)	0.40	Some students are consistently better than others, but the different activities require different skills. Thus, the student effect size is modest.
Day-to-day difference in running-wheel activity of mice (effect size calculated for the number of wheel revolutions per day performed on 12 consecutive days by 6 mice)	0.54	The amount of running varies less from day to day in the same animal than it varies from animal to animal.
Day-to-day difference in room temperature (effect size calculated for 12 daily measurements of ambient temperature at six different locations inside an air-conditioned room)	0.68	On warm days, the temperature in the room rises a bit, but different locations inside the room are affected similarly. Thus, the effect size of day-to-day variation is relatively large.
Circadian rhythm of body temperature of rats, dogs, or squirrels (effect size calculated for 12 measurements per day on 6 consecutive days)	0.82	The effect size of circadian rhythmicity is quite large, at least for body temperature.
Mean ambient temperature in New York City recorded monthly (effect size calculated for 12 monthly measurements per year on 6 consecutive years from 1997 to 2002)	0.98	Although temperature varies somewhat from year to year, the variation between summer and winter (each year) is much greater. Thus, the effect size of month is very large.
The thermal conductivity of metals (effect size calculated for 12 different metals measured 6 different times under constant ambient temperature and atmospheric pressure)	1.00	The different metals have different, nonoverlapping conductivities. Also a metal's conductivity is immutable (if temperature and pressure are held constant). Thus, the effect size is maximal.

[a] For consistency, all phenomena involve 12 treatment levels with six cases per level. In each instance, the effect size is the standard deviation of the 12 means divided by the standard deviation of all 72 data points. This computation of effect size as σ_M/σ is similar but not identical to that described in J. Cohen's *Statistical Power Analysis for the Behavioral Sciences* (Hillsdale, NJ: Lawrence Erlbaum Associates, 1988). Here, effect size can vary from 0 (no effect) to 1 (maximal effect).

of other daily biological rhythms—is not just a curious phenomenon that adds color to more fundamental physiological processes. Instead, circadian rhythmicity is an essential component of physiological regulation, as will be discussed in greater detail in Chapter 10.

5.2.1 Locomotor Activity

Moving from one place to another is a most common action among animals (Figure 5.12). That animal locomotor activity exhibits daily rhythmicity is perhaps the best-established fact in circadian physiology. Daily rhythms of locomotor activity have been documented in a large number of species of invertebrates,[80–110] including the harvestman (*Rhaucus vulneratus*),[110] which is not a spider, not a scorpion, but still an arachnid (Figure 5.13). Among the vertebrates, daily rhythms of locomotor activity have been documented in reptiles,[111–116] fishes,[15,117–129] and birds.[130–148] In mammals, the majority of studies has been conducted on rodents, including laboratory rats,[9,30,149–189] domestic mice,[190–229] hamsters,[230–243] squirrels,[244–250] degus,[251–259] molerats,[260–264] voles,[17,265,266] guinea pigs,[267–269] Nile grass rats,[270–277] and other species.[19,278–304] Other mammals whose daily activity patterns have been well described include bats,[305,306] tree shrews,[307,308] rabbits,[309–312] cats,[313–315] dogs,[316,317] sheep,[318–321] horses,[321–323] bears,[324,325] various marsupials,[326–330] and other species.[331–346] Many studies have been conducted on primates,[347–366] including marmoset monkeys[367–373] and humans.[374–388]



FIGURE 5.12 A tern on flight. Flying animals, like animals on land and water, exhibit a daily rhythm of activity. (Copyright ArtToday, Tucson, AZ.)



FIGURE 5.13 The other arachnid. The harvestman (*Rhaucus vulneratus*) is a member of the opilione group, the third major group of arachnids (the other two groups being those of spiders and scorpions). (Photo courtesy of Andrea Rojas.)

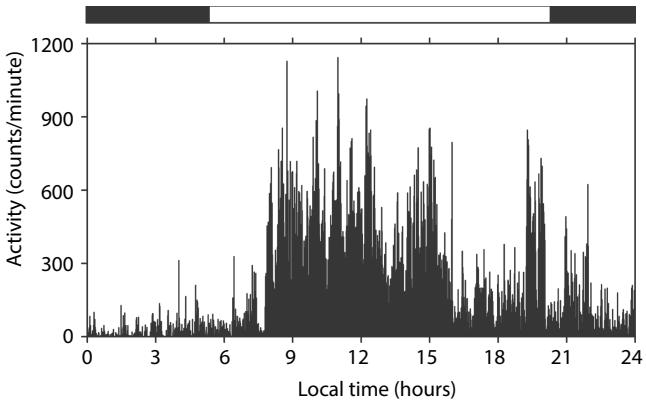


FIGURE 5.14 Moving in Chicago. The graph shows the physical activity (in 1-minute resolution averaged over six consecutive days) of a 36-year-old woman from Chicago living her regular life. The horizontal dark and white bars at the top of the graph indicate the duration of the dark and light phases of the prevailing light–dark cycle, respectively. (Data from Sani, M. et al., *Chronobiol. Int.*, 32, 650, 2015.)

The activity records of a 36-year-old woman from Chicago are shown in Figure 5.14. To eliminate excessive variability due to daily idiosyncrasies, the data from six consecutive days were averaged minute by minute. It can be clearly seen that activity is low from midnight to about 7 or 8 a.m., goes up for most of the day, and returns to a low level around 10 or 11 p.m.

Sample records of activity of individuals of five species of small mammals are shown in Figure 5.15. Flying squirrels (*Glaucomys volans*) and golden hamsters (*Mesocricetus auratus*) are nocturnal animals and exhibit much higher levels of activity during the night than during the day. In contrast, tree shrews (*Tupaia belangeri*) and Richardson's ground squirrels (*Urocitellus richardsonii*) are diurnal animals and exhibit much higher levels of activity during the day than during the night. Degus (*Octodon degus*) are preponderantly diurnal but do not exhibit clear-cut daily rhythms as the other four species do. Whereas interspecies differences in the daily pattern of activity are of great interest to naturalists, most laboratory researchers are especially fond of species that show robust daily rhythmicity, because in these species even minor effects of experimental disruptions can be reliably measured. In this respect, the domestic mouse (*Mus musculus*) is an ideal species, particularly if locomotor activity is monitored through running wheels. As shown in Figure 5.16, the actogram of locomotor activity of a mouse is exceptionally "clean," with all activity concentrated in a short segment of the daily cycle,

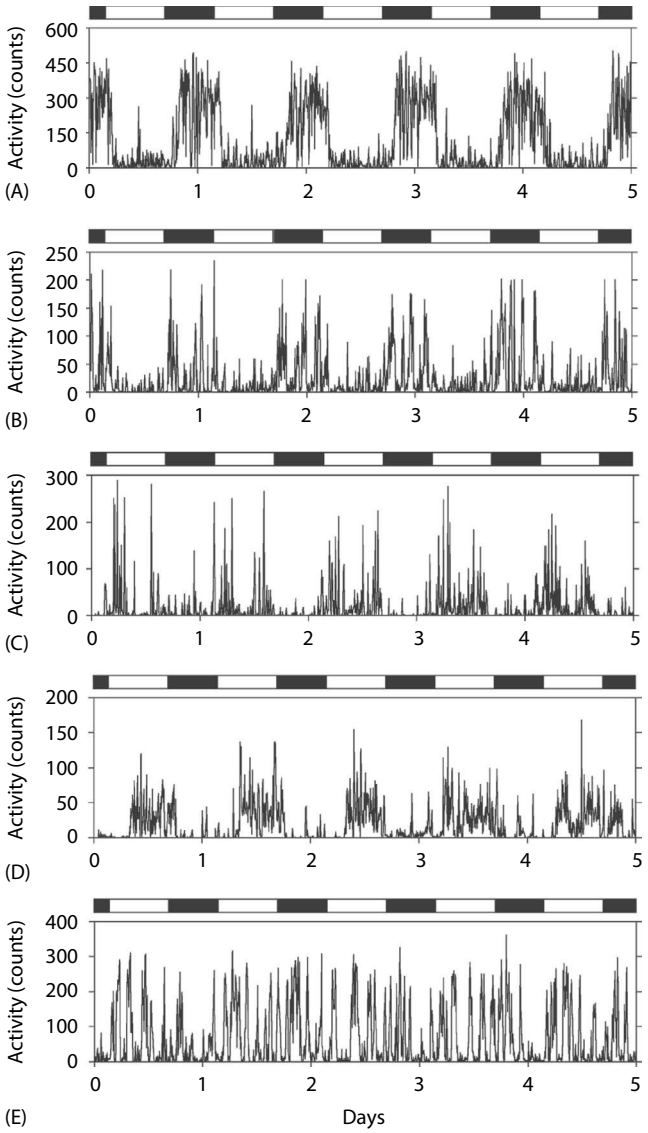


FIGURE 5.15 Rhythms of activity. Shown are representative 5-day segments of the records of locomotor activity (measured by telemetry with 6-minute resolution) of individuals of five different species of small mammals. The horizontal white and dark bars at the top of each graph indicate the duration of the light and dark phases of the prevailing light–dark cycle, respectively. Most of the activity of the flying squirrel (A) and golden hamster (B) is restricted to the dark phase, whereas most of the activity of the tree shrew (C) and Richardson's ground squirrel (D) is restricted to the light phase. The activity rhythm of the degu (E) is not as robust as that of the other four species. (Data from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999; unpublished data from the archives of the Refinetti lab.)

almost exclusively at night. Nonetheless, notice that the mouse does not run continuously throughout the night; instead, it takes several breaks after an initial long bout of 2 or 3 hours. Based on the number of wheel revolutions, and the diameter of the wheel, it can be calculated that a typical mouse runs the equivalent of 6 km (4 miles) each night.[199] As mentioned in Chapter 3, the daily segment of activity is called α (*alpha*), whereas the segment of rest is called ρ (*rho*).

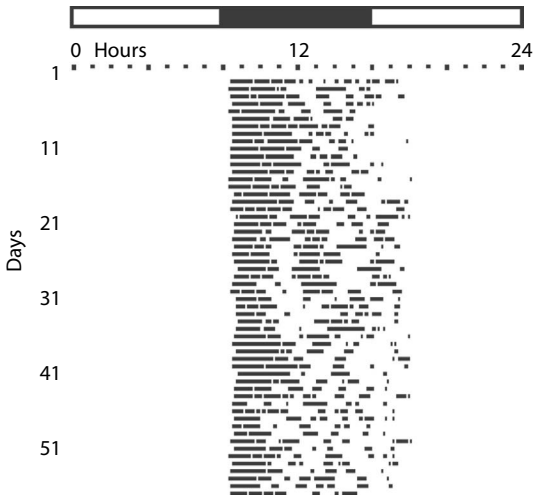


FIGURE 5.16 Clean data. This actogram of the running-wheel activity records of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle with 8 hours of darkness per day (LD 16:8) exemplifies the robustness of the rhythm of running-wheel activity. (If you are not familiar with actograms, look back at Figure 3.19 in Chapter 3.) (Data from the archives of the Refinetti lab.)

The daily distribution of activity differs not only from species to species but also from individual to individual in the same species. Examples of activity patterns of individual Nile grass rats (*Arvicanthis niloticus*) averaged over 10 consecutive days are shown in Figure 5.17. This species exhibits more interindividual variability than do mice. The records in Panels A and B reflect a clearly diurnal but bimodal pattern of activity, with a peak at dawn and another peak at dusk. The records in Panels E and F reflect a much more compact pattern of activity that is relatively constant throughout the light phase, whereas the records in Panels C and D have a pattern that is intermediary between the other two patterns. It has been reported that not all Nile grass rats are diurnal when housed with running wheels,[271,272] but the animals that were observed to be nocturnal seemed to have been members of an idiosyncratic subgroup. In my laboratory, the activity rhythms of all of 54 Nile grass rats housed with running wheels have been predominantly diurnal.[273,274,389] A third research team observed predominantly diurnal patterns of running-wheel activity with considerable crepuscular activity for 1 or 2 hours before lights on and 1 or 2 hours after lights off.[390] Observations in the wild in Kenya were consistent with the laboratory data in revealing a predominantly diurnal pattern of activity.[391] It should be pointed out that the presence of crepuscular activity—that is, a bimodal pattern of activity with peaks around dawn and dusk—is very common in a variety of species,[392] even though there is usually a predominance of activity either during the day or during the night. This matter is discussed in greater detail in Chapter 9.

Interspecies comparisons of the daily activity pattern are facilitated by the quantification of rhythm robustness, as previously described in Chapter 3. Figure 5.18 presents the mean robustness of the running-wheel activity rhythms of six species of laboratory rodents. Laboratory rats

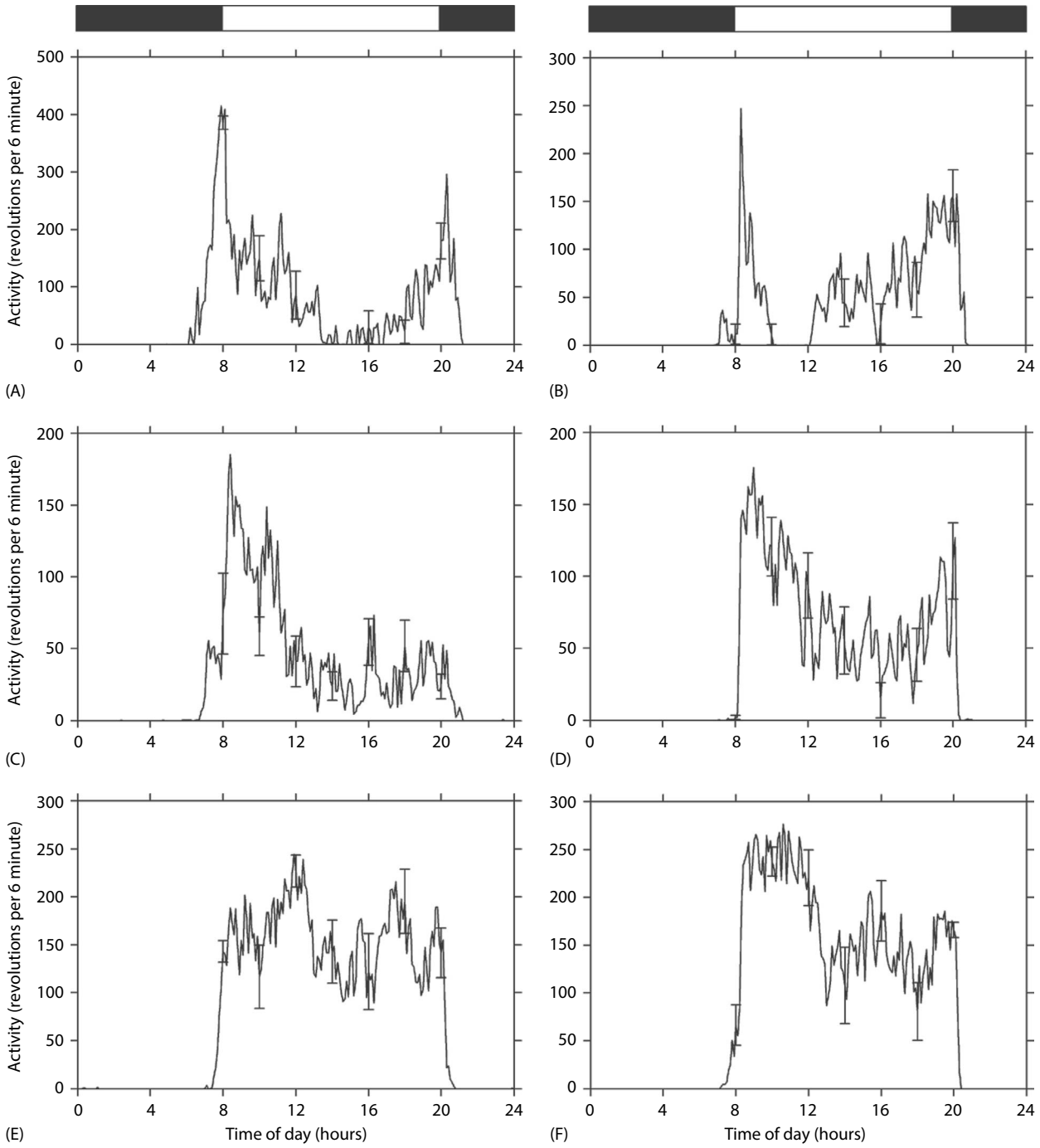


FIGURE 5.17 Intraspecies comparison of activity patterns. The graphs show the daily distributions of running-wheel activity of six Nile grass rats (*Arvicanthis niloticus*) maintained under a light–dark cycle with 12 hours of light per day (LD 12:12). In each graph, the data points are the means of 10 consecutive days for each 6-minute interval. To avoid cluttering, the standard errors of the means are plotted only at 2-hour intervals. Although all six animals (Panels A through F) exhibit robust daily rhythmicity, with activity predominantly during the light phase, the detailed pattern of activity varies from animal to animal. (Data from the archives of the Refinetti lab.)

(*Rattus norvegicus*) and Mongolian gerbils (*Meriones unguiculatus*) exhibit weak, though statistically significant, rhythmicity. The activity rhythms of Siberian hamsters (*Phodopus sungorus*) are slightly more robust. Nile grass rats and domestic mice exhibit equally strong rhythmicity. Syrian hamsters (golden hamsters, *M. auratus*) have the most robust rhythms of all laboratory rodents. If it were not for the fact that other aspects of the physiology and genetics of mice are much better known than those of golden hamsters, the latter would probably be the favorite species of circadian physiologists. At this time, golden hamsters are used often but much less frequently than mice. A search of the U.S. National Library of Medicine's PubMed database in May 2015, restricted to articles containing the term *circadian*,

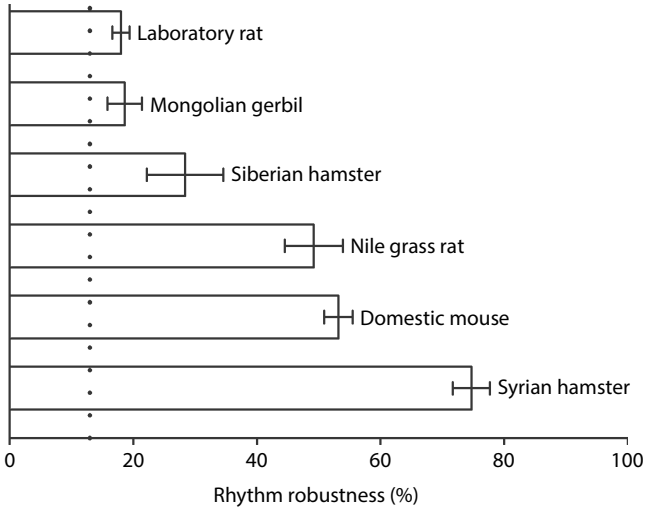


FIGURE 5.18 Interspecies comparison of rhythm robustness. The graph shows the mean robustness (±SE) of the running-wheel activity rhythm of several laboratory rodent species (based on samples of 5–15 animals per species). The dotted line indicates the lowest robustness for statistically significant rhythmicity. The existence of interspecies differences is evident. (Data from the archives of the Refinetti lab.)

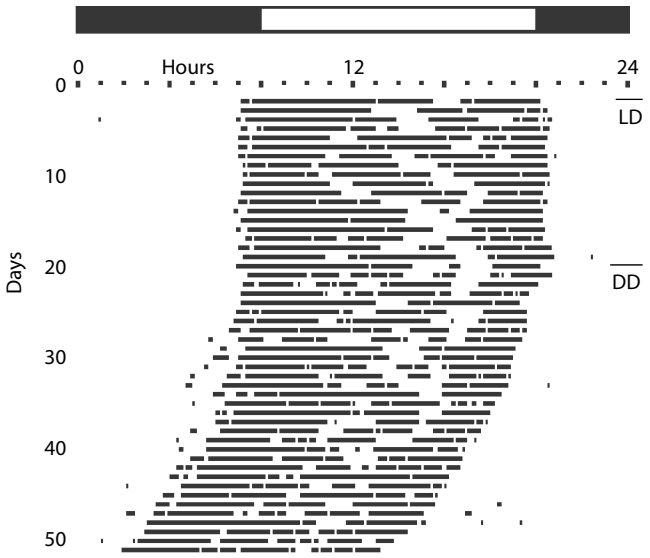


FIGURE 5.19 Circadian rhythms freerun under constant conditions. This actogram shows the rhythm of running-wheel activity of a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle for 20 days and in constant darkness for the following 30 days. Ambient temperature was constant at 24°C, and food was freely available, throughout the 50 days. Although the period of the activity rhythm was 24.0 hours in the presence of the light–dark cycle, it shortened to 23.8 hours in the absence of the environmental cue. (Data from the archives of the Refinetti lab.)

yielded 6470 articles of studies conducted on mice but only 1120 articles of studies conducted on golden hamsters.

With very few exceptions that may have been due to methodological deficiencies, daily rhythms of activity have been consistently found to persist in constant environmental conditions, which suggests that they are endogenously generated *circadian* rhythms. Of course, persistence of rhythmicity in the absence of a light–dark cycle does not prove that the rhythm is endogenously generated. The rhythm might very well be controlled by any of millions of other geophysical variables that oscillate daily as the Earth rotates around its axis (such as ambient temperature, humidity, magnetic fields, etc.). But then, how can we make sure that absolute constant conditions have been established in our study? We cannot. Does this mean that we cannot truly tell whether there is real endogenous rhythmicity? Well, actually, luck is on our side. It happens that the rhythms recorded from animals maintained in constant darkness in the absence of other obvious time cues (such as cycles of ambient temperature or food availability) do not repeat themselves exactly every 24 hours. The period of these rhythms is almost always slightly different from 24.0 hours. Therefore, the rhythms must be "free-running", as uncontrolled environmental variables cannot be resetting the rhythms each day—if they were, the period of the rhythms would be exactly 24.0 hours, as determined by the period of the Earth's rotation. They must be genuine *circadian rhythms*.

One example of a free-running circadian rhythm is given in Figure 5.19. A Nile grass rat was initially kept under a 24-hour light–dark cycle with 12 hours of light and 12 hours of darkness (abbreviated as LD 12:12 or, less frequently, as 12L:12D). During this time, the animal started running on the wheel about an hour before lights on each day and kept running almost continuously until lights off. After 20 days, the lights were turned off permanently, and the grass rat was kept in continuous darkness (abbreviated as DD, because it can be thought of as a light–dark cycle with two dark phases). At this point, the activity rhythm started to freerun with a period of 23.8 hours, as indicated by the slow leftward drift of activity onsets. The freerun strongly suggests that the rhythm is endogenously generated. However, if you are a very skeptical person, you might argue that some unknown geophysical cycle with 23.8-hour period could be responsible for the observed rhythmicity. Thus, we need to look at Figure 5.20 now. Three grass rats were housed in adjacent isolation boxes in the same laboratory, at the same time. After a week under LD 12:12, they were "released" into DD. Notice that the free-running period of one of the animals is shorter than 24.0 hours, that of another is exactly 24.0 hours, and that of the third animal is longer than 24.0 hours. Therefore, the observed periods cannot possibly be caused by a 23.8-hour environmental cycle. They must be endogenously generated, and different animals must have slightly different endogenous periods. As we will see in Chapter 6, this can be confirmed further by the use of specific genetic mutations.

Free-running rhythms of locomotor activity have been documented in a large number of species of invertebrates,[80,81,83,85,86,89,93,95,96,98–106,108,109,283,393–399] reptiles,[112–116,400–403] fishes,[118,119,121,123,124,127,404] and birds.[131–135,137,138,140–142,145–148,405] In mammals, most studies have been conducted on rodents, including laboratory rats,[23,150,156,157,160,161,163,170–172,174,175,179,182,185,186,188,189,406–410] domestic mice,[194–196,199,202,205–207,210–223,226–228,411–419] hamsters,[231, 236,237,242,420–427] squirrels,[245,246,248,428,429] degus,[254,255,259,430]

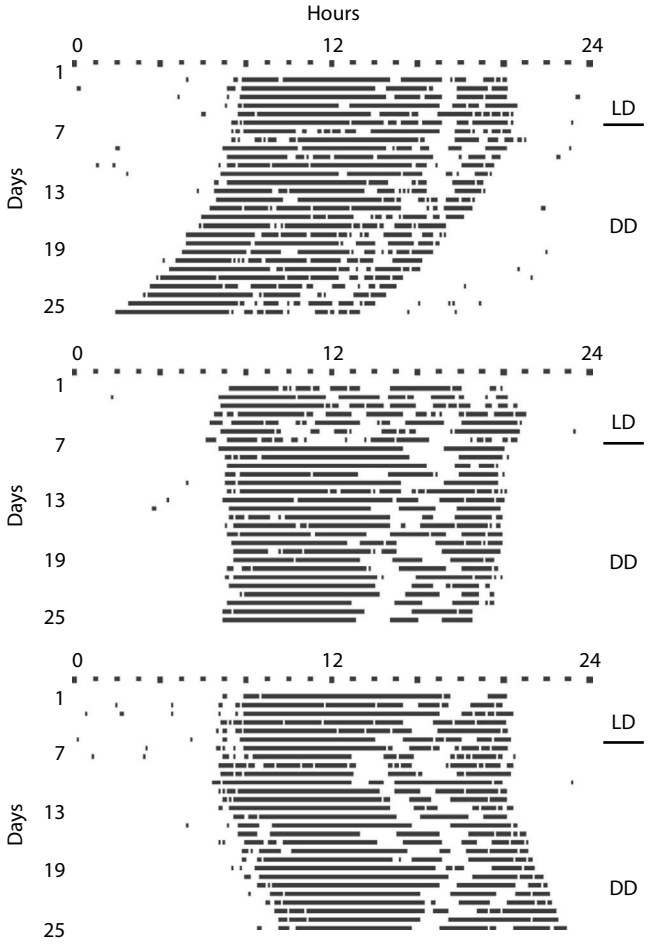


FIGURE 5.20 Excluding other environmental cues. These actograms show the records of running-wheel activity of three Nile grass rats kept under a light–dark cycle for a week and in constant darkness for 2 weeks in adjacent cages in the same laboratory. Even though all potential geophysical time cues were the same for the three animals, one of them exhibited a free-running period shorter than 24.0 hours, another exhibited a period of 24.0 hours, and the third one exhibited a period longer than 24.0 hours. Therefore, the rhythmicity must be endogenously generated. (Data from the archives of the Refinetti lab.)

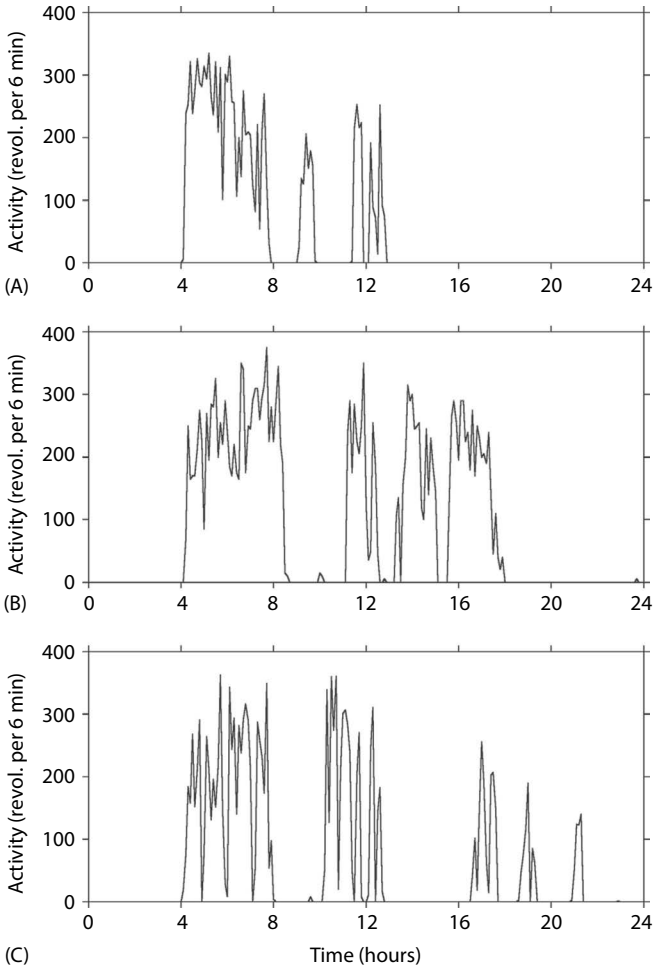


FIGURE 5.21 Activity patterns in constant darkness. The graphs show one-day sections of the records of running-wheel activity of (A) a golden hamster (*Mesocricetus auratus*), (B) a Nile grass rat (*Arvicanthis niloticus*), and (C) a domestic mouse (*Mus musculus*) maintained in constant darkness. The patterns of activity, which are representative of the patterns exhibited by other members of these species, are clearly different. The golden hamster concentrates its daily activity in an interval of about 8 hours, whereas the grass rat and the mouse distribute their activity over wider intervals. All three animals, and especially the mouse, take several breaks during the active phase of their cycles. (Data from the archives of the Refinetti lab.)

Nile grass rats,[270,271,273,274] chipmunks,[301,302,431] and other species.[260–263,265,266,268,300,432–434] Other mammals whose free-running activity patterns have been described include rabbits,[309,311] cats,[313,314] dogs,[316,317] tree shrews,[307,308] and other species.[258,264,277–280,282,284,287,289,303–306,318,325,327,330,331,336,339,435] Many studies have been conducted on primates,[347–349, 354–356,360–363,365,371,436–439] including humans.[374,376,377,380,382,383,440–445]

When animals are free-running in constant darkness, without the influence of environmental light–dark cycles, it becomes quite relevant to compare the duration of the active phases (α) of different species. For how long are the animals active in each circadian cycle? Records of representative individuals of three rodent species are shown in Figure 5.21. The free-running periods of the three animals were not identical (τ = 24.0 hours for the golden hamster, τ = 23.5 hours for the grass rat and the mouse), but the small difference in τ does not invalidate a comparison of values of α. Notice that the hamster is active for only about 9 hours each day, whereas the grass rat is active for 14 hours, and the mouse for 17 hours. The concept of α is a little misleading because animals are not continually active throughout the active phase—the total durations of actual activity in Figure 5.21 are 6 hours for the hamster, 10.5 hours for the grass rat, and 9.5 hours for the mouse. However, with this caveat, α is a useful parameter for interspecies comparisons of the activity rhythm. Mean values of α for 74 species are shown in Table 5.2. The species are listed in alphabetical order of Latin names, and you may consult the list of "Organisms Used" (at the end of the book) if you need help identifying the common names. The list in Table 5.2 is not exhaustive, but α ranges from as little as 8 hours in

TABLE 5.2
Duration of the Active Phase (α) of the Circadian Cycle in the Absence of an Environmental Light–Dark Cycle[a]

Species[b]	α (Hours)	Sources
Acomys cahirinus	14	[280]
Acomys russatus	14	[279]
Anopheles gambiae	16	[96]
Aotus azarai	14	[348]
Aotus lemurinus	14	[1037]
Aotus trivirgatus	15	[486]
Apis cerana	12	[101,104]
Apis mellifera	10	[99,100,397,912]
Apteronemobius asahinai	16	[398]
Armadillidium vulgare	11	[86]
Arvicanthis ansorgei	14	[282–284,432]
Arvicanthis niloticus	14	[274,277,1038,1039]
Bombus terrestris	15	[102,103]
Caenorhabditis elegans	16	[80]
Callithrix jacchus	13	[1040]
Carassius auratus	13	[118,1041]
Cavia porcellus	15	[268]
Chaetodipus intermedius	10	[278]
Clethrionomys rutilus	10	[265]
Columba livia	14	[134]
Coturnix coturnix	11	[476,477]
Cryptomys hottentotus	12	[264]
Ctenomys knighti	16	[304]
Danio rerio	11	[119,121,404]
Dasyuroides byrnei	12	[330]
Dimorphostylis asiatica	9	[393]
Dipodomys merriami	11	[401]
Drosophila melanogaster	16	[93,95,1042]
Eulemur fulvus	18	[348]
Eutamias sibiricus	12	[431]
Felis catus	17	[314]
Funambulus pennanti	12	[435,1043]
Gekko gecko	16	[114]
Georychus capensis	12	[261,262]
Glaucomys volans	10	[245]
Glyphiulus cavernicolus	12	[106]
Hipposideros speoris	11	[306,331]
Homo sapiens	16	[374,376,377,382,383,440,441, 444,445,489,504,562,695,862, 1044–1050]
Iguana iguana	12	[112,113,534]
Lemniscomys barbarus	14	[303]
Leucophaea maderae	13	[1051]
Limulus polyphemus	12	[509]
Lycosa tarantula	10	[108]
Macaca mulatta	12	[354]
Macaca nemestrina	15	[356]
Meriones unguiculatus	16	[275,1039]
Mesocricetus auratus	9	[236,237,1039,1052–1061]
Microcebus murinus	13	[360–363,438]
Microtus arvalis	9	[266]
Molossus molossus	12	[305]
Mus booduga	12	[433,434]
Mus musculus	14	[194,196,199,207,210–214,216, 217,219,221,222,226–228,411, 413,415–418,1039,1055,1057, 1062–1071]
Mus terricolor	10	[287,1072]
Octodon degus	15	[252,254,259,275,430,1039,1073]
Oryctolagus cuniculus	14	[311]
Otolemur garnettii	12	[349]
Ovis aries	15	[318]
Passer domesticus	12	[140,141,1074]
Phodopus sungorus	9	[1039,1075,1076]
Platynereis dumerilii	10	[81]
Podarcis sicula	10	[533]
Protophormia terraenovae	8	[1077]
Rattus norvegicus	12	[156,161,163,175,182,186,283,406, 408, 410,460,1039,1078–1083]
Saimiri sciureus	12	[439,487]
Sceloporus occidentalis	12	[1084]
Sigmodon hispidus	10	[289]
Sminthopsis macroura	12	[327]
Spalacopus cyanus	12	[300]
Sturnus vulgaris	14	[132,133]
Sylvia borin	10	[148]
Tamias striatus	9	[301]
Tinca tinca	18	[123]
Tupaia belangeri	12	[307]
Ursus arctos	10	[325]

[a] As a rule, determinations were made in constant darkness. In a few cases, values measured in constant darkness were not available, and the values shown were obtained in constant dim light. The values shown are the means for each species. Some species exhibit greater interindividual variability than others.

[b] For common English equivalents of scientific names of the species, see the table of "Organisms Used" at the end of the book.

Protophormia terraenovae (an insect, the blow fly) to as much as 18 hours in *Tinca tinca* (a fish, the tench).

An important consideration in the study of free-running rhythms is how to keep track of the time when events take place. Under a light–dark cycle, one can easily say, for instance, that an animal had a meal 3 hours after lights on. This is not possible, however, when the animal is in constant darkness. For thousands of years, humans have divided each day into 24 equal segments of 1 hour, each hour into 60 equal segments of 1 minute, and each minute into 60 equal segments



FIGURE 5.22 Grazing antelopes. Animals in the wild, as well as in the laboratory, exhibit a daily rhythm of ingestive behavior. (Courtesy of National Image Library, United States Fish and Wildlife Service.)

of 1 second.[446,447] This system of time measurement applies to everyone and is immutable in the scale of decades and centuries. In contrast, the periods of circadian rhythms vary from individual to individual as well as along an individual's life. Therefore, a separate system of time measurement—based on the duration of the circadian cycle of each individual—is necessary. To facilitate comparison with solar time, a *circadian hour* is defined as 1/24 of the circadian period. Thus, there are 24 circadian hours in each circadian cycle. If, for instance, an organism has a circadian period of 26 hours, then each circadian hour lasts 65 solar minutes rather than 60 solar minutes. Since there is no sunrise or sunset inside the organism, an arbitrary time point must be chosen as the beginning of a new circadian cycle. Traditionally, the time of initiation of activity (the *activity onset* time) is defined as circadian time zero (or CT 0). To preserve the distinction between day-active organisms and night-active organisms, activity is assumed to start 12 circadian hours later in night-active organisms. Thus, for nocturnal organisms, the activity onset time is defined as circadian time twelve (that is, CT 12 instead of CT 0). For both nocturnal and diurnal organisms, *subjective day* goes from CT 0 to CT 12, and *subjective night* goes from CT 12 to CT 24 (= CT 0), but nocturnal organisms are active during subjective night and diurnal organisms during subjective day. A group of researchers proposed the use of a single definition of circadian time for both nocturnal and diurnal organisms,[448] but so far, no one has adopted the proposal.

5.2.2 Feeding and Excretion

Eating is an essential behavior for all animals (Figure 5.22). Drinking is also essential for many terrestrial animals, although some are capable of extracting water from wet food or even from dry food.[449] The circadian organization of behavior is only one of several factors that determine the timing of meals,[450] but, as was the case for locomotor activity, daily and/or circadian rhythmicity of feeding has been documented in a large number of species, particularly laboratory rats,[9,25,149,150,152,159,161,163,164,168,176,451–463], domestic mice,[213,215,222,411,413,415,419,464–468] other rodents,[266–268,289,469] fishes,[127,470,471] birds,[132,136,138,141–143,147,472–478] and other animals.[10,309,310,342,479–485] The latter include nonhuman primates[347,353,355,365,366,436,437,486–488] and humans.[489–495] An example of a free-running rhythm of feeding is shown in Figure 5.23. A laboratory rat was maintained in constant darkness, and its feeding and wheel-running activities were monitored. Notice that the feeding pattern is quite different from that of humans. Rats nibble throughout subjective night (when they are active) instead of having three consolidated meals per day (breakfast, lunch, and dinner). On the other hand, rats are similar to humans in that they can learn to enjoy an occasional alcoholic drink, and some even become alcoholics. When offered access to a 20% alcohol solution at different times of the day, rats ingest greater volumes early in the night.[496] Considering that early night for a rat is equivalent to early morning for humans, rats must be different from humans after all. Humans are more sensitive to both the stimulatory and sedative effects of alcohol early in the night.[497]

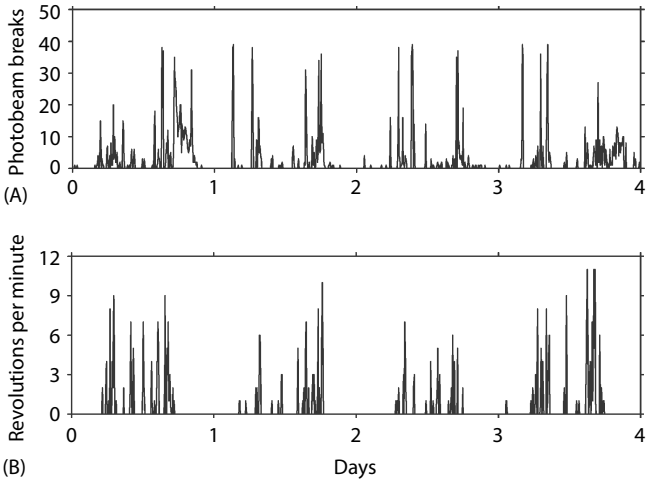


FIGURE 5.23 Feeding rhythm. The graphs show 4-day segments of the records of (A) feeding and (B) running-wheel activity of a laboratory rat (*Rattus norvegicus*) kept in constant darkness. Feeding was monitored by an infrared sensor attached to the food hopper. The records indicate that ingestive behavior is relatively spread out through subjective night with peaks of feeding activity occurring approximately at the same time as peaks of locomotor activity. (Data from the archives of the Refinetti lab.)

We will postpone a detailed discussion of the definition of circadian rhythmicity to later chapters, but it must be pointed out here that the determination of the endogenous nature of a rhythm is not always equivalent to the determination of the autonomous nature of the rhythm. The documentation of free-running rhythms in an organism is convincing evidence that *the organism* is capable of generating circadian rhythms. However, when various rhythms are studied, there is no obvious reason to believe that *each rhythm* is independently generated. For instance, if both locomotor activity and feeding exhibit circadian rhythmicity, it may well be that the two rhythms are independently generated, but it is also possible that the feeding rhythm is a by-product of the activity rhythm, or that the activity rhythm somehow derives from the feeding rhythm, or even that both rhythms derive from a third rhythm. Thus, even though all free-running rhythms recorded in an organism can be called *circadian*, only by empirical research can we determine whether each rhythm is autonomously generated or is *externally* driven by another rhythm. This issue will be discussed in Chapter 9.

The goal of feeding is to extract energy from the food. However, not everything in the ingested food can be assimilated. Also, the processing of assimilated products generates unusable metabolites, so that excretion of feces and urine is a necessity of life (Figure 5.24). Although circadian physiologists have not dedicated as much attention to excretion as to feeding and locomotor activity, daily and/or circadian rhythms of excretion have been documented in various mammalian species,[25,267,309,310,316,366,419,455,498,499] including humans.[380,382,500–505] An example of a daily rhythm of urinary excretion is given in Figure 5.25. A tree shrew (*T. belangeri*) was maintained under a light–dark cycle (LD 12:12) with food and water freely available, and its urinary output was monitored. It is clear that micturition occurs almost exclusively during the day in this diurnal species.



FIGURE 5.24 An old outhouse. Excretion (urination and defecation) exhibits daily rhythmicity. (Copyright ArtToday, Tucson, AZ.)

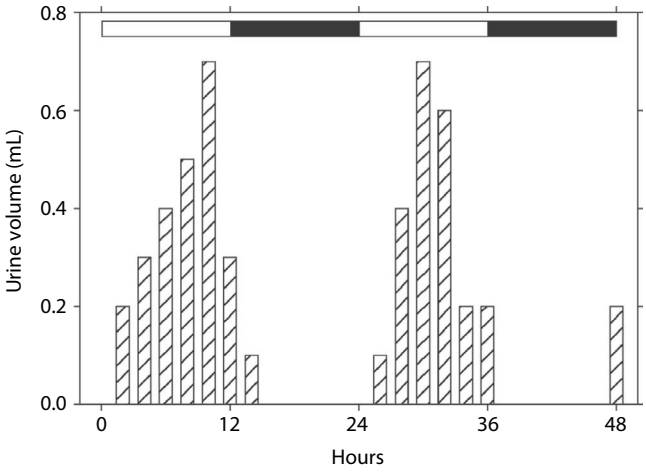


FIGURE 5.25 Daily rhythm of urinary excretion. The graph shows the pattern of urinary excretion of a tree shrew (*Tupaia belangeri*) maintained under a light–dark cycle with 12 hours of light per day (as indicated by the horizontal white and dark bars). The animal was housed in a metabolic chamber connected to a fraction collector. Practically all urinary excretion is restricted to the light phase of the light–dark cycle in this diurnal animal. (Data collected by R. Refinetti at M. Menaker's laboratory at the University of Virginia, 1991.)

5.2.3 Sensation and Perception

Considering the importance of the senses as the sources of information about the environment, circadian physiologists have dedicated amazingly little attention to daily and circadian variations in sensory processes. In human subjects, a few studies have shown that a given noxious stimulus (such as immersion of a hand in freezing water) is perceived as more painful at night than during the day.[506,507] Patients suffering from fibromyalgia (widespread muscle pain) report more pain in the early morning,[508] although the daily variation in pain in this case could be due to a daily variation in tissue inflammation rather than to a daily variation in pain perception.

Daily variations in the sensitivity of the visual, auditory, and chemical senses have been occasionally investigated,[509–515] but the majority of studies has concentrated on pain. An example is shown in Figure 5.26. Golden hamsters were placed on a hot plate (50°C) at different times of the day, and the latency of the reaction to the stimulus (licking of the paw) was measured. The data from two representative hamsters are shown in the figure. Notice that there is clear daily rhythmicity, with shorter latencies (and, therefore, greater pain sensation) during the dark phase of the light–dark cycle, which is when the animals are active. Notice also that the response latency falls

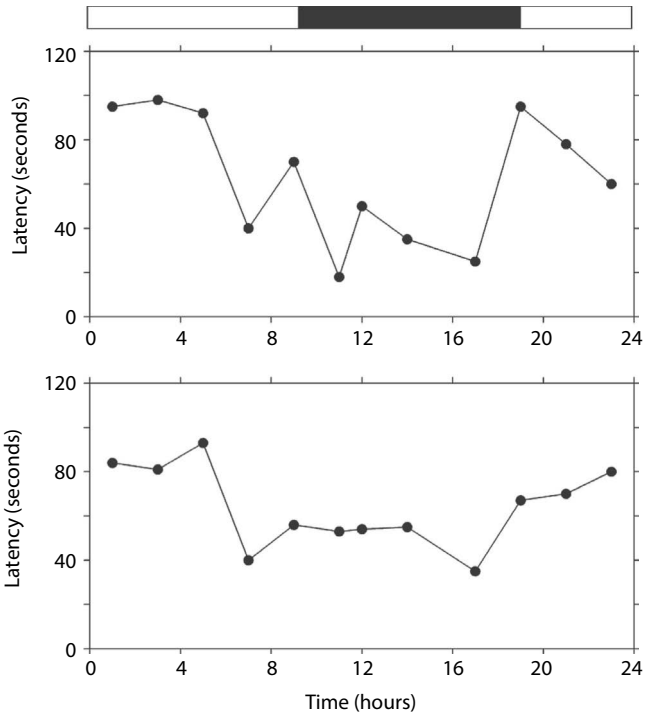


FIGURE 5.26 Daily rhythm of nociception. The graphs show the daily variation in pain sensitivity (nociception) of two golden hamsters maintained under a light–dark cycle (as indicated by the horizontal bars at the top). The data points correspond to measures of latency of the paw-licking response evoked by heating of the floor (50°C or 122°F). Latencies are shorter (suggesting greater sensitivity) during the dark phase of the light–dark cycle in both specimens of this nocturnal species. (Data from Pickard, G.E., *Brain Res.*, 425, 395, 1987.)

before the lights go off, which suggests that the process has an endogenous component. Indeed, rhythmicity in response latency was observed in hamsters free-running in constant light.[516] Curiously, the latencies were shorter during subjective day rather than during subjective night, which is inconsistent with the findings obtained when the tests were conducted under the light–dark cycle. Inconsistent results were obtained even between studies conducted under light–dark cycles in rats and mice: shorter latencies have been reported to occur at night,[517–519] during the day,[520] or with two daily troughs—one during the day and the other during the night.[521] A study in horses, which are diurnal animals, revealed shorter response latencies at the end of the day.[522] Human sensitivity to tooth pain has been reported to be highest late at night.[523] A study in mice identified daily oscillation in pain reactions in tests conducted during the spring but not in tests conducted during the winter.[524] Evidently, further studies are necessary to clarify the inconsistencies.

In contrast to the paucity of studies on daily rhythmicity of sensory processes *stricto senso*, many studies have been conducted on the daily rhythmicity of temperature sensation as reflected in the thermoregulatory behavior of temperature selection. Consistent daily variation in the selection of ambient temperature along a temperature gradient has been documented in invertebrates,[16,84,525–528] fishes,[15,529–532]

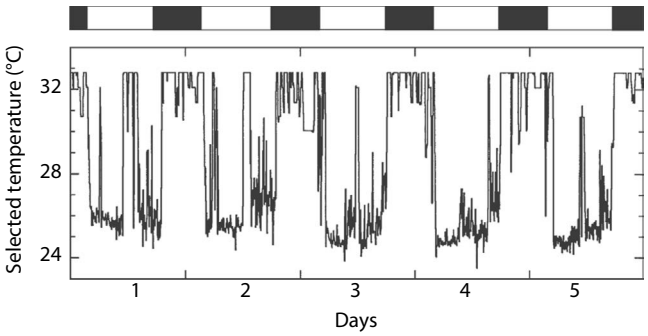


FIGURE 5.27 Daily rhythm of temperature selection. The graph shows the daily rhythm of temperature selection of a tree shrew (*Tupaia belangeri*) maintained under a light–dark cycle (as indicated by the horizontal bars at the top). The animal was housed in a long cage with a gradient of ambient temperature from 14°C to 33°C. Tree shrews, which are diurnal, consistently select higher temperatures during the night than during the day. (Data from Refinetti, R., *Physiol. Behav.*, 63, 517, 1998.)

reptiles,[114,533–545] rodents,[30,169,234,244,253,297,546–551] and other mammals.[24,552,553] An example is shown in Figure 5.27. A tree shrew was maintained under a light–dark cycle (LD 14:10) in a temperature gradient similar to the one described in Chapter 2. The animal consistently selected higher temperatures at night than during the day. Why a diurnal animal should select higher temperatures during the night, instead of during the day, is not immediately obvious, and this issue will be discussed in detail in Chapter 10.

Reptiles, such as lizards (Figure 5.28), rely much more than do mammals on the selection of adequate thermal environments in order to optimize bodily functions. Consequently, their temperature selection behavior has been studied extensively.[114,533–545,554] Figure 5.29 shows the temperature selection rhythm of an iguana lizard (*Iguana iguana*) kept in constant light. The lizard was maintained in a cage at 22°C and was able to move on and off a "hot rock" (i.e., a small electric heater).



FIGURE 5.28 Basking lizard. Many animals select cooler or warmer environments at different times of the day in a consistent, rhythmic manner. (Copyright ArtToday, Tucson, AZ.)

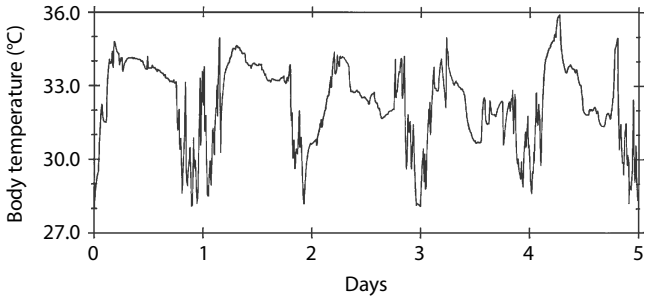


FIGURE 5.29 Circadian rhythm of temperature selection. The graph shows the body temperature rhythm of a small iguana lizard (*Iguana iguana*) maintained under constant light in a room at 22°C. The animal could change its body temperature by moving on and off a "hot rock" (i.e., an electric heater). The records show a clear oscillation in temperature with a period close to 24 hours. (Data collected by R. Refinetti at M. Menaker's Laboratory at the University of Virginia, 1991.)

Because lizards are cold-blooded animals, the circadian rhythm of surface-temperature selection resulted in a circadian rhythm of body temperature (as measured by a temperature-sensitive radio transmitter implanted in the abdomen).

Time perception is another phenomenon that has received considerable attention. Humans often use watches and clocks to measure the passage of time (Figure 5.30).



FIGURE 5.30 What time is it? One reason why humans routinely use clocks to measure the passage of time may be that our perception of time intervals varies with the time of day. (Copyright ArtToday, Tucson, AZ.)

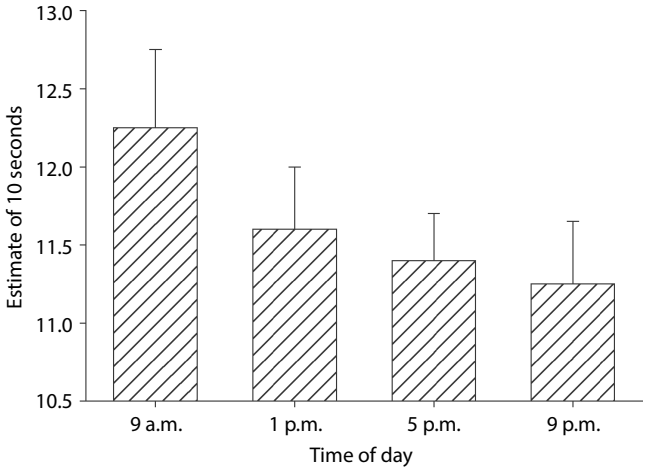


FIGURE 5.31 It's shorter than you think! When people are asked to estimate the duration of a 10-second interval, they always overestimate it. The extent of the overestimation is greater earlier in the day. The data shown in this graph are the means (±SE) for 14 young men under a constant routine protocol in the laboratory. (Data from Kuriyama, K. et al., *Neurosci. Res.*, 46, 23, 2003.)

These instruments allow us to time events without having to constantly think about them. However, humans and other animals are quite capable of estimating the passage of time in the range of seconds, minutes, or hours.[555] Interestingly, this capability seems to depend on circadian rhythms. The dependence occurs at two levels. First, the ability to estimate short durations of time exhibits daily and circadian rhythmicity. An example is shown in Figure 5.31. Fourteen young men were individually kept in bed rest from 7 a.m. to 11 p.m. and were asked to estimate the duration of 10 seconds at four different times of the day. They overestimated the duration at all times, but the overestimation was greatest in the morning.[556] Greater overestimation in the early morning than at other times of the day was found also in other studies where subjects were asked to estimate the duration of 10 seconds[557] or of an hour.[558] However, in one study, the highest estimate was found to occur in the early night instead,[382] and in another the peak times were not consistent across subjects, even though all subjects exhibited daily rhythmicity in their estimates.[559] In yet another study, the researchers investigated the "personal tempo" of subjects asked to tap their fingers at their preferred rate. The average rate of 2.3 taps per second exhibited a daily variation of about 30% with a peak at 7 p.m.[560] Thus, it seems well established that time perception exhibits daily rhythmicity, but further studies are necessary to determine the precise waveform of the rhythm.

The other level at which time perception depends on circadian rhythms has to do with free-running periods. Estimates of the duration of an hour were found to be longer when the subjects were free-running with circadian periods longer than 24 hours,[561,562] which means that we perceive an hour as 1/24 of a day, not necessarily as 60 minutes. As shown in Figure 5.32, this is true for estimates of an hour but not for estimates of brief intervals of a few seconds. As a matter of fact, a large body of research on humans and other animals

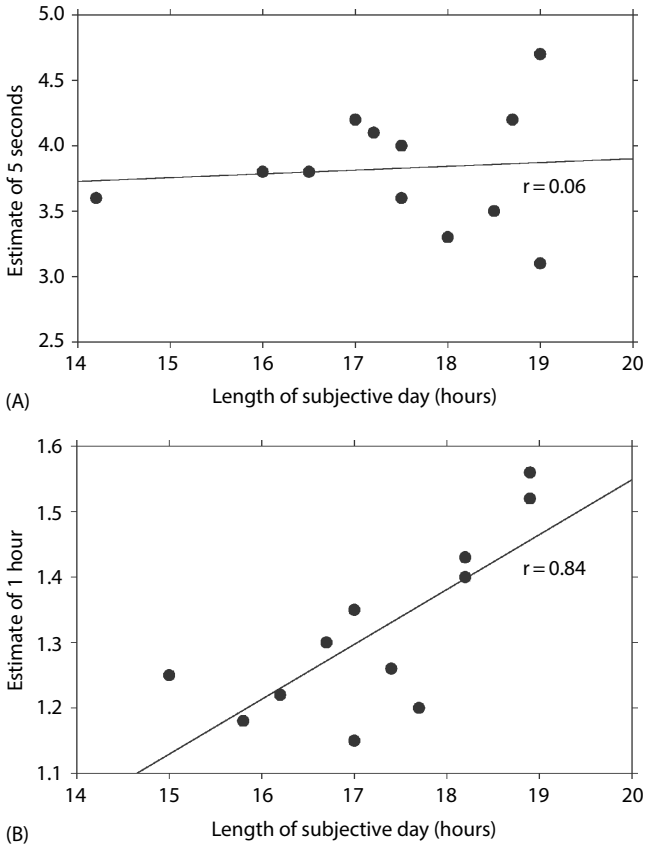


FIGURE 5.32 Time is relative. Circadian rhythms of human subjects maintained under constant conditions normally freerun with a period longer than 24.0 hours. When the circadian period is long, both subjective day and subjective night are longer. Subjects experiencing long subjective days estimate the duration of 1 hour as being proportionally longer (B), but their estimates of the duration of 5 seconds are not significantly affected by the length of subjective day (A). In both panels, *r* is the correlation coefficient for length of subjective day and estimate of duration. (Data from Aschoff, J., *J. Biol. Rhythms*, 13, 437, 1998.)

indicates that short intervals are timed by a mechanism distinct from the one that times circadian rhythms,[563] and a study on mice has shown that the timing of short intervals is not affected by surgical destruction of the master circadian pacemaker in the brain.[564] Estimates of short intervals are affected by body temperature, suggesting that the clock runs faster at higher temperatures,[556,557,565,566] whereas estimates of an hour or longer are temperature compensated.[562]

Researchers who investigate short-interval timing are usually not circadian physiologists and have their own theories about how timing is achieved. A very popular theory, called *scalar timing theory*, assumes the existence of a central clock that beats with a constant frequency, an accumulator that counts the beats between two external events, and a memory register with which the accumulator counts are compared.[567,568] Some researchers, however, feel that a central clock is not necessary[569] and that timing may be "distributed," meaning that different brain circuits are involved in the timing of task-specific and modality-specific processes.[570] The involvement of different timing circuits in the estimation of different durations is also suggested by the observation of discontinuities in the process—that is, some time intervals are perceived with lower sensitivity than others.[571]

5.2.4 Learning and Other Processes

Surprisingly few investigators have studied daily or circadian oscillations in learning and memory.[572–578] The results from a very well-conducted study in mice are shown in Figure 5.33. Mice were subjected to a procedure of fear conditioning, in which a 30-second tone is repeatedly presented along with a 2-second foot shock. Mice naturally respond to a foot shock by *freezing* (i.e., becoming immobile), so that they gradually learn to freeze in response to the tone (which initially did not by itself induce freezing). Notice that the mice approached an asymptotic level of performance after about five training sessions and, more importantly, that animals taught during the day learned better than animals taught during the night. Not shown in the figure is the fact that both learning and memory–recall were better during the day (when mice are normally resting) and that similar results were obtained in free-running animals.[574] Results from another interesting study, this one conducted in the marine snail *Aplysia californica*, are shown in Figure 5.34. This study dealt with *long-term sensitization* of the siphon-withdrawal reflex. Essentially, the animals naturally respond to a noxious stimulus (such as a shock) by retracting the siphon and, if the stimulus is presented repeatedly, the duration of the retraction is gradually increased. Training and testing were conducted in constant darkness. The data in Panel A were obtained when the snails were trained at different circadian times and tested 24 hours later. The performance was best at CT 9, but you may wonder

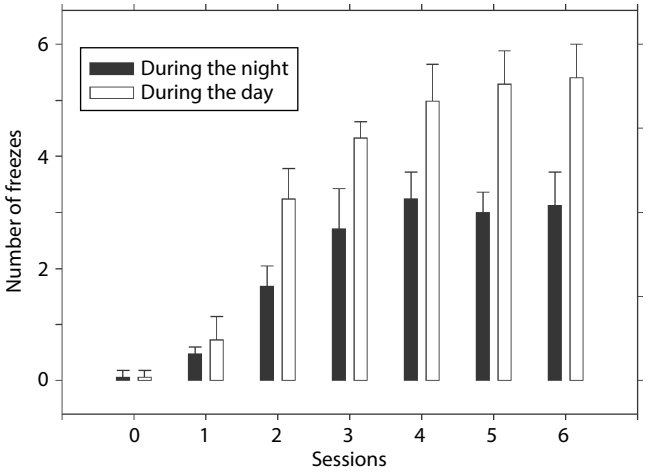


FIGURE 5.33 Daily rhythm of learning ability. Mice can be taught to fear an originally innocuous auditory tone if the tone is repeatedly associated with an electric shock to the foot (fear conditioning). A convenient fear response is *freezing* (immobilization). The graph shows that learning is more effective during the day than during the night. Each bar corresponds to the mean (±SE) of 8 mice. (Data from Chaudhury, D. and Colwell, C.S., *Behav. Brain Res.*, 133, 95, 2002.)

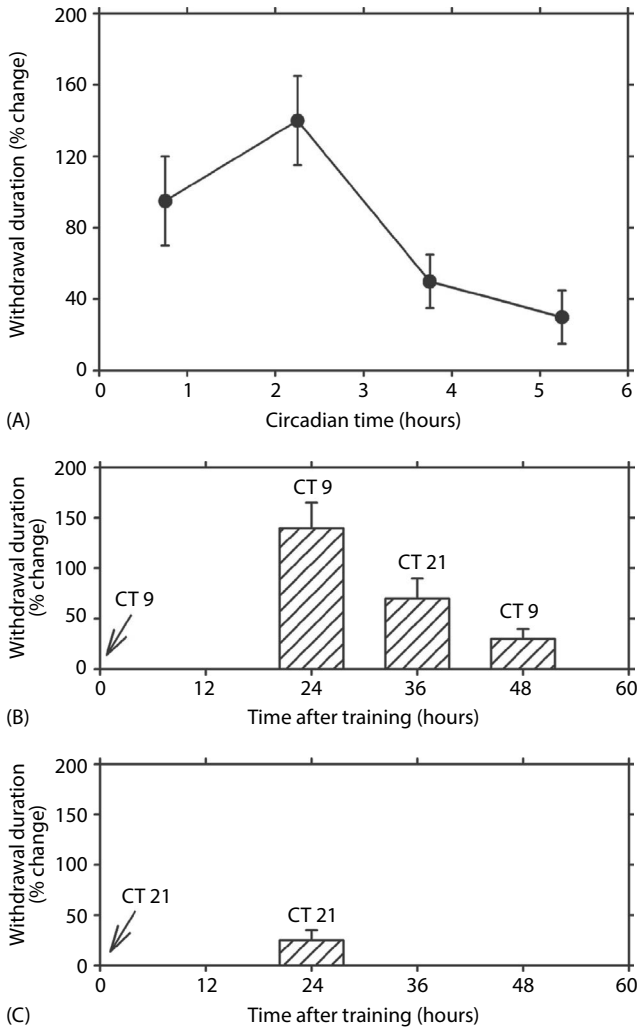


FIGURE 5.34 Circadian rhythm of learning ability. The marine snail *Aplysia californica* exhibits a simple form of learning called long-term sensitization, in which the duration of the siphon-withdrawal reflex in response to a noxious stimulus (electric shock) increases after repeated presentation of the stimulus. In snails maintained in constant darkness, sensitization is greater during the first part of the circadian cycle than during the second part (A). As in any form of learning, there is decay over time (B), but learning is better at circadian time nine (CT 9) than at circadian time twenty one (CT 21) when tests are conducted the same number of hours after training (B and C). (Data from Fernandez, R.I. et al., *Proc. Natl. Acad. Sci. USA*, 100, 14415, 2003).

whether this is the time when learning is best or memory–recall is best. Thus, the data in Panel B were obtained when the snails were trained at CT 9 and then tested at either CT 9 or CT 21 in the following circadian cycle. Performance was better at CT 9 than at CT 21, but there was an obvious loss of memory over time, as indicated by the performance 48 hours after the training session. Still, the performance at CT 21 was better than when the animals were trained at CT 21 and tested at CT 21 a day later (Panel C). Thus, the function in Panel A was indeed due to a circadian variation in learning, not in memory–recall. The snails do learn better at CT 9. Because these snails are diurnal, they learn better during their active phase,[575] unlike mice, which—as we have just seen—learn better during the rest phase of the circadian cycle.[574] Zebrafish, which are diurnal, were also shown to learn better during their active phase.[579]

Daily rhythmicity has been documented in many other behaviors in a variety of species. For example, some species of ants exhibit a daily rhythm of brood translocation by moving pupae from a cooler to a warmer location within the nest—and back—each day.[580,581] Tunas, seals, penguins, eels, and turtles exhibit a daily rhythm of ocean diving.[582–588] A species of South American toad exhibits daily rhythmicity of skin color change.[589] Hydrozoans of the genus *Hydra* display a daily rhythm of body contraction.[590] Flies, rodents, and humans mate preferentially at particular times of the day.[44,45,270,591] Mother rats and mother rabbits also nurse their pups preferentially at particular times of the day.[310,592] Marmosets exhibit a daily rhythm of autogrooming.[11,368] Male hamsters temporarily placed together in the same cage engage in more sparring at night than during the day.[593] Mice kept in groups in a cold environment huddle together more often during the day than during the night.[191] Particularly curious is the pattern of roaring in red deer (*Cervus elaphus*). During the breeding season, male red deer emit lion-like vocalizations, and these vocalizations exhibit daily rhythmicity,[594] as shown in Figure 5.35.

In humans, daily rhythmicity in alertness is particularly well documented. Probably as no surprise to you, it has been consistently found that alertness is lowest at wake-up time.[595–602] Actually, if people are kept awake and tested during their usual sleep time (with the appropriate controls for sleep disruption), their alertness is the worst during the 4 hours preceding the usual wake-up time.[603] Other cognitive functions known to exhibit daily rhythmicity include mathematical calculation

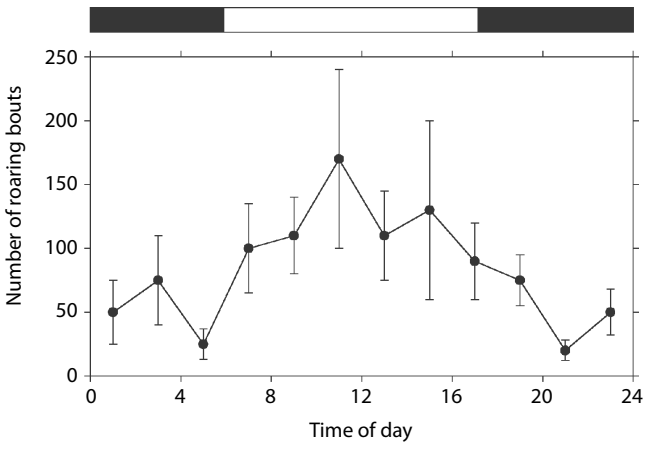


FIGURE 5.35 Roar like a lion. Red deer (*Cervus elaphus*) emit vocal sounds that resemble the roaring of lions. The graph shows the daily distribution of roaring bouts of a male red deer. Each data point is the median (±half ranges) of values collected over 2 weeks. The horizontal dark and white bars at the top indicate the duration of darkness and sunlight, respectively. Roaring bouts are clearly more common during daytime than during nighttime. (Data from Pépin, D. et al., *Appl. Anim. Behav. Sci.*, 74, 233, 2001.)

performance and memory recall.[595,597,599,604] Regular changes in mood have also been described, although there is disagreement about the details. A common distinction in mood assessment is that between *positive affect* and *negative affect*.[605] Positive affect can vary from joviality/enthusiasm to drowsiness/dullness, whereas negative affect varies from distress/hostility to calm/rest. Although studies have consistently found daily rhythmicity in positive affect, rhythmicity in negative affect has been found in some studies[606–608] but not in others.[609–611]

5.3 AUTONOMIC RHYTHMS

Behavioral processes are under voluntary control and, at least in humans, involve conscious awareness. Autonomic processes, on the other hand, are carried out automatically, often without evoking conscious awareness. Therefore, autonomic rhythms are theoretically more tightly controlled by the circadian system than are behavioral rhythms. In practice, however, autonomic rhythms can often be disrupted by behavioral processes. For instance, the rhythms of body temperature and of cardiovascular function can be disrupted by vigorous exercise during their expected troughs, and the rhythm of melatonin secretion can be disrupted by exposure to light during its expected peak. There certainly is no single rhythm that can be considered the ideal expression of the state of the circadian pacemaker. Some rhythms are more robust than others, and some rhythms are more easily measured than others, but all rhythms are presumably important for the operation of the organism. In this section, we will discuss many autonomic rhythms, including the rhythms of body temperature, cardiovascular function, melatonin secretion, cortisol secretion, metabolism, and sleep.

5.3.1 Body Temperature

In circadian physiology, the term *body temperature* is used rather loosely, most often as a synonym of *body core temperature*. Because most heat-producing organs are located in the core of the body, and because ambient temperature is normally lower than core temperature, there is actually a temperature gradient in the body. This means that different organs have different temperatures.[612–616] Regional differences in temperature are evident even at the body surface, as illustrated by the thermograph in Figure 5.36. In this black-and-white image, higher temperatures are generally indicated by lighter shades of gray, whereas lower temperatures are indicated by darker shades. You can see that the hands, feet, and head (insulated by hair) are barely visible because of their relatively low temperatures. Even in a thermally neutral environment (i.e., ambient temperature of approximately 26°C, or 79°F), human skin temperature is usually below 33°C, while core temperature is about 37°C (98.6°F). Thus, what *thermal physiologists* usually call *body* temperature is a weighted average of *core* and *skin* temperatures. Although core temperature is itself an intangible concept (which is usually estimated by the measurement of rectal temperature), and although skin temperature varies considerably from one body site to another, a

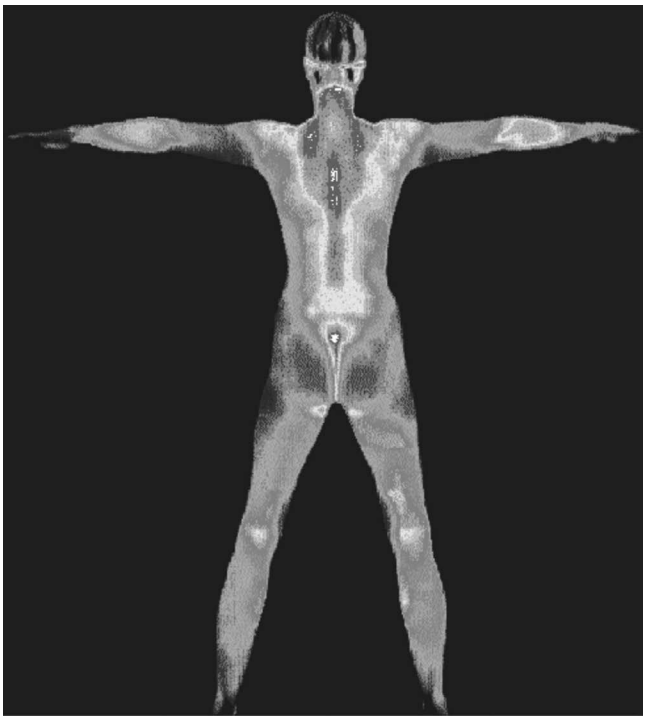


FIGURE 5.36 Show me your heat! This whole-body infrared thermography image of a human patient shows regional differences in skin temperature. Higher temperatures are indicated by lighter shades of gray. (Image courtesy of Meditherm, Medical Monitoring Systems, Lake Oswego, OR.)

reasonable approximation of body temperature (T_b) is given by $0.8T_r + 0.2T_s$, where T_r is rectal temperature and T_s is mean skin temperature.[617] Without meaning to disrespect thermal physiologists, this strict definition of body temperature will *not* be used here. Instead, *body temperature* will be used in the loose sense of "temperature of a central part of the body," not even necessarily that of the upper rectum. Aside from allegiance to traditional usage in circadian physiology, this is justified by the fact that, although input from both the skin and body core is used by the thermoregulatory system, control of peripheral temperature is often sacrificed in favor of control of central temperature. We will return to this issue in Chapters 10 and 11.

Daily rhythmicity of body temperature has been extensively documented in laboratory rats,[9,30,150–152,155,158,159,161,162,166,169–174,176,178–182,187,283,462,546,618–645] as well as in domestic mice,[192,193,197,198,200,201,203–205,207–209,216,224–227,548,646–655] golden hamsters,[230,232–235,237,241,423,656–658] and many other rodent species.[19,240,244,248,250–255,263,269–271,276,279,281,290,293,295–298,659–677] A large number of studies has also been conducted on primates,[348,350,351,353,357–360,362,363,366,372,486–488,678–684] including humans,[5,6,27,34,374,380–382,384,385,500–502,505,560,596,601,610,685–714] as well as in dogs,[316,715–719] cats,[313–315,720] goats,[721–725] sheep,[319,726–731] horses,[321,732–737] cattle,[10,738–742] other mammals,[307,326,328,329,332,334–336,339,343,743–759] and many species of birds.[131,134,135,137–139,145,473,616,760–769] Although only mammals and birds are truly warm-blooded animals—and, therefore, can generate body temperature rhythms in homogeneous

thermal environments—other animals are capable of generating body temperature rhythms by selecting different ambient temperatures at different times of the day, as previously discussed in this chapter. Interestingly, at least one reptile—the green iguana (*I. iguana*)—is capable of generating a small-amplitude rhythm of body temperature even when housed in a homogeneous thermal environment.[112,403]

An example of a daily rhythm of body temperature in birds is shown in Figure 5.37. A hen (*Gallus domesticus*) was kept under a 24-hour light–dark cycle, and its body temperature was continuously recorded. Notice the presence of clear daily rhythmicity, with higher temperatures consistently attained during the light phase of the light–dark cycle in this diurnal species. As we saw previously in Chapter 4, hens also exhibit an estrous cycle of body temperature, which is not synchronized to the light–dark cycle. Thus, small but sharp peaks in temperature are seen at the time of ovulation (as indicated by the inverted triangles). This superposition of the estrous cycle on the daily rhythm causes small changes in the waveform of the rhythm, causing it to be less reproducible than the rhythm observed in roosters. The estrous cycles of other species also disrupt the daily rhythm of body temperature, even though not as much as in species that ovulate every day. For this reason, most studies of daily and circadian rhythms of body temperature (or of locomotor activity, for that matter) are conducted on males.

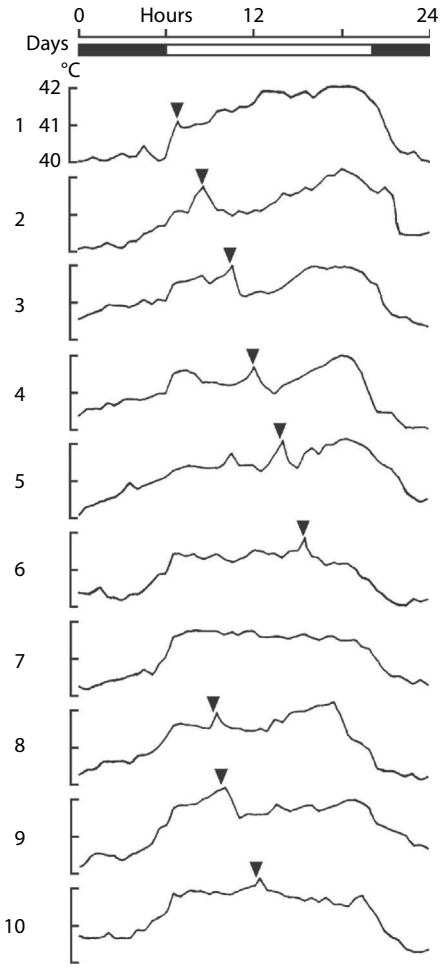


FIGURE 5.37 Daily rhythm of body temperature in chicken. This actogram-like graph shows a 10-day segment of the records of body temperature of a hen (*Gallus domesticus*) measured by telemetry. The bird was under a light–dark cycle, as indicated by the horizontal bars at the top. A daily rhythm of body temperature is clearly seen. Although the daily rhythm is synchronized to the light–dark cycle (with higher temperatures during the light phase in this diurnal animal), small peaks associated with ovulation (inverted black triangles) define an additional free-running rhythm with a period longer than 24.0 hours. Notice that the ovulation rhythm is gated by the daily rhythm, so that ovulation does not occur on the day when it would take place during the dark phase of the light–dark cycle (day 7). (Data from Kadono, H. et al., *J. Appl. Physiol.*, 51, 1145, 1981.)

Examples of body temperature rhythms of male individuals of five mammalian species are shown in Figure 5.38. To facilitate visualization of the daily patterns of oscillation, the data are plotted in 2-hour intervals (thus eliminating

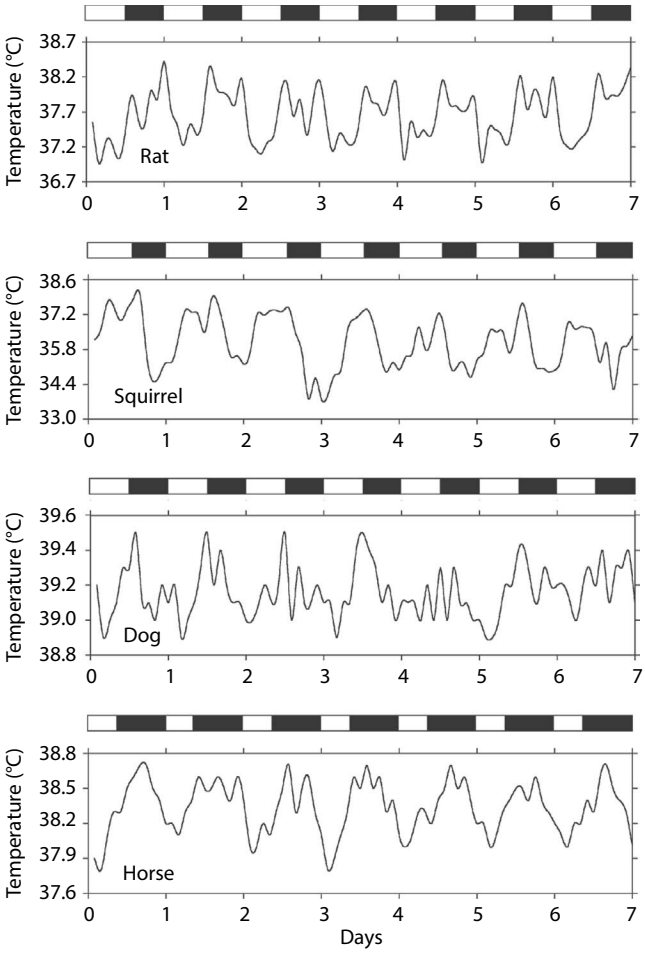


FIGURE 5.38 Daily rhythms of body temperature in mammals. The graphs show 7-day segments (with 2-hour resolution) of the records of body core temperature of representative individuals of four mammalian species. The white and dark horizontal bars at the top of each graph indicate the duration of the light and dark phases of the prevailing light–dark cycles. Interspecies differences in waveform and acrophase are evident in the temperature records. (Data from Refinetti, R. and Piccione, G., *J. Therm. Biol.*, 30, 139, 2005.)

high-frequency ultradian oscillations). Despite some "noise" that is characteristic of biological systems, regular daily oscillations of body temperature can be seen in all five records. Consistently with its nocturnal habits, the laboratory rat exhibits a rhythm with high temperatures during the night. Conversely, the rhythm of the diurnal squirrel is characterized by high temperatures during the day. The rhythms of the dog and the horse (both of which are diurnal) have a somewhat more triangular shape and seem to peak at night. Notice, however, that in these species, there is a consistent elevation of body temperature throughout the day and a fall during the night.

A closer look at interspecies differences is possible in Figure 5.39, where the data are plotted with greater temporal resolution (6 minutes) and the Y-axis is scaled uniformly

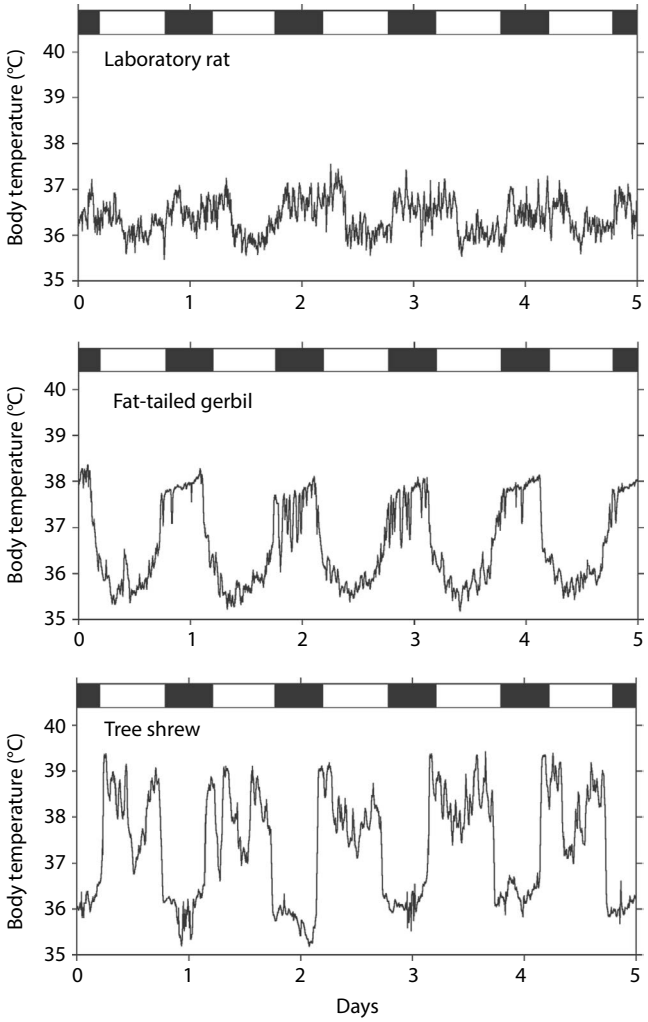


FIGURE 5.39 Interspecies differences in the amplitude of the temperature rhythm. The graphs show 5-day segments (with 6-minute resolution) of the telemetric records of body core temperature of representative individuals of three mammalian species. The white and dark horizontal bars at the top indicate the duration of the light and dark phases of the prevailing light–dark cycle. Interspecies differences are evident not only in the waveform and acrophase but also in the amplitude of the rhythms. (Adapted from Refinetti, R., *J. Therm. Biol.*, 24, 477, 1999.)

for the different species. Again, the rhythms of the nocturnal animals (laboratory rat and fat-tailed gerbil) are characterized by higher temperatures during the night, whereas the rhythm of the diurnal animal (tree shrew) is characterized by higher temperatures during the day. Also evident are differences in waveform: square for the rat, rectangular for the gerbil, and bimodal for the tree shrew. In addition, the amplitudes of the rhythms differ among the species: the daily range of oscillation of the temperature rhythm is less than 2°C for the rat but more than 4°C for the tree shrew. Table 5.3 lists the mean level, range of oscillation, and acrophase (peak time) of the body temperature rhythms of 78 species of mammals and birds. As will be discussed in greater detail in Chapter 10, the mean level of the body temperature rhythm tends to be higher by more than 1°C in large-sized species than in small-sized ones, although there is considerable interspecies variability. Also, the body temperature of birds tends to be more than 3°C higher than that of mammals (on average, 41°C and 37.5°C, respectively), and the temperature of marsupial mammals tends to be about 3°C lower than that of placental mammals. The range of oscillation also varies with body size across species: the range is almost 2°C narrower in large species than in small ones—although, again, there is considerable interspecies variability. As for the acrophase, it usually occurs at night in nocturnal animals and during the day in diurnal animals, but it does not seem to be related to body size, except that few large mammals are nocturnal.

How reliable are interindividual and interspecies differences in the parameters of the body temperature rhythm? It varies. As was the case for locomotor activity, the robustness of the rhythms varies from species to species. Cattle (Figure 5.40) exhibit the most robust rhythm of body temperature of all species tested so far,[738] and their rhythmic parameters can be reliably distinguished from those of other species. When a species exhibits low rhythm robustness, however, interspecies comparisons are less reliable. Nonetheless, one can obtain a realistic impression of the reproducibility of determinations *within* a species by inspecting Figure 5.41. Records of body temperature of 10 individual golden hamsters maintained under a 24-hour light–dark cycle (LD 14:10) are shown. Although there are some obvious intraspecies differences, mainly in the mean level and waveform of the rhythm, the similarities are more conspicuous than the differences. Records of different individuals of a species are generally more similar to each other than to the records of individuals of another species. This allows the creation of *educed rhythms*—that is, one-day patterns that reasonably describe the rhythmicity of most members of a species. An educed rhythm of human body temperature is shown in Figure 5.42. The data were obtained from 8 healthy men living a normal sedentary life in a hospital ward with access to three meals a day, at 08:15 a.m., 12:30 p.m., and 5:30 p.m. The mean level of the rhythm is 37.0°C, the range of excursion is 0.9°C, and the acrophase occurs at 5:15 p.m. If you wake up at about 8 o'clock each morning, your own body temperature rhythm should resemble the one in the figure (see Exercise 1.3). If you wake up much earlier or much later, your rhythm should still

TABLE 5.3
Parameters of the Daily Rhythm of Body Temperature

Species[a]	Mean Level (°C)	Range (°C)	Acrophase (Hours)[b]	Sources
Acomys russatus	36.7	2.0	18	[279,659]
Aethomys namaquensis	36.4	3.0	18	[281,672]
Antechinus stuartii	36.5	3.1	19	[328]
Aotus trivirgatus	37.8	1.4	18	[486]
Apodemus flavicollis	37.4	1.7	17	[290]
Apodemus mystacinus	38.4	2.2	17	[659]
Arvicanthis ansorgei	38.6	3.0	6	[283]
Arvicanthis niloticus	37.6	2.0	6	[270,271,276]
Bettongia gaimardi	37.4	1.7	22	[743]
Bos taurus	38.8	1.0	15	[10,738–741]
Callithrix jacchus	37.5	3.0	6	[372,683]
Camelus dromedarius	37.4	1.0	10	[759]
Canis familiaris	39.0	0.6	11	[716–719]
Capra hircus	38.8	0.7	13	[721,723–725,730]
Cebus albifrons	37.2	2.7	6	[678]
Columba livia	40.6	2.1	6	[134,136,760]
Coturnix coturnix	41.0	1.3	15	[763]
Cynomys ludovicianus	37.4	2.5	7	[332]
Dasypus novemcinctus	35.5	2.6	18	[339]
Dasyurus viverrinus	36.5	3.6	18	[329]
Didelphis marsupialis	35.5	2.5	19	[744]
Didelphis virginiana	35.4	4	20	[744]
Dryomys laniger	36.4	5.6	16	[673]
Equus caballus	37.9	1.0	14	[732,735,736]
Erinaceus europaeus	35.4	1.2	16	[745]
Eulemur fulvus	38	0.9	18	[348]
Felis catus	38.2	1.0	16	[313–315,720]
Gallus domesticus	40.5	1.4	8	[138,139,473,767]
Glaucomys volans	37.1	2.1	17	[244]
Heterocephalus glaber	33.8	3.8	15	[334]
Homo sapiens	36.9	1.1	10	[34,374,384,501,502,685,689, 691–693,695–697, 701–703,706,711,1085,1086]
Ictidomys tridecemlineatus	36.6	4.6	7	[618,669]
Isoodon macrouros	36.2	2.5	16	[744]
Isoodon obesulus	36.5	2.5	13	[756]
Lasiorhinus latifrons	35.3	2.9	16	[326]
Macaca fuscata	37	2.4	9	[350]
Macaca mulatta	37.3	1.5	10	[351,353,679,778]
Macaca nemestrina	38.0	2.0	8	[357]
Macropus giganteus	34.6	2.8	19	[746]
Macropus rufus	36.3	1.7	17	[746]
Marmota monax	37.7	1.3	10	[293]
Meleagris gallopavo	40.2	1.2	12	[131]
Mephitis mephitis	36.4	1.3	12	[656]
Meriones unguiculatus	37.2	2.7	11	[296,618]
Mesocricetus auratus	36.9	2.1	17	[230,234,618,658]
Microcebus murinus	36.6	2.4	17	[358–360,362,363]
Monodelphis domestica	34.3	6.5	15	[757]
Mus musculus	36.5	2.0	18	[192,193,198,207,216,224,226,548,619, 646, 647,650,654,1065]
Myrmecobius fasciatus	35	5.8	10	[747]
Nasua nasua	37.5	1.9	7	[748]
Octodon degus	37.0	2.0	7	[251–253,255,618]

(*Continued*)

TABLE 5.3 (*Continued*)
Parameters of the Daily Rhythm of Body Temperature

Species[a]	Mean Level (°C)	Range (°C)	Acrophase (Hours)[b]	Sources
Odocoileus hemionus	38.3	1.8	12	[343]
Oryctolagus cuniculus	39.4	0.9	16	[656,749]
Ovis aries	39.5	0.9	10	[726,727,730]
Pachyuromys duprasi	36.5	2.5	18	[297]
Papio hamadryas	37.9	1.7	8	[684]
Petaurus breviceps	37	3.2	18	[758]
Procyon lotor	38.1	1.4	1	[656]
Rattus norvegicus	37.3	1.6	18	[23,30,150,152,153,155,158,159,161,162,170,173,182, 283,618–622,625,630,634,639,642,643,646,773,775]
Rhabdomys pumilio	36.6	2.0	5	[672]
Saimiri sciureus	37.6	2.2	7	[436,680,681]
Sarcophilus harrisii	35.7	4.2	18	[329]
Sminthopsis macroura	36.2	5.5	18	[1087]
Spalax ehrenbergi	36.4	1.5	5	[263]
Spermophilus beecheyi	36.4	2.4	5	[668]
Spermophilus lateralis	36.5	4.0	6	[248]
Spermophilus xanthoprymnus	37.9	4.0	7	[677]
Struthio camelus	39.1	1.8	9	[616]
Suncus murinus	35.0	6.0	14	[335]
Sus scrofa	39.1	0.8	12	[336,750,754]
Thallomys nigricauda	36.8	2.1	18	[670]
Thallomys paedulcus	36.6	2.9	18	[281]
Trichosurus vulpecula	37.4	2.9	16	[744]
Tupaia belangeri	37.7	4.6	5	[244,307]
Tyto alba	40.3	2.2	17	[769]
Urocitellus parryii	36.9	4.4	8	[675]
Urocitellus richardsonii	36.1	3.3	10	[618,676]
Vombatus ursinus	34.7	1.4	18	[753]

[a] For common English equivalents of scientific names of the species, see the table of "Organisms Used" at the end of the book.
[b] The acrophase is given as number of hours after lights on (sunrise) for animals maintained under a light–dark cycle with 12 hours of light and 12 hours of darkness per day.



FIGURE 5.40 A dairy cow. Domestic cattle (*Bos taurus*) exhibit the most robust daily rhythm of body temperature of all animal species studied so far. (Photo by Keith Weller; Agricultural Research Service, United States Department of Agriculture.)

resemble the one in the figure, but the acrophase will probably be much earlier or much later, respectively.

Free-running circadian rhythms of body temperature have been documented in birds,[131,134,135,137–139,145,473,762,763,765,770,771] rodents,[150,161,170–172,174,179,182,205,207,227,237,254,255,263,270,279,283,420,422,423,427,625,641,648,669,675,772–777] primates,[348,360,362,363,436,486–488,679,682,778] including humans[374,380,382,440–444,695,703,779,780] and other mammals.[307,313,314,336,339,720,726,732,759,781–783] Figure 5.43 shows an example. A thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) was kept under constant illumination (LL) in an environmental chamber at a constant temperature of 24°C, and its body temperature was monitored by telemetry. Notice that body temperature oscillates regularly with a circadian period shorter than 24 hours (i.e., 23.3 hours).

5.3.2 Cardiovascular Function

Daily rhythmicity in cardiovascular function (heart rate and blood pressure) has been studied most often in rodents,[30,158,164,167,168,177,179,181,183,193,201,226,241,269,295,633,784–794] but also in various

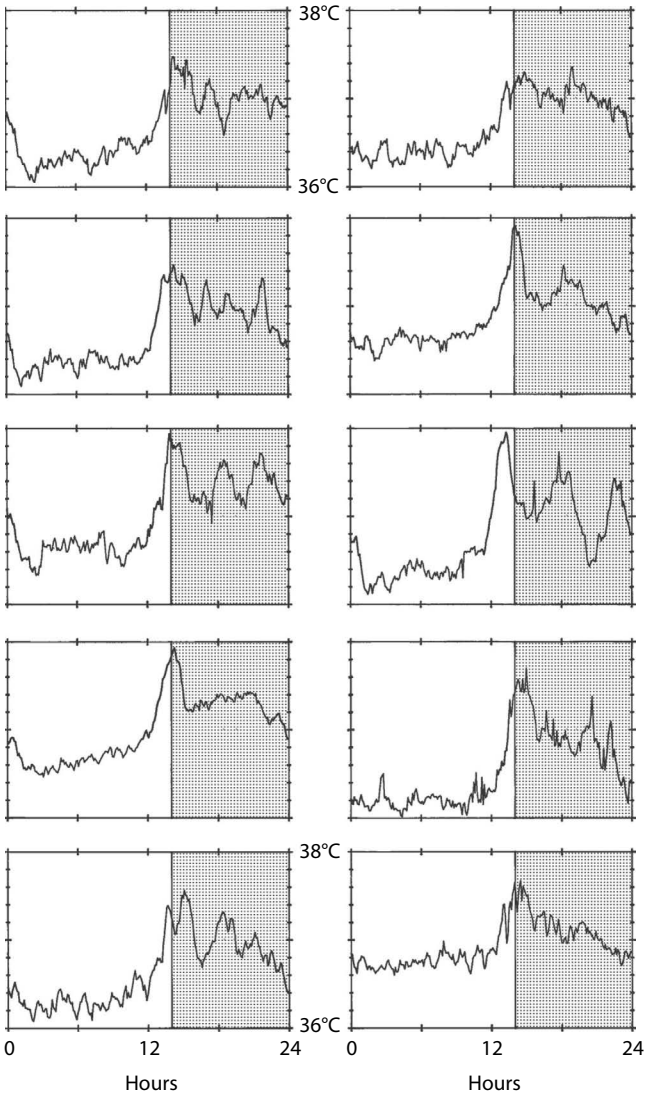


FIGURE 5.41 Intraspecies consistency of the temperature rhythm. The graphs show 24-hour segments (with 6-minute resolution) of the records of body core temperature of 10 individual golden hamsters (*Mesocricetus auratus*) maintained in an environment kept at 24°C under a light–dark cycle with 14 hours of light and 10 hours of darkness per day. Although individual differences can be seen, the rhythmic parameters are similar in all 10 animals. (Data from the archives of the Refinetti lab.)

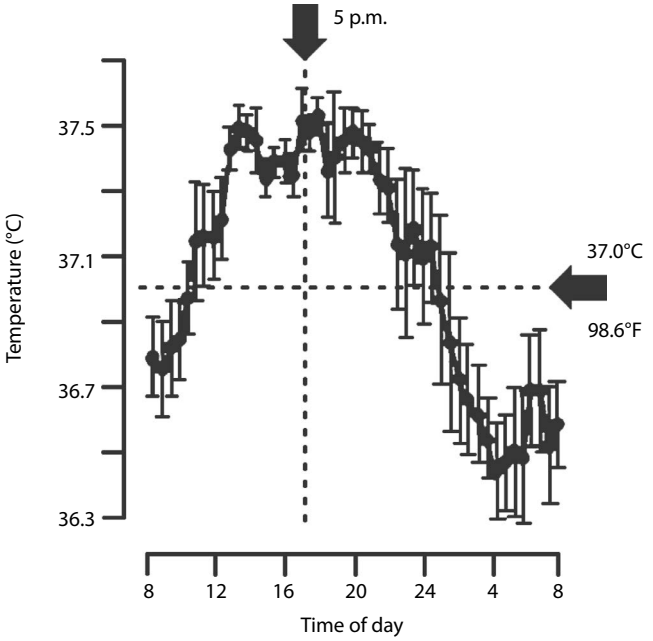


FIGURE 5.42 Human body temperature rhythm. The graph shows the typical rhythm of core body temperature of humans (*Homo sapiens*) living a normal, sedentary life with 3 meals per day (breakfast at 8:15 a.m.) and 8 hours of sleep at night. The data points correspond to the mean (±SE) temperature of 8 men recorded at 15-minute intervals. The mean level of the rhythm is 37.0°C, the range of oscillation is 0.9°C, and the acrophase is 5:15 p.m. (Data from Scales W.E. et al., *J. Appl. Physiol.*, 65, 1840, 1988.)

species of birds and mammals,[139,321,335,353,369,678,719,729,795–799] including humans.[27,381,382,502,560,697,702,800–812] Although very few studies have differentiated daily rhythms from circadian rhythms, free-running rhythms have occasionally been documented.[179,382,427,813]

We saw in Chapter 4 that the heart exhibits an ultradian rhythm of pulsation (*heart rate*) driven by a pacemaker in the sinoatrial node. This ultradian rhythm is modulated by the circadian system, so that heart rate oscillates daily. An example is shown in Figure 5.44. Heart rate measurements were taken from a Mongrel dog at hourly intervals for several days. Notice that heart rate increases each day from about 55 beats per minute to about 80 beats per minute.

Blood pressure is, of course, the pressure in the blood that flows through arteries and veins. What is normally called blood pressure, however, is *arterial* blood pressure, which is essentially the result of contractions of the heart. As shown in Figure 5.45, contraction of the left ventricle (ventricular systole) raises arterial pressure to approximately 120 mm Hg in a healthy human adult. As the ventricle relaxes (diastole), arterial pressure goes down to approximately 80 mm Hg. The mean arterial pressure (systolic plus diastolic, divided by two) is 100 mm Hg. In other species, the normal values of systolic and diastolic pressure are different, but the process is similar. Figure 5.46 shows mean values of systolic and diastolic arterial pressures of a group of 11 laboratory rats. Notice that both measures are elevated at night and lower during the day.

5.3.3 Melatonin and Cortisol Secretion

As mentioned in Chapter 4, *melatonin* is a hormone that is synthesized mainly in the *pineal gland*—but also in the eyes—and is secreted into the general circulation. Melatonin is not a particularly important hormone for any of the major physiological systems, but it has received great attention from circadian physiologists because of its central role in photoperiodism, which affects multiple physiological systems in photoperiodic organisms. As will be discussed in Chapters 12 and 13, melatonin also plays an important role in the modulation of circadian rhythms, and the pineal gland is an important circadian

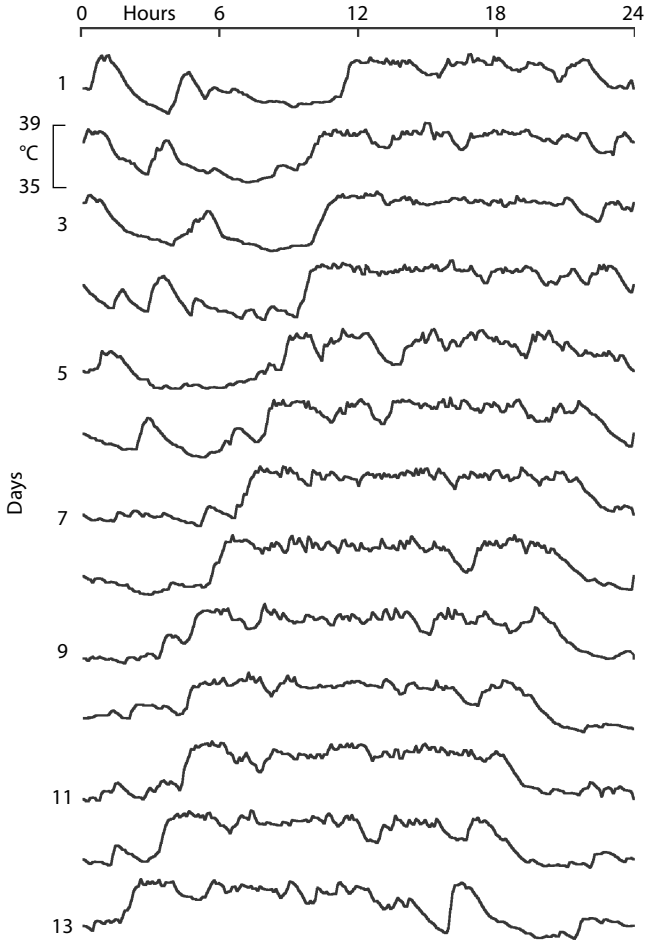


FIGURE 5.43 Circadian rhythm of body temperature. This actogram-like graph shows a 13-day segment of the records of body temperature of a 13-lined ground squirrel (*Ictidomys tridecemlineatus*) maintained in constant light. Temperature was recorded by telemetry with 6-minute resolution. A free-running rhythm with a period of 23.3 hours can be easily identified. (Data from Refinetti, R., *Physiol. Zool.*, 69, 270, 1996.)

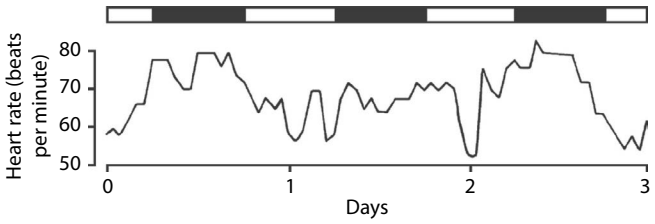


FIGURE 5.44 Daily rhythm of heart rate. The graph shows a 3-day segment of the records of heart rate of a Mongrel dog (*Canis familiaris*). Measurements were made by telemetry at hourly intervals. The duration of the prevailing light–dark cycle is shown by the horizontal light and dark bars. A clear daily rhythm, with high values during the night, can be observed. (Data from Ashkar, E., *Am. J. Physiol.*, 236, R231, 1979.)

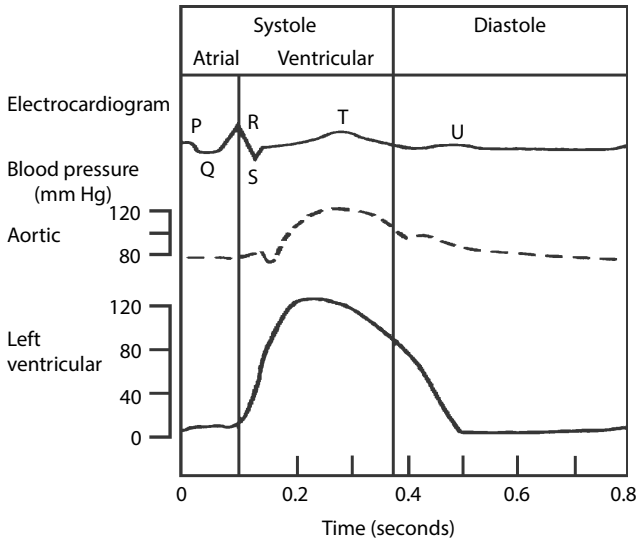


FIGURE 5.45 Systolic and diastolic blood pressure. Systolic blood pressure is the pressure measured during contraction of the left ventricle of the heart (systole). Diastolic blood pressure is the pressure measured during relaxation of the ventricle (diastole). (Adapted from Ganong, W.F., *Rev. Med. Physiol.*, 20th edn., Lange Medical, New York, 2001.)

pacemaker in many nonmammalian vertebrates. Daily rhythmicity of melatonin secretion can be measured directly at the mammalian pineal gland *in vivo* by microdialysis.[814–816] More commonly, researchers measure melatonin carried in the blood. Daily rhythmicity of blood melatonin concentration has been documented in fishes,[18,122,817–819] reptiles,[403,820,821] birds,[20,146,822–824] and mammals,[318,320,321,737,825–835] including

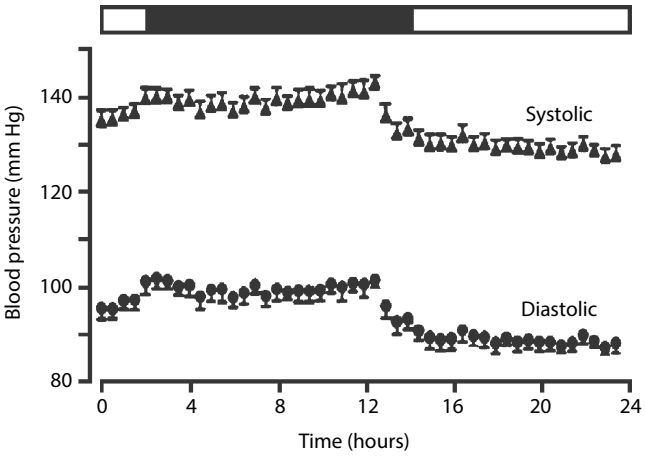


FIGURE 5.46 Daily rhythm of blood pressure. The graph shows the daily variation in systolic and diastolic blood pressure of laboratory rats (normotensive Wistar-Kyoto strain) measured at 30-minute intervals through chronically implanted catheters. Each data point corresponds to the mean (±SE) of 11 rats. The duration of the prevailing light–dark cycle is shown by the horizontal light and dark bars. Blood pressure is consistently higher during the dark phase of the light–dark cycle in this nocturnal species. (Data from Henry, R. et al., *Hypertension*, 16, 422, 1990.)

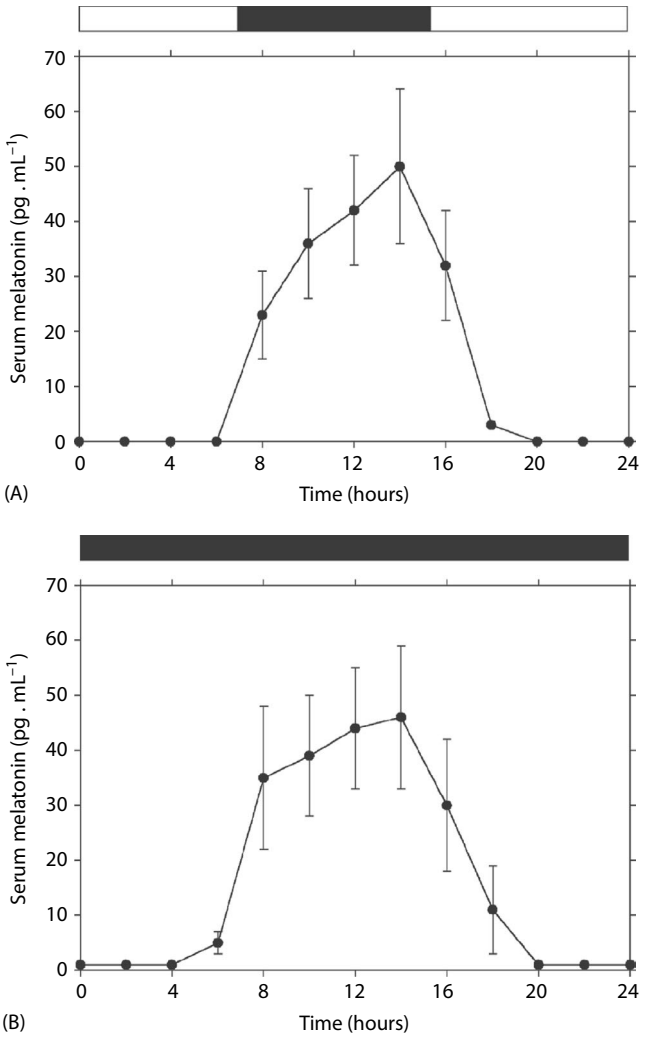


FIGURE 5.47 Daily and circadian rhythms of melatonin secretion. In goats (*Capra hircus*), as in many other animals, the hormone melatonin is secreted rhythmically under a light–dark cycle (A) as well as in constant darkness (B). Each data point corresponds to the mean (±SE) of seven goats. Melatonin secretion was measured as the concentration of melatonin in the serum at 2-hour intervals. (Data from Alila-Johansson, A. et al., *J. Biol. Rhythms*, 16, 254, 2001.)

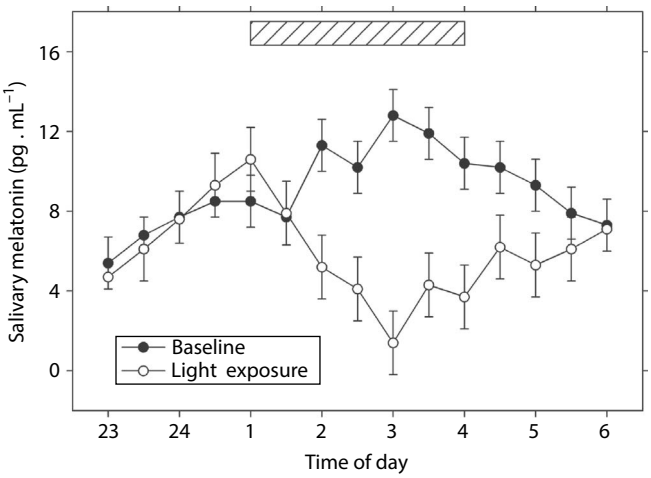


FIGURE 5.48 Melatonin suppression by light. Although melatonin secretion follows a circadian rhythm in the absence of an environmental light–dark cycle, environmental light does have a suppressive effect on melatonin secretion. The data points in the graph are the means (±SE) of seven human subjects maintained under constant dim light (baseline) or exposed to bright light for 3 hours (light exposure). Melatonin secretion was measured as the concentration of melatonin in the saliva at 30-minute intervals. The hatched horizontal bar indicates the duration of the bright light exposure. Notice that both curves exhibit circadian rhythmicity but that the curve for the light exposure condition exhibits also light-induced suppression. (Data from Hébert, M. et al., *J. Pineal Res.*, 33, 198, 2002.)

rodents[240,836–849] and humans.[378,606,699,700,704,705,714,850–859] An example is shown in Panel A of Figure 5.47. Seven goats (*Capra hircus*) were maintained under a light–dark cycle, and blood samples were collected at 2-hour intervals for the determination of melatonin concentration in the serum. Notice that there is a clear daily rhythm of melatonin concentration, with greater concentrations being attained during the night. Of course, the daily rhythm could be induced by the alternation of light and darkness, particularly because melatonin secretion is inhibited by light regardless of time of day, as shown in Figure 5.48. The data in this figure are from a different species (humans), but it is evident that light exposure at night can drastically inhibit melatonin secretion. Thus, in order to determine whether the rhythmicity in melatonin secretion is not merely the result of photic inhibition during the day, measurements must be conducted in constant darkness. Panel B in Figure 5.47 (with data from goats again) confirms the persistence of rhythmicity in constant darkness. Circadian rhythmicity of melatonin secretion (under constant environmental conditions) has been documented in many studies in various species.[146,206,318,403,759,821–824,828,837,839,841,848,849,852,860–865]

Unlike the case of other variables mentioned in this chapter, melatonin has been shown *not* to exhibit true circadian (endogenous) rhythmicity in some species. In horses, two studies measuring blood concentration of melatonin found strong rhythmicity while the animals were under a light–dark cycle but not when they were under constant darkness, even though other variables in the same animals remained rhythmic under constant darkness.[737,833] In two species of fish, melatonin exhibited 24-hour rhythmicity under even very dim light–dark cycles but not under constant darkness.[818,819] In reindeer, the strong rhythmicity of melatonin detected in the blood when the animals were under a light–dark cycle vanished after only a few days in constant darkness.[834] When samples of connective tissue (*fibroblasts*) were collected from reindeer and compared with samples taken from mice, the mouse samples exhibited 24-hour rhythmicity *in vitro* but the reindeer samples did not.[835] The authors of the latter study suggested that short-lived seasonal mammals (such as hamsters) may have strong circadian clocks and weak circannual clocks, whereas long-lived seasonal mammals (such as reindeer) may have weak circadian clocks and strong circannual clocks.[835]

Studies on human subjects very often use the rhythm of melatonin secretion to estimate the phase of the

circadian clock. Melatonin concentration is measured in plasma, urine, or saliva, and the time when melatonin concentration rises above a certain level is taken as a phase marker (called the dim light melatonin onset [DLMO]).[866] Phase estimates based on the DLMO are highly correlated with estimates based on the rhythms of activity and body temperature,[867] and some authors find the DLMO to be the best circadian phase marker.[868] Although collection of salivary samples is minimally invasive, it does require that the subject be awake and willing to provide the samples. It has been recently proposed that measurement of skin temperature on the wrist, which can be accomplished with a small data logger capable of recording data unobtrusively for many days and nights, may serve as a good circadian phase marker.[869] Circadian phase determined by records of wrist temperature has been shown to be reasonably similar to circadian phase determined by records of salivary melatonin,[870] although ambulatory monitoring seems to require the inclusion of several variables measured through various devices in order to achieve the accuracy provided by salivary melatonin.[385]

Cortisol is a hormone secreted by the cortex of the adrenal gland. It plays an important role in the metabolism of glucose and proteins and has anti-inflammatory properties.[871,872] The adrenal cortex secretes many other hormones, including *corticosterone*, which is structurally very similar to cortisol. Many rodents secrete corticosterone almost exclusively, whereas humans secrete seven times as much cortisol as corticosterone. Unlike the secretion of melatonin, the secretion of cortisol/corticosterone is not suppressed by photic stimulation; however, it is affected by various internal and external factors, the best known of which is *stress*. Daily and/or circadian rhythmicity in cortisol/corticosterone secretion has been documented in nonmammalian[18,20,820] and mammalian[364,522,778,830,831,873–875] vertebrates, particularly rats,[170,175,176,187,637,790,843,845,876–885] mice,[203,204,213,215,886–891] other rodents,[839,863,892,893] and humans.[376,501,596,606,705–707,853–860,862,894–903] Figure 5.49 shows the average daily rhythm of cortisol secretion (as measured by serum cortisol concentration) of 31 young men. Notice that serum concentration starts to rise in the middle of the night, reaches the daily peak at wake-up time, and falls throughout the day.

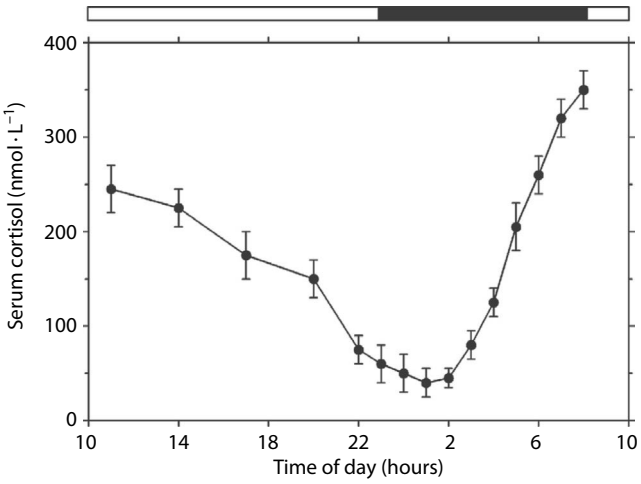


FIGURE 5.49 Daily rhythm of cortisol secretion. The graph shows the daily variation in serum cortisol concentration of human subjects. Each data point corresponds to the mean (±SE) of 31 young men. The duration of the prevailing light–dark cycle is shown by the horizontal light and dark bars. Serum cortisol concentration starts rising in the middle of the night and reaches a peak at wake-up time. (Data from Selmaoui, B. and Touitou, Y., *Life Sci.*, 73, 3339, 2003.)

5.3.4 Metabolism and Sleep

Living organisms need energy to sustain life. Whether energy is obtained directly from sunlight by photosynthesis or indirectly by the breakdown of nutrients, substances must be metabolized. Daily and/or circadian rhythmicity in *metabolism* has been documented in plants and invertebrates,[526,904–912] lower vertebrates,[539,913,914] birds,[136,767,769,915–917] and mammals,[334,339,436,746,748,752,918–921] including rats[151,152,154,162,164,166,462,636,644,773,922–926] and other rodents[205,208,235,297,298,466,659,662,666,670,927–930] and humans.[502,707,931] Figure 5.50 shows a 4-day segment of the circadian rhythm of metabolism of a laboratory rat. Aside from considerable biological noise, one can see a regular pattern of rising and falling metabolic rate with a period close to 24 hours.

Low metabolic rates in animals are usually associated with lower activity levels and often also with *sleep*. Although not all animals sleep,[932] sleep is a pervasive function in the animal kingdom,[933] from flies[934,935] to whales.[936] The number of hours of sleep needed per day varies greatly from species to species. As illustrated in Figure 5.51, some bats sleep as much as 20 hours each day, whereas horses usually sleep only 3 hours.

Regardless of how many hours a species spends on sleep each day, episodes of sleep are not randomly distributed over time. On the contrary, electroencephalographic monitoring of sleep indicates a very unequal distribution of sleep stages, as exemplified in Figure 5.52. The figure shows the percentages of slow-wave sleep (SWS) and rapid-eye-movement

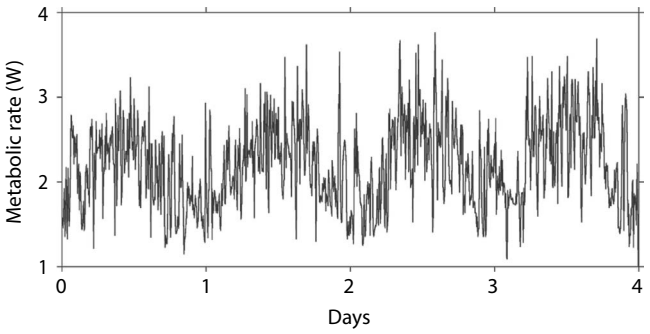


FIGURE 5.50 Circadian rhythm of metabolism. The graph shows a 4-day segment of the records of whole-organism metabolism of a laboratory rat (*Rattus norvegicus*) housed at 24°C in constant darkness. Metabolic rate was computed from measurements of oxygen consumption performed at 6-minute intervals. A clear circadian rhythm, accompanied by large ultradian oscillations, can be seen. (Data from Refinetti, R., *Exp. Physiol.*, 88, 423, 2003.)

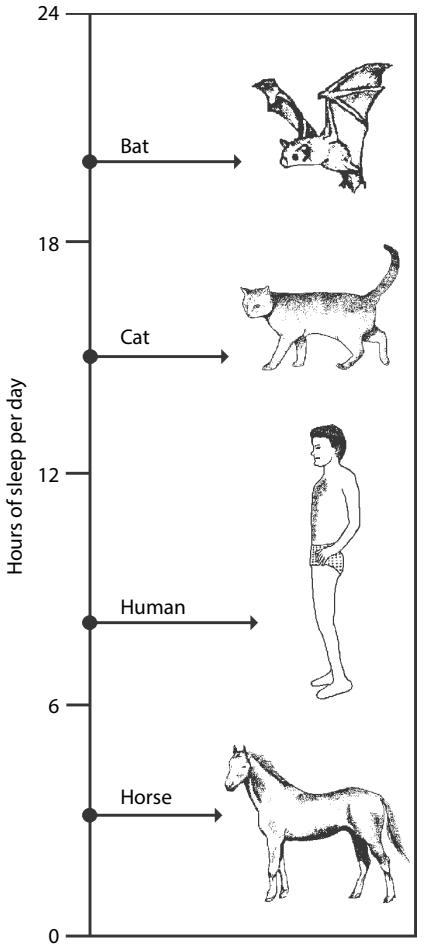


FIGURE 5.51 How much do you sleep? The usual number of hours of sleep per day varies greatly from species to species, as exemplified by these four species. (Data from Siegel, J.M., *Science*, 294, 1058, 2001.)

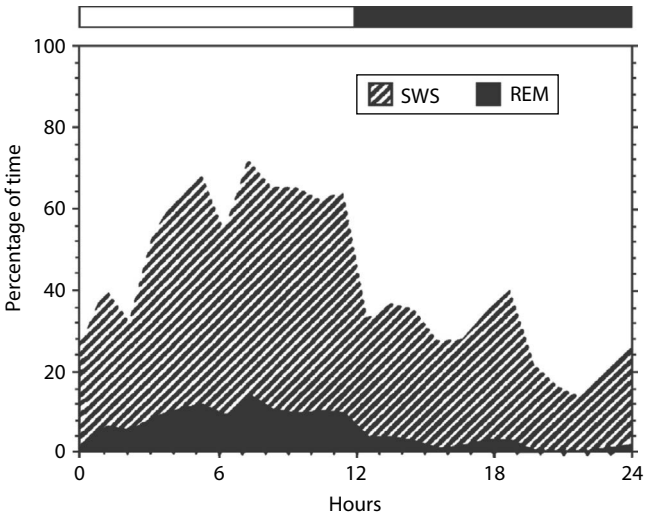


FIGURE 5.52 Daily rhythm of sleep stages. The graph shows the daily variation in the percentage of time spent each day on slow-wave sleep and rapid-eye-movement sleep by laboratory rats (*Rattus norvegicus*). Shown are the mean values for six adult rats (7 months old) at hourly intervals. The duration of the prevailing light–dark cycle is shown by the horizontal light and dark bars. As nocturnal animals, rats sleep more during the day than during the night. Notice that, unlike humans, rats do not consolidate their sleep into a single 8-hour interval. (Data from Li, H. and Satinoff, E., *Am. J. Physiol.*, 269, R208, 1995.)

(REM) sleep of laboratory rats over the 24-hour cycle. In this nocturnal species, both SWS and REM sleep are much more frequent during the light phase than during the dark phase of the light–dark cycle. Daily or circadian rhythmicity of electroencephalographically monitored sleep has been documented in a variety of animals, including rodents,[31,156,169,172,197,251,413,415,624,640,648,774,937–944] humans,[441,695,705,708,864,945–947] and other vertebrates.[135,372,720,755,761,948–950]

Whether or not one actually sleeps on a given day, subjective sleepiness still exhibits daily rhythmicity. For example, Figure 5.53 shows the mean sleepiness scores of two groups of six people kept in bed but awake for 24 consecutive hours. To partially control the effect of sleep deprivation, one group started the protocol in the morning (squares) and the other in the evening (triangles). Thus, one group missed sleep at the end of the protocol and the other at the beginning. The two curves are very similar, showing greater sleepiness around the usual sleep time. Therefore, sleep deprivation did not significantly alter the circadian rhythmicity of sleepiness. Well, it did a little bit. Notice that there is not a sharp reduction in sleepiness between 10:00 and 18:00 (at the end of the

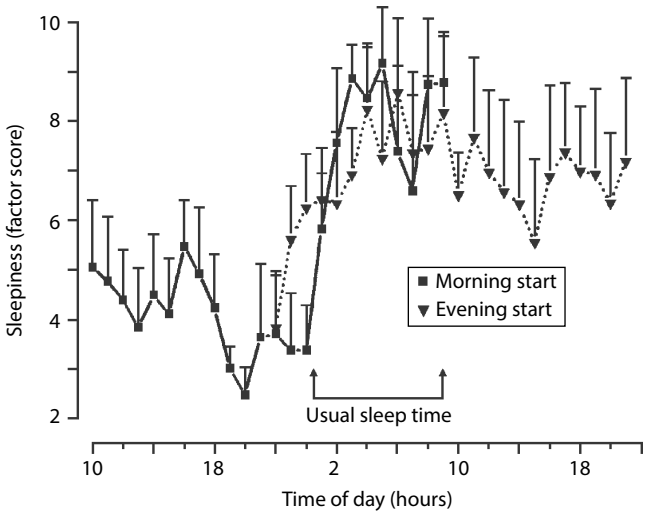


FIGURE 5.53 Circadian rhythm of sleepiness. The graph shows the variation of subjective sleepiness of human subjects in a constant routine protocol (that is, in bed rest, with no sleep allowed, and with small meals provided every hour) for over 24 consecutive hours. Each data point corresponds to the mean (±SE) of 6 subjects. As a control for sleep deprivation, data collection started in the morning for one group of subjects (morning start) and in the evening for another group (evening start). An increase in sleepiness at the usual sleep time is evident. (Data from Varkevisser, M. and Kerkhof, G.A., *Chronobiol. Int.*, 20, 109, 2003.)

protocol) in the evening-start group. As will be discussed in greater detail in Chapter 10, sleepiness (and actual sleep, if allowed) is under the control of both a circadian process and a restorative process.

5.3.5 Other Functions

Daily and/or circadian rhythmicity has been described in many other autonomic functions, including the secretion of reproductive hormones,[739,778,876,900,902,951–957] ingestive/digestive hormones,[364,878,895,896,958–962] and thyroid hormones.[692,854,860,882,896,926,963] Daily rhythms in breathing have been documented as well,[166,636,707,964] as illustrated in Figure 5.54. Also documented have been daily changes in properties of the visual system,[965–974] as exemplified by the iguana electroretinogram data in Figure 5.55. Notice that the rhythm of electrical activity can be observed both when the animals are kept under a light–dark cycle and when they are kept in constant darkness.

Among many other rhythms that have been described are the rhythms of growth and various cellular processes in bacteria,[975–977] resistance to herbicides in weeds,[978] photosynthetic capacity in algae and plants,[7,905,979–982] root pressure in the sunflower plant,[983] amount of carbohydrates and amino acids in the leaves of the potato plant,[984] leaf movement in the mustard plant,[985] mold growth,[986,987] mitosis in mammalian cells,[657,890,988] bioluminescence (glow) in protists and insects,[989,990] egg laying and eclosion of insects from the pupal stage,[991–996] heat resistance in invertebrates and vertebrates,[14,635,997] blood eosinophil count in mammals,[627,998,999] human salivary flow rate,[1000] human oral malodor,[1001] nutrient content of asinine milk,[1002] human height and body composition,[1003–1006] human colonic motility,[1007] glycogen concentration,[887,1008,1009] cholesterol synthesis,[312,723,959,963,1010–1016] intraocular pressure,[1017–1021] susceptibility to anesthetics,[1022–1024] dentin/enamel increment,[1025,1026] bone cell activity,[1027] alcohol-induced hypothermia,[1028] gastric acid secretion,[1029,1030] concentration of serum fat-soluble vitamins,[1031] glucose tolerance,[1032] contents of stomach and intestine,[1033] blood coagulation,[1034] and concentration of plasma aldosterone.[1035,1036] Figure 5.56 provides an example of the rhythm of photosynthetic capacity in the alga *Phaeodactylum tricornutum*. There can be no photosynthesis without light, so that daily rhythmicity of photosynthesis under a light–dark cycle is ineluctable. To evaluate

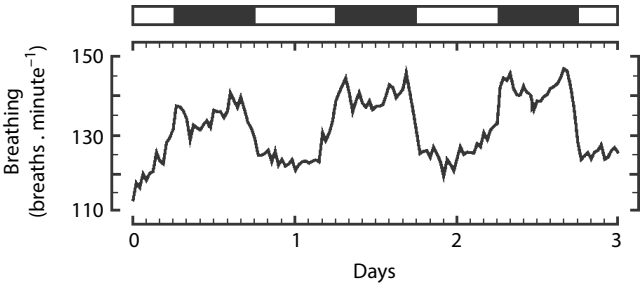


FIGURE 5.54 Daily rhythm of breathing rate. The graph shows the daily variation in the breathing rate of laboratory rats (*Rattus norvegicus*) on three successive days. Shown are the mean values for 23 rats with 15-minute resolution. The duration of the prevailing light–dark cycle is shown by the horizontal light and dark bars. As nocturnal animals, rats breathe at a higher rate during the night than during the day. (Data from Mortola, J.P. and Seifert, E.L., *Respir. Physiol. Neurobiol.*, 131, 91, 2002.)

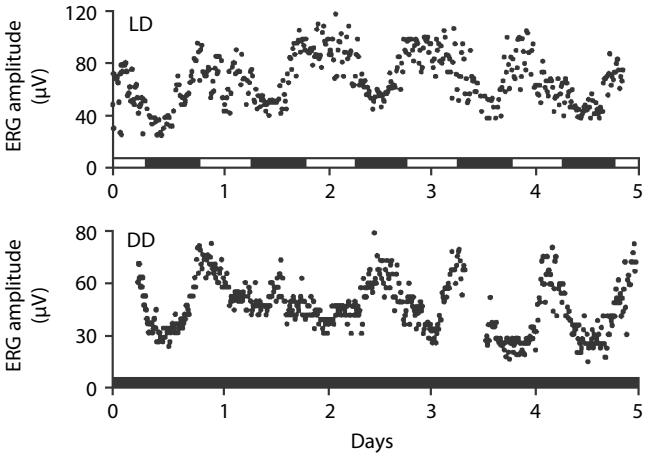


FIGURE 5.55 Daily and circadian rhythms of activity of retinal cells. The graph shows the oscillation of the amplitude of the b-wave of the electroretinogram (the compound electrical activity of neurons in the retina of the eye) of two green iguanas (*Iguana iguana*), one kept under a light–dark cycle and one kept in constant darkness, as indicated by the horizontal bars adjacent to the abscissa. In this diurnal lizard, the amplitude of the ERG is greater during the day than during the night. The rhythm freeruns in constant darkness. (Data from Miranda-Anaya, M. et al., *J. Biol. Rhythms*, 15, 163, 2000.)

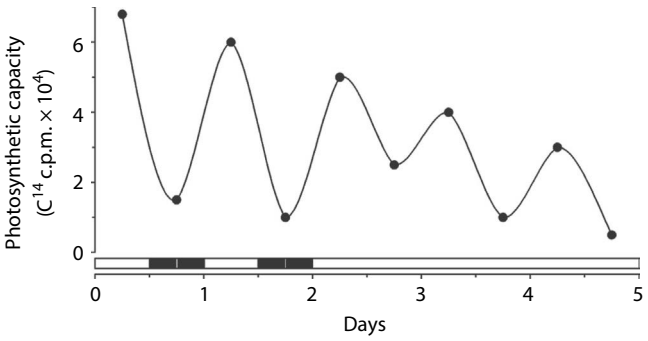


FIGURE 5.56 Daily and circadian rhythms of photosynthetic capacity. By necessity, photosynthesis cannot occur in the absence of light. Consequently, all plants exhibit a daily rhythm of photosynthetic activity. More interestingly, a daily rhythm of photosynthetic capacity can be demonstrated if nocturnal measurements are conducted during a brief exposure to light. The graph shows the daily variation in photosynthetic capacity of the alga *Phaeodactylum tricornutum* as measured by the assimilation of C^{14} from the medium in 15-minute sessions during the day and during the night (with 15 minutes of light during the nocturnal sessions). When the alga was placed in constant light (400 lux), the rhythm freeran. Ambient temperature was constant at 15°C throughout the study. (Data from Palmer, J.D. et al., *Nature*, 203, 1087, 1964.)

photosynthetic *capacity*, the authors presented 15 minutes of light in the middle of the dark phase and compared the measurements with those obtained during a 15-minute interval in the middle of the light phase. This revealed a daily rhythm of photosynthetic capacity (first 2 days of the records). Circadian rhythmicity persisted in constant light (next 3 days).

Although the various daily and circadian rhythms described in this chapter do not constitute an exhaustive list of all rhythms that have been recorded to date, they are hopefully sufficient to document the pervasiveness of circadian rhythmicity in behavioral and autonomic processes. In Section III of the book, we will examine the endogenous and environmental mechanisms responsible for this rhythmicity.

SUMMARY

1. The Earth's rotation around its axis generates daily environmental cycles. The daily environmental cycle of greatest importance to organisms is the alternation of light and darkness. A *civil day* lasts 24.0 hours and includes a seasonally variable interval of light (*day*), a variable interval of darkness (*night*), and two *twilights* (dawn and dusk). Many human populational activities exhibit daily rhythmicity in synchrony with the civil day.
2. Biological processes that cycle in 24-hour intervals are called *daily rhythms*. A daily rhythm that is endogenously generated and is modulated by 24-hour environmental cycles is called a *circadian rhythm*. Many behavioral processes of individual organisms exhibit daily and/or circadian rhythmicity, including locomotor activity, feeding, excretion, sensory processing, and learning capability. Rhythms of locomotor activity have been the most thoroughly studied behavioral rhythms.
3. Many autonomic processes of individual organisms exhibit daily and/or circadian rhythmicity, including the control of body temperature, cardiovascular function, melatonin secretion, cortisol secretion, metabolism, and sleep. Rhythms of body temperature have been the most thoroughly studied autonomic rhythms.

EXERCISES

5.1 Daily rhythm of heart rate. Measuring rhythms in your own body is perhaps the best way to gain an intuitive feel for the ubiquity of circadian rhythms. In Exercise 1.2, you measured your daily rhythm of body temperature. In this exercise, you will measure your daily rhythm of variation in heart rate. At rest, your heart beats about 70 times per minute; however, the number of beats per minute varies with the time of day. All you need is a stopwatch (or a regular watch that displays seconds) and a sheet of paper to record the data. Try to take your pulse every hour for two or more consecutive days. If you have difficulty in taking the pulse at the wrist, try taking it at your neck (and, if even this does not work, borrow a stethoscope and take the pulse at the chest). You may count the beats for a whole minute or count them for only 30 seconds and then multiply the number by two. Because heart rate is more strongly affected by physical exertion and by psychological stress than by the circadian system, you must make sure to be calm and not physically active for at least 15 minutes before a measurement. Occasionally, you may ask a friend to take your pulse while you sleep at night (but tell him or her not to wake you up, otherwise your pulse may be disturbed). At the end, draw a graphic showing heart rate (Y-axis) as a function of time (X-axis). You should be able to observe a clear daily rhythm.

5.2 Determining mean value, amplitude, and acrophase. In this exercise, we will use the program **Acro** to determine the mean level, amplitude, and acrophase of circadian rhythms. As explained in Section 3.2.2, this program fits a cosine wave to the data and determines the acrophase as the peak of the cosine wave. The mean level and amplitude of the rhythm are determined by analysis of the actual data. If you have not done the computer exercises in previous chapters, you should be reminded that you first need to double-click on the **Circadian** icon to open the program banner and then click on **Acro** (the seventh icon from the left) to start it.

1. Start the program **Acro**.
2. Choose **Load data from disk**.
3. Select the **Data** subfolder by double-clicking on it.
4. Select the data file **A16** by clicking on it. This file contains the records of body temperature of a fat-tailed gerbil measured by telemetry every 6 minutes for a week under a 24-hour light–dark cycle. As usual, the file contains only values for the ordinate. You may want to inspect the data set with **Plot** before proceeding.
5. Back to **Acro**, click on **OK** (or double-click on the file name) to load **A16**.
6. Click on **File contains data only**.
7. Since the data were collected every **6** minutes and the file starts at midnight (or **0** hour), we can use the default values. Click on **OK**.
8. Since the animal was under a 24-hour light–dark cycle, we can assume that the **Period of the cycle** is 24.0 hours and use the default value (**24**). If the period is different from 24.0 hours, there will be more (or less) than one full cycle in each 24 hours, and the program must know about it in order to make the appropriate adjustments.
9. The **Number of cycles** panel allows you to specify how many cycles (which is the same as number of days if the period is 24.0 hours) to use in the analysis. If you specify **One cycle**, only the first cycle will be loaded from the disk file. If you specify **More than one cycle**, you will be asked how many

cycles should be used and how many cycles should be skipped before loading the cycles to be used (just in case the cycles you want to use are not at the beginning of the file). The various cycles are averaged into a single cycle. Let us choose **One cycle** first. Click on it.

10. In the unlikely case that you want to change the program's choice of mean level and amplitude, you can do so at the **Confirm parameters** panel. Unless you have a very good reason to change the parameters, you should leave them as they are. Notice also the box entitled "Compute also threshold." It allows you to request that the program calculates not only the acrophase but also the time at which the data series passes above an arbitrary value (or threshold) set by you. We will not use this option in this exercise. If we were to use it, we would simply click on the check box. Click on **OK** now.
11. The **Results** panel shows you the mean (35.68°C), amplitude (1.715°C), and acrophase (1.6 hours or 1:36 a.m.) of the rhythm. Although I have not told you so yet, the lights were on from 5 a.m. to 7 p.m., which means that the acrophase is 3.4 hours before lights on for this nocturnal rodent. Also shown is the 95% confidence interval for the acrophase. Based on the data for this 1 day, we can be 95% confident that the true acrophase of the body temperature rhythm of this animal is between 0.09 and 3.11 hours (i.e., between 00:05 and 03:07 hours). An index of goodness of fit of the cosine wave to the data and the probability associated with this index are also shown. In this case, the index is 0.113, which is statistically significant at the 0.001 level (meaning that the fit is very good). The graph on the right allows you to subjectively assess the acrophase and the goodness of fit.
12. Since the file contains 7 days of data, let us repeat the procedure using 7 days rather than just 1 day. Click on **Start a whole new analysis**.
13. Click on **Load data from disk**, then on **OK**, then on **File contains data only**, then on **OK**, then on **More than one cycle**. Click on the **Number of cycles to be averaged** box, delete the number **1**, and type the number **7**. Then, click on **OK**. At the **Confirm parameters** panel, click on **OK**.
14. Because we used more than one cycle, the program was able to provide 95% confidence intervals not only for the acrophase but also for the mean and the amplitude. Notice that the computed acrophase is 1.1 hours (rather than 1.6 hours), and the goodness-of-fit index is 0.04 (even better than before). This is natural, as a more accurate calculation of acrophase is possible when more data are available.
15. Click on *Same parameters, new file*. Select file **A17**. This file is similar to the previous one except that the animal whose body temperature was recorded was a tree shrew (which is diurnal) rather than a fat-tailed gerbil.
16. Since we told the program to use the same parameters with a different file, we do not need to enter all the information again (the low and high values—which, of course, are not the same in the two files—are automatically replaced). Click on *OK* to see the results.
17. The computed acrophase is 11.7 hours (11:42 a.m.), which is 6.7 hours after lights on. Not surprisingly, the body temperature rhythm of the diurnal tree shrew peaks during the day, whereas the rhythm of the nocturnal gerbil peaked at night.
18. Finally, let us analyze a file with unequally spaced data. Click on *Start a whole new analysis*, then on *Load data from disk*, then select the data file **A18**, and click on *OK*. This file contains the records of locomotor activity of a thirteen-lined ground squirrel measured by telemetry every 6 minutes for 1 day under a 24-hour light–dark cycle (lights on from 3 a.m. to 5 p.m.). Each line of the file contains a time stamp (the clock time in decimal format) and the activity count.
19. Click on *File contains times and data*. Leave the *Period of cycle* as **24** and choose *One cycle*. As usual, just click on *OK* at the *Confirm parameters* panel.
20. The *Results* panel indicates that the acrophase is 9.3 hours (or 9:18 a.m.). This is 4.3 hours after lights on, which is consistent with the diurnal habits of this squirrel species.

5.3 Calculating circadian period by the chi-square periodogram procedure. In this exercise, we will use the program **Tau** (the 8th icon from the left in the Circadian banner) to calculate the period of circadian rhythms using the chi-square periodogram procedure described in Section 3.2.3. The program requires equally spaced data points in a data file without time tags.

1. Start the program **Tau**.
2. Notice that there are five panels: the *Source* panel (where you identify the file to be analyzed, as in previous programs), the *Data* panel (where you specify the format of the data file), the *Period* panel (where you pick the range of periods to be tested and the resolution to be used), the *Destination panel* (where you choose the place where the results should be displayed), and the screen display panel (currently blank).
3. In the *Source* panel, select the Data subfolder and then the data file **A08**. This file contains artificial data constructed as a cosine wave with a period of 23.5 hours (you inspected it using **Plot** in Exercise 3.4). Do not change the defaults in the *Data*, *Period*, or *Destination* panels. Although the file contains data collected over 20 days, we will use only the first 10 days. Click on *OK*.

4. In the display panel, a Q_P value is given for each potential period between 20.0 and 26.0 hours. The higher the Q_P value, the closer to real is the period associated with it. In this case, the highest Q_P is associated with a period of 23.5 hours (as it should be, since the data set was artificially constructed to have a period of 23.5 hours). Of course, the highest Q_P in a periodogram may not be absolutely (statistically) high. This is where the chi-square test comes in. As shown at the top of the panel, a Q_P of 2350 is significantly different from noise at a significance level lower than 0.0001.
5. Select data file **A09**. This is also an artificial file, but 60% of the data points in the preceding file were replaced with random noise in the range of oscillation. Click on *OK*. The highest Q_P is now only 750 (down from 2350), and it is off by a decimal unit (23.6 hours rather than 23.5 hours). The level of significance is still better than 0.0001, however.
6. Now select data file **A10**. This file contains 85% noise. Click on *OK*. Do not be surprised that the best Q_P is associated with a period of 25.5 hours. This happened by chance, as indicated by the nonsignificance reported right below ($p > 0.05$). Is 85% noise too much noise? Not necessarily. Click on the *Bins to use* scroll bar in the *Data* panel. A window will open telling you that, as in any statistical test, you can improve the power of the chi-square periodogram by increasing the sample size. Keep clicking on the scroll bar until you reach 4800 (which is the maximal number of bins available in the file). This corresponds to 20 days rather than 10. Click on *OK*. The period estimate is now very close to the correct one (23.4 hours as compared to 23.5 hours) and the p value is now below 0.001. By increasing the number of data points, we increased the statistical power of the test.
7. Let us now analyze a time series made up of real (biological) data. Select the data file **A24**. This file contains the records of running-wheel activity of a mouse maintained under a light–dark cycle for 16 days and in constant darkness for 17 days. Inspect the file as an actogram in **Plot** and notice that the period of the activity rhythm seems to be 24.0 hours under the light–dark cycle but much shorter than 24.0 hours in constant darkness.
8. Back to **Tau**, file **A24** should already be selected. Now set the *Bins to use* back to **2400** (to limit the analysis to the first 10 days) and then click on *OK*. As expected, the program detects significant rhythmicity with a period of 24.0 hours.
9. Now set *Bins to skip* to **3840**, so that the 16 days under the light–dark cycle are excluded. Click on *OK* again. The program confirms our expectation of significant rhythmicity with a period shorter than 24.0 hours (i.e., 23.4 hours).

5.4 Calculating circadian period by the Lomb–Scargle periodogram procedure. This exercise is similar to Exercise 5.3. The only difference is that we will be dealing with data files containing unequally spaced data points, which will require the use of a different program. **LSP** (the 9th icon from the left in the Circadian banner) calculates the period of circadian rhythms using the Lomb–Scargle periodogram procedure.

1. Start the program **LSP**.
2. Notice that there are five steps to be followed: you identify the data file in *Step 1*, specify the data format in *Step 2*, set the parameters of analysis in *Step 3*, execute the analysis in *Step 4*, and read the results in *Step 5*.
3. Select the data file **A11** in *Step 1*. This file contains artificial data constructed as a cosine wave with a period of 23.5 hours. Unlike the file **A08** that we used in the previous exercise, this file contains time stamps. Thus, in *Step 2*, you must specify that the file contains *Times and data*. You do not need to alter the values in *Step 3*. Move on to *Step 4* and click on *Compute*.
4. In *Step 5*, you can see that, as expected, the data set was judged to be rhythmic with a period of 23.5 hours. The value of the PN statistic (1199) is much larger than the criterion value for significance at the 0.01 level (12). Thus, the rhythmicity is statistically significant. To see the values of PN associated with each period in the chosen range of 18.0–30.0 hours, click on *See all*. After you have seen enough, click on *Close Window* to return to the main window.
5. Next, select the file **A12**. This is also an artificial file, but 60% of the data points in the preceding file were replaced with random noise in the range of oscillation. Click on *Compute*. As you can see, the calculated period is only slightly off mark (23.55 instead of 23.50 hours), and the PN value (288) is still greater than the criterion for significance at the 0.01 level.
6. Now select the data file **A13** (which contains a cosine wave with 85% noise) and click on *Compute*. This time, the calculated period is clearly wrong (28.07 hours instead of 23.50 hours). The reason for this is that the program was unable to detect significant rhythmicity in the data set (notice that the best PN was only 8, which is less than the criterion for significance).
7. You may remember from Exercise 5.3 that the chi-square periodogram procedure was able to detect rhythmicity in a data set with 85% noise. Is the Lomb–Scargle periodogram procedure more susceptible to loss of sensitivity in the presence of noise? Well, we did some tricks to help the chi-square periodogram procedure, so it is just fair that

we do some tricks here also. Let us start by using one of the files that we used in Exercise 5.3. Select the file **A10**. Because this file does not contain time tags, you must also select *Data only* in *Step 2*. Then click on *Compute*.

8. The reported period is slightly off mark (23.31 instead of 23.50 hours), and the largest PN is smaller than the criterion for significance (10 as compared to 12). Now let us try a few tricks. In Step 3, set the *Lower period* to **21**, the *Higher period* to **26**, and the *Significance level* to **0.05**. Then click on *Compute*.
9. *Voilà!* There is significant rhythmicity! The period is still slightly off mark (23.31 instead of 23.50), but the highest PN value now exceeds the criterion for significance at the 0.05 level (which is the least stringent level of significance commonly accepted but is still acceptable).
10. Finally, let us analyze some real (biological) data. Switch back to *Times and data* in *Step 2*. Then select data file **A14** in *Step 1*. This file contains records of body temperature of a laboratory rat maintained in constant darkness for 7 days. Measurements were made every 6 minutes, but, because 10% of the measurements are missing, the data set must be considered unequally spaced (and the file must have time tags). Click on *Compute*.
11. The results indicate a circadian period of 24.35 hours, with a PN value of 354 (which is much larger than the criterion value at the 0.05 level of significance). If you have not inspected **A14** using **Plot**, you may wish to do it now to make sure that the period calculated by **LSP** is consistent with your subjective evaluation of it.

5.5 Calculating all circadian parameters by the cosinor procedure. This exercise is similar to Exercises 5.2, 5.3, and 5.4 but is more comprehensive. The iterative cosinor procedure implemented in the program **Cosinor** (the 10th icon from the left in the Circadian banner) calculates the period of circadian rhythms at the same time as it calculates the mean level (mesor), amplitude, and acrophase. It also provides a measure of rhythm robustness.

1. Start the program **Cosinor**.
2. Notice that, similarly to Tau and LSP, there are five panels in the program window: you identify the data file in the *Source* panel, specify the data format in the *File type* panel, set the parameters of analysis in the *Data* and *Period* panels, and stipulate the destination for the results in the *Destination* panel.
3. For comparison with Tau and LSP, choose data file **A10**. Remember that this data file contains artificial data constructed as a cosine wave with a period of 23.5 hours and 85% noise. You may keep most default settings as they are, but, in the *Period* panel, change *Start at* to 18 and *End at* to 30 to make the range of periods the same as the one we used with LSP in the previous exercise. Then click on *OK*.
4. In the Results panel on the right, notice that, like LSP, Cosinor identified a period of 23.3 hours (thus, reasonably close to the true period of 23.5 hours). Unlike LSP or Tau, however, Cosinor found the rhythmicity to be statistically significant with $p < 0.001$. Thus, Cosinor is more *powerful* (more capable of identifying rhythmicity) than the other two programs. The Results panel also provides the robustness of the rhythm (rather low in this case, at 0.7%) as well as the mesor (4.9581), amplitude (0.3791), and acrophase (–192°). The acrophase is given only in degrees because the period is different from 24.0 hours (see below).
5. Choose file **A24**. This file contains running-wheel activity records of a mouse maintained under a 24-hour light–dark cycle for longer than the first 10 days. Leave the *Bins to Use* at the default value of 2400 and click on *OK*.
6. While inspecting the Results panel, notice that the acrophase is reported not only in degrees of angle but also in hours (both in decimal format and in clock format). For these data, the acrophase occurs at 14:59. Computation of the acrophase is based on the assumption that the first data point was measured at time 0.0. If your own data set starts at a different time of day, you can indicate it at the bottom of the *Data* panel before you click on *OK* to run the analysis.
7. Now choose file **A11**. This file has time tags, so you must click on *File contains data with time tags* in the *File Type* panel before proceeding.
8. Click on *OK* and inspect the Results panel. Because the data correspond to a perfect cosine wave, rhythm robustness is 100%. The correct period (23.5 hours) is identified.

SUGGESTIONS FOR FURTHER READING

As mentioned in the preceding chapters, the source articles listed in the "Literature Cited" section may be consulted for detailed information about topics covered in this chapter. For more general further reading, the following sources may be useful:

Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.) (2001). *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum.

A dense but authoritative volume with contributions from experts in virtually all aspects of circadian rhythmicity.

Kramer, A. and Merrow, M. (Eds.) (2013). *Circadian Clocks* (*Handbook of Experimental Pharmacology*, Vol. 217). New York: Springer.

Like the volume edited by Takahashi and colleagues, this volume is a dense but authoritative volume with contributions from experts in virtually all aspects of circadian rhythmicity. Because of being published 12 years later, it has the advantage of including much more material about the molecular bases of circadian rhythmicity.

Foster, R. and Kreitzman, L. (2004). *Rhythms of Life: The Biological Clocks That Control the Daily Lives of Every Living Thing*. London, U.K.: Profile.

A delightful book! In contrast to the two books listed earlier, this short book written by a scientist and a professional writer is targeted at general audiences. Naturally, topics are covered much less systematically and in much less detail, but the book is much more readable as an excellent introduction to the field.

Binkley, S. (1997). *Biological Clocks: Your Owner's Manual*. Amsterdam, the Netherlands: Harwood Academic.

Another general audience book. Written 7 years before Foster and Kreitzman's book and with a different perspective, this book may be of interest to some readers.

Hall, A. J. W. and McWatters, H. G. (Eds.) (2006). *Endogenous Plant Rhythms* (*Annual Plant Reviews*, Vol. 21). New York: Wiley/Blackwell.

An edited book with contributions from experts dealing mainly with circadian rhythmicity and photoperiodism in plants.

WEBSITES TO EXPLORE

Circadian Rhythm Laboratory (Refinetti): http://www.circadian.org.
Circadian Rhythms and Sleep Laboratory (Mistlberger): http://www.sfu.ca/psyc/faculty/mistlberger/Lab.html.
Journal of Circadian Rhythms: http://www.jcircadianrhythms.com.
Laboratory of Circadian and Sleep Medicine (Colwell): http://www.mrrc.npi.ucla.edu/ccolwell/.
Medical Chronobiology Program at Harvard Medical School: https://sleep.med.harvard.edu/research/labs/54/.
Millar Research Group (University of Warwick): http://www.amillar.org.
SciVee (Videos of interviews with circadian biologists): http://www.scivee.tv/browse?brfl=Clocks.

REFERENCES

1. Pritchard, D. R. (Ed.) (1994). *The American Heritage Dictionary*, 3rd edn. Boston, MA: Houghton Mifflin.
2. Halberg, F. (1959). Physiologic 24-hour periodicity: General and procedural considerations with reference to the adrenal cycle. *Zeitschrift für Vitamin-, Hormon- und Fermentforschung* 10: 225–296.
3. Brown, F. A., Hastings, J. W., and Palmer, J. D. (1970). *The Biological Clock: Two Views*. New York: Academic Press.
4. Aschoff, J. (1981). A survey on biological rhythms. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 3–10.
5. Ogle, W. (1866). On the diurnal variations in the temperature of the human body in health. *St. George's Hospital Reports* 1: 221–245.
6. Mellette, H. C., Hutt, B. K., Askovitz, S. I., and Horvath, S. M. (1951). Diurnal variations in body temperature. *Journal of Applied Physiology* 3: 665–675.
7. Palmer, J. D., Livingston, L., and Zusy, F. D. (1964). A persistent diurnal rhythm in photosynthetic capacity. *Nature* 203: 1087–1088.
8. Lobban, M. C. and Tredre, B. E. (1967). Diurnal rhythms of renal excretion and body temperature in aged subjects. *Journal of Physiology* 188: 48P–49P.
9. De Castro, J. M. (1978). Diurnal rhythms of behavioral effects on core temperature. *Physiology and Behavior* 21: 883–886.
10. Araki, C. T., Nakamura, R. M., and Kam, L. W. G. (1987). Diurnal temperature sensitivity of dairy cattle in a naturally cycling environment. *Journal of Thermal Biology* 12: 23–26.
11. Menezes, A. A. L., Moreira, L. F. S., Queiroz, J. W., Menna-Barreto, L. S., and Benedito-Silva, A. A. (1994). Diurnal variation and distribution of grooming behavior in captive common marmoset families (*Callithrix jacchus*). *Brazilian Journal of Medical and Biological Research* 27: 61–65.
12. Pennartz, C. M. A., de Jeu, M. T. G., Bos, N. P. A., Schaap, J., and Geurtsen, A. M. S. (2002). Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. *Nature* 416: 286–290.
13. Crystal, M. M. (1971). Diel periodicity of mating in laboratory-adapted screwworm flies relative to photoperiod. *Journal of Medical Entomology* 30: 747–748.
14. Johnson, C. R. (1972). Diel variation in the thermal tolerance of *Litoria gracilenta* (Anura: Hylidae). *Comparative Biochemistry and Physiology A* 41: 727–730.
15. Reynolds, W. W., Casterlin, M. E., Matthey, J. K., Millington, S. T., and Ostrowski, A. C. (1978). Diel patterns of preferred temperature and locomotor activity in the goldfish *Carassius auratus*. *Comparative Biochemistry and Physiology A* 59: 225–227.
16. Bückle-Ramírez, L. F., Díaz-Herrera, F., Correa-Sandoval, F., Barón-Sevilla, B., and Hernández-Rodríguez, M. (1994). Diel thermoregulation of the crawfish *Procambarus clarkii* (Crustacea: Cambaridae). *Journal of Thermal Biology* 19: 419–422.
17. Halle, S. (1995). Diel pattern of locomotor activity in populations of root voles, *Microtus oeconomus*. *Journal of Biological Rhythms* 10: 211–224.
18. Pavlidis, M., Greenwood, L., Paalavuo, M., Molsa, H., and Laitinen, J. T. (1999). The effect of photoperiod on diel rhythms in serum melatonin, cortisol, glucose, and electrolytes in the common dentex, *Dentex dentex*. *General and Comparative Endocrinology* 113: 240–250.
19. Kronfeld-Schor, N., Dayan, T., Elvert, R., Haim, A., Zisapel, N., and Heldmaier, G. (2001). On the use of the time axis for ecological separation: Diel rhythms as an evolutionary constraint. *American Naturalist* 158: 451–457.
20. Tarlow, E. M., Hau, M., Anderson, D. J., and Wikelski, M. (2003). Diel changes in plasma melatonin and corticosterone concentrations in tropical Nazca boobies (*Sula granti*) in relation to moon phase and age. *General and Comparative Endocrinology* 133: 297–304.
21. Maurel, E. (1884). Expériences sur les variations nycthémérales de la température normale. *Comptes Rendus des Séances de la Société de Biologie de Paris* 37: 588.
22. Popovic, V., Popovic, P., and Petrovic, V. (1956). La thyroïde et le rythme nycthéméral. *Comptes Rendus des Séances de la Societé de Biologie de Strasbourg* 150: 1249–1251.

23. Heusner, A. (1959). Variation nycthémérale de la température centrale chez le rat adapté à la neutralité thermique. *Comptes Rendus de Séances de la Société de Biologie de Strasbourg* 153: 1258–1261.
24. Cabanac, M., Hildebrandt, G., Massonnet, B., and Strempel, H. (1976). A study of the nycthemeral cycle of behavioural temperature regulation in man. *Journal of Physiology* 257: 275–291.
25. Barney, C. C., Vergoth, C., Renkema, L. A., and Meeuwsen, K. W. (1995). Nycthemeral variation in thermal dehydration-induced thirst. *Physiology and Behavior* 58: 329–335.
26. Amar, J., Vernier, I., Rossignol, E., Lenfant, V., Conte, J. J., and Chamontin, B. (1997). Influence of nycthemeral blood pressure pattern in treated hypertensive patients on hemodialysis. *Kidney International* 51: 1863–1866.
27. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C., and Van Hoecke, J. (2001). Nycthemeral variations in core temperature and heart rate: Continuous cycling exercise versus continuous rest. *International Journal of Sports Medicine* 22: 553–557.
28. Refinetti, R. and Laney, M. Y. (2003). Nycthemeral variation in intra-aural temperature and attention/memory but not in physical vigor of assiduous members of a fitness club. *Biological Rhythm Research* 34: 115–121.
29. Halberg, F. (1953). Some physiological and clinical aspects of 24-hour periodicity. *Journal-Lancet* 73: 20–32.
30. Gordon, C. J. (1994). 24-Hour control of body temperature in rats. I. Integration of behavioral and autonomic effectors. *American Journal of Physiology* 267: R71–R77.
31. Deboer, T., Vyazovskiy, V. V., and Tobler, I. (2000). Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 429–436.
32. van Houwelingen, C. A. J. and Beersma, D. G. M. (2001). Seasonal changes in 24-h patterns of suicide rates: A study on train suicides in The Netherlands. *Journal of Affective Disorders* 66: 215–223.
33. Mormont, M. C., Waterhouse, J., Bleuzen, P., Giacchetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset, J. L., Touitou, Y., and Lévi, F. (2000). Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clinical Cancer Research* 6: 3038–3045.
34. Cagnacci, A., Arangino, S., Tuveri, F., Paoletti, A. M., and Volpe, A. (2002). Regulation of the 24-h body temperature rhythm of women in luteal phase: Role of gonadal steroids and prostaglandins. *Chronobiology International* 19: 721–730.
35. Daubenmire, R. F. (1974). *Plants and Environment*. New York: Wiley.
36. Miller, A. and Thompson, J. C. (1983). *Elements of Meteorology*, 4th edn. Columbus, OH: Charles E. Merrill.
37. Trewartha, G. T. and Horn, L. H. (1980). *An Introduction to Climate*, 5th edn. New York: McGraw-Hill.
38. Couderc, P. (1971). Calendar. *Encyclopedia Americana* 5: 184–190.
39. Audoin, C. and Guinot, B. (2001). *The Measurement of Time* (Trans. by S. Lyle). Cambridge, U.K.: Cambridge University Press.
40. Zaha, M. A. (1972). Shedding some needed light on optical measurements. *Electronics* Nov. 6: 91–96.
41. Walmsley, L., Hanna, L., Mouland, J., Martial, F., West, A., Smedley, A. R., Bechtold, D. A., Webb, A. R., Lucas, R. J., and Brown, T. M. (2015). Colour as a signal for entraining the mammalian circadian clock. *PLoS Biology* 13: e1002127.
42. Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., Salinger, M. J. et al. (1997). Maximum and minimum temperature trends for the globe. *Science* 277: 364–367.
43. Laumann, E. O., Gagnon, J. H., Michael, R. T., and Michaels, S. (1994). *The Social Organization of Sexuality: Sexual Practices in the United States*. Chicago, IL: University of Chicago Press.
44. Palmer, J. D., Udry, J. R., and Morris, N. M. (1982). Diurnal and weekly, but no lunar rhythm in human copulation. *Human Biology* 54: 111–121.
45. Refinetti, R. (2005). Time for sex: Nycthemeral distribution of human sexual behavior. *Journal of Circadian Rhythms* 3: art. 4.
46. Jankowski, K. S., Díaz-Morales, J. F., and Randler, C. (2014). Chronotype, gender, and time for sex. *Chronobiology International* 31: 911–916.
47. Barak, Y., Stein, D., Ring, A., Ticher, A., and Elizur, A. (1997). Patterns of first intercourse: A survey among Israeli women. *Biological Rhythm Research* 28: 36–41.
48. Fraser, W. D., McLean, F. H., and Usher, R. H. (1989). Diurnal variation in admission to hospital of women in labour. *Canadian Journal of Surgery* 32: 33–35.
49. Ruffieux, C., Marazzi, A., and Paccaud, F. (1992). The circadian rhythm of the perinatal mortality rate in Switzerland. *American Journal of Epidemiology* 135: 936–951.
50. Anderka, M., Declercq, E. R., and Smith, W. (2000). A time to be born. *American Journal of Public Health* 90: 124–126.
51. Goldstick, O., Weissman, A., and Drugan, A. (2003). The circadian rhythm of "urgent" operative deliveries. *Israel Medical Association Journal* 5: 564–566.
52. Åkerstedt, T., Kecklund, G., and Hörte, L. G. (2001). Night driving, season, and the risk of highway accidents. *Sleep* 24: 401–406.
53. Haikonen, H. and Summala, H. (2001). Deer-vehicle crashes: Extensive peak at 1 hour after sunset. *American Journal of Preventive Medicine* 21: 209–213.
54. Yasseri, T., Sumi, R., and Kertész, J. (2012). Circadian patterns of Wikipedia editorial activity: A demographic analysis. *PLOS ONE* 7: e30091.
55. Suvakov, M., Mitrovic, M., Gligorijevic, V., and Tadic, B. (2013). How the online social networks are used: Dialogues-based structure of MySpace. *Journal of the Royal Society Interface* 10: 20120819.
56. Maldonado, G. and Kraus, J. F. (1991). Variation in suicide occurrence by time of day, day of week, month, and lunar phase. *Suicide and Life-Threatening Behavior* 21: 174–187.
57. Gallerani, M., Avato, F. M., Dal Monte, D., Caracciolo, S., Fersini, C., and Manfredini, R. (1996). The time for suicide. *Psychological Medicine* 26: 867–870.
58. Preti, A. and Miotto, P. (2001). Diurnal variations in suicide by age and gender in Italy. *Journal of Affective Disorders* 65: 253–261.
59. Carroll, R., Metcalfe, C., Gunnell, D., Mohamed, F., and Eddleston, M. (2012). Diurnal variation in probability of death following self-poisoning in Sri Lanka: Evidence for chronotoxicity in humans. *International Journal of Epidemiology* 41: 1821–1828.
60. Silla, A. and Luoma, J. (2012). Main characteristics of train-pedestrian fatalities on Finnish railroads. *Accident Analysis and Prevention* 45: 61–66.
61. Fromm, R. E., Levine, R. L., and Pepe, P. E. (1992). Circadian variation in the time of request for helicopter transport of cardiac patients. *Annals of Emergency Medicine* 21: 1196–1199.

62. Spielberg, C., Falkenhahn, D., Willich, S. N., Wegscheider, K., and Völler, H. (1996). Circadian, day-of-week, and seasonal variability in myocardial infarction: Comparison between working and retired patients. *American Heart Journal* 132: 579–585.
63. Kentsch, M., Rodemerk, U., Ittel, T. H., Müller-Esch, G., and Mitusch, R. (2002). Tag-Nacht-Variabilität der Prähospital-Phase des akuten Myokardinfarktes. *Zeitschrift für Kardiologie* 91: 637–641.
64. Manfredini, R., La Cecilia, O., Boari, B., Steliu, J., Michelini, V., Carli, P., Zanotti, C., Bigoni, M., and Gallerani, M. (2002). Circadian pattern of emergency calls: Implications for ED organization. *American Journal of Emergency Medicine* 20: 282–286.
65. López-Messa, J. B., Garmendia-Leiza, J. R., Aguilar-García, M. D., Andrés-de-Llano, J. M., Alberola-López, C., and Ardura-Fernández, J. (2004). Factores de riesgo cardiovascular en el ritmo circadiano del infarto agudo de miocardio. *Revista Española de Cardiología* 57: 850–858.
66. Manfredini, R., Boari, B., Bressan, S., Gallerani, M., Salmi, R., Portaluppi, F., and Mehta, R. H. (2004). Influence of circadian rhythm on mortality after myocardial infarction: Data from a prospective cohort of emergency calls. *American Journal of Emergency Medicine* 22: 555–559.
67. Savopoulos, C., Ziakas, A., Hatzitolios, A., Delivoria, C., Kounanis, A., Mylonas, S., Tsougas, M., and Psaroulis, D. (2006). Circadian rhythm in sudden cardiac death: A retrospective study of 2,665 cases. *Angiology* 57: 197–204.
68. Reavey, M., Saner, H., Paccaud, F., and Marques-Vidal, P. (2013). Exploring the periodicity of cardiovascular events in Switzerland: Variation in deaths and hospitalizations across seasons, day of the week and hour of the day. *International Journal of Cardiology* 168: 2195–2200.
69. Kleinpeter, G., Schatzer, R., and Böck, F. (1995). Is blood pressure really a trigger for the circadian rhythm of subarachnoid hemorrhage? *Stroke* 26: 1805–1810.
70. Casetta, I., Granieri, E., Fallica, E., la Cecilia, O., Paolino, E., and Manfredini, R. (2002). Patient demographic and clinical features and circadian variation in onset of ischemic stroke. *Archives of Neurology* 59: 48–53.
71. Gupta, A. and Shetty, H. (2005). Circadian variation in stroke: A prospective hospital-based study. *International Journal of Clinical Practice* 59: 1272–1275.
72. Rosenberg, J., Pedersen, M. H., Ramsing, T., and Kehlet, H. (1992). Circadian variation in unexpected postoperative death. *British Journal of Surgery* 79: 1300–1302.
73. Laskey, W. K., Selzer, F., Holmes, D. R., Wilensky, R. L., Cohen, H. A., Williams, D. O., Kip, K. E., and Detre, K. M. (2005). Temporal variation in in-hospital mortality with percutaneous coronary intervention: A report from the National Heart, Lung and Blood Institute Dynamic Registry. *American Heart Journal* 150: 569–576.
74. Soriani, S., Fiumana, E., Manfredini, R., Boari, B., Battistella, P. A., Canetta, E., Pedretti, S., and Borgna-Pignatti, C. (2006). Circadian and seasonal variation of migraine attacks in children. *Headache* 46: 1571–1574.
75. Manfredini, R., Vanni, A., Peron, L., Cecilia, O. la, Smolensky, M. H., and Grassi, L. (2001). Day–night variation in aggressive behavior among psychiatric inpatients. *Chronobiology International* 18: 503–511.
76. Reinberg, O., Reinberg, A., Téhard, B., and Mechkouri, M. (2002). Accidents in children do not happen at random: Predictable time-of-day incidence of childhood trauma. *Chronobiology International* 19: 615–631.
77. Koch, H. J., Vogel, M., and Raschka, C. (2003). Circadian rhythm of accidents in children: A basic activity periodicity. *Chronobiology International* 20: 157–159.
78. Reinberg, O., Reinberg, A., and Mechkouri, M. (2005). 24-Hour, weekly, and annual patterns in traumatic and non-traumatic surgical pediatric emergencies. *Chronobiology International* 22: 353–381.
79. Parks, D. K., Yetman, R. J., McNeese, M. C., Burau, K., and Smolensky, M. H. (2000). Day–night pattern in accidental exposures to blood-borne pathogens among medical students and residents. *Chronobiology International* 17: 61–70.
80. Saigusa, T., Ishizaki, S., Watabiki, S., Ishii, N., Tanakadate, A., Tamai, Y., and Hasegawa, K. (2002). Circadian behavioural rhythm in *Caenorhabditis elegans*. *Current Biology* 12: R46–RR47.
81. Zantke, J., Ishikawa-Fujiwara, T., Arboleda, E., Lohs, C., Schipany, K., Hallay, N., Straw, A. D., Todo, T., and Tessmar-Raible, K. (2013). Circadian and circalunar clock interactions in a marine annelid. *Cell Reports* 5: 99–113.
82. Honegger, H. W. (1976). Locomotor activity in *Uca crenulata*, and the response to two Zeitgebers, light–dark and tides. In: DeCoursey, P. J. (Ed.), *Biological Rhythms in the Marine Environment*. Columbia, SC: University of South Carolina Press, pp. 93–102.
83. Chiesa, J. J., Aguzzi, J., García, J. A., Sardà, F., and de la Iglesia, H. O. (2010). Light intensity determines temporal niche switching of behavioral activity in deep-water *Nephrops norvegicus* (Crustacea: Decapoda). *Journal of Biological Rhythms* 25: 277–287.
84. Crawshaw, L. I. (1971). Temperature selection and activity in the crayfish, *Orconectes immunis*. *Journal of Comparative Physiology* 95: 315–322.
85. Cloudsley-Thompson, J. L. (1952). Studies in diurnal rhythms. II. Changes in the physiological responses of the woodlouse *Oniscus asellus* to environmental stimuli. *Journal of Experimental Biology* 29: 295–303.
86. Refinetti, R. (2000). Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 73: 575–583.
87. Aguzzi, J., Company, J. B., and Abelló, P. (2004). Locomotor activity rhythms of continental slope *Nephrops norvegicus* (Decapoda: Nephropidae). *Journal of Crustacean Biology* 24: 282–290.
88. Bernardi, N. (1990). Temperature influence upon food ingestion and spontaneous locomotion of the freshwater prawn *Macrobrachium acanthurus*. *Journal of Thermal Biology* 15: 33–36.
89. Dreisig, H. (1980). Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia* 44: 376–389.
90. Pivnick, K. A. and McNeil, J. N. (1986). Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). *Ecology* 67: 1024–1035.
91. Shaw, P. J., Cirelli, C., Greenspan, R. J., and Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. *Science* 287: 1834–1837.
92. Hamblen-Coyle, M. J., Wheeler, D. A., Rutila, J. E., Rosbash, M., and Hall, J. C. (1992). Behavior of period-altered circadian rhythm mutants of *Drosophila* in light:Dark cycles (Diptera: Drosophilidae). *Journal of Insect Behavior* 5: 417–446.
93. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., and Tomioka, K. (2004). *Drosophila cry^b* mutation reveals two circadian clocks that drive locomotor rhythm and have different responsiveness to light. *Journal of Insect Physiology* 50: 479–488.

94. Rieger, D., Fraunholz, C., Popp, J., Bichler, D., Dittmann, R., and Helfrich-Förster, C. (2007). The fruit fly *Drosophila melanogaster* favors dim light and times its activity peaks to early dawn and late dusk. *Journal of Biological Rhythms* 22: 387–399.
95. Rieger, D., Wülbeck, C., Rouyer, F., and Helfrich-Förster, C. (2009). *Period* gene expression in four neurons is sufficient for rhythmic activity of *Drosophila melanogaster* under dim light conditions. *Journal of Biological Rhythms* 24: 271–282.
96. Jones, M. D. R., Hill, M., and Hope, A. M. (1967). The circadian flight activity of the mosquito *Anopheles gambiae*: Phase setting by the light regime. *Journal of Experimental Biology* 47: 503–511.
97. Meyer-Peters, H. (1993). Seasonality of circadian locomotor activity in an insect. *Journal of Comparative Physiology A* 171: 713–724.
98. Sharma, V. K., Lone, S. R., Mathew, D., Goel, A., and Chandrashekaran, M. K. (2004). Possible evidence for shift work schedules in the media workers of the ant species *Camponotus compressus*. *Chronobiology International* 21: 297–308.
99. Moore, D. and Rankin, M. A. (1985). Circadian locomotor rhythms in individual honeybees. *Physiological Entomology* 10: 191–197.
100. Frisch, B. and Koeniger, N. (1994). Social synchronization of the activity rhythms of honeybees within a colony. *Behavioral Ecology and Sociobiology* 35: 91–98.
101. Fuchikawa, T. and Shimizu, I. (2008). Parametric and non-parametric entrainment of circadian locomotor rhythm in the Japanese honeybee *Apis cerana japonica*. *Biological Rhythm Research* 39: 57–67.
102. Yerushalmi, S., Bodenhaimer, S., and Bloch, G. (2006). Developmentally determined attenuation in circadian rhythms links chronobiology to social organization in bees. *Journal of Experimental Biology* 209: 1044–1051.
103. Stelzer, R. J., Stanewsky, R., and Chittka, L. (2010). Circadian foraging rhythms of bumblebees monitored by radio-frequency identification. *Journal of Biological Rhythms* 25: 257–267.
104. Shimizu, I., Kawai, Y., Taniguchi, M., and Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
105. North, R. D. (1993). Entrainment of the circadian rhythm of locomotor activity in wood ants by temperature. *Animal Behaviour* 45: 393–397.
106. Koilraj, A. J., Sharma, V. K., Marimuthu, G., and Chandrashekaran, M. K. (2000). Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae: Spirostreptida). *Chronobiology International* 17: 757–765.
107. Ndoutoume-Ndong, A., Rojas-Rousse, D., and Allemand, R. (2006). Rythmes d'activité locomotrice chez deux insectes parasitoïdes sympatriques: *Eupelmus orientalis* et *Eupelmus vuilleti* (Hyménoptère, Eupelmidae). *Comptes Rendus Biologies* 329: 476–482.
108. Ortega-Escobar, J. (2002). Circadian rhythms of locomotor activity in *Lycosa tarentula* (Araneae, Lycosidae) and the pathways of ocular entrainment. *Biological Rhythm Research* 33: 561–576.
109. Edgar, A. L. and Yuan, H. A. (1968). Daily locomotory activity in *Phalangium opilio* and seven species of *Leiobunum* (Arthropoda: Phalangida). *Bios* 39: 167–176.
110. Rojas-Garcia, A., Manzanilla, O. V., Huertas-Vargas, D., and Hernández, L. F. G. (2011). Ritmo de actividad diaria del opilión *Rhaucus* cf. *vulneratus* Simon, 1879 (Opiliones: Cosmetidae). *Boletin de la Sociedad Entolomológica Aragonesa* 48: 458–460.
111. Crawshaw, L. I., Johnston, M. H., and Lemons, D. E. (1980). Acclimation, temperature selection, and heat exchange in the turtle, *Chrysemys scripta*. *American Journal of Physiology* 238: R443–R446.
112. Tosini, G. and Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *Journal of Biological Rhythms* 10: 248–255.
113. Bartell, P. A., Miranda-Anaya, M., and Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
114. Refinetti, R. and Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.
115. Bertolucci, C., Sovrano, V. A., Magnone, M. C., and Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
116. Ellis, D. J., Firth, B. T., and Belan, I. (2009). Thermocyclic and photocyclic entrainment of circadian locomotor activity rhythms in sleepy lizards, *Tiliqua rugosa*. *Chronobiology International* 26: 1369–1388.
117. Fingerman, S. W. (1976). Circadian rhythms of brain 5-hydroxytryptamine and swimming activity in the teleost *Fundulus grandis*. *Comparative Biochemistry and Physiology C* 54: 49–53.
118. Iigo, M. and Tabata, M. (1996). Circadian rhythms of locomotor activity in the goldfish *Carassius auratus*. *Physiology and Behavior* 60: 775–781.
119. Hurd, M. W., Debruyne, J., Straume, M., and Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiology and Behavior* 65: 465–472.
120. Rihel, J., Prober, D. A., Arvanites, A., Lam, K., Zimmerman, S., Jang, S., Haggarty, S. J. et al. (2010). Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science* 327: 348–351.
121. Gandhi, A. V., Mosser, E. A., Oikonomou, G., and Prober, D. A. (2015). Melatonin is required for the circadian regulation of sleep. *Neuron* 85: 1193–1199.
122. Bayarri, M. J., Muñoz-Cueto, J. A., López-Olmeda, J. F., Vera, L. M., Rol-de-Lama, M. A., Madrid, J. A., and Sánchez-Vázquez, F. J. (2004). Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). *Physiology and Behavior* 81: 577–583.
123. Herrero, M. J., Madrid, J. A., and Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
124. Chen, W. M. and Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
125. Whitney, N. M., Papastamatiou, Y. P., Holland, K. N., and Lowe, C. G. (2007). Use of an acceleration data logger to measure diel activity patterns in captive whitetip reef sharks, *Triaenodon obesus*. *Aquatic Living Resources* 20: 299–305.
126. Schulz, U. H. and Leuchtenberger, C. (2006). Activity patterns of South American silver catfish (*Rhamdia quelen*). *Brazilian Journal of Biology* 66: 565–574.

127. Kasai, M. and Kiyohara, S. (2010). Food- and light-entrainable oscillators control feeding and locomotor activity rhythms, respectively, in the Japanese catfish, *Plotosus japonicus*. *Journal of Comparative Physiology A* 196: 901–912.
128. Montoya, A., López-Olmeda, J. F., Lopez-Capel, A., Sánchez-Vásquez, F. J., and Pérez-Ruzafa, A. (2012). Impact of a telemetry-transmitter implant on daily behavioral rhythms and physiological stress indicators in gilthead seabream (*Sparus aurata*). *Marine Environmental Research* 79: 48–54.
129. Payne, N. L., van der Meulen, D. E., Gannon, R., Semmens, J. M., Suthers, I. M., Gray, C. A., and Taylor, M. D. (2012). Rain reverses diel activity rhythms in an estuarine teleost. *Proceedings of the Royal Society B* 280: 20122363.
130. Tieleman, B. I. and Williams, J. B. (2002). Effects of food supplementation on behavioural decisions of hoopoe-larks in the Arabian Desert: Balancing water, energy and thermoregulation. *Animal Behaviour* 63: 519–529.
131. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W., and Bacon, W. L. (2000). Circadian rhythm of the preovulatory surge of luteinizing hormone and its relationships to rhythms of body temperature and locomotor activity in turkey hens. *Biology of Reproduction* 62: 1452–1458.
132. Ebihara, S. and Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
133. King, V. M., Bentley, G. E., and Follett, B. K. (1997). A direct comparison of photoperiodic time measurement and the circadian system in European starlings and Japanese quail. *Journal of Biological Rhythms* 12: 431–442.
134. Oshima, I. and Ebihara, S. (1988). The measurement and analysis of circadian locomotor activity and body temperature rhythms by a computer-based system. *Physiology and Behavior* 43: 115–119.
135. Berger, R. J. and Phillips, N. H. (1994). Constant light suppresses sleep and circadian rhythms in pigeons without consequent sleep rebound in darkness. *American Journal of Physiology* 267: R945–R952.
136. Rashotte, M. E., Basco, P. S., and Henderson, R. P. (1995). Daily cycles in body temperature, metabolic rate, and substrate utilization in pigeons: Influence of amount and timing of food consumption. *Physiology and Behavior* 57: 731–746.
137. Oshima, I., Yamada, H., Goto, M., Sato, K., and Ebihara, S. (1989). Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. *Journal of Comparative Physiology A* 166: 217–226.
138. Aschoff, J. and von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
139. Winget, C. M. and Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
140. Eskin, A. (1971). Some properties of the system controlling the circadian activity rhythm of sparrows. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 55–80.
141. Hau, M. and Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
142. Heigl, S. and Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
143. Kumar, V. and Gwinner, E. (2005). Pinealectomy shortens resynchronisation times of house sparrow (*Passer domesticus*) circadian rhythms. *Naturwissenschaften* 92: 419–422.
144. Trivedi, A. K., Rani, S., and Kumar, V. (2006). Natural daylight restricted to twilights delays the timing of testicular regression but does not affect the timing of the daily activity rhythm of the house sparrow (*Passer domesticus*). *Journal of Circadian Rhythms* 4: art. 5.
145. Underwood, H. and Edmonds, K. (1995). The circadian rhythm of thermoregulation in Japanese quail. II. Multioscillator control. *Journal of Biological Rhythms* 10: 234–247.
146. Kumar, V., Gwinner, E., and Van't Hof, T. J. (2000). Circadian rhythms of melatonin in European starlings exposed to different lighting conditions: Relationship with locomotor and feeding rhythms. *Journal of Comparative Physiology A* 186: 205–215.
147. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
148. Bartell, P. A. and Gwinner, E. (2005). A separate circadian oscillator controls nocturnal migratory restlessness in the songbird *Sylvia borin*. *Journal of Biological Rhythms* 20: 538–549.
149. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K., and Shiraishi, T. (1988). Disruption of light-dark cycle of feeding and drinking behavior, and ambulatory activity induced by development of obesity in the Zucker rat. *International Journal of Obesity* 12: 481–490.
150. Mistlberger, R. E., Lukman, H., and Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: Assessment and intervention. *Appetite* 30: 255–267.
151. Shido, O., Sakurada, S., and Nagasaka, T. (1991). Effect of heat acclimation on diurnal changes in body temperature and locomotor activity in rats. *Journal of Physiology* 433: 59–71.
152. Shido, O., Sakurada, S., Kohda, W., and Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
153. Satinoff, E., Kent, S., and Hurd, M. (1991). Elevated body temperature in female rats after exercise. *Medicine and Science in Sports and Exercise* 23: 1250–1253.
154. Keesey, R. E., Swiergiel, A. H., and Corbett, S. W. (1990). Contribution of spontaneous activity to daily energy expenditure of adult obese and lean Zucker rats. *Physiology and Behavior* 48: 327–331.
155. Morley, R. M., Conn, C. A., Kluger, M. J., and Vander, A. J. (1990). Temperature regulation in biotelemetered spontaneously hypertensive rats. *American Journal of Physiology* 258: R1064–R1069.
156. Borbély, A. A. and Neuhaus, H. U. (1978). Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. *Journal of Comparative Physiology* 128: 37–46.
157. Albers, H. E., Gerall, A. A., and Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
158. Meinrath, M. and D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
159. Kittrell, E. M. W. and Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.
160. Isobe, Y., Aoki, K., Furuyama, F., and Hashitani, T. (1992). Effect of warm or cold on circadian rhythm of running wheel activity and body temperature in spontaneously hypertensive rats. *Medical Principles and Practice* 3: 63–71.

161. Ikeda, M. and Inoué, S. (1998). Simultaneous recording of circadian rhythms of brain and intraperitoneal temperatures and locomotor and drinking activities in the rat. *Biological Rhythm Research* 29: 142–150.
162. Nagashima, K., Nakai, S., Matsue, K., Konishi, M., Tanaka, M., and Kanosue, K. (2003). Effects of fasting on thermoregulatory processes and the daily oscillations in rats. *American Journal of Physiology* 284: R1486–R1493.
163. Francis, A. J. P. and Coleman, G. J. (1988). The effect of ambient temperature cycles upon circadian running and drinking activity in male and female laboratory rats. *Physiology and Behavior* 43: 471–477.
164. Williams, T. D., Chambers, J. B., May, O. L., Henderson, R. P., Rashotte, M. E., and Overton, J. M. (2000). Concurrent reductions in blood pressure and metabolic rate during fasting in the unrestrained SHR. *American Journal of Physiology* 278: R255–R262.
165. López, L., González-Pardo, H., Cimadevilla, J. M., Cavas, M., Aller, M. A., Arias, J., and Arias, J. L. (2002). Cytochrome oxidase activity of the suprachiasmatic nucleus and pineal gland in rats with portacaval shunt. *Experimental Neurology* 173: 275–282.
166. Seifert, E. L. and Mortola, J. P. (2002). The circadian pattern of breathing in conscious adult rats. *Respiration Physiology* 129: 297–305.
167. Witte, K., Grebmer, W., Scalbert, E., Delagrande, P., Guardiola-Lemaître, B., and Lemmer, B. (1998). Effects of melatoninergic agonists on light-suppressed circadian rhythms in rats. *Physiology and Behavior* 65: 219–224.
168. Witte, K. and Lemmer, B. (1999). Development of inverse circadian blood pressure pattern in transgenic hypertensive TGR(mREN2)27 rats. *Chronobiology International* 16: 293–303.
169. Ray, B., Mallick, H. N., and Kumar, V. M. (2004). Changes in thermal preference, sleep-wakefulness, body temperature and locomotor activity of rats during continuous recording for 24 hours. *Behavioural Brain Research* 154: 519–526.
170. Honma, K. and Hiroshige, T. (1978). Internal synchronization among several circadian rhythms in rats under constant light. *American Journal of Physiology* 235: R243–R249.
171. Honma, K. and Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
172. Ikeda, M., Sagara, M., and Inoué, S. (2000). Continuous exposure to dim illumination uncouples temporal patterns of sleep, body temperature, locomotion and drinking behavior in the rat. *Neuroscience Letters* 279: 185–189.
173. Benstaali, C., Bogdan, A., and Touitou, Y. (2002). Effect of a short photoperiod on circadian rhythms of body temperature and motor activity in old rats. *Pflügers Archiv* 444: 73–79.
174. Deprés-Brummer, P., Lévi, F., Metzger, G., and Touitou, Y. (1995). Light-induced suppression of the rat circadian system. *American Journal of Physiology* 268: R1111–R1116.
175. Honma, K., von Goetz, C., and Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.
176. Fischette, C. T., Edinger, H. M., and Siegel, A. (1981). Temporary desynchronization among circadian rhythms with lateral fornix ablation. *Brain Research* 229: 85–101.
177. Lemmer, B., Hauptfleisch, S., and Witte, K. (2000). Loss of 24 h rhythm and light-induced c-fos mRNA expression in the suprachiasmatic nucleus of the transgenic hypertensive TGR(mRen2)27 rat and effects on cardiovascular rhythms. *Brain Research* 883: 250–257.
178. Scales, W. E. and Kluger, M. J. (1987). Effect of antipyretic drugs on circadian rhythm in body temperature of rats. *American Journal of Physiology* 253: R306–R313.
179. Warren, W. S. and Cassone, V. M. (1995). The pineal gland: Photoreception and coupling of behavioral, metabolic, and cardiovascular circadian outputs. *Journal of Biological Rhythms* 10: 64–79.
180. Gordon, C. J. and Rezvani, A. H. (2001). Genetic selection of rats with high and low body temperatures. *Journal of Thermal Biology* 26: 223–229.
181. Yang, Y. and Gordon, C. J. (1996). Ambient temperature limits and stability of temperature regulation in telemetered male and female rats. *Journal of Thermal Biology* 21: 353–363.
182. Murakami, D. M., Horwitz, B. A., and Fuller, C. A. (1995). Circadian rhythms of temperature and activity in obese and lean Zucker rats. *American Journal of Physiology* 269: R1038–R1043.
183. Lemmer, B., Mattes, A., Böhm, M., and Ganten, D. (1993). Circadian blood pressure variation in transgenic hypertensive rats. *Hypertension* 22: 97–101.
184. Fenn, M. G. P. and Macdonald, D. W. (1995). Use of middens by red foxes: Risk reverses rhythms of rats. *Journal of Mammalogy* 76: 130–136.
185. Chiesa, J. J., Araujo, J. F., and Díez-Noguera, A. (2006). Method for studying behavioural activity patterns during long-term recordings using a force-plate actometer. *Journal of Neuroscience Methods* 158: 157–168.
186. Numano, R., Yamazaki, S., Umeda, N., Samura, T., Sujino, M., Takahashi, R., Ueda, M. et al. (2006). Constitutive expression of the Period1 gene impairs behavioral and molecular circadian rhythms. *Proceedings of the National Academy of Sciences United States of America* 103: 3716–3721.
187. Angeles-Castellanos, M., Salgado-Delgado, R., Rodriguez, K., Buijs, R. M., and Escobar, C. (2010). The suprachiasmatic nucleus participates in food entrainment: A lesion study. *Neuroscience* 165: 1115–1126.
188. Stryjek, R., Modlinska, K., Turlejski, K., and Pisula, W. (2013). Circadian rhythm of outside-nest activity in wild (WWCPS), albino and pigmented laboratory rats. *PLOS ONE* 8: e66055.
189. Tapia-Osorio, A., Salgado-Delgado, R., Angeles-Castellanos, M., and Escobar, C. (2013). Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behavioural Brain Research* 252: 1–9.
190. Mount, L. E. and Willmott, J. V. (1967). The relation between spontaneous activity, metabolic rate and the 24 hour cycle in mice at different environmental temperatures. *Journal of Physiology* 190: 371–380.
191. Batchelder, P., Kinney, R. O., Demlow, L., and Lynch, C. B. (1983). Effects of temperature and social interactions on huddling behavior in *Mus musculus*. *Physiology and Behavior* 31: 97–102.
192. Weinert, D. and Waterhouse, J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. *Physiology and Behavior* 63: 837–843.
193. Kramer, K., Voss, H. P., Grimbergen, J., and Bast, A. (1998). Circadian rhythms of heart rate, body temperature, and locomotor activity in freely moving mice measured with radio telemetry. *Lab Animal* 27(8): 23–26.
194. Possidente, B., McEldowney, S., and Pabon, A. (1995). Aging lengthens circadian period for wheel-running activity in C57BL mice. *Physiology and Behavior* 57: 575–579.
195. Davis, F. C. and Menaker, M. (1981). Development of the mouse circadian pacemaker: Independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.

196. Abe, H., Honma, S., Honma, K., Suzuki, T., and Ebihara, S. (1999). Functional diversities of two activity components of circadian rhythm in genetical splitting mice (CS strain). *Journal of Comparative Physiology A* 184: 243–251.
197. Sei, H., Oishi, K., Morita, Y., and Ishida, N. (2001). Mouse model for morningness/eveningness. *NeuroReport* 12: 1461–1464.
198. Irizarry, R. A., Tankersley, C., Frank, R., and Flanders, S. (2001). Assessing homeostasis through circadian patterns. *Biometrics* 57: 1228–1237.
199. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
200. Weinert, D. and Waterhouse, J. (1999). Daily activity and body temperature rhythms do not change simultaneously with age in laboratory mice. *Physiology and Behavior* 66: 605–612.
201. Tankersley, C. G., Irizarry, R., Flanders, S. E., Rabold, R., and Frank, R. (2003). Unstable heart rate and temperature regulation predict mortality in AKR/J mice. *American Journal of Physiology* 284: R742–R750.
202. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
203. Filipski, E., King, V. M., Etienne, M. C., Li, X. M., Claustrat, B., Granda, T. G., Milano, G., Hastings, M. H., and Lévi, F. (2004). Persistent twenty-four hour changes in liver and bone marrow despite suprachiasmatic nuclei ablation in mice. *American Journal of Physiology* 287: R844–R851.
204. Filipski, E., King, V. M., Li, X. M., Granda, T. G., Mormont, M. C., Liu, X. H., Claustrat, B., Hastings, M. H., and Lévi, F. (2002). Host circadian clock as a control point in tumor progression. *Journal of the National Cancer Institute* 94: 690–697.
205. Nagashima, K., Matsue, K., Konishi, M., Iidaka, C., Miyazaki, K., Ishida, N., and Kanosue, K. (2005). The involvement of Cry1 and Cry2 genes in the regulation of the circadian body temperature rhythm in mice. *American Journal of Physiology* 288: R329–R335.
206. Mendoza, J., Graff, C., Dardente, H., Pévet, P., and Challet, E. (2005). Feeding cues alter clock gene oscillations and photic responses in the suprachiasmatic nuclei of mice exposed to a light–dark cycle. *Journal of Neuroscience* 25: 1514–1522.
207. Castillo, M. R., Hochstetler, K. J., Greene, D. M., Firmin, S. I., Tavernier, R. J., Raap, D. K., and Bult-Ito, A. (2005). Circadian rhythm of core body temperature in two laboratory mouse lines. *Physiology and Behavior* 86: 538–545.
208. Satoh, Y., Kawai, H., Kudo, N., Kawashima, Y., and Mitsumoto, A. (2006). Time-restricted feeding entrains daily rhythms of energy metabolism in mice. *American Journal of Physiology* 290: R1276–R1283.
209. Mendoza, J., Pévet, P., and Challet, E. (2008). High-fat feeding alters the clock synchronization to light. *Journal of Physiology* 586: 5901–5910.
210. Bullough, J. D., Figueiro, M. G., Possidente, B. P., Parsons, R. H., and Rea, M. S. (2005). Additivity in murine circadian phototransduction. *Zoological Science* 22: 223–227.
211. Doyle, S. E., Castrucci, A. M., McCall, M., Provencio, I., and Menaker, M. (2006). Nonvisual light responses in the *Rpe65* knockout mouse: Rod loss restores sensitivity to the melanopsin system. *Proceedings of the National Academy of Sciences United States of America* 103: 10432–10437.
212. Kopp, C., Ressel, V., Wigger, E., and Tobler, I. (2006). Influence of estrous cycle and ageing on activity patterns in two inbred mouse strains. *Behavioural Brain Research* 167: 165–174.
213. Kohsaka, A., Laposky, A. D., Ramsey, K. M., Estrada, C., Joshu, C., Kobayashi, Y., Turek, F. W., and Bass, J. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabolism* 6: 414–421.
214. DeBruyne, J. P., Noton, E., Lambert, C. M., Maywood, E. S., Weaver, D. R., and Reppert, S. M. (2006). A clock shock: Mouse CLOCK is not required for circadian oscillator function. *Neuron* 50: 465–477.
215. Sheward, W. J., Maywood, E. S., French, K. L., Horn, J. M., Hastings, M. H., Seckl, J. R., Holmes, M. C., and Harmar, A. J. (2007). Entrainment to feeding but not to light: Circadian phenotype of $VPAC_2$ receptor-null mice. *Journal of Neuroscience* 27: 4351–4358.
216. Liu, C., Li, S., Liu, T., Borjigin, J., and Lin, J. D. (2007). Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. *Nature* 447: 477–481.
217. Green, C. B., Douris, N., Kojima, S., Strayer, C. A., Fogerty, J., Lourim, D., Keller, S. R., and Besharse, J. C. (2007). Loss of nocturnin, a circadian deadenylase, confers resistance to hepatic steatosis and diet-induced obesity. *Proceedings of the National Academy of Sciences United States America* 104: 9888–9893.
218. Xu, Y., Toh, K. L., Jones, C. R., Shin, J. Y., Fu, Y. H., and Ptácek, L. J. (2007). Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell* 128: 59–70.
219. Sans-Fuentes, M. A., Díez-Noguera, A., and Cambras, T. (2010). Light responses of the circadian system in leptin deficient mice. *Physiology and Behavior* 99: 487–494.
220. Meng, Q. J., Logunova, L., Maywood, E. S., Gallego, M., Yoo, S. H., Takahashi, J. S., Virshup, D. M., Boot-Handford, R. P., Hastings, M. H., and Loudon, A. S. I. (2008). Setting clock speed in mammals: The CK1ε *tau* mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. *Neuron* 58: 78–88.
221. van der Veen, D. R. and Archer, S. N. (2010). Light-dependent behavioral phenotypes in PER3-deficient mice. *Journal of Biological Rhythms* 25: 3–8.
222. Power, A., Hughes, A. T. L., Samuels, R. E., and Piggins, H. D. (2010). Rhythm-promoting actions of exercise in mice with deficient neuropeptide signaling. *Journal of Biological Rhythms* 25: 235–246.
223. Refinetti, R. (2010). Entrainment of circadian rhythm by ambient temperature cycles in mice. *Journal of Biological Rhythms* 25: 247–256.
224. Helwig, B. G., Ward, J. A., Blaha, M. D., and Leon, L. R. (2012). Effect of intraperitoneal radiotelemetry instrumentation on voluntary wheel running and surgical recovery in mice. *Journal of the American Association for Laboratory Animal Science* 51: 1–9.
225. Helwig, B. G., Ward, J. A., Blaha, M. D., and Leon, L. R. (2012). Effect of intraperitoneal radiotelemetry instrumentation on voluntary wheel running and surgical recovery in mice. *Journal of the American Association for Laboratory Animal Science* 51: 600–608.
226. Wolff, G., Duncan, M. J., and Esser, K. A. (2013). Chronic phase advance alters circadian physiological rhythms and peripheral molecular clocks. *Journal of Applied Physiology* 115: 373–382.
227. Studholme, K. M., Gompf, H. S. and Morin, L. P. (2013). Brief light stimulation during the mouse nocturnal activity phase simultaneously induces a decline in core temperature and locomotor activity followed by EEG-determined sleep. *American Journal of Physiology* 304: R459–R471.

228. Kuljis, D. A., Loh, D. H., Truong, D., Vosko, A. M., Ong, M. L., McClusky, R., Arnold, A. P., and Colwell, C. S. (2013). Gonadal- and sex-chromosome-dependent sex differences in the circadian system. *Endocrinology* 154: 1501–1512.
229. Mulder, C. K., Papantoniou, C., Gerkema, M. P., and van der Zee, E. A. (2014). Neither the SCN nor the adrenals are required for circadian time-place learning in mice. *Chronobiology International* 31: 1075–1092.
230. Conn, C. A., Borer, K. T., and Kluger, M. J. (1990). Body temperature rhythm and response to pyrogen in exercising and sedentary hamsters. *Medicine and Science in Sports and Exercise* 22: 636–642.
231. Davis, F. C., Stice, S., and Menaker, M. (1987). Activity and reproductive state in the hamster: Independent control by social stimuli and a circadian pacemaker. *Physiology and Behavior* 40: 583–590.
232. Golombek, D. A., Ortega, G., and Cardinali, D. P. (1993). Wheel running raises body temperature and changes the daily cycle in golden hamsters. *Physiology and Behavior* 53: 1049–1054.
233. Refinetti, R. (1994). Contribution of locomotor activity to the generation of the daily rhythm of body temperature in golden hamsters. *Physiology and Behavior* 56: 829–831.
234. Refinetti, R. (1995). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behavioral Neuroscience* 109: 523–527.
235. Brown, C. M. and Refinetti, R. (1996). Daily rhythms of metabolic heat production, body temperature, and locomotor activity in golden hamsters. *Journal of Thermal Biology* 21: 227–230.
236. Weinert, D., Fritzche, P., and Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
237. Boulos, Z., Macchi, M., Houpt, T. A., and Terman, M. (1996). Photic entrainment in hamsters: Effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
238. Tokura, H. and Oishi, T. (1985). Circadian locomotor activity rhythm under the influences of temperature cycle in the Djungarian hamster, *Phodopus sungorus*, entrained by 12 hour light–12 hour dark cycle. *Comparative Biochemistry and Physiology A* 81: 271–275.
239. Anchordoquy, H. C. and Lynch, G. R. (2000). Timing of testicular recrudescence in Siberian hamsters is unaffected by pinealectomy or long-day photoperiod after 9 weeks in short days. *Journal of Biological Rhythms* 15: 406–416.
240. Steinlechner, S., Stieglitz, A., and Ruf, T. (2002). Djungarian hamsters: A species with a labile circadian pacemaker? Arrhythmicity under a light–dark cycle induced by short light pulses. *Journal of Biological Rhythms* 17: 248–258.
241. Hashimoto, H., Moritani, N., and Saito, T. R. (2004). Comparative study on circadian rhythms of body temperature, heart rate, and locomotor activity in three species of hamsters. *Experimental Animals* 53: 43–46.
242. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2007). Circadian effects of light no brighter than moonlight. *Journal of Biological Rhythms* 22: 356–367.
243. Gattermann, R., Johnston, R. E., Yigit, N., Fritzsche, P., Larimer, S., Özkurt, S., Neumann, K. et al. (2008). Golden hamsters are nocturnal in captivity but diurnal in nature. *Biology Letters* 4: 253–255.
244. Refinetti, R. (1998). Body temperature and behavior of tree shrews and flying squirrels in a thermal gradient. *Physiology and Behavior* 63: 517–520.
245. DeCoursey, P. (1960). Phase control of activity in a rodent. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 49–55.
246. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
247. Everts, L. G., Strijkstra, A. M., Hut, R. A., Hoffmann, I. E., and Millesi, E. (2004). Seasonal variation in daily activity patterns of free-ranging European ground squirrels (*Spermophilus citellus*). *Chronobiology International* 21: 57–71.
248. Lee, T. M., Holmes, W. G., and Zucker, I. (1990). Temperature dependence of circadian rhythms in golden-mantled ground squirrels. *Journal of Biological Rhythms* 5: 25–34.
249. Lee, T. M. and Zucker, I. (1995). Seasonal variations in circadian rhythms persist in gonadectomized golden-mantled ground squirrels. *Journal of Biological Rhythms* 10: 188–195.
250. Refinetti, R. (1995). Body temperature and behaviour of golden hamsters (*Mesocricetus auratus*) and ground squirrels (*Spermophilus tridecemlineatus*) in a thermal gradient. *Journal of Comparative Physiology A* 177: 701–705.
251. Kas, M. J. H. and Edgar, D. M. (1998). Crepuscular rhythms of EEG sleep–wake in a hystricomorph rodent, *Octodon degus*. *Journal of Biological Rhythms* 13: 9–17.
252. Labyak, S. E. and Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
253. Refinetti, R. (1996). Rhythms of body temperature and temperature selection are out of phase in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 60: 959–961.
254. Kas, M. J. H. and Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.
255. Goel, N. and Lee, T. M. (1995). Social cues accelerate reentrainment of circadian rhythms in diurnal female *Octodon degus* (Rodentia: Octodontidae). *Chronobiology International* 12: 311–323.
256. Labyak, S. E., Lee, T. M., and Goel, N. (1997). Rhythm chronotypes in a diurnal rodent, *Octodon degus*. *American Journal of Physiology* 273: R1058–R1066.
257. Ocampo-Garcés, A., Hernández, F., Mena, W., and Palacios, A. G. (2005). Wheel-running and rest activity pattern interaction in two octodontids (*Octodon degus*, *Octodon bridgesi*). *Biological Research* 38: 299–305.
258. Ocampo-Garcés, A., Mena, W., Hernández, F., Cortés, N., and Palacios, A. G. (2006). Circadian chronotypes among wild-captured west Andean octodontids. *Biological Research* 39: 209–220.
259. Hummer, D. L., Jechura, T. J., Mahoney, M. M., and Lee, T. M. (2007). Gonadal hormone effects on entrained and free-running circadian activity rhythms in the developing diurnal rodent *Octodon degus*. *American Journal of Physiology* 292: R586–R597.
260. Lovegrove, B. G., Heldmaier, G., and Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
261. Oosthuizen, M. K., Cooper, H. M., and Bennett, N. C. (2003). Circadian rhythms of locomotor activity in solitary and social species of African mole-rats (Family: Bathyergidae). *Journal of Biological Rhythms* 18: 481–490.
262. Lovegrove, B. G. and Papenfus, M. E. (1995). Circadian activity rhythms in the solitary Cape molerat (*Georychus capensis*: Bathyergidae) with some evidence of splitting. *Physiology and Behavior* 58: 679–685.

263. Goldman, B. D., Goldman, S. L., Riccio, A. P., and Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
264. Schöttner, K., Oosthuizen, M. K., Broekman, M., and Bennett, N. C. (2006). Circadian rhythms of locomotor activity in the Lesotho mole-rat, *Cryptomys hottentotus* subspecies from Sani Pass, South Africa. *Physiology and Behavior* 89: 205–212.
265. Tavernier, R. J., Largen, A. L., and Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
266. Gerkema, M. P., Groos, G. A., and Daan, S. (1990). Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. *Journal of Biological Rhythms* 5: 81–95.
267. Jilge, B. (1985). The rhythm of food and water ingestion, faeces excretion and locomotor activity in the guinea pig. *Zeitschrift für Versuchstierkunde* 27: 215–225.
268. Büttner, D. (1992). Social influences on the circadian rhythm of locomotor activity and food intake of guinea pigs. *Journal of Interdisciplinary Cycle Research* 23: 100–112.
269. Akita, M., Ishii, K., Kuwahara, M., and Tsubone, H. (2001). The daily pattern of heart rate, body temperature, and locomotor activity in guinea pigs. *Experimental Animals* 50: 409–415.
270. McElhinny, T. L., Smale, L., and Holekamp, K. E. (1997). Patterns of body temperature, activity, and reproductive behavior in a tropical murid rodent, *Arvicanthis niloticus*. *Physiology and Behavior* 62: 91–96.
271. Blanchong, J. A., McElhinny, T. L., Mahoney, M. M., and Smale, L. (1999). Nocturnal and diurnal rhythms in the unstriped Nile rate, *Arvicanthis niloticus*. *Journal of Biological Rhythms* 14: 364–377.
272. Schwartz, M. D. and Smale, L. (2005). Individual differences in rhythms of behavioral sleep and its neural substrates in Nile grass rats. *Journal of Biological Rhythms* 20: 526–537.
273. Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. *Physiology and Behavior* 82: 285–294.
274. Refinetti, R. (2004). Parameters of photic resetting of the circadian system of a diurnal rodent, the Nile grass rat. *Acta Scientiae Veterinariae* 32: 1–6.
275. Refinetti, R. (2006). Variability of diurnality in laboratory rodents. *Journal of Comparative Physiology A* 192: 701–714.
276. Schrader, J. A., Walaszczyk, E. J., and Smale, L. (2009). Changing patterns of daily rhythmicity across reproductive states in diurnal female Nile grass rats (*Arvicanthis niloticus*). *Physiology and Behavior* 98: 547–556.
277. Gall, A. J., Smale, L., Yan, L., and Nunez, A. A. (2013). Lesions of the intergeniculate leaflet lead to a reorganization in circadian regulation and a reversal in masking responses to photic stimuli in the Nile grass rat. *PLOS ONE* 8: e67387.
278. Stewart, M. C. and Reeder, W. G. (1968). Temperature and light synchronization experiments with circadian activity rhythms in two color forms of the rock pocket mouse. *Physiological Zoology* 41: 149–156.
279. Cohen, R. and Kronfeld-Schor, N. (2006). Individual variability and photic entrainment of circadian rhythms in golden spiny mice. *Physiology and Behavior* 87: 563–574.
280. Weber, E. T. and Hohn, V. M. (2005). Circadian activity rhythms in the spiny mouse, *Acomys cahirinus*. *Physiology and Behavior* 86: 427–433.
281. Lovegrove, B. G. and Heldmaier, G. (1994). The amplitude of circadian body temperature rhythms in three rodents (*Aethomys namaquensis*, *Thallomys paedulcus* and *Cryptomys damarensis*) along the arboreal-subterranean gradient. *Australian Journal of Zoology* 42: 65–78.
282. Slotten, H. A., Krekling, S., and Pévet, P. (2005). Photic and nonphotic effects on the circadian activity rhythm in the diurnal rodent *Arvicanthis ansorgei*. *Behavioural Brain Research* 165: 91–97.
283. Cuesta, M., Clesse, D., Pévet, P., and Challet, E. (2009). From daily behavior to hormonal and neurotransmitter rhythms: Comparison between diurnal and nocturnal rat species. *Hormones and Behavior* 55: 338–347.
284. Mendoza, J., Gourmelen, S., Dumont, S., Sage-Ciocca, D., Pévet, P., and Challet, E. (2012). Setting the main circadian clock of a diurnal mammal by hypocaloric feeding. *Journal of Physiology* 590: 3155–3168.
285. Eccard, J. A., Meyer, J., and Sundell, J. (2004). Space use, circadian activity pattern, and mating system of the nocturnal tree rat *Thallomys nigricauda*. *Journal of Mammalogy* 85: 440–445.
286. Pita, R., Mira, A., and Beja, P. (2011). Circadian activity rhythms in relation to season, sex and interspecific interactions in two Mediterranean voles. *Animal Behaviour* 81: 1023–1030.
287. Basu, P. and Singaravel, M. (2013). Accurate and precise circadian locomotor activity rhythms in male and female Indian pygmy field mice, *Mus terricolor*. *Biological Rhythm Research* 44: 531–539.
288. Basu, P., Singaravel, M., and Refinetti, R. (2015). Experimental quantification of improvement during circadian wheel running in the Indian field mouse, *Mus terricolor*: Theoretical uses. *Biological Rhythm Research* 46: 173–180.
289. Johnston, P. G. and Zucker, I. (1983). Lability and diversity of circadian rhythms of cotton rats, *Sigmodon hispidus*. *American Journal of Physiology* 244: R338–RR346.
290. Gebczynski, A. K. and Taylor, J. R. E. (2004). Daily variation of body temperature, locomotor activity and maximum nonshivering thermogenesis in two species of small rodents. *Journal of Thermal Biology* 29: 123–131.
291. Mrosovsky, N., Melnyk, R. B., Lang, K., Hallonquist, J. D., Boshes, M., and Joy, J. E. (1980). Infradian cycles in dormice (*Glis glis*). *Journal of Comparative Physiology A* 137: 315–339.
292. Roxburgh, L. and Perrin, M. R. (1994). Temperature regulation and activity pattern of the round-eared elephant shrew *Macroscelides proboscideus*. *Journal of Thermal Biology* 19: 13–20.
293. Hayes, S. R. (1976). Daily activity and body temperature of the southern woodchuck, *Marmota monax monax*, in northwestern Arkansas. *Journal of Mammalogy* 57: 291–299.
294. Nelissen, M. and Nelissen-Joris, N. (1975). On the diurnal rhythm of activity of *Meriones unguiculatus* (Milne-Edwards, 1867). *Acta Zoologica et Patologica Antverpiensia* 61: 25–30.
295. Weinert, D., Nevill, A., Weinandy, R., and Waterhouse, J. (2003). The development of new purification methods to assess the circadian rhythm of body temperature in Mongolian gerbils. *Chronobiology International* 20: 249–270.
296. Weinert, D., Weinandy, R., and Gattermann, R. (2007). Photic and non-photic effects on the daily activity pattern of Mongolian gerbils. *Physiology and Behavior* 90: 325–333.
297. Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology A* 119: 295–300.

298. Sacher, G. A. and Duffy, P. H. (1978). Age changes in rhythms of energy metabolism, activity, and body temperature in *Mus* and *Peromyscus*. In: Samis, H. V. and Capobianco, S. (Eds.), *Aging and Biological Rhythms*. New York: Plenum, pp. 105–124.
299. Rezende, E. L., Cortés, A., Bacigalupe, L. D., Nespolo, R. F., and Bozinovic, F. (2003). Ambient temperature limits aboveground activity of the subterranean rodent *Spalacopus cyanus*. *Journal of Arid Environments* 55: 63–74.
300. Begall, S., Daan, S., Burda, H., and Overkamp, G. J. F. (2002). Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83: 153–158.
301. Kramm, K. R. and Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.
302. Richter, C. P. (1978). Evidence for existence of a yearly clock in surgically and self-blinded chipmunks. *Proceedings of the National Academy of Sciences United States of America* 75: 3517–3521.
303. Lahmam, M., El M'rabet, A., Ouarour, A., Pévet, P., Challet, E. and Vuillez, P. (2008). Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent *Lemniscomys barbarus*. *Chronobiology International* 25: 882–904.
304. Flôres, D. E. F., Tomotani, B. M., Tachinardi, P., Oda, G. A., and Valentinuzzi, V. S. (2013). Modeling natural photic entrainment in a subterranean rodent (*Ctenomys knighti*), the tuco-tuco. *PLOS ONE* 8: e68243.
305. Erkert, H. G. (2004). Extremely low threshold for photic entrainment of circadian activity rhythms in molossid bats (*Molossus molossus*; Chiroptera, Molossidae). *Mammalian Biology* 69: 361–374.
306. Joshi, D. S. and Vanlalnghaka, C. (2005). Non-parametric entrainment by natural twilight in the microchiropteran bat, *Hipposideros speoris*, inside a cave. *Chronobiology International* 22: 631–640.
307. Refinetti, R. and Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
308. Meijer, J. H., Daan, S., Overkamp, G. J. F., and Hermann, P. M. (1990). The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *Journal of Biological Rhythms* 5: 1–16.
309. Jilge, B. (1991). Restricted feeding: A nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
310. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
311. Kennedy, G. A., Hudson, R., and Armstrong, S. M. (1994). Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiology and Behavior* 55: 385–389.
312. Piccione, G., Caola, G., and Refinetti, R. (2007). Daily rhythms of liver-function indicators in rabbits. *Journal of Physiological Sciences* 57: 101–105.
313. Johnson, R. F. and Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
314. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., and Johnson, R. F. (1987). A two-peak circadian system in body temperature and activity in the domestic cat, *Felis catus*. *Journal of Thermal Biology* 12: 27–37.
315. Hilmer, S., Algar, D., Plath, M., and Schleucher, E. (2010). Relationship between daily body temperature and activity patterns of free-ranging feral cats in Australia. *Journal of Thermal Biology* 35: 270–274.
316. Hawking, F., Lobban, M. C., Gammage, K., and Worms, M. J. (1971). Circadian rhythms (activity, temperature, urine and microfilariae) in dog, cat, hen, duck, Thamnomys and Gerbillus. *Journal of Interdisciplinary Cycle Research* 2: 455–473.
317. Siwak, C. T., Tapp, P. D., Zicker, S. C., Murphey, H. L., Muggenburg, B. A., Head, E., and Cotman, C. W. (2003). Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience* 117: 813–824.
318. Ebling, F. J. P., Lincoln, G. A., Wollnik, F., and Anderson, N. (1988). Effects of constant darkness and constant light on circadian organization and reproductive responses in the ram. *Journal of Biological Rhythms* 3: 365–384.
319. Mohr, E. G. and Krzywanek, H. (1995). Endogenous oscillator and regulatory mechanisms of body temperature in sheep. *Physiology and Behavior* 57: 339–347.
320. Lincoln, G., Messager, S., Andersson, H., and Hazlerigg, D. (2002). Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: Evidence for an internal coincidence timer. *Proceedings of the National Academy of Sciences United States of America* 99: 13890–13895.
321. Piccione, G., Caola, G., and Refinetti, R. (2005). Temporal relationships of 21 physiological variables in horse and sheep. *Comparative Biochemistry and Physiology A* 142: 389–396.
322. Gill, J. (1991). A new method for continuous recording of motor activity in horses. *Comparative Biochemistry and Physiology A* 99: 333–341.
323. Scheibe, K. M., Berher, A., Langbein, J., Streich, W. J., and Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biological Rhythm Research* 30: 216–233.
324. Paisley, S. and Garshelis, D. L. (2006). Activity patterns and time budgets of Andean bears (*Tremarctos ornatus*) in the Apolobamba Range of Bolivia. *Journal of Zoology* 268: 25–34.
325. Ware, J. V., Nelson, O. L., Robbins, C. T., and Jansen, H. T. (2012). Temporal organization of activity in the brown bear (*Ursus arctos*): Roles of circadian rhythms, light, and food entrainment. *American Journal of Physiology* 303: R890–R902.
326. Wells, R. T. (1978). Thermoregulation and activity rhythms in the hairy-nosed wombat, *Lasiorhinus latifrons* (Owen), (Vombatidae). *Australian Journal of Zoology* 26: 639–651.
327. Francis, A. J. P. and Coleman, G. J. (1990). Ambient temperature cycles entrain the free-running circadian rhythms of the stripe-faced dunnart, *Sminthopsis macroura*. *Journal of Comparative Physiology A* 167: 357–362.
328. Körtner, G. and Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiology and Behavior* 58: 31–36.
329. Jones, M. E., Grigg, G. C., and Beard, L. A. (1997). Body temperatures and activity patterns of Tasmanian devils (*Sarcophilus harrisii*) and eastern quolls (*Dasyurus viverrinus*) through a subalpine winter. *Physiological Zoology* 70: 53–60.
330. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
331. Marimuthu, G., Rajan, S., and Chandrashekaran, M. K. (1981). Social entrainment of the circadian rhythm in the flight activity of the microchiropteran bat *Hipposideros speoris*. *Behavioral Ecology and Sociobiology* 8: 147–150.

332. Bakko, E. B., Porter, W. P. and Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
333. Kowalczyk, R., Jedrzejewska, B., and Zalewski, A. (2003). Annual and circadian activity patterns of badgers (*Meles meles*) in Bialowieza Primeval Forest (eastern Poland) compared with other Palaearctic populations. *Journal of Biogeography* 30: 463–472.
334. Riccio, A. P. and Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
335. Ishii, K., Uchino, M., Kuwahara, M., Tsubone, H., and Ebukuro, S. (2002). Diurnal fluctuations of heart rate, body temperature and locomotor activity in the house musk shrew (*Suncus murinus*). *Experimental Animals* 51: 57–62.
336. Ingram, D. L. and Mount, L. E. (1973). The effects of food intake and fasting on 24-hourly variations in body temperature in the young pig. *Pflügers Archiv* 339: 299–304.
337. Lancia, R. A., Dodge, W. E., and Larson, J. S. (1982). Winter activity patterns of two radio-marked beaver colonies. *Journal of Mammalogy* 63: 598–606.
338. Francis, A. J. P., de Alwis, C., Peach, L., and Redman, J. R. (1999). Circadian activity rhythms in the Australian platypus, *Ornithorhynchus anatinus* (Monotremata). *Biological Rhythm Research* 30: 91–103.
339. Harlow, H. J., Phillips, J. A., and Ralph, C. L. (1982). Circadian rhythms and the effects of exogenous melatonin in the nine-banded armadillo, *Dasypus novemcinctus*: A mammal lacking a distinct pineal gland. *Physiology and Behavior* 29: 307–313.
340. Merrill, S. B. and Mech, L. D. (2003). The usefulness of GPS telemetry to study wolf circadian and social activity. *Wildlife Society Bulletin* 31: 947–960.
341. Jácomo, A. T. A., Silveira, L. and Diniz-Filho, J. A. F. (2004). Niche separation between the maned wolf (*Chrysocyon brachyurus*), the crab-eating fox (*Dusicyon thous*) and the hoary fox (*Dusicyon vetulus*) in central Brazil. *Journal of Zoology* 262: 99–106.
342. Xia, C., Liu, W., Xu, W., Yang, W., Xu, F., and Blank, D. (2013). Diurnal time budgets and activity rhythms of the Asiatic wild ass *Equus hemionus* in Xinjiang, Western China. *Pakistan Journal of Zoology* 45: 1241–1248.
343. Sargeant, G. A., Eberhardt, L. E., and Peek, J. M. (1994). Thermoregulation by mule deer (*Odocoileus hemionus*) in arid rangelands of southcentral Washington. *Journal of Mammalogy* 75: 536–544.
344. Ensing, E. P., Ciuti, S., de Wijs, F. A. L. M., Lentferink, D. H., ten Hoedt, A., Boyce, M. S., and Hut, R. A. (2014). GPS based daily activity patterns in European red deer and North American elk (*Cervus elaphus*): Indication for a weak circadian clock in ungulates. *PLOS ONE* 9: e106997.
345. van Oort, B. E. H., Tyler, N. J. C., Gerkema, M. P., Folkow, L., and Stokkan, K. A. (2007). Where clocks are redundant: Weak circadian mechanisms in reindeer living under polar photic conditions. *Naturwissenschaften* 94: 183–194.
346. Hetem, R. S., Strauss, W. M., Fick, L. G., Maloney, S. K., Meyer, L. C. R., Shobrak, M., Fuller, A., and Mitchell, D. (2012). Activity re-assignment and microclimate selection of free-living Arabian oryx: Responses that could minimise the effects of climate change on homeostasis? *Zoology* 115: 411–416.
347. Rauth-Widmann, B., Fuchs, E., and Erkert, H. G. (1996). Infradian alteration of circadian rhythms in owl monkeys (*Aotus lemurinus griseimembra*): An effect of estrous? *Physiology and Behavior* 59: 11–18.
348. Erkert, H. G. and Cramer, B. (2006). Chronobiological background to cathemerality: Circadian rhythms in *Eulemur fulvus albifrons* (Prosimii) and *Aotus azarai boliviensis* (Anthropoidea). *Folia Primatologica* 77: 87–103.
349. Erkert, H. G., Gburek, V., and Scheideler, A. (2006). Photic entrainment and masking of prosimian circadian rhythms (*Otolemur garnettii*, Primates). *Physiology and Behavior* 88: 39–46.
350. Takasu, N., Nigi, H., and Tokura, H. (2002). Effects of diurnal bright/dim light intensity on circadian core temperature and activity rhythms in the Japanese macaque. *Japanese Journal of Physiology* 52: 573–578.
351. Tapp, W. N. and Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
352. Fuller, C. A., Hoban-Higgins, T. M., Klimovitsky, V. Y., Griffin, D. W., and Alpatov, A. M. (1996). Primate circadian rhythms during spaceflight: Results from Cosmos 2044 and 2229. *Journal of Applied Physiology* 81: 188–193.
353. Barger, L. K., Hoban-Higgins, T. M., and Fuller, C. A. (2008). Assessment of circadian rhythms throughout the menstrual cycle of female rhesus monkeys. *American Journal of Primatology* 70: 19–25.
354. Masuda, K. and Zhdanova, I. V. (2010). Intrinsic activity rhythms in *Macaca mulatta*: Their entrainment to light and melatonin. *Journal of Biological Rhythms* 25: 361–371.
355. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
356. Tokura, H. and Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
357. Weed, M. R. and Hienz, R. D. (2006). Effects of morphine on circadian rhythms of motor activity and body temperature in pig-tailed macaques. *Pharmacology, Biochemistry and Behavior* 84: 487–496.
358. Génin, F. and Perret, M. (2003). Daily hypothermia in captive grey mouse lemurs (*Microcebus murinus*): Effects of photoperiod and food restriction. *Comparative Biochemistry and Physiology B* 136: 71–81.
359. Aujard, F. and Vasseur, F. (2001). Effect of ambient temperature on the body temperature rhythm of male gray mouse lemurs (*Microcebus murinus*). *International Journal of Primatology* 22: 43–56.
360. Perret, M. and Aujard, F. (2001). Daily hypothermia and torpor in a tropical primate: Synchronization by 24-h light–dark cycle. *American Journal of Physiology* 281: R1925–R1933.
361. Schilling, A., Richard, J. P., and Servière, J. (1999). Duration of activity and period of circadian activity-rest rhythm in a photoperiod-dependent primate, *Microcebus murinus*. *Comptes Rendus de l'Académie des Sciences* 322: 759–770.
362. Perret, M., Gomez, D., Barbosa, A., Aujard, F., and Théry, M. (2010). Increased late night response to light controls the circadian pacemaker in a nocturnal primate. *Journal of Biological Rhythms* 25: 186–196.
363. Perret, M., Aujard, F., Séguy, M., and Schilling, A. (2003). Olfactory bulbectomy modifies photic entrainment and circadian rhythms of body temperature and locomotor activity in a nocturnal primate. *Journal of Biological Rhythms* 18: 392–401.
364. Zeitzer, J. M., Buckmaster, C. L., Parker, K. J., Hauck, C. M., Lyons, D. M., and Mignot, E. (2003). Circadian and homeostatic regulation of hypocretin in a primate model: Implications for the consolidation of wakefulness. *Journal of Neuroscience* 23: 3555–3560.

365. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
366. Moore-Ede, M. C., Kass, D. A., and Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
367. Häster, L. and Erkert, H. G. (1993). Alteration of circadian period length does not influence the ovarian cycle length in common marmosets, *Callithrix j. jacchus* (Primates). *Chronobiology International* 10: 165–175.
368. Menezes, A. A. L., Moreira, L. F. S., Azevedo, C. V. M., Costa, S. F., and Castro, C. S. S. (1993). Behavioral rhythms in the captive common marmoset (*Callithrix jacchus*) under natural environmental conditions. *Brazilian Journal of Medical and Biological Research* 26: 741–745.
369. Schnell, C. R. and Wood, J. M. (1995). Measurement of blood pressure and heart rate by telemetry in conscious unrestrained marmosets. *Laboratory Animals* 29: 258–261.
370. de Castro, C. S. S., de Menezes, A. L., and Moreira, L. F. S. (2003). Locomotor activity rhythm in free-ranging common marmosets (*Callithrix jacchus*). *Biological Rhythm Research* 34: 23–30.
371. Silva, M. A. A., Albuquerque, A. M., and Araujo, J. F. (2005). Light–dark cycle synchronization of circadian rhythm in blind primates. *Journal of Circadian Rhythms* 3: art. 10.
372. Hoffmann, K., Coolen, A., Schlumbohm, C., Meerlo, P., and Fuchs, E. (2012). Remote long-term registrations of sleep–wake rhythms, core body temperature and activity in marmoset monkeys. *Behavioural Brain Research* 235: 113–123.
373. Melo, P., Gonçalves, B., Menezes, A., and Azevedo, C. (2013). Socially adjusted synchrony in the activity profiles of common marmosets in light–dark conditions. *Chronobiology International* 30: 818–827.
374. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
375. Binkley, S., Tome, M. B., Crawford, D., and Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
376. Kennaway, D. J. and Van Dorp, C. F. (1991). Free-running rhythms of melatonin, cortisol, electrolytes, and sleep in humans in Antarctica. *American Journal of Physiology* 260: R1137–R1144.
377. Steel, G. D., Callaway, M., Suedfeld, P., and Palinkas, L. (1995). Human sleep–wake cycles in the high Arctic: Effects of unusual photoperiodicity in a natural setting. *Biological Rhythm Research* 26: 582–592.
378. Yoneyama, S., Hashimoto, S., and Honma, K. (1999). Seasonal changes of human circadian rhythms in Antarctica. *American Journal of Physiology* 277: R1091–R1097.
379. Natale, V. (2002). Circadian motor asymmetries in humans. *Neuroscience Letters* 320: 102–104.
380. Aschoff, J., Gerecke, U., and Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
381. Gander, P. H., Connell, L. J., and Graeber, R. C. (1986). Masking of the circadian rhythm of heart rate and core temperature by the rest-activity cycle in man. *Journal of Biological Rhythms* 1: 119–135.
382. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F., and Pauly, J. E. (Eds.), *Chronobiology*. Tokyo, Japan: Igaku Shoin, pp. 451–459.
383. Endo, T., Honma, S., Hashimoto, S., and Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
384. Edwards, B., Waterhouse, J., Reilly, T., and Atkinson, G. (2002). A comparison of the suitabilities of rectal, gut, and insulated axilla temperatures for measurement of the circadian rhythm of core temperature in field studies. *Chronobiology International* 19: 579–597.
385. Kolodyazhniy, V., Späti, J., Frey, S., Götz, T., Wirz-Justice, A., Kräuchi, K., Cajochen, C., and Wilhelm, F. H. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. *Journal of Biological Rhythms* 26: 55–67.
386. Herder, E. and Siehndel, P. (2012). Daily and weekly patterns in human mobility. *CEUR Workshop Proceedings* 872: art. 3.
387. Sun, L., Axhausen, K. W., Lee, D. H., and Huang, X. (2013). Understanding metropolitan patterns of daily encounters. *Proceedings of the National Academy of Sciences United States of America* 110: 13774–13779.
388. Sani, M., Refinetti, R., Jean-Louis, G., Pandi-Perumal, S. R., Durazo-Arvizu, R. A., Dugas, L. R., Kafensztok, R. et al. (2015). Daily activity patterns of 2316 men and women from five countries differing in socioeconomic development. *Chronobiology International* 32: 650–656.
389. Refinetti, R. (2004). The Nile grass rat as a laboratory animal. *Lab Animal* 33(9): 54–57.
390. Redlin, U. and Mrosovsky, N. (2004). Nocturnal activity in a diurnal rodent (*Arvicanthis niloticus*): The importance of masking. *Journal of Biological Rhythms* 19: 58–67.
391. Blanchong, J. A. and Smale, L. (2000). Temporal patterns of activity of the unstriped Nile rat, *Arvicanthis niloticus*. *Journal of Mammalogy* 81: 595–599.
392. Aschoff, J. (1966). Circadian activity pattern with two peaks. *Ecology* 47: 657–662.
393. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
394. Dainton, B. H. (1954). The activity of slugs. I. The induction of activity by changing temperatures. *Journal of Experimental Biology* 31: 165–187.
395. Page, T. L. (1985). Circadian organization in cockroaches: Effects of temperature cycles on locomotor activity. *Journal of Insect Physiology* 31: 235–242.
396. Toma, D. P., Bloch, G., Moore, D., and Robinson, G. E. (2000). Changes in *period* mRNA levels in the brain and division of labor in honey bee colonies. *Proceedings of the National Academy of Sciences United States of America* 97: 6914–6919.
397. Bloch, G., Shemesh, Y., and Robinson, G. E. (2006). Seasonal and task-related variation in free running activity rhythms in honey bees (*Apis mellifera*). *Insectes Sociaux* 53: 115–118.
398. Takekata, H., Matsuura, Y., Goto, S. G., Satoh, A., and Numata, H. (2012). RNAi of the circadian clock gene *period* disrupts the circadian rhythm but not the circatidal rhythm in the mangrove cricket. *Biology Letters* 8: 488–491.
399. Williams, B. G. and Naylor, E. (1967). Spontaneously induced rhythm of tidal periodicity in laboratory-reared *Carcinus*. *Journal of Experimental Biology* 47: 229–234.
400. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
401. Lowe, C. H., Hinds, D. S., Lardner, P. J., and Justice, K. E. (1967). Natural free-running period in vertebrate animal populations. *Science* 156: 531–534.

402. Janik, D. S., Pickard, G. E., and Menaker, M. (1990). Circadian locomotor rhythms in the desert iguana. II. Effects of electrolytic lesions to the hypothalamus. *Journal of Comparative Physiology A* 166: 811–816.
403. Tosini, G. and Menaker, M. (1998). Multioscillatory circadian organization in a vertebrate, *Iguana iguana*. *Journal of Neuroscience* 18: 1105–1114.
404. Debruyne, J., Hurd, M. W., Gutiérrez, L., Kaneko, M., Tan, Y., Wells, D. E., and Cahill, G. M. (2004). Isolation and phenogenetics of a novel circadian rhythm mutant in zebrafish. *Journal of Neurogenetics* 18: 403–428.
405. Pohl, H. (1993). Does aging affect the period of the circadian pacemaker in vertebrates? *Naturwissenschaften* 80: 478–481.
406. Sridaran, R. and McCormack, C. E. (1980). Parallel effects of light signals on the circadian rhythms of running activity and ovulation in rats. *Journal of Endocrinology* 85: 111–120.
407. Weber, A. L. and Adler, N. T. (1978). Dissociation of persistent estrus and a free-running activity cycle in different light cycles. *Biology of Reproduction* 19: 425–430.
408. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S., and Takahashi, K. (1988). Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiology and Behavior* 42: 87–91.
409. Bauer, M. S. (1992). Irradiance responsivity and unequivocal type-1 phase responsivity of rat circadian activity rhythms. *American Journal of Physiology* 263: R1110–R1114.
410. Stokes, M. A., Kent, S., and Armstrong, S. M. (1999). The effect of multiple pulses of light on the circadian phase response of the rat. *Journal of Biological Rhythms* 14: 172–184.
411. Edgar, D. M., Kilduff, T. S., Martin, C. E., and Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior* 50: 373–378.
412. Hofstetter, J. R., Grahame, N. J., and Mayeda, A. R. (2003). Circadian activity rhythms in high-alcohol-preferring and low-alcohol-preferring mice. *Alcohol* 30: 81–85.
413. Edgar, D. M., Martin, C. E. and Dement, W. C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *Journal of Biological Rhythms* 6: 185–199.
414. Yoshimura, T., Nishio, M., Goto, M., and Ebihara, S. (1994). Differences in circadian photosensitivity between retinally degenerate CBA/J mice (*rd/rd*) and normal CBA/M mice (+/+). *Journal of Biological Rhythms* 9: 51–60.
415. Edgar, D. M. and Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
416. Muñoz, M., Peirson, S. N., Hankins, M. W., and Foster, R. G. (2005). Long-term constant light induces constitutive elevated expression of mPER2 protein in the murine SCN: A molecular basis for Aschoff's rule? *Journal of Biological Rhythms* 20: 3–14.
417. Marchant, E. G. and Mistlberger, R. E. (1996). Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. *Physiology and Behavior* 60: 657–663.
418. Yang, Y., Duguay, D., Bédard, N., Rachalski, A., Baquiran, G., Na, C. H., Fahrenkrug, J. et al. (2012). Regulation of behavioral circadian rhythms and clock protein PER1 by the deubiquitinating enzyme USP2. *Biology Open* 1: 789–801.
419. Malloy, J. N., Paulose, J. K., Li, Y., and Cassone, V. M. (2012). Circadian rhythms of gastrointestinal function are regulated by both central and peripheral oscillators. *American Journal of Physiology* 303: G461–G473.
420. Refinetti, R. (1996). Ultradian rhythms of body temperature and locomotor activity in wild-type and tau-mutant hamsters. *Animal Biology* 5: 111–115.
421. Fitzgerald, K. M. and Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences United States of America* 73: 2923–2927.
422. Refinetti, R. and Menaker, M. (1992). The circadian rhythm of body temperature of normal and tau-mutant golden hamsters. *Journal of Thermal Biology* 17: 129–133.
423. DeCoursey, P. J., Pius, S., Sandlin, C., Wethey, D., and Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiology and Behavior* 65: 457–463.
424. Zucker, I., Fitzgerald, K. M., and Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.
425. Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology A* 162: 35–46.
426. Oklejewicz, M., Hut, R. A., Daan, S., Loudon, A. S. I., and Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.
427. Refinetti, R. and Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
428. DeCoursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science* 131: 33–35.
429. Mrosovsky, N., Boshes, M., Hallonquist, J. D., and Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.
430. Lee, T. M. and Labyak, S. E. (1997). Free-running rhythms and light- and dark-pulse phase response curves for diurnal *Octodon degus* (Rodentia). *American Journal of Physiology* 273: R278–R286.
431. Honma, S. and Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
432. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P., and Challet, E. (2003). Circadian profile and photic regulation of clock genes in the suprachiasmatic nucleus of a diurnal mammal, *Arvicanthis ansorgei*. *Neuroscience* 116: 583–591.
433. Sharma, V. K. (1996). Light-induced phase response curves of the circadian activity rhythm in individual field mice, *Mus booduga*. *Chronobiology International* 13: 401–409.
434. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
435. Kumar, D. and Singaravel, M. (2014). Phase and period responses to short light pulses in a wild diurnal rodent, *Funambulus pennanti*. *Chronobiology International* 31: 320–327.
436. Robinson, E. L. and Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
437. Boulos, Z., Frim, D. M., Dewey, L. K., and Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.

438. Pifferi, F., Dal-Pan, A., Menaker, M., and Aujard, F. (2011). Resveratrol dietary supplementation shortens the free-running circadian period and decreases body temperature in a prosimian primate. *Journal of Biological Rhythms* 26: 271–275.
439. Aschoff, J. and Tokura, H. (1986). Circadian activity rhythms in squirrel monkeys: Entrainment by temperature cycles. *Journal of Biological Rhythms* 1: 91–99.
440. Chandrashekaran, M. K., Marimuthu, G., and Geetha, L. (1997). Correlations between sleep and wake in internally synchronized and desynchronized circadian rhythms in humans under prolonged isolation. *Journal of Biological Rhythms* 12: 26–33.
441. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C., and Knauer, R. S. (1980). Human sleep: Its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
442. Zulley, J., Wever, R., and Aschoff, J. (1981). The dependence of onset and duration of sleep on the circadian rhythm of rectal temperature. *Pflügers Archiv* 391: 314–318.
443. Lund, R. (1974). Personality factors and desynchronization of circadian rhythms. *Psychosomatic Medicine* 36: 224–228.
444. Campbell, S. S., Dawson, D., and Zulley, J. (1993). When the human circadian system is caught napping: Evidence for endogenous rhythms close to 24 hours. *Sleep* 16: 638–640.
445. Eastman, C. I., Molina, T. A., Dziepak, M. E., and Smith, M. R. (2012). Blacks (African Americans) have shorter free-running circadian periods than Whites (Caucasian Americans). *Chronobiology International* 29: 1072–1077.
446. Jespersen, J. and Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency*, 2nd edn. Mineola, NY: Dover.
447. Barnett, J. E. (1998). *Time's Pendulum: From Sundials to Atomic Clocks*. New York: Plenum.
448. Daan, S., Merrow, M., and Roenneberg, T. (2002). External time, internal time. *Journal of Biological Rhythms* 17: 107–109.
449. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th edn. New York: Cambridge University Press.
450. Strubbe, J. H. and Woods, S. C. (2004). The timing of meals. *Psychological Review* 111: 128–141.
451. Drewnowski, A., Grinker, J. A., Gruen, R., and Sullivan, A. C. (1985). Effects of inhibitors of carbohydrate absorption or lipid metabolism on meal patterns of Zucker rats. *Pharmacology, Biochemistry and Behavior* 23: 811–821.
452. Balagura, S. and Coscina, D. V. (1968). Periodicity of food intake in the rat as measured by an operant response. *Physiology and Behavior* 3: 641–643.
453. Alingh Prins, A., de Jong-Nagelsmit, A., Keijser, J., and Strubbe, J. H. (1986). Daily rhythms of feeding in the genetically obese and lean Zucker rats. *Physiology and Behavior* 38: 423–426.
454. Cunningham, J. T., Beltz, T., Johnson, R. F., and Johnson, A. K. (1992). The effects of ibotenate lesions of the median preoptic nucleus on experimentally-induced and circadian drinking behavior in rats. *Brain Research* 580: 325–330.
455. Velasco-Plaza, A., Granda, T. G., and Cachero, M. T. G. (1993). Circadian rhythms of food and water intake and urine excretion in diabetic rats. *Physiology and Behavior* 54: 665–670.
456. Zucker, I. (1971). Light-dark rhythms in rat eating and drinking behavior. *Physiology and Behavior* 6: 115–126.
457. Strubbe, J. H., Spiteri, N. J., and Alingh Prins, A. J. (1986). Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats. *Physiology and Behavior* 36: 647–651.
458. Galbicka, G., Smurthwaite, S., Riggs, R., and Tang, L. W. (1997). Daily rhythms in a complex operant: Targeted percentile shaping of run lengths in rats. *Physiology and Behavior* 62: 1165–1169.
459. Rosenwasser, A. M., Boulos, Z., and Terman, M. (1983). Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiology and Behavior* 30: 353–359.
460. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
461. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M., and Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
462. Shido, O., Sugano, Y., and Nagasaka, T. (1986). Circadian change of heat loss in response to change in core temperature in rats. *Journal of Thermal Biology* 11: 199–202.
463. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K., Uchimura, K., and Asano, C. (1992). Advance shift of feeding circadian rhythm induced by obesity progression in Zucker rats. *American Journal of Physiology* 263: R1169–R1175.
464. Chen, X., McClusky, R., Chen, J., Beaven, S. W., Tontonoz, P., Arnold, A. P., and Reue, K. (2012). The number of X chromosomes causes sex differences in adiposity in mice. *PLoS Genetics* 8: e1002709.
465. Eckel-Mahan, K. L., Patel, V. R., Mohney, R. P., Vignola, K. S., Baldi, P., and Sassone-Corsi, P. (2012). Coordination of the transcriptome and metabolome by the circadian clock. *Proceedings of the National Academy of Sciences USA* 109: 5541–5546.
466. Kaiyala, K. J., Morton, G. J., Thaler, J. P., Meek, T. H., Tylee, T., Ogimoto, K., and Wisse, B. E. (2012). Acutely decreased thermoregulatory energy expenditure or decreased activity energy expenditure both acutely reduce food intake in mice. *PLOS ONE* 7: e41473.
467. Gamsby, J. J. and Gulick, D. (2015). Chronic shifts in the length and phase of the light cycle increase intermittent alcohol drinking in C57BL/6J mice. *Frontiers in Behavioral Neuroscience* 9: art. 9.
468. Froy, O., Chapnik, N., and Miskin, R. (2008). The suprachiasmatic nuclei are involved in determining circadian rhythms during restricted feeding. *Neuroscience* 155: 1152–1159.
469. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A., and Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
470. Valverde, J. C., López, P. M., and Ruiz, J. C. (2005). Effect of periodical water current on the phasing of demand feeding rhythms in sea bass (*Dicentrarchus labrax* L.). *Physiology and Behavior* 85: 394–403.
471. Sánchez-Vázquez, F. J., Madrid, J. A., and Zamora, S. (1995). Circadian rhythms of feeding activity in sea bass, *Dicentrarchus labrax* L.: Dual phasing capacity of diel demand-feeding pattern. *Journal of Biological Rhythms* 10: 256–266.
472. Shapiro, C. J. and Weathers, W. W. (1981). Metabolic and behavioral responses of American kestrels to food deprivation. *Comparative Biochemistry and Physiology A* 68: 11–114.

473. Kadono, H., Besch, E. L., and Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
474. Houdelier, C., Guyomarc'h, C., Lumineau, S., and Richard, J. P. (2002). Circadian rhythms of oviposition and feeding activity in Japanese quail: Effects of cyclic administration of melatonin. *Chronobiology International* 19: 1107–1119.
475. Lumineau, S. and Guyomarc'h, C. (2000). Circadian rhythm of activity during the annual phases in the European quail, *Coturnix coturnix*. *Comptes Rendus de l'Académie des Sciences* 323: 793–799.
476. Guyomarc'h, C., Lumineau, S., and Richard, J. P. (1998). Circadian rhythm of activity in Japanese quail in constant darkness: Variability of clarity and possibility of selection. *Chronobiology International* 15: 219–230.
477. Lumineau, S. and Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
478. Reierth, E. and Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
479. Best, J. B. (1960). Diurnal cycles and cannibalism in planaria. *Science* 131: 1884–1885.
480. Casey, T. M., Joos, B., Fitzgerald, T. D., Yurlina, M. E., and Young, P. A. (1988). Synchronized group foraging, thermoregulation, and growth of Eastern tent caterpillars in relation to microclimate. *Physiological Zoology* 61: 372–377.
481. Ryer, C. H. and Boehlert, G. W. (1983). Feeding chronology, daily ration, and the effects of temperature upon gastric evacuation in the pipefish, *Syngnathus fuscus*. *Environmental Biology of Fishes* 9: 301–306.
482. Chen, W. M., Naruse, M., and Tabata, M. (2002). Circadian rhythms and individual variability of self-feeding activity in groups of rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* 33: 491–500.
483. Sánchez-Vázquez, F. J., Iigo, M., Madrid, J. A., and Tabata, M. (2000). Pinealectomy does not affect the entrainment to light nor the generation of the circadian demand-feeding rhythms of rainbow trout. *Physiology and Behavior* 69: 455–461.
484. Bovet, J. (1994). Missed opportunities in the study of activity and body temperature of beaver (*Castor canadensis*). *Canadian Journal of Zoology* 72: 567–569.
485. Bobbert, A. C. and Riethoven, J. J. (1991). Feedback in the rabbit's central circadian system, revealed by the changes in its free-running food intake pattern induced by blinding, cervical sympathectomy, pinealectomy, and melatonin administration. *Journal of Biological Rhythms* 6: 263–278.
486. Hoban, T. M., Levine, A. H., Shane, R. B., and Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
487. Fuller, C. A. and Edgar, D. M. (1986). Effects of light intensity on the circadian temperature and feeding rhythms in the squirrel monkey. *Physiology and Behavior* 36: 687–691.
488. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Feeding time synchronizes primate circadian rhythms. *Physiology and Behavior* 18: 775–779.
489. Green, J., Pollak, C. P., and Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
490. De Castro, J. M. (2001). Heritability of diurnal changes in food intake in free-living humans. *Nutrition* 17: 713–720.
491. De Castro, J. M. (1987). Circadian rhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology and Behavior* 40: 437–446.
492. Scheer, F. A. J., Morris, C. J., and Shea, S. A. (2013). The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. *Obesity* 21: 421–423.
493. Setas, C. D., Pinhão, S. C., Carvalho, D. M., Correia, F. C., and Medina, J. L. (2004). Avaliação da ingestão energética circadiana num grupo de trabalhadores da segurança social do Porto. *Acta Médica Portuguesa* 17: 417–426.
494. De Graaf, C., Jas, P., van der Kooy, K., and Leenen, R. (1993). Circadian rhythms of appetite at different stages of a weight loss programme. *International Journal of Obesity* 17: 521–526.
495. Waterhouse, J., Kao, S., Edwards, B., Weinert, D., Atkinson, G., and Reilly, T. (2005). Transient changes in the pattern of food intake following a simulated time-zone transition to the East across eight time zones. *Chronobiology International* 22: 299–319.
496. Gauvin, D. V., Baird, T. J., Vanacek, S. A., Briscoe, R. J., Vallett, M., and Holloway, F. A. (1997). Effects of time-of-day and photoperiod phase shifts on voluntary ethanol consumption in rats. *Alcoholism: Clinical and Experimental Research* 21: 817–825.
497. Van Reen, E., Rupp, T. L., Acebo, C., Seifer, R., and Carskadon, M. A. (2013). Biphasic effects of alcohol as a function of circadian phase. *Sleep* 36: 137–145.
498. Roelfsema, F., van der Heide, D., and Smeenk, D. (1980). Circadian rhythms of urinary electrolyte excretion in freely moving rats. *Life Sciences* 27: 2303–2309.
499. Schmidt, F., Yoshimura, Y., Ni, R. X., Kneesel, S., and Constantinou, C. E. (2001). Influence of gender on the diurnal variation of urine production and micturition characteristics of the rat. *Neurourology and Urodynamics* 20: 287–295.
500. Elliott, A. L., Mills, J. N., Minors, D. S., and Waterhouse, J. M. (1972). The effect of real and simulated time-zone shifts upon the circadian rhythms of body temperature, plasma 11-hydroxycorticosteroids, and renal excretion in human subjects. *Journal of Physiology* 221: 227–257.
501. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N., and Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.
502. Kräuchi, K. and Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *American Journal of Physiology* 267: R819–R829.
503. Le Marchand, L., Wilkens, L. R., Harwood, P., and Cooney, R. V. (1992). Use of breath hydrogen and methane as markers of colonic fermentation in epidemiologic studies: Circadian patterns of excretion. *Environmental Health Perspectives* 98: 199–202.
504. Aschoff, J. (1994). The timing of defecation within sleep-wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
505. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.
506. Hildebrandt, G., Strempel, H., and Will, H. D. (1992). Circadian variations of the subjective habituation to cold pain. *Journal of Interdisciplinary Cycle Research* 23: 222–224.
507. Koch, H. J. and Raschka, C. (2004). Diurnal variation of pain perception in young volunteers using the tourniquet pain model. *Chronobiology International* 21: 171–173.

508. Bellamy, N., Sothern, R. B. and Campbell, J. (2004). Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. *Journal of Rheumatology* 31: 379–389.
509. Barlow, R. B. (1983). Circadian rhythms in the *Limulus* visual system. *Journal of Neuroscience* 3: 856–870.
510. Barrozo, R. B., Minoli, S. A., and Lazzari, C. R. (2004). Circadian rhythm of behavioural responsiveness to carbon dioxide in the blood-sucking bug *Triatoma infestans* (Heteroptera: Reduviidae). *Journal of Insect Physiology* 50: 249–254.
511. Page, T. L. and Koelling, E. (2003). Circadian rhythm in olfactory response in the antennae controlled by the optic lobe in the cockroach. *Journal of Insect Physiology* 49: 697–707.
512. Chabot, C. C. and Taylor, D. H. (1992). Circadian modulation of the rat acoustic startle response. *Behavioral Neuroscience* 106: 846–852.
513. Bassi, C. J. and Powers, M. K. (1986). Daily fluctuations in the detectability of dim lights by humans. *Physiology and Behavior* 38: 871–877.
514. Nordin, S., Lotsch, J., Murphy, C., Hummel, T., and Kobal, G. (2003). Circadian rhythm and desensitization in chemosensory event-related potentials in response to odorous and painful stimuli. *Psychophysiology* 40: 612–619.
515. Carskadon, M. A., Saletin, J. M., Van Reen, E., Bartz, A., Hart, C., Raynor, H., and Herz, R. S. (2015). Circadian influences on smell and taste detection thresholds: Preliminary results from adolescents. *Sleep* 38: A67.
516. Pickard, G. E. (1987). Circadian rhythm of nociception in the golden hamster. *Brain Research* 425: 395–400.
517. Martínez-Gómez, M., Cruz, Y., Salas, M., Hudson, R., and Pacheco, P. (1994). Assessing pain threshold in the rat: Changes with estrus and time of day. *Physiology and Behavior* 55: 651–657.
518. Christina, A. J. M., Merlin, N. J., Vijaya, C., Jayaprakash, S., and Murugesh, N. (2004). Daily rhythm of nociception in rats. *Journal of Circadian Rhythms* 2: art. 2.
519. Cui, Y., Sugimoto, K., Araki, N., Sudoh, T., and Fujimura, A. (2005). Chronopharmacology of morphine in mice. *Chronobiology International* 22: 515–522.
520. Rosenfeld, J. P. and Rice, P. E. (1979). Diurnal rhythms in nociceptive thresholds of rats. *Physiology and Behavior* 23: 419–420.
521. Konecka, A. M. and Sroczynska, I. (1998). Circadian rhythm of pain in male mice. *General Pharmacology* 31: 809–810.
522. Hamra, J. G., Kamerling, S. G., Wolfsheimer, K. J., and Bagwell, C. A. (1993). Diurnal variation in plasma ir-β-endorphin levels and experimental pain thresholds in the horse. *Life Sciences* 53: 121–129.
523. Pöllmann, L. and Harris, P. H. P. (1978). Rhythmic changes in pain sensitivity in teeth. *International Journal of Chronobiology* 5: 459–464.
524. Perissin, L., Facchin, P., and Porro, C. A. (2003). Tonic pain response in mice: Effects of sex, season and time of day. *Life Sciences* 72: 897–907.
525. Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemistry and Physiology A* 77: 513–517.
526. Díaz-Iglesias, E., Díaz-Herrera, F., Re-Araujo, A. D., Báez-Hidalgo, M., López-Zenteno, M., Valdés-Sánchez, G., and López-Murillo, A. K. (2004). Temperatura preferida y consumo de oxígeno circadiano de la langosta roja, *Panulirus interruptus* (Randall, 1842). *Ciencias Marinas* 30: 169–178.
527. Grodzicki, P. and Caputa, M. (2005). Social versus individual behaviour: A comparative approach to thermal behaviour of the honeybee (*Apis mellifera* L.) and the American cockroach (*Periplaneta americana* L.). *Journal of Insect Physiology* 51: 315–322.
528. Kaneko, H., Head, L. M., Ling, J., Tang, X., Liu, Y., Hardin, P. E., Emery, P., and Hamada, F. N. (2012). Circadian rhythm of temperature preference and its neural control in *Drosophila*. *Current Biology* 22: 1851–1857.
529. Neill, W. H., Magnuson, J. J., and Chipman, G. G. (1972). Behavioral thermoregulation by fishes: A new experimental approach. *Science* 176: 1443–1445.
530. Beitinger, T. L. and Magnuson, J. J. (1975). Influence of social rank and size on thermoselection behavior of bluegill (*Lepomis macrochirus*). *Journal of the Fisheries Research Board of Canada* 32: 2133–2136.
531. Kavaliers, M. and Ralph, C. L. (1980). Pineal involvement in the control of behavioral thermoregulation of the white sucker, *Catostomus commersoni*. *Journal of Experimental Zoology* 212: 301–303.
532. Schurmann, H. and Christiansen, J. S. (1994). Behavioral thermoregulation and swimming activity of two arctic teleosts (subfamily Gadinae): The Polar cod (*Boreogadus saida*) and the navaga (*Eleginus navaga*). *Journal of Thermal Biology* 19: 207–212.
533. Innicenti, A., Minutini, L., and Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
534. Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. *Journal of Comparative Physiology A* 179: 135–142.
535. Sievert, L. M. and Hutchison, V. H. (1988). Light versus heat: Thermoregulatory behavior in a nocturnal lizard (*Gekko gecko*). *Herpetologica* 44: 266–273.
536. Brown, R. P. (1996). Thermal biology of the gecko *Tarentola boettgeri*: Comparisons among populations from different elevations within Gran Canaria. *Herpetologica* 52: 396–405.
537. Regal, P. J. (1967). Voluntary hypothermia in reptiles. *Science* 155: 1551–1553.
538. Cowgell, J. and Underwood, H. (1979). Behavioral thermoregulation in lizards: A circadian rhythm. *Journal of Experimental Zoology* 210: 189–194.
539. Rismiller, P. D. and Heldmaier, G. (1991). Seasonal changes in daily metabolic patterns of *Lacerta viridis*. *Journal of Comparative Physiology B* 161: 482–488.
540. Christian, K. A., Tracy, C. R., and Porter, W. P. (1985). Inter- and intra-individual variation in body temperatures of the Galapagos land iguana (*Conolophus pallidus*). *Journal of Thermal Biology* 10: 47–50.
541. Sievert, L. M. and Hutchison, V. H. (1989). Influences of season, time of day, light and sex on the thermoregulatory behaviour of *Crotaphytus collaris*. *Journal of Thermal Biology* 14: 159–165.
542. Jarling, C., Scarperi, M., and Bleichert, A. (1989). Circadian rhythm in the temperature preference of the turtle, *Chrysemys scripta elegans*, in a thermal gradient. *Journal of Thermal Biology* 14: 173–178.
543. Shine, R. and Lambeck, R. (1990). Seasonal shifts in the thermoregulatory behaviour of Australian blacksnakes, *Pseudechis porphyriacus* (Serpentes: Elapidae). *Journal of Thermal Biology* 15: 301–305.
544. Skinner, D. C. (1991). Effect of intraperitoneal melatonin injections on thermoregulation in the Transvaal girdled lizard, *Cordylus vittifer*. *Journal of Thermal Biology* 16: 179–184.

545. Sievert, L. M. and Paulissen, M. A. (1996). Temperature selection and thermoregulatory precision of bisexual and parthenogenetic *Cnemidophorus* lizards from southern Texas. *Journal of Thermal Biology* 21: 15–20.
546. Briese, E. (1985). Rats prefer ambient temperatures out of phase with their body temperature circadian rhythm. *Brain Research* 345: 389–393.
547. Gordon, C. J., Becker, P., and Ali, J. S. (1998). Behavioral thermoregulatory responses of single- and group-housed mice. *Physiology and Behavior* 65: 255–262.
548. Lee, H., Iida, T., Mizuno, A., Suzuki, M., and Caterina, M. J. (2005). Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. *Journal of Neuroscience* 25: 1304–1310.
549. Murakami, H. and Kinoshita, K. (1977). Spontaneous activity and heat avoidance of mice. *Journal of Applied Physiology* 43: 573–576.
550. Gordon, C. J. (1993). Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiology and Behavior* 53: 257–263.
551. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T., and Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *Journal of Physiology* 526: 417–424.
552. Beste, R., Kaiser, K., Issing, K., and Hensel, H. (1978). Perception of local thermal stimuli in the course of day. *Pflügers Archiv* 377(S): R56.
553. Baldwin, B. A. and Ingram, D. L. (1968). Factor influencing behavioral thermoregulation in the pig. *Physiology and Behavior* 3: 409–415.
554. Christian, K. and Bedford, G. (1996). Thermoregulation by the spotted tree monitor, *Varanus scalaris*, in the seasonal tropics of Australia. *Journal of Thermal Biology* 21: 67–73.
555. Meck, W. H. (2003). Introduction: The persistence of time. In: Meck, W. H. (Ed.), *Functional and Neural Mechanisms of Interval Timing*. Boca Raton, FL: CRC Press, pp. xvii–xli.
556. Kuriyama, K., Uchiyama, M., Suziki, H., Tagaya, H., Ozaki, A., Aritake, S., Kamei, Y., Nishikawa, T., and Takahashi, K. (2003). Circadian fluctuation of time perception in healthy human subjects. *Neuroscience Research* 46: 23–31.
557. Miró, E., Cano, M. C., Espinosa-Fernández, L., and Buela-Casal, G. (2003). Time estimation during prolonged sleep deprivation and its relation to activation measures. *Human Factors* 45: 148–159.
558. Campbell, S. S., Murphy, P. J., and Boothroyd, C. E. (2001). Long-term time estimation is influenced by circadian phase. *Physiology and Behavior* 72: 589–593.
559. Pati, A. K. and Gupta, S. (1994). Time estimation circadian rhythm in shift workers and diurnally active humans. *Journal of Biosciences* 19: 325–330.
560. Moussay, S., Dosseville, F., Gauthier, A., Larue, J., Sesboüe, B., and Davenne, D. (2002). Circadian rhythms during cycling exercise and finger-tapping task. *Chronobiology International* 19: 1137–1149.
561. Aschoff, J. (1992). On the dilatability of subjective time. *Perspectives in Biology and Medicine* 35(2): 276–280.
562. Aschoff, J. (1998). Human perception of short and long time intervals: Its correlation with body temperature and the duration of wake time. *Journal of Biological Rhythms* 13: 437–442.
563. Hills, T. T. (2003). Toward a unified theory of animal event timing. In: Meck, W. H. (Ed.), *Functional and Neural Mechanisms of Interval Timing*. Boca Raton, FL: CRC Press, pp. 77–111.
564. Lewis, P. A., Miall, R. C., Daan, S., and Kacelnik, A. (2003). Interval timing in mice does not rely upon the circadian pacemaker. *Neuroscience Letters* 348: 131–134.
565. Hancock, P. A. (1993). Body temperature influence on time perception. *Journal of General Psychology* 120: 197–215.
566. Wearden, J. H. and Penton-Voak, I. S. (1995). Feeling the heat: Body temperature and the rate of subjective time revisited. *Quarterly Journal of Experimental Psychology* 48B: 129–141.
567. Church, R. M. (2003). A concise introduction to scalar timing theory. In: Meck, W. H. (Ed.), *Functional and Neural Mechanisms of Interval Timing*. Boca Raton, FL: CRC Press, pp. 3–22.
568. Allman, M. J., Teki, S., Griffiths, T. D., and Meck, W. H. (2014). Properties of the internal clock: First- and second-order principles of subjective time. *Annual Review of Psychology* 65: 743–771.
569. Dragoi, V., Staddon, J. E. R., Palmer, R. G., and Buhusi, C. V. (2003). Interval timing as an emergent learning property. *Psychological Review* 110: 126–144.
570. Mauk, M. D. and Buonomano, D. V. (2004). The neural basis of temporal processing. *Annual Review of Neuroscience* 27: 307–340.
571. Crystal, J. D. (2003). Nonlinearities in sensitivity to time: Implications for oscillator-based representations of interval and circadian clocks. In: Meck, W. H. (Ed.), *Functional and Neural Mechanisms of Interval Timing*. Boca Raton, FL: CRC Press, pp. 61–76.
572. Valentinuzzi, V. S., Kolker, D. E., Vitaterna, M. H., Ferrari, E. A. M., Takahashi, J. S., and Turek, F. W. (2001). Effect of circadian phase on context and cued fear conditioning in C57BL/6J mice. *Animal Learning and Behavior* 29: 133–142.
573. Oklejewicz, M., van der Zee, E. A., Gerkema, M. P., and Daan, S. (2001). Memory retention in wild-type and tau mutant Syrian hamsters. *Behaviour* 138: 789–796.
574. Chaudhury, D. and Colwell, C. S. (2002). Circadian modulation of learning and memory in fear-conditioned mice. *Behavioural Brain Research* 133: 95–108.
575. Fernandez, R. I., Lyons, L. C., Levenson, J., Khabour, O., and Eskin, A. (2003). Circadian modulation of long-term sensitization in *Aplysia*. *Proceedings of the National Academy of Sciences United States of America* 100: 14415–14420.
576. Winocur, G. and Hasher, L. (2004). Age and time-of-day effects on learning and memory in a non-matching-to-sample test. *Neurobiology of Aging* 25: 1107–1115.
577. Wagatsuma, A., Sugai, R., Chono, K., Azami, S., Hatakeyama, D., Sadamoto, H., and Ito, E. (2004). The early snail acquires the learning: Comparison of scores for conditioned taste aversion between morning and afternoon. *Acta Biologica Hungarica* 55: 149–155.
578. Cajochen, C., Knoblauch, V., Wirz-Justice, A., Kräuchi, K., Graw, P., and Wallach, D. (2004). Circadian modulation of sequence learning under high and low speed pressure conditions. *Behavioural Brain Research* 151: 167–176.
579. Rawashdeh, O., de Borsetti, N. H., Roman, G., and Cahill, G. M. (2007). Melatonin suppresses nighttime memory formation in zebrafish. *Science* 318: 1144–1146.
580. Roces, F. (1995). Variable thermal sensitivity as output of a circadian clock controlling the bimodal rhythm of temperature choice in the ant *Camponotus mus*. *Journal of Comparative Physiology A* 177: 637–643.
581. Roces, F. and Núñez, J. A. (1996). A circadian rhythm of thermal preference in the ant *Camponotus mus*: Masking and entrainment by temperature cycles. *Physiological Entomology* 21: 138–142.

582. Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A. et al. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293: 1310–1314.
583. Bennett, K. A., McConnell, B. J., and Fedak, M. A. (2001). Diurnal and seasonal variations in the duration and depth of the longest dives in southern elephant seals (*Mirounga leonina*): Possible physiological and behavioral constraints. *Journal of Experimental Biology* 204: 649–662.
584. Kunnasranta, M., Hyvärinen, H., Häkkinen, J., and Koskela, J. T. (2002). Dive types and circadian behaviour patterns of Saimaa ringed seals *Phoca hispida saimensis* during the open-water season. *Acta Theriologica* 47: 63–72.
585. Handrich, Y., Bevan, R. M., Charrassin, J. B., Butler, P. J., Pütz, K., Woakes, A. J., Lage, J., and Le Maho, Y. (1997). Hypothermia in foraging king penguins. *Nature* 388: 64–67.
586. Butler, P. J. (2001). Diving beyond the limits. *News in Physiological Sciences* 16: 222–227.
587. Aarestrup, K., Okland, F., Hansen, M. M., Righton, D., Gargan, P., Castonguay, M., Bernatchez, L. et al. (2009). Oceanic spawning migration of the European eel (*Anguilla anguilla*). *Science* 325: 1660.
588. Fielder, D. P. (2012). Seasonal and diel dive performance and behavioral ecology of the bimodally respiring freshwater turtle *Myuchelys bellii* of eastern Australia. *Journal of Comparative Physiology A* 198: 129–143.
589. Filadelfi, A. M. C., Vieira, A., and Louzada, F. M. (2005). Circadian rhythm of physiological color change in the amphibian *Bufo ictericus* under different photoperiods. *Comparative Biochemistry and Physiology A* 142: 370–375.
590. Passano, L. M. and McCullough, C. B. (1964). Co-ordinating systems and behaviour in *Hydra*. I. Pacemaker system of the periodic contractions. *Journal of Experimental Biology* 41: 643–664.
591. Sakai, T. and Ishida, N. (2001). Circadian rhythms of female mating activity governed by clock genes in *Drosophila*. *Proceedings of the National Academy of Sciences United States of America* 98: 9221–9225.
592. Leon, M., Adels, L., Coopersmith, R., and Woodside, B. (1984). Diurnal cycle of mother-young contact in Norway rats. *Physiology and Behavior* 32: 999–1003.
593. Lerwill, C. J. (1977). Diurnal variations in the agonistic behaviour of the golden hamster (*Mesocricetus auratus*). *Journal of Zoology* 182: 159–161.
594. Pépin, D., Cargnelutti, B., Gonzalez, G., Joachim, J., and Reby, D. (2001). Diurnal and seasonal variations of roaring activity of farmed red deer stags. *Applied Animal Behaviour Science* 74: 233–239.
595. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M., and Czeisler, C. A. (1992). Short-term memory, alertness and performance: A reappraisal of their relationship to body temperature. *Journal of Sleep Research* 1: 24–29.
596. Van Dongen, H. P. A., Kerkhof, G. A., and Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
597. Kraemer, S., Danker-Hopfe, H., Dorn, H., Schmidt, A., Ehlert, I., and Herrmann, W. M. (2000). Time-of-day variations of indicators of attention: Performance, physiologic parameters, and self-assessment of sleepiness. *Biological Psychiatry* 48: 1069–1080.
598. De Gennaro, L., Ferrara, M., Curcio, G., and Bertini, M. (2001). Visual search performance across 40 h of continuous wakefulness: Measures of speed and accuracy and relation with oculomotor performance. *Physiology and Behavior* 74: 197–204.
599. Wright, K. P., Hull, J. T., and Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *American Journal of Physiology* 283: R1370–R1377.
600. Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M., and Tisak, J. (2002). Investigation of morning-evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.
601. Varkevisser, M. and Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: Validating a self-constructed test battery. *Chronobiology International* 20: 109–121.
602. Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A., and Cajochen, C. (2004). Circadian and wake-dependent modulation of fastest and slowest reaction times during psychomotor vigilance task. *Physiology and Behavior* 80: 695–701.
603. Cohen, D. A., Wang, W., Wyatt, J. K., Krinauer, R. E., Dijk, D. J., Czeisler, C. A., and Klerman, E. B. (2010). Uncovering residual effects of chronic sleep loss on human performance. *Science Translational Medicine* 2: 14ra3.
604. Youngstedt, S. D., Perlis, M. L., O'Brien, P. M., Palmer, C. R., Smith, M. T., Orff, H. J., and Kripke, D. F. (2003). No association of sleep with total daily physical activity in normal sleepers. *Physiology and Behavior* 78: 395–401.
605. Watson, D. and Clark, L. A. (1997). Measurement and mismeasurement of mood: Recurrent and emergent issues. *Journal of Personality Assessment* 68: 267–297.
606. Mitsutake, G., Otsuka, K., Cornélissen, G., Herold, M., Günther, R., Dawes, C., Burch, J. B., Watson, D., and Halberg, F. (2001). Circadian and infradian rhythms in mood. *Biomedicine and Pharmacotherapy* 55: 94–100.
607. Mitsutake, G., Cornélissen, G., Otsuka, K., Dawes, C., Burch, J., Rawson, M. J., Siegelová, J. et al. (2002). Relationship between positive and negative moods and blood pressure in a clinically healthy man. *Scripta Medica* 75: 315–320.
608. Golder, S. A. and Macy, M. W. (2011). Diurnal and seasonal mood vary with work, sleep, and daylength across diverse cultures. *Science* 333: 1878–1881.
609. Watson, D., Wiese, D., Vaidya, J., and Tellegen, A. (1999). The two general activation systems of affect: Structural findings, evolutionary considerations, and psychobiological evidence. *Journal of Personality and Social Psychology* 76: 820–838.
610. Murray, G., Allen, N. B., and Trinder, J. (2002). Mood and the circadian system: Investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
611. Murray, G., Nicholas, C. L., Kleiman, J., Dwyer, R., Carrington, M. J., Allen, N. B., and Trinder, J. (2009). Nature's clocks and human mood: The circadian system modulates reward motivation. *Emotion* 9: 705–716.
612. Kiley, J. P., Eldridge, F. L., and Millhorn, D. E. (1984). Brain, blood and rectal temperature during whole body cooling. *Comparative Biochemistry and Physiology A* 79: 631–634.
613. Brinnel, H. and Cabanac, M. (1989). Tympanic temperature is a core temperature in humans. *Journal of Thermal Biology* 14: 47–53.
614. Kolka, M. A., Quigley, M. D., Blanchard, L. A., Toyota, D. A., and Stephenson, L. A. (1993). Validation of a temperature telemetry system during moderate and strenuous exercise. *Journal of Thermal Biology* 18: 203–210.
615. Audet, D. and Thomas, D. W. (1996). Evaluation of the accuracy of body temperature measurement using external radio transmitters. *Canadian Journal of Zoology* 74: 1778–1781.

616. Fuller, A., Kamerman, P. R., Maloney, S. K., Mitchell, G., and Mitchell, D. (2003). Variability in brain and arterial blood temperatures in free-ranging ostriches in their natural habitat. *Journal of Experimental Biology* 206: 1171–1181.
617. Minard, D. (1970). Body heat content. In: Hardy, J. D., Gagge, A. P., and Stolwijk, J. A. J. (Eds.), *Physiological and Behavioral Temperature Regulation*. Springfield, IL: Charles C. Thomas, pp. 345–357.
618. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
619. Kluger, M. J., Conn, C. A., Franklin, B., Freter, R., and Abrams, G. D. (1990). Effect of gastrointestinal flora on body temperature of rats and mice. *American Journal of Physiology* 258: R552–R557.
620. Georgiev, J. (1978). Influence of environmental conditions and handling on the temperature rhythm of the rat. *Biotelemetry and Patient Monitoring* 5: 229–234.
621. Berkey, D. L., Meeuwsen, K. W., and Barney, C. C. (1990). Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. *American Journal of Physiology* 258: R743–R749.
622. Peloso, E., Wachulec, M., and Satinoff, E. (2002). Stress-induced hyperthermia depends on both time of day and light condition. *Journal of Biological Rhythms* 17: 164–170.
623. Bruguerolle, B. and Roucoules, X. (1994). Time-dependent changes in body temperature rhythm induced in rats by brewer's yeast injection. *Chronobiology International* 11: 180–186.
624. Yoshida, Y., Fujiki, N., Nakajima, T., Ripley, B., Matsumura, H., Yoneda, H., Mignot, E., and Nishino, S. (2001). Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light–dark cycle and sleep–wake activities. *European Journal of Neuroscience* 14: 1075–1081.
625. Refinetti, R. (1990). Experimentally induced disruption of the diurnal rhythm of body temperature of the rat. *Biotemas* 3(2): 47–58.
626. Fioretti, M. C., Riccardi, C., Menconi, E., and Martini, L. (1974). Control of the circadian rhythm of body temperature in the rat. *Life Sciences* 14: 2111–2119.
627. Halberg, F., Zander, H. A., Houglum, M. W., and Mühlemann, H. R. (1954). Daily variations in tissue mitoses, blood eosinophils and rectal temperatures of rats. *American Journal of Physiology* 177: 361–366.
628. Thornhill, J. A., Hirst, M., and Gowdey, C. W. (1978). Measurement of diurnal core temperatures of rats in operant cages by AM telemetry. *Canadian Journal of Physiology and Pharmacology* 56: 1047–1050.
629. Abrams, R. and Hammel, H. T. (1965). Cyclic variations in hypothalamic temperature in unanesthetized rats. *American Journal of Physiology* 208: 698–702.
630. Miles, G. H. (1962). Telemetering techniques for periodicity studies. *Annals of the New York Academy of Sciences* 98: 858–865.
631. Tanaka, H., Yanase, M., Kanosue, K., and Nakayama, T. (1990). Circadian variation of thermoregulatory responses during exercise in rats. *American Journal of Physiology* 258: R836–R841.
632. Roussel, B., Chouvet, G., and Debilly, G. (1976). Rythmes circadiens des températures internes et ambiance thermique chez le rat. *Pflügers Archiv* 365: 183–189.
633. Tornatzky, W. and Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior* 53: 983–993.
634. Yoda, T., Crawshaw, L. I., Yoshida, K., Su, L., Hosono, T., Shido, O., Sakurada, S., Fukuda, Y., and Kanosue, K. (2000). Effects of food deprivation on daily changes in body temperature and behavioral thermoregulation in rats. *American Journal of Physiology* 278: R133–R139.
635. Isobe, Y., Takaba, S., and Ohara, K. (1980). Diurnal variation of thermal resistance in rats. *Canadian Journal of Physiology and Pharmacology* 58: 1174–1179.
636. Stephenson, R., Liao, K. S., Hamrahi, H., and Horner, R. L. (2001). Circadian rhythms and sleep have additive effects on respiration in the rat. *Journal of Physiology* 536: 225–235.
637. Krieger, D. T. (1974). Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* 95: 1195–1201.
638. Murphy, H. M., Wideman, C. H., and Nadzam, G. R. (2003). A laboratory animal model of human shift work. *Integrative Physiological and Behavioral Science* 38: 316–328.
639. Refinetti, R., Ma, H., and Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
640. Li, H. and Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
641. Spencer, F., Shirer, H. W., and Yochim, J. M. (1976). Core temperature in the female rat: Effect of pinealectomy or altered lighting. *American Journal of Physiology* 231: 355–360.
642. Severinsen, T. and Øritsland, N. A. (1991). Endotoxin induced prolonged fever in rats. *Journal of Thermal Biology* 16: 167–171.
643. Smith, S. E., Ramos, F. A., Refinetti, R., Farthing, J. P., and Paterson, P. G. (2013). Protein-energy malnutrition induces an aberrant acute-phase response and modifies the circadian rhythm of core temperature. *Applied Physiology, Nutrition and Metabolism* 38: 844–853.
644. Maskrey, M., Wiggins, P. R., and Frappell, P. B. (2001). Behavioral thermoregulation in obese and lean Zucker rats in a thermal gradient. *American Journal of Physiology* 281: R1675–R1680.
645. Tsai, L., Tsai, Y., Huang, K., Huang, Y., and Tzeng, J. (2005). Repeated light–dark shifts speed up body weight gain in male F344 rats. *American Journal of Physiology* 289: E212–E217.
646. Leon, L. R., Walker, L. D., DuBose, D. A., and Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: Impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
647. Keeney, A. J., Hogg, S., and Marsden, C. A. (2001). Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. *Physiology and Behavior* 74: 177–184.
648. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D., and Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
649. Berezkin, M. V., Kudinova, V. F., Batygov, A. N., Ponomareva, L. E., and Zhukova, G. N. (1989). Effect of lighting conditions on circadian rhythm of rectal temperature in mice. *Bulletin of Experimental Biology and Medicine* 106: 1337–1340.
650. Conn, C. A., Franklin, B., Freter, R., and Kluger, M. J. (1991). Role of gram-negative and gram-positive gastrointestinal flora in temperature regulation of mice. *American Journal of Physiology* 261: R1358–R1363.
651. Connolly, M. S. and Lynch, C. B. (1983). Classical genetic analysis of circadian body temperature rhythms in mice. *Behavior Genetics* 13: 491–500.

652. Nomoto, S., Ohta, M., Kanai, S., Yoshida, Y., Takiguchi, S., Funakoshi, A., and Miyasaka, K. (2004). Absence of the cholecystokinin-A receptor deteriorates homeostasis of body temperature in response to changes in ambient temperature. *American Journal of Physiology* 287: R556–R561.
653. Gebczynski, A. K. (2005). Daily variation of thermoregulatory costs in laboratory mice selected for high and low basal metabolic rate. *Journal of Thermal Biology* 30: 187–193.
654. Gerhart-Hines, Z., Feng, D., Emmett, M. J., Everett, L. J., Loro, E., Briggs, E. R., Bugge, A. et al. (2013). The nuclear receptor Rev-erbα controls circadian thermogenic plasticity. *Nature* 503: 410–413.
655. Conti, B., Sanchez-Alavez, M., Winsky-Sommerer, R., Morale, M. C., Lucero, J., Brownell, S., Fabre, V. et al. (2006). Transgenic mice with a reduced core body temperature have an increased life span. *Science* 314: 825–828.
656. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
657. Chaudhry, A. P., Halberg, F., Keenan, C. E., Harner, R. N., and Bittner, J. J. (1958). Daily rhythms in rectal temperature and in epithelial mitoses of hamster pinna and pouch. *Journal of Applied Physiology* 12: 221–224.
658. Watts Jr., R. H. and Refinetti, R. (1996). Circadian modulation of cold-induced thermogenesis in the golden hamster. *Biological Rhythm Research* 27: 87–94.
659. Rubal, A., Choshniak, I., and Haim, A. (1992). Daily rhythms of metabolic rate and body temperature of two murids from extremely different habitats. *Chronobiology International* 9: 341–349.
660. Elvert, R., Kronfeld, N., Dayan, T., Haim, A., Zisapel, N., and Heldmaier, G. (1999). Telemetric field studies of body temperature and activity rhythms of *Acomys russatus* and *A. cahirinus* in the Judean Desert of Israel. *Oecologia* 119: 484–492.
661. Haim, A., Yedidia, I., Haim, D., and Zisapel, N. (1994). Photoperiodicity in daily rhythms of body temperature, food and energy intake of the golden spiny mouse (*Acomys russatus*). *Israel Journal of Zoology* 40: 145–150.
662. Haim, A. and Zisapel, N. (1995). Oxygen consumption and body temperature rhythms in the golden spiny mouse: Responses to changes in day length. *Physiology and Behavior* 58: 775–778.
663. Haim, A., McDevitt, R. M., and Speakman, J. R. (1995). Thermoregulatory responses to manipulations of photoperiod in wood mice *Apodemus sylvaticus* from high latitudes. *Journal of Thermal Biology* 20: 437–443.
664. Saarela, S. and Hissa, R. (1993). Metabolism, thermogenesis and daily rhythm of body temperature in the wood lemming, *Myopus schisticolor. Journal of Comparative Physiology B* 163: 546–555.
665. Halberg, E., Halberg, F., Timm, R. M., Regal, P. J., Cugigni, P., and de Remigis, P. (1982). Socially-related and spontaneous circadian thermo-acrophase shifts in *Rhabdomys pumilio*: Complications for chronopharmacologists. In: Takahashi, R., Halberg, F. and Walker, C. A. (Eds.), *Toward Chronopharmacology*. Oxford, U.K.: Pergamon, pp. 357–368.
666. Haim, A., Ellison, G. T. H., and Skinner, J. D. (1988). Thermoregulatory circadian rhythms in the pouched mouse (*Saccostomus campestris*). *Comparative Biochemistry and Physiology A* 91: 123–127.
667. Haim, A. (1996). Food and energy intake, non-shivering thermogenesis and daily rhythm of body temperature in the bushy-tailed gerbil *Sekeetamys calurus*: The role of photoperiod manipulations. *Journal of Thermal Biology* 21: 37–42.
668. Muchlinski, A. E., Baldwin, B. C., Padick, D. A., Lee, B. Y., Salguero, H. S., and Gramajo, R. (1998). California ground squirrel body temperature regulation patterns measured in the laboratory and in the natural environment. *Comparative Biochemistry and Physiology A* 120: 365–372.
669. Refinetti, R. (1996). The body temperature rhythm of the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus. Physiological Zoology* 69: 270–275.
670. Haim, A., Downs, C. T., and Raman, J. (2001). Effects of adrenergic blockade on the daily rhythms of body temperature and oxygen consumption of the black-tailed tree rat (*Thallomys nigricauda*) maintained under different photoperiods. *Journal of Thermal Biology* 26: 171–177.
671. Levy, O., Dayan, T., Rotics, S. and Kronfeld-Schor, N. (2012). Foraging sequence, energy intake and torpor: An individual-based field study of energy balancing in desert golden spiny mice. *Ecology Letters* 15: 1240–1248.
672. Lovegrove, B. G. (2009). Modification and miniaturization of Thermochron iButtons for surgical implantation into small animals. *Journal of Comparative Physiology B* 179: 451–458.
673. Gür, M. K., Bulut, S., Gür, H., and Refinetti, R. (2014). Body temperature patterns and use of torpor in an alpine glirid species, the woolly dormouse. *Acta Theriologica* 59: 299–309.
674. Long, R. A., Martin, T. J., and Barnes, B. M. (2005). Body temperature and activity patterns in free-living Arctic ground squirrels. *Journal of Mammalogy* 86: 314–322.
675. Williams, C. T., Sheriff, M. J., Schmutz, J. A., Kohl, F., Øivind, T., Buck, C. L., and Barnes, B. M. (2011). Data logging of body temperatures provides precise information on phenology of reproductive events in a free-ling Arctic hibernator. *Journal of Comparative Physiology B* 181: 1101–1109.
676. Scott, G. W., Fisher, K. C., and Love, J. A. (1974). A telemetric study of the abdominal temperature of a hibernator, *Spermophilus richardsonii*, maintained under constant conditions of temperature and light during the active season. *Canadian Journal of Zoology* 52: 653–658.
677. Gür, M. K., Refinetti, R., and Gür, H. (2009). Daily rhythmicity and hibernation in the Anatolian ground squirrel under natural and laboratory conditions. *Journal of Comparative Physiology B* 179: 155–164.
678. Winget, C. M., Card, D. H., and Hetherington, N. W. (1968). Circadian oscillations of deep-body temperature and heart rate in a primate (*Cebus albafrons*). *Aerospace Medicine* 39: 350–353.
679. Simpson, S. and Galbraith, J. J. (1906). Observations on the normal temperature of the monkey and its diurnal variation, and on the effect of changes in the daily routine on this variation. *Transactions of the Royal Society of Edinburgh* 45: 65–104.
680. Fuller, C. A. (1984). Circadian brain and body temperature rhythms in the squirrel monkey. *American Journal of Physiology* 246: R242–R246.
681. Fuller, C. A., Sulzman, F. M., and Moore-Ede, M. C. (1985). Role of heat loss and heat production in generation of the circadian temperature rhythm of the squirrel monkey. *Physiology and Behavior* 34: 543–546.
682. Fuller, C. A., Sulzman, F. M., and Moore-Ede, M. C. (1978). Thermoregulation is impaired in an environment without circadian time cues. *Science* 199: 794–796.
683. Hetherington, C. M. (1978). Circadian oscillations of body temperature in the marmoset, *Callithrix jacchus. Laboratory Animals* 12: 107–108.
684. Mitchell, D., Fuller, A., and Maloney, S. K. (2009). Homeothermy and primate bipedalism: Is water shortage or solar radiation the main threat to baboon (*Papio hamadryas*) homeothermy? *Journal of Human Evolution* 56: 439–446.

685. Wever, R. and Zink, R. A. (1971). Fortlaufende Registrierung der Rectaltemperatur des Menschen unter extremen Bedingungen. *Pflügers Archiv* 327: 186–190.
686. Stephenson, L. A., Wenger, C. B., O'Donovan, B. H., and Nadel, E. R. (1984). Circadian rhythm in sweating and cutaneous blood flow. *American Journal of Physiology* 246: R321–R324.
687. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.
688. Benedict, F. G. (1904). Studies in body temperature. I. Influence of the inversion of the daily routine; the temperature of night-workers. *American Journal of Physiology* 11: 145–169.
689. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
690. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
691. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K., and Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
692. Souetre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B., and Darcourt, G. (1988). Twenty-four-hour profiles of body temperature and plasma TSH in bipolar patients during depression and during remission and in normal control subjects. *American Journal of Psychiatry* 145: 1133–1137.
693. Scales, W. E., Vander, A. J., Brown, M. B., and Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
694. Marotte, H. and Timbal, J. (1981). Circadian rhythm of temperature in man: Comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
695. Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
696. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T., and Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
697. Kleitman, N. and Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
698. Cisse, F., Martineaud, R., and Martineaud, J. P. (1991). Circadian cycles of central temperature in hot climate in man. *Archives Internationales de Physiologie, de Biochimie et de Biophysique* 99: 155–159.
699. Shanahan, T. L. and Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *Journal of Clinical Endocrinology and Metabolism* 73: 227–235.
700. Honma, K., Honma, S., Kohsaka, M., and Fukuda, N. (1992). Seasonal variation in the human circadian rhythm: Dissociation between sleep and temperature rhythm. *American Journal of Physiology* 262: R885–R891.
701. Barrett, J., Lack, L., and Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
702. Lee, Y. H. and Tokura, H. (1993). Circadian rhythm of human rectal and skin temperatures under the influences of three different kinds of clothing. *Journal of Interdisciplinary Cycle Research* 24: 33–42.
703. Pollak, C. P. and Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
704. Honma, K., Honma, S., Nakamura, K., Sasaki, M., Endo, T., and Takahashi, T. (1995). Differential effects of bright light and social cues on reentrainment of human circadian rhythms. *American Journal of Physiology* 268: R528–R535.
705. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J., and Van Cauter, E. (1997). Sleepiness, performance, and neuroendocrine function during sleep deprivation: Effects of exposure to bright light or exercise. *Journal of Biological Rhythms* 12: 245–258.
706. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J. et al. (2001). Sleep, performance, circadian rhythms, and light–dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
707. Spengler, C. M., Czeisler, C. A., and Shea, S. A. (2000). An endogenous circadian rhythm of respiratory control in humans. *Journal of Physiology* 526: 683–694.
708. Gradisar, M. and Lack, L. (2004). Relationships between the circadian rhythms of finger temperature, core temperature, sleep latency, and subjective sleepiness. *Journal of Biological Rhythms* 19: 157–163.
709. Heikens, M. J., Gorbach, A. M., Eden, H. S., Savastano, D. M., Chen, K. Y., Skarulis, M. C., and Yanovski, J. A. (2011). Core body temperature in obesity. *American Journal of Clinical Nutrition* 93: 963–967.
710. Grimaldi, D., Provini, F., Pierangeli, G., Mazzella, N., Zamboni, G., Marchesini, G., and Cortelli, P. (2015). Evidence of a diurnal thermogenic handicap in obesity. *Chronobiology International* 32: 299–302.
711. Baker, F. C., Waner, J. I., Vieira, E. F., Taylor, S. R., Driver H. S., and Mitchell, D. (2001). Sleep and 24 hour body temperatures: A comparison in young men, naturally cycling women and women taking hormonal contraceptives. *Journal of Physiology* 530: 565–574.
712. Mackowiak, P. A., Wasserman, S. S., and Levine, M. M. (1992). A critical appraisal of 98.6°F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich. *Journal of the American Medical Association* 268: 1578–1580.
713. Shechter, A., Boudreau, P., Varin, F., and Boivin, D. B. (2011). Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. *Journal of Biological Rhythms* 26: 260–270.
714. Bratzke, D., Rolke, B., Ulrich, R., and Peters, M. (2007). Central slowing during the night. *Psychological Science* 18: 456–461.
715. Rawson, R. O., Stolwijk, J. A. J., Graichen, H., and Abrams, R. (1965). Continuous radio telemetry of hypothalamic temperatures from unrestrained animals. *Journal of Applied Physiology* 20: 321–325.
716. Refinetti, R. and Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.
717. Piccione, G., Giudice, E., Fazio, F., and Refinetti, R. (2011). Association between obesity and reduced body temperature in dogs. *International Journal of Obesity* 35: 1011–1018.
718. Piccione, G., Fazio, F., Giudice, E., and Refinetti, R. (2009). Body size and the daily rhythm of body temperature in dogs. *Journal of Thermal Biology* 34: 171–175.
719. Piccione, G., Caola, G., and Refinetti, R. (2005). Daily rhythms of blood pressure, heart rate, and body temperature in fed and fasted male dogs. *Journal of Veterinary Medicine A* 52: 377–381.

720. Kuwabara, N., Seki, K., and Aoki, K. (1986). Circadian, sleep and brain temperature rhythms in cats under sustained daily light-dark cycles and constant darkness. *Physiology and Behavior* 38: 283–289.
721. Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D., and Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflügers Archiv* 436: 659–666.
722. Ayo, J. O., Oladele, S. B., Ngam, S., Fayomi, A., and Afolayan, S. B. (1998). Diurnal fluctuations in rectal temperature of the Red Sokoto goat during the harmattan season. *Research in Veterinary Science* 66: 7–9.
723. Piccione, G., Caola, G., and Refinetti, R. (2003). Circadian rhythms of body temperature and liver function in fed and food-deprived goats. *Comparative Biochemistry and Physiology A* 134: 563–572.
724. Jessen, C. and Kuhnen, G. (1996). Seasonal variations of body temperature in goats living in an outdoor environment. *Journal of Thermal Biology* 21: 197–204.
725. Mphahlele, N. R., Fuller, A., Roth, J., and Kamerman, P. R. (2004). Body temperature, behavior, and plasma cortisol changes induced by chronic infusion of *Staphylococcus aureus* in goats. *American Journal of Physiology* 287: R863–R869.
726. Recabarren, S. E., Vergara, M., Llanos, A. J., and Serón-Ferré, M. (1987). Circadian variation of rectal temperature in newborn sheep. *Journal of Developmental Physiology* 9: 399–408.
727. Bligh, J., Ingram, D. L., Keynes, R. D., and Robinson, S. G. (1965). The deep body temperature of an unrestrained Welsh mountain sheep recorded by a radiotelemetric technique during a 12-month period. *Journal of Physiology* 176: 136–144.
728. Mohr, E. and Krzywanek, H. (1990). Variations of core-temperature rhythms in unrestrained sheep. *Physiology and Behavior* 48: 467–473.
729. Lowe, T. E., Cook, C. J., Ingram. J. R., and Harris, P. J. (2001). Impact of climate on thermal rhythm in pastoral sheep. *Physiology and Behavior* 74: 659–664.
730. Piccione, G., Caola, G., and Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
731. Piccione, G., Gianesella, M., Morgante, M., and Refinetti, R. (2013). Daily rhythmicity of core and surface temperatures of sheep kept under thermoneutrality or in the cold. *Research in Veterinary Science* 95: 261–265.
732. Piccione, G., Caola, G., and Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
733. Piccione, G., Caola, G., and Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
734. Refinetti, R. and Piccione, G. (2005). Intra- and inter-individual variability in the circadian rhythm of body temperature of rats, squirrels, dogs, and horses. *Journal of Thermal Biology* 30: 139–146.
735. Green, A. R., Gates, R. S., and Lawrence, L. M. (2005). Measurement of horse core body temperature. *Journal of Thermal Biology* 30: 370–377.
736. Smith, J. E., Barnes, A. L., and Maloney, S. K. (2006). A nonsurgical method allowing continuous core temperature monitoring in mares for extended periods, including during endurance exercise. *Equine Veterinary Journal Supplement* 36: 65–69.
737. Piccione, G., Giannetto, C., Bertolucci, C., and Refinetti, R. (2013). Daily rhythmicity of circulating melatonin is not endogenously generated in the horse. *Biological Rhythm Research* 44: 143–149.
738. Piccione, G., Caola, G., and Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
739. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L., and Bitman, J. (1999). Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. *Domestic Animal Endocrinology* 16: 41–55.
740. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A., and Littledike, E. T. (1990). Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. *Journal of Animal Science* 68: 2658–2665.
741. Hahn, G. L., Chen, Y. R., Nienaber, J. A., Eigenberg, R. A., and Parkhurst, A. M. (1992). Characterizing animal stress through fractal analysis of thermoregulatory responses. *Journal of Thermal Biology* 17: 115–120.
742. Vaidya, M. M., Kumar, P., and Singh, S. V. (2012). Circadian changes in heat storage and heat loss through sweating and panting in Karan Fries cattle during different seasons. *Biological Rhythm Research* 43: 137–146.
743. Rose, R. W., Swain, R., and Bryant, S. L. (1990). Body temperature: Rhythm and regulation in the Tasmanian bettong (*Bettongia gaimardi*) (Marsupialia: Potoroidae). *Comparative Biochemistry and Physiology A* 97: 573–576.
744. Gemmell, R. T., Turner, S. J., and Krause, W. J. (1997). The circadian rhythm of body temperature of four marsupials. *Journal of Thermal Biology* 22: 301–307.
745. Fowler, P. A. and Racey, P. A. (1990). Daily and seasonal cycles of body temperature and aspects of heterothermy in the hedgehog *Erinaceus europaeus*. *Journal of Comparative Physiology B* 160: 299–307.
746. McCarron, H. C. K., Buffenstein, R., Fanning, F. D., and Dawson, T. J. (2001). Free-ranging heart rate, body temperature and energy metabolism in eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*) in the arid regions of South East Australia. *Journal of Comparative Physiology B* 171: 401–411.
747. Cooper, C. E. and Withers, P. C. (2004). Patterns of body temperature variation and torpor in the numbat, *Myrmecobius fasciatus* (Marsupialia: Myrmecobiidae). *Journal of Thermal Biology* 29: 277–284.
748. Chevillard-Hugot, M. C., Müller, E. F., and Kulzer, E. (1980). Oxygen consumption, body temperature and heart rate in the coati (*Nasua nasua*). *Comparative Biochemistry and Physiology A* 65: 305–309.
749. Varosi, S. M., Brigmon, R. L., and Besch, E. L. (1990). A simplified telemetry system for monitoring body temperature in small animals. *Laboratory Animal Science* 40: 299–302.
750. Macari, M. (1983). Efeito do cruzamento de suínos sobre o comportamento termoregulador. *Ciência e Cultura* 35: 1145–1150.
751. Bligh, J. and Harthoorn, A. M. (1965). Continuous radiotelemetric records of the deep body temperature of some unrestrained African mammals under near-natural conditions. *Journal of Physiology* 176: 145–162.
752. Hildwein, G. and Kayser, C. (1970). Relation entre la température colonique et la consommation d'oxygène d'un insectivore, le Tenrec, au cours du nycthémère. *Comptes Rendus des Séances de la Societé de Biologie de Strasbourg* 164: 429–432.

753. Peters, D. G. and Rose, R. W. (1979). The oestrous cycle and basal body temperature in the common wombat (*Vombatus ursinus*). *Journal of Reproduction and Fertility* 57: 453–460.
754. Hanneman, S. K., McKay, K., Costas, G., and Rosenstrauch, D. (2005). Circadian temperature rhythm of laboratory swine. *Comparative Medicine* 55: 249–255.
755. Coolen, A., Hoffmann, K., Barf, R. P., Fuchs, E., and Meerlo, P. (2012). Telemetric study of sleep architecture and sleep homeostasis in the day-active tree shrew *Tupaia belangeri*. *Sleep* 35: 879–888.
756. Warnecke, L., Withers, P. C., Schleucher, E., and Maloney, S. K. (2007). Body temperature variation of free-ranging and captive southern brown bandicoots *Isoodon obesulus* (Marsupialia: Paramelidae). *Journal of Thermal Biology* 32: 72–77.
757. Busse, S., Lutter, D., Heldmaier, G., Jastroch, M., and Meyer, C. W. (2014). Torpor at high ambient temperature in a neotropical didelphid, the grey short-tailed opossum (*Monodelphis domestica*). *Naturwissenschaften* 101: 1003–1006.
758. Christian, N. and Geiser, F. (2007). To use or not to use torpor? Activity and body temperature predictors. *Naturwissenschaften* 94: 483–487.
759. El Allali, K., Achaâban, M. R., Bothorel, B., Piro, M., Bouâouda, H., El Allouchi, M., Ouassat, M., Malan, A., and Pévet, P. (2013). Entrainment of the circadian clock by daily ambient temperature cycles in the camel (*Camelus dromedarius*). *American Journal of Physiology* 304: R1044–R1052.
760. Rashotte, M. E. and Henderson, D. (1988). Coping with rising food costs in a closed economy: Feeding behavior and nocturnal hypothermia in pigeons. *Journal of the Experimental Analysis of Behavior* 50: 441–456.
761. Rashotte, M. E., Pastukhov, I. F., Poliakov, E. L., and Henderson, R. P. (1998). Vigilance states and body temperature during the circadian cycle in fed and fasted pigeons (*Columbia livia*). *American Journal of Physiology* 275: R1690–R1702.
762. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. I. Effector mechanisms. *Pflügers Archiv* 386: 173–179.
763. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
764. Bobr, L. W. and Sheldon, B. L. (1977). Analysis of ovulation-oviposition patterns in the domestic fowl by telemetry measurement of deep body temperature. *Australian Journal of Biological Sciences* 30: 243–257.
765. Kadono, H. and Besch, E. L. (1980). Influence of laying cycle on body temperature rhythm in the domestic hen. In: Tanabe, Y. (Ed.), *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin, Germany: Springer, pp. 91–99.
766. Wilson, H. R., Mather, F. B., Brigmon, R. L., Besch, E. L., Dugan, V. P., and Boulos, N. Z. (1989). Feeding time and body temperature interactions in broiler breeders. *Poultry Science* 68: 608–616.
767. Michels, H., Herremans, M., and Decuypere, E. (1985). Light–dark variations of oxygen consumption and subcutaneous temperature in young *Gallus domesticus*: Influence of ambient temperature and depilation. *Journal of Thermal Biology* 10: 13–20.
768. Smit, B. and McKechnie, A. E. (2010). Do owls use torpor? Winter thermoregulation in free-ranging pearl-spotted owlets and African scops-owls. *Physiological and Biochemical Zoology* 83: 149–156.
769. Thouzeau, C., Duchamp, C., and Handrich, Y. (1999). Energy metabolism and body temperature of barn owls fasting in the cold. *Physiological and Biochemical Zoology* 72: 170–178.
770. Winget, C. M., Averkin, E. G., and Fryer, T. B. (1965). Quantitative measurement by telemetry of ovulation and oviposition in the fowl. *American Journal of Physiology* 209: 853–858.
771. MacMillen, R. E. and Trost, C. H. (1967). Nocturnal hypothermia in the Inca dove, *Scardafella inca*. *Comparative Biochemistry and Physiology* 23: 243–253.
772. Pickard, G. E., Kahn, R., and Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiology and Behavior* 32: 763–766.
773. Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429.
774. Eastman, C. and Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
775. Deprés-Brummer, P., Metzger, G., and Lévi, F. (1996). Analyses des rythmes de la température corporelle du rat en libre cours. *Pathologie Biologie* 44: 150–156.
776. Kas, M. J. H. and Edgar, D. M. (2000). Photic phase response curve in *Octodon degus*: Assessment as a function of activity phase preference. *American Journal of Physiology* 278: R1385–R1389.
777. Halberg, F. and Visscher, M. B. (1954). Some physiologic effects of lighting. In: Tenobergen, J. E. (Ed.), *Proceedings of the First International Photobiological Congress*. Wageningen, the Netherlands: Veenman and Zonen, pp. 396–398.
778. Honnebier, M. B. O. M., Jenkins, S. L., and Nathanielsz, P. W. (1992). Circadian timekeeping during pregnancy: Endogenous phase relationships between maternal plasma hormones and the maternal body temperature rhythm in pregnant rhesus monkeys. *Endocrinology* 131: 2051–2058.
779. Reinberg, A., Halberg, F., Ghata, J., and Siffre, M. (1966). Spectre thermique (rythmes de la température rectale) d'une femme adulte avant, pendant et après son isolement souterrain de trois mois. *Comptes Rendus de l'Académie des Sciences* 262: 782–785.
780. Colin, J., Timbal, J., Boutelier, C., Houdas, Y., and Siffre, M. (1968). Rhythm of the rectal temperature during a 6-month free-running experiment. *Journal of Applied Physiology* 25: 170–176.
781. Menaker, M. (1959). Endogenous rhythms of body temperature in hibernating bats. *Nature* 184: 1251–1252.
782. Menaker, M. (1961). The free running period of the bat clock: Seasonal variations at low body temperature. *Journal of Cellular and Comparative Physiology* 57: 81–86.
783. Jilge, B., Kuhnt, B., Landerer, W., and Rest, S. (2001). Circadian temperature rhythms in rabbit pups and in their does. *Laboratory Animals* 35: 364–373.
784. Kramer, K., Grimbergen, J., Brockway, B., Brockway, B., and Voss, H. P. (1999). Circadian respiratory rate rhythms in freely moving small laboratory animals using radio telemetry. *Lab Animal* 28(4): 38–41.
785. Witte, K., Engelhardt, S., Janssen, B. J. A., Lohse, M., and Lemmer, B. (2004). Circadian and short-term regulation of blood pressure and heart rate in transgenic mice with cardiac overexpression of the β_1-adrenoceptor. *Chronobiology International* 21: 205–216.
786. Buenafe, A. C., Zwickey, H., Moes, N., Oken, B., and Jones, R. E. (2008). A telemetric study of physiologic changes in mice with induced autoimmune encephalomyelitis. *Lab Animal* 37: 361–368.

787. Henry, R., Casto, R., and Printz, M. P. (1990). Diurnal cardiovascular patterns in spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* 16: 422–428.
788. Smith, T. L., Coleman, T. G., Stanek, K. A., and Murphy, W. R. (1987). Hemodynamic monitoring for 24 h in unanesthetized rats. *American Journal of Physiology* 253: H1335–H1341.
789. Su, D. F., Julien, C., Kandza, P., and Sassard, J. (1987). Variation circadienne de la sensibilité du baroréflexe chez le rat conscient. *Comptes Rendus de l'Académie des Sciences* 305: 683–686.
790. Brown, G. M. and Seggie, J. (1988). Effects of antidepressants on entrainment of circadian rhythms. *Progress in Neuropsychopharmacology and Biological Psychiatry* 12: 299–306.
791. Sato, K., Chatani, F., and Sato, S. (1995). Circadian and short-term variabilities in blood pressure and heart rate measured by telemetry in rabbits and rats. *Journal of the Autonomic Nervous System* 54: 235–246.
792. Halberg, J., Halberg, E., Hayes, D. K., Smith, R. D., Halberg, F., Delea, C. S., Danielson, R. S., and Bartter, F. C. (1979). Schedule shifts, life quality and quantity modeled by murine blood pressure elevation and arthropod life span. *International Journal of Chronobiology* 7: 17–64.
793. Scheer, F. A. J. L., Ter Horst, G. J., van der Vliet, J. and Buijs, R. M. (2001). Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *American Journal of Physiology* 280: H1391–H1399.
794. Lemmer, B., Schiffer, S., Witte, K., and Gorbey, S. (2005). Inverse blood pressure rhythm of transgenic hypertensive TGR(mREN2)27 rats: Role of norepinephrine and expression of tyrosine-hydroxylase and reuptake$_1$-transporter. *Chronobiology International* 22: 473–488.
795. Matsunaga, T., Harada, T., Mitsui, T., Inokuma, M., Hashimoto, M., Miyauchi, M., Murano, H., and Shibutani, Y. (2001). Spectral analysis of circadian rhythms in heart rate variability of dogs. *American Journal of Veterinary Research* 62: 37–42.
796. Ashkar, E. (1979). Twenty-four-hour pattern of circulation by radiotelemetry in the unrestrained dog. *American Journal of Physiology* 236: R231–R236.
797. Mishina, M., Watanabe, T., Matsuoka, S., Shibata, K., Fujii, K., Maeda, H., and Wakao, Y. (1999). Diurnal variations of blood pressure in dogs. *Journal of Veterinary Medical Sciences* 61: 643–647.
798. Nolan, E. R., Girand, M., Bailie, M., and Yeragani, V. K. (2004). Circadian changes in the QT variability index in the Beagle dog. *Clinical and Experimental Pharmacology and Physiology* 31: 783–785.
799. Eloranta, E., Norberg, H., Nilsson, A., and Säkkinen, H. (2002). Individually coded telemetry: A tool for studying heart rate and behaviour in reindeer calves. *Acta Veterinaria Scandinavica* 43: 135–144.
800. Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K., and Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s–44s.
801. Scheer, F. A. J. L., van Doornen, L. J. P., and Buijs, R. M. (1999). Light and diurnal cycle affect human heart rate: Possible role for the circadian pacemaker. *Journal of Biological Rhythms* 14: 202–212.
802. Hermida, R. C., Ayala, D. E., Fernández, J. R., Mojón, A., Alonso, I., and Calvo, C. (2002). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiology International* 19: 461–481.
803. Kumagai, Y., Shiga, T., Sunaga, K., Cornélissen, G., Ebihara, A., and Halberg, F. (1992). Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 19: 43–58.
804. Leary, A. C., Struthers, A. D., Donnan, P. T., MacDonald, T. M., and Murphy, M. B. (2002). The morning surge in blood pressure and heart rate is dependent on levels of physical activity after waking. *Journal of Hypertension* 20: 865–870.
805. Viola, A. U., Simon, C., Ehrhart, J., Geny, B., Piquard, F., Muzet, A., and Brandenberger, G. (2002). Sleep processes exert a predominant influence on the 24-h profile of heart rate variability. *Journal of Biological Rhythms* 17: 539–547.
806. O'Sullivan, C., Duggan, J., Atkins, N., and O'Brien, E. (2003). Twenty-four-hour ambulatory blood pressure in community-dwelling elderly men and women aged 60–102 years. *Journal of Hypertension* 21: 1641–1647.
807. Van Eekelen, A. P. J., Houtveen, J. H., and Kerkhof, G. A. (2004). Circadian variation in cardiac autonomic activity: Reactivity measurements to different types of stressors. *Chronobiology International* 21: 107–129.
808. Hermida, R. C., Fernández, J. R., Ayala, D. E., Mojón, A., Alonso, I., and Calvo, C. (2004). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiology International* 21: 147–160.
809. Hadtstein, C., Wühl, E., Soergel, M., Witte, K., and Schaefer, F. (2004). Normative values for circadian and ultradian cardiovascular rhythms in childhood. *Hypertension* 43: 547–554.
810. Taillard, J., Sanchez, P., Lemoine, P., and Mouret, J. (1990). Heart rate circadian rhythm as a biological marker of desynchronization in major depression: A methodological and preliminary report. *Chronobiology International* 7: 305–316.
811. Hermida, R. C., Calvo, C., Ayala, D. E., Domínguez, M. J., Covelo, M., Fernández, J. R., Fontao, M. J., and López, J. E. (2004). Administration-time-dependent effects of doxazosin GITS on ambulatory blood pressure of hypertensive subjects. *Chronobiology International* 21: 277–296.
812. Thomas, C., Wood, G. C., Langer, R. D., and Stewart, W. F. (2008). Elevated blood pressure in primary care varies in relation to circadian and seasonal changes. *Journal of Human Hypertension* 22: 755–760.
813. Hu, K., Ivanov, P. C., Hilton, M. F., Chen, Z., Ayers, R. T., Stanley, H. E., and Shea, S. A. (2004). Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. *Proceedings of the National Academy of Sciences United States of America* 101: 18223–18227.
814. Sun, X., Liu, T., Deng, J., and Borjigin, J. (2003). Long-term in vivo pineal microdialysis. *Journal of Pineal Research* 35: 118–124.
815. Liu, T. and Borjigin, J. (2005). N-acetyltransferase is not the rate-limiting enzyme of melatonin synthesis at night. *Journal of Pineal Research* 39: 91–96.
816. Liu, T. and Borjigin, J. (2006). Relationship between nocturnal serotonin surge and melatonin onset in rodent pineal gland. *Journal of Circadian Rhythms* 4: art. 12.
817. Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K., and Tabata, M. (2003). Variations in plasma melatonin levels of the rainbow trout (*Oncorhynchus mykiss*) under various light and temperature conditions. *Zoological Science* 20: 1011–1016.
818. Strand, J. E. T., Aarseth, J. J., Hanebrekke, T. L., and Jørgensen, E. H. (2008). Keeping track of time under ice and snow in a sub-arctic lake: Plasma melatonin rhythms in Arctic charr overwintering under natural conditions. *Journal of Pineal Research* 44: 227–233.

819. Rahman, M. S., Kim, B. H., Takemura, A., Park, C. B., and Lee, Y. D. (2004). Effects of moonlight exposure on plasma melatonin rhythms in the seagrass rabbitfish, *Siganus canaliculatus*. *Journal of Biological Rhythms* 19: 325–334.
820. Jessop, T. S., Limpus, C. J., and Whittier, J. M. (2002). Nocturnal activity in the green sea turtle alters daily profiles of melatonin and corticosterone. *Hormones and Behavior* 41: 357–365.
821. Bertolucci, C., Foà, A., and Van't Hof, T. J. (2002). Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. *Hormones and Behavior* 41: 414–419.
822. Hasegawa, M. and Ebihara, S. (1992). Circadian rhythms of pineal melatonin release in the pigeon measured by in vivo microdialysis. *Neuroscience Letters* 148: 89–92.
823. Brandstätter, R., Kumar, V., Abraham, U., and Gwinner, E. (2000). Photoperiodic information acquired and stored *in vivo* retained *in vitro* by a circadian oscillator, the avian pineal gland. *Proceedings of the National Academy of Sciences United States of America* 97: 12324–12328.
824. Zawilska, J. B., Lorenc, A., Berezinska, M., Vivien-Roels, B., Pévet, P., and Skene, D. J. (2006). Diurnal and circadian rhythms in melatonin synthesis in the turkey pineal gland and retina. *General and Comparative Endocrinology* 145: 162–168.
825. Thrun, L. A., Moenter, S. M., O'Callaghan, D., Woodfill, C. J. I., and Karsch, F. J. (1995). Circannual alterations in the circadian rhythm of melatonin secretion. *Journal of Biological Rhythms* 10: 42–54.
826. Guerin, M. V., Deed, J. R., Kennaway, D. J., and Matthews, C. D. (1995). Plasma melatonin in the horse: Measurements in natural photoperiod and in acutely extended darkness throughout the year. *Journal of Pineal Research* 19: 7–15.
827. Aarseth, J. J., Van't Hof, T. J., and Stokkan, K. A. (2003). Melatonin is rhythmic in newborn seals exposed to continuous light. *Journal of Comparative Physiology B* 173: 37–42.
828. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2001). Seasonal variation in endogenous serum melatonin profiles in goats: A difference between spring and fall? *Journal of Biological Rhythms* 16: 254–263.
829. García, A., Landete-Castillejos, T., Zarazaga, L., Garde, J., and Gallego, L. (2003). Seasonal changes in melatonin concentrations in female Iberian red deer (*Cervus elaphus hispanicus*). *Journal of Pineal Research* 34: 161–166.
830. Andersson, H., Johnston, J. D., Messager, S., Hazlerigg, D., and Lincoln, G. (2005). Photoperiod regulates clock gene rhythms in the ovine liver. *General and Comparative Endocrinology* 142: 357–363.
831. Haritou, S. J. A., Zylstra, R., Ralli, C., Turner, S., and Tortonese, D. J. (2008). Seasonal changes in circadian peripheral plasma concentrations of melatonin, serotonin, dopamine and cortisol in aged horses with Cushing's disease under natural photoperiod. *Journal of Neuroendocrinology* 20: 988–996.
832. Kilmer, D. M., Sharp, D. C., Berglund, L. A., Grubaugh, W., McDowell, K. J., and Peck, L. S. (1982). Melatonin rhythms in Pony mares and foals. *Journal of Reproduction and Fertility Supplement* 32: 303–307.
833. Murphy, B. A., Martin, A. M., Furney, P., and Elliott, J. A. (2011). Absence of a serum melatonin rhythm under acutely extended darkness in the horse. *Journal of Circadian Rhythms* 9: art. 3.
834. Stokkan, K. A., van Oort, B. E. H., Tyler, N. J. C., and Loudon, A. S. I. (2007). Adaptations for life in the Arctic: Evidence that melatonin rhythms in reindeer are not driven by a circadian oscillator but remain acutely sensitive to environmental photoperiod. *Journal of Pineal Research* 43: 289–293.
835. Lu, W., Meng, Q. J., Tyler, N. J. C., Stokkan, K. A., and Loudon, A. S. I. (2010). A circadian clock is not required in an Arctic mammal. *Current Biology* 20: 533–537.
836. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J., and Simonneaux, V. (2003). Mechanisms regulating the marked seasonal variation in melatonin synthesis in the European hamster pineal gland. *American Journal of Physiology* 284: R1043–R1052.
837. Maywood, E. S., Hastings, M. H., Max, M., Ampleford, E., Menaker, M., and Loudon, A. S. I. (1993). Circadian and daily rhythms of melatonin in the blood and pineal gland of free-running and entrained Syrian hamsters. *Journal of Endocrinology* 136: 65–73.
838. Pitrosky, B., Kirsch, R., Vivien-Roels, B., Georg-Bentz, I., Canguilhem, B., and Pévet, P. (1995). The photoperiodic response in Syrian hamster depends upon a melatonin-driven circadian rhythm of sensitivity to melatonin. *Journal of Neuroendocrinology* 7: 889–895.
839. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N., and Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
840. Vivien-Roels, B., Malan, A., Rettori, M. C., Delagrange, P., Jeanniot, J. P., and Pévet, P. (1998). Daily variations in pineal melatonin concentrations in inbred and outbred mice. *Journal of Biological Rhythms* 13: 403–409.
841. Nakahara, D., Nakamura, M., Iigo, M., and Okamura, H. (2003). Bimodal circadian secretion of melatonin from the pineal gland in a living CBA mouse. *Proceedings of the National Academy of Sciences United States of America* 100: 9584–9589.
842. Stepien, J. M. and Kennaway, D. J. (2001). Phase response relationships between light pulses and melatonin rhythms in rats. *Journal of Biological Rhythms* 16: 234–242.
843. Challet, E., Pévet, P., Vivien-Roels, B., and Malan, A. (1997). Phase-advanced daily rhythms of melatonin, body temperature, and locomotor activity in food-restricted rats fed during daytime. *Journal of Biological Rhythms* 12: 65–79.
844. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M., and Honma, K. (2000). Clock genes outside the suprachiasmatic nucleus involved in manifestation of locomotor activity rhythm in rats. *European Journal of Neuroscience* 12: 4206–4214.
845. Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., van der Vliet, J., van Heijningen, C., Simonneaux, V., Pévet, P., and Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: Inhibitory and stimulatory mechanisms. *European Journal of Neuroscience* 17: 221–228.
846. McEachron, D. L., Kripke, D. F., Sharp, F. R., Lewy, A. J., and McClellan, D. E. (1985). Lithium effects on selected circadian rhythms in rats. *Brain Research Bulletin* 15: 347–350.
847. Barassin, S., Saboureau, M., Kalsbeek, A., Bothorel, B., Vivien-Roels, B., Malan, A. Buijs, R. M., Guardiola-Lemaitre, B., and Pévet, P. (1999). Interindividual differences in the pattern of melatonin secretion of the Wistar rat. *Journal of Pineal Research* 27: 193–201.
848. Vasicek, C. A., Malpaux, B., Fleming, P. A., and Bennett, N. C. (2005). Melatonin secretion in the Mashona mole-rat, *Cryptomys darlingi*: Influence of light on rhythmicity. *Physiology and Behavior* 83: 689–697.
849. Fukuhara, C., Aguzzi, J., Bullock, N., and Tosini, G. (2005). Effect of long-term exposure to constant dim light on the circadian system of rats. *Neurosignals* 14: 117–125.

850. Martin, S. K. and Eastman, C. I. (2002). Sleep logs of young adults with self-selected sleep times predict the dim light melatonin onset. *Chronobiology International* 19: 695–707.
851. Voultsios, A., Kennaway, D. J., and Dawson, D. (1997). Salivary melatonin as a circadian phase marker: Validation and comparison to plasma melatonin. *Journal of Biological Rhythms* 12: 457–466.
852. Wright, K. P., Hughes, R. J., Kronauer, R. E., Dijk, D. J., and Czeisler, C. A. (2001). Intrinsic near-24-h pacemaker period determines limits of circadian entrainment to a weak synchronizer in humans. *Proceedings of the National Academy of Sciences United States of America* 98: 14027–14032.
853. Jelínková-Vondrasová, D., Hájek, I., and Illnerová, H. (1999). Adjustment of the human circadian system to changes of the sleep schedule under dim light at home. *Neuroscience Letters* 265: 111–114.
854. Lindblom, N., Heiskala, H., Hätönen, T., Mustanoja, S., Alfthan, H., Alila-Johansson, A., and Laakso, M. L. (2000). No evidence for extraocular light induced phase shifting of human melatonin, cortisol and thyrotropin rhythms. *NeuroReport* 11: 713–717.
855. Roden, M., Koller, M., Pirich, K., Vierhapper, H., and Waldhauser, F. (1993). The circadian melatonin and cortisol secretion pattern in permanent night shift workers. *American Journal of Physiology* 265: R261–R267.
856. Whitson, P. A., Putcha, L., Chen, Y. M., and Baker, E. (1995). Melatonin and cortisol assessment of circadian shifts in astronauts before flight. *Journal of Pineal Research* 18: 141–147.
857. Rajaratnam, S. M. W., Dijk, D. J., Middleton, B., Stone, B. M., and Arendt, J. (2003). Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *Journal of Clinical Endocrinology and Metabolism* 88: 4303–4309.
858. Selmaoui, B. and Touitou, Y. (2003). Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: A study of three different 24-h cycles over six weeks. *Life Sciences* 73: 3339–3349.
859. Cutolo, M., Maestroni, G. J. M., Otsa, K., Aakre, O., Villaggio, B., Capellino, S., Montagna, P. et al. (2005). Circadian melatonin and cortisol levels in rheumatoid arthritis patients in winter time: A north and south Europe comparison. *Annals of the Rheumatic Diseases* 64: 212–216.
860. Goichot, B., Weibel, L., Chapotot, F., Gronfier, C., Piquard, F., and Brandenberger, G. (1998). Effect of the shift of the sleep–wake cycle on three robust endocrine markers of the circadian clock. *American Journal of Physiology* 275: E243–E248.
861. Kelly, T. L., Neri, D. F., Grill, J. T., Ryman, D., Hunt, P. D., Dijk, D. J., Shanahan, T. L., and Czeisler, C. A. (1999). Nonentrained circadian rhythms of melatonin in submariners scheduled to an 18-hour day. *Journal of Biological Rhythms* 14: 190–196.
862. Hack, L. M., Lockley, S. W., Arendt, J., and Skene, D. J. (2003). The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects. *Journal of Biological Rhythms* 18: 420–429.
863. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M., and Loudon, A. S. I. (1999). Free running circadian rhythms of melatonin, luteinizing hormone, and cortisol in Syrian hamsters bearing the circadian *tau* mutation. *Endocrinology* 140: 758–764.
864. Frey, S., Birchler-Pedross, A., Hostetter, M., Brunner, P., Götz, T., Müunch, M., Blatter, K., Knoblauch, V., Wirz-Justice, A., and Cajochen, C. (2012). Young women with major depression live on higher homeostatic sleep pressure than healthy controls. *Chronobiology International* 29: 278–294.
865. Cain, S. W., Dennison, C. F., Zeitzer, J. M., Guzik, A. M., Khalsa, S. B. S., Santhi, N., Schoen, M. W., Czeisler, C. A., and Duffy, J. F. (2010). Sex differences in phase angle of entrainment and melatonin amplitude in humans. *Journal of Biological Rhythms* 25: 288–296.
866. Benloucif, S., Burgess, H. J., Klerman, E. B., Lewy, A. J., Middleton, B., Murphy, P. J., Parry, B. L., and Revell, V. L. (2008). Measuring melatonin in humans. *Journal of Clinical Sleep Medicine* 4: 66–69.
867. Lim, A. S. P., Chang, A. M., Shulman, J. M., Raj, T., Chibnik, L. B., Cain, S. W., Benoist, C. et al. (2012). A common polymorphism near *PER1* and the timing of human behavioral rhythms. *Annals of Neurology* 72: 324–334.
868. Klerman, E. B., Gershengorn, H. B., Duffy, J. F., and Kronauer, R. E. (2002). Comparisons of the variability of three markers of the human circadian pacemaker. *Journal of Biological Rhythms* 17: 181–193.
869. Sarabia, J. A., Rol, M. A., Mendiola, P., and Madrid, J. A. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate for a new index of the circadian system. *Physiology and Behavior* 95: 570–580.
870. Bonmati-Carrion, M. A., Middleton, B., Revell, V., Skene, D. J., Rol, M. A., and Madrid, J. A. (2014). Circadian phase assessment by ambulatory monitoring in humans: Correlation with dim light melatonin onset. *Chronobiology International* 31: 37–51.
871. Barrett, K. E., Barman, S. M., Boitano, S., and Brooks, H. L. (2012). *Ganong's Review of Medical Physiology*, 24th edn. New York: McGraw-Hill.
872. Melmed, S., Polonsky, K. S., Larsen, P. R., and Kronenberg, H. M. (Eds.) (2011). *Williams Textbook of Endocrinology*, 12th edn. Philadelphia, PA: Saunders.
873. Irvine, C. H. G. and Alexander, S. L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology* 11: 227–238.
874. Gordon, M. E. and McKeever, K. H. (2005). Diurnal variation of ghrelin, leptin, and adiponectin in Standardbred mares. *Journal of Animal Science* 83: 2365–2371.
875. Suzuki, M., Uchida, S., Ueda, K., Tobayama, T., Katsumata, E., Yoshioda, M., and Aida, K. (2003). Diurnal and annual changes in serum cortisol concentrations in Indo-Pacific bottlenose dolphins *Tursiops aduncus* and killer whales *Orcinus orca*. *General and Comparative Endocrinology* 132: 427–433.
876. Greco, A. M., Gambardella, P., Sticchi, R., D'Aponte, D., di Renzo, G., and de Franciscis, P. (1989). Effects of individual housing on circadian rhythms of adult rats. *Physiology and Behavior* 45: 363–366.
877. Marcilhac, A., Maurel, D., Anglade, G., Ixart, G., Mekaouche, M., Héry, F., and Siaud, P. (1997). Effects of bilateral olfactory bulbectomy on circadian rhythms of ACTH, corticosterone, motor activity and body temperature in male rats. *Archives of Physiology and Biochemistry* 105: 552–559.
878. Kalsbeek, A., Fliers, E., Romijn, J. A., la Fleur, S. E., Wortel, J., Bakker, O., Endert, E., and Buijs, R. M. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685.
879. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O., and Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
880. Ruiter, M., La Fleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A., and Buijs, R. M. (2003). The daily rhythm in plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. *Diabetes* 52: 1709–1715.

881. Mitome, M., Honma, S., Yoshihara, T., and Honma, K. (1994). Prefeeding increase in paraventricular NE release is regulated by a feeding-associated rhythm in rats. *American Journal of Physiology* 266: E606–E611.
882. Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J., and Buijs, R. M. (2000). Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology* 141: 3832–3841.
883. La Fleur, S. E., Kalsbeek, A., Wortel, J., and Buijs, R. M. (1999). A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *Journal of Neuroendocrinology* 11: 643–652.
884. Cailotto, C., La Fleur, S. E., Van Heijningen, C., Wortel, J., Kalsbeek, A., Feenstra, M., Pévet, P., and Buijs, R. M. (2005). The suprachiasmatic nucleus controls the daily variation of plasma glucose via the autonomic output to the liver: Are the clock genes involved? *European Journal of Neuroscience* 22: 2531–2540.
885. Cavigelli, S. A., Monfort, S. L., Whitney, T. K., Mechref, Y. S., Novotny, M., and McClintock, M. K. (2005). Frequent serial fecal corticoid measures from rats reflect circadian and ovarian corticosterone rhythms. *Journal of Endocrinology* 184: 153–163.
886. Zvonic, S., Ptitsyn, A. A., Conrad, S. A., Scott, L. K., Floyd, Z. E., Kilroy, G., Wu, X., Goh, B. C., Mynatt, R. L., and Gimble, J. M. (2006). Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* 55: 962–970.
887. Halberg, F., Barnum, C. P., Silber, R. H., and Bittner, J. J. (1958). 24-Hour rhythms at several levels of integration in mice on different lighting regimens. *Proceedings of the Society for Experimental Biology* 97: 897–900.
888. LeGates, T. A., Altimus, C. M., Wang, H., Lee, H. K., Yang, S., Zhao, H., Kirkwood, A., Weber, E. T., and Hattar, S. (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature* 491: 594–598.
889. Filipski, E., Delaunay, F., King, V. M., Wu, M. W., Claustrat, B., Gréchez-Cassiau, A., Guettier, C., Hastings, M. H., and Lévi, F. (2004). Effects of chronic jet lag on tumor progression in mice. *Cancer Research* 64: 7879–7885.
890. Scheving, L. E., Tsai, T. H., Powell, E. W., Pasley, J. N., Halberg, F., and Dunn, J. (1983). Bilateral lesions of suprachiasmatic nuclei affect circadian rhythms in [^{3}H]-thymidine incorporation into deoxyribonucleic acid in mouse intestinal tract, mitotic index of corneal epithelium, and serum corticosterone. *Anatomical Record* 205: 239–249.
891. Minami, Y., Kasukawa, T., Kakazu, Y., Iigo, M., Sugimoto, M., Ikeda, S., Yasui, A., van der Horst, G. T., Soga, T., and Ueda, H. R. (2009). Measurement of internal body time by blood metabolomics. *Proceedings of the National Academy of Sciences United States of America* 106: 9890–9895.
892. Verhagen, L. A. W., Pévet, P., Saboureau, M., Sicard, B., Nesme, B., Claustrat, B., Buijs, R. M., and Kalsbeek, A. (2004). Temporal organization of the 24-h corticosterone rhythm in the diurnal murid rodent *Arvicanthis ansorgei* Thomas 1910. *Brain Research* 995: 197–204.
893. Mohawk, J. A., Cashen, K., and Lee, T. M. (2005). Inhibiting cortisol response accelerates recovery from a photic phase shift. *American Journal of Physiology* 288: R221–R228.
894. Van Eekelen, A. P. J., Kerkhof, G. A., and van Amsterdam, J. G. C. (2003). Circadian variation in cortisol reactivity to acute stressor. *Chronobiology International* 20: 863–878.
895. Dzaja, A., Dalal, M. A., Himmerich, H., Uhr, M., Pollmächer, T., and Schuld, A. (2004). Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *American Journal of Physiology* 286: E963–E967.
896. Holmbäck, U., Forslund, A., Lowden, A., Forslund, J., Åkerstedt, T., Lennernäs, M., Hambreus, L., and Stridsberg, M. (2003). Endocrine responses to nocturnal eating: Possible implications for night work. *European Journal of Nutrition* 42: 75–83.
897. Bacon, P. A., Daly, J. R., Myles, A. B., and Savage, O. (1968). Hypothalamo-pituitary-adrenal function in patients on long-term adrenocorticotrophin therapy. *Annals of the Rheumatic Diseases* 27: 7–13.
898. Chua, E. C., Shui, G., Lee, I. T., Lau, P., Tan, L. C., Yeo, S. C., Lam, B. D. et al. (2013). Extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans. *Proceedings of the National Academy of Sciences United States of America* 110: 14468–14473.
899. Weibel, L., Spiegel, K., Follenius, M., Ehrhart, J., and Branderberger, G. (1996). Internal dissociation of the circadian markers of the cortisol rhythm in night workers. *American Journal of Physiology* 270: E608–E613.
900. Bao, A. M., Ji, Y. F., van Someren, E. J. W., Hofman, M. A., Liu, R. Y., and Zhou, J. N. (2004). Diurnal rhythms of free estradiol and cortisol during the normal menstrual cycle in women with major depression. *Hormones and Behavior* 45: 93–102.
901. Koenigsberg, H. W., Teicher, M. H., Mitropoulou, V., Navalta, C., New, A. S., Trestman, R., and Siever, L. J. (2004). 24-h monitoring of plasma norepinephrine, MHPG, cortisol, growth hormone and prolactin in depression. *Journal of Psychiatric Research* 38: 503–511.
902. Matchock, R. L., Dorn, L. D., and Susman, E. J. (2007). Diurnal and seasonal cortisol, testosterone, and DHEA rhythms in boys and girls during puberty. *Chronobiology International* 24: 969–990.
903. Levi, F. A., Canon, C., Touitou, Y., Sulon, J., Mechkouri, M., Ponsart, E. D., Touboul, J. P. et al. (1988). Circadian rhythms in circulating T lymphocyte subtypes and plasma testosterone, total and free cortisol in five healthy men. *Clinical and Experimental Immunology* 71: 329–335.
904. Rascher, U., Hütt, M. T., Siebke, K., Osmond, B., Beck, F., and Lüttge, U. (2001). Spatiotemporal variation of metabolism in a plant circadian rhythm: The biological clock as an assembly of coupled individual oscillators. *Proceedings of the National Academy of Sciences United States of America* 98: 11801–11805.
905. Chia-Looi, A. and Cumming, B. G. (1972). Circadian rhythms of dark respiration, flowering, net photosynthesis, chlorophyll content, and dry weight changes in *Chenopodium rubrum*. *Canadian Journal of Botany* 50: 2219–2226.
906. Hillman, W. S. (1970). Carbon dioxide output as an index of circadian timing in *Lemna* photoperiodism. *Plant Physiology* 45: 273–279.
907. Gromysz-Kalkowska, K. and Szubartowska, E. (1984). Oxygen consumption in two terrestrial species of crustaceans (Isopoda). *Bulletin of the Polish Academy of Sciences, Biological Sciences* 32: 47–56.
908. Gromysz-Kalkowska, K., Oder, M., and Szubartowska, E. (1984). Oxygen consumption in *Armadillidium nasutum* Buddle-Lund (Isopoda). *Folia Biologica Kraków* 32: 89–108.
909. Aguzzi, J., Company, J. B., Sardà, F., and Abelló, P. (2003). Circadian oxygen consumption patterns in continental slope *Nephrops norvegicus* (Decapoda: Nephropidae) in the western Mediterranean. *Journal of Crustacean Biology* 23: 749–757.

910. Kronenberg, F. and Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *Journal of Comparative Physiology* 148: 65–76.
911. Takahashi-del-Bianco, M., Benedito-Silva, A. A., Hebling, J. A., Marques, N., and Marques, M. D. (1992). Circadian oscillatory patterns of oxygen uptake in individual workers of the ant *Camponotus rufipes*. *Physiological Entomology* 17: 377–383.
912. Moritz, R. F. A. and Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.
913. Chakraborty, S. C., Ross, L. G., and Ross, B. (1992). The effect of photoperiod on the resting metabolism of carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology A* 101: 77–82.
914. Moran, D., Softley, R., and Warrant, E. J. (2014). Eyeless Mexican cavefish save energy by eliminating the circadian rhythm in metabolism. *PLOS ONE* 9: e107877.
915. Aschoff, J. and Pohl, H. (1970). Rhythmic variations in energy metabolism. *Federation Proceedings* 29: 1541–1552.
916. Prinzinger, R., Schäfer, T., and Schuchmann, K. L. (1992). Energy metabolism, respiratory quotient and breathing parameters in two convergent small bird species: The fork-tailed sunbird and the Chilean hummingbird. *Journal of Thermal Biology* 17: 71–79.
917. Lovegrove, B. G. and Smith, G. A. (2003). Is 'nocturnal hypothermia' a valid physiological concept in small birds? A study on Bronze Mannikins *Spermestes cucullatus*. *Ibis* 145: 547–557.
918. Randolph, J. C. (1980). Daily metabolic patterns of short-tailed shrews (*Blarina*) in three natural seasonal temperature regimes. *Journal of Mammalogy* 61: 628–638.
919. Henken, A. M., Brandsma, H. A., van der Hel, W., and Verstegen, M. W. A. (1993). Circadian rhythm in heat production of limit-fed growing pigs of several breeds kept at and below thermal neutrality. *Journal of Animal Science* 71: 1434–1440.
920. Verstegen, M. W. A., van der Hel, W., Duijghuisen, R., and Geers, R. (1986). Diurnal variation in thermal demand of growing pigs. *Journal of Thermal Biology* 11: 131–135.
921. Kemp, B., de Greef-Lammers, F. J., Verstegen, M. W. A., and van der Hel, W. (1990). Some aspects of daily pattern in thermal demand of breeding boars. *Journal of Thermal Biology* 15: 103–108.
922. Armitage, G., Harris, R. B. S., Hervey, G. R., and Tobin, G. (1984). The relationship between energy expenditure and environmental temperature in congenitally obese and non-obese Zucker rats. *Journal of Physiology* 350: 197–207.
923. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G., and Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
924. Carlisle, H. J., Wilkinson, C. W., Laudenslager, M. L., and Keith, L. D. (1979). Diurnal variation of heat intake in ovariectomized, steroid-treated rats. *Hormones and Behavior* 12: 232–242.
925. Heusner, A. (1956). Mise en évidence d'une variation nycthémérale de la calorification indépendante du cycle de l'activité chez le rat. *Comptes Rendus des Séances de la Société de Biologie de Strasbourg* 150: 1246–1249.
926. Hervey, G. R., Hussain, S. H., Rayfield, K. M., Tobin, G., and Whitaker, E. M. (1987). The role of thyroid hormones in the metabolic response to cold in the rat. *Journal of Physiology* 386: 73P.
927. Tschöp, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407: 908–913.
928. Stupfel, M., Gourlet, V., Court, L., Mestries, J., Parramon, A., and Mérat, P. (1987). Periodic analysis of ultradian (40 min < tau < 24 h) respiratory variations in laboratory vertebrates of various circadian activities. *Chronobiologia* 14: 365–375.
929. Vogt, F. D. and Lynch, G. R. (1982). Influence of ambient temperature, nest availability, huddling, and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus*. *Physiological Zoology* 55: 56–63.
930. Haim, A., Saarela, S., Hohtola, E., and Zisapel, N. (2004). Daily rhythms of oxygen consumption, body temperature, activity and melatonin in the Norwegian lemming *Lemmus lemmus* under northern summer photoperiod. *Journal of Thermal Biology* 29: 629–633.
931. Dauncey, M. J. (1980). Metabolic effects of altering the 24-h energy intake in man using direct and indirect calorimetry. *British Journal of Nutrition* 43: 257–269.
932. Kavanau, J. L. (1998). Vertebrates that never sleep: Implications for sleep's basic function. *Brain Research Bulletin* 46: 269–279.
933. Cirelli, C. and Tononi, G. (2008). Is sleep essential? *PLoS Biology* 6: e216.
934. Ganguly-Fitzgerald, I., Donlea, J., and Shaw, P. J. (2006). Waking experience affects sleep need in *Drosophila*. *Science* 313: 1775–1781.
935. Gilestro, G. F., Tononi, G., and Cirelli, C. (2009). Widespread changes in synaptic markers as a function of sleep and wakefulness in *Drosophila*. *Science* 324: 109–112.
936. Lyamin, O. I., Mukhametov, L. M., Siegel, J. M., Nazarenko, E. A., Polyakova, I. G., and Shpak, O. V. (2002). Unihemispheric slow wave sleep and the state of the eyes in a white whale. *Behavioural Brain Research* 129: 125–129.
937. Naylor, E., Bergmann, B. M., Krauski, K., Zee, P. C., Takahashi, J. S., Vitaterna, M. H., and Turek, F. W. (2000). The circadian clock mutation alters sleep homeostasis in the mouse. *Journal of Neuroscience* 20: 8138–8143.
938. Wisor, J. P., O'Hara, B. F., Terao, A., Selby, C. P., Kilduff, T. S., Sancar, A., Edgar, D. M., and Franken, P. (2002). A role for *cryptochromes* in sleep regulation. *BMC Neuroscience* 3: art. 20.
939. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P., and Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
940. Franken, P., Dijk, D. J., Tobler, I., and Borbély, A. A. (1991). Sleep deprivation in rats: Effects on EEG power spectra, vigilance states, and cortical temperature. *American Journal of Physiology* 261: R198–R208.
941. Adrien, J., Dugovic, C., and Martin, P. (1991). Sleep-wakefulness patterns in the helpless rat. *Physiology and Behavior* 49: 257–262.
942. Stephan, F. K. and Nunez, A. A. (1977). Elimination of circadian rhythms in drinking, activity, sleep, and temperature by isolation of the suprachiasmatic nuclei. *Behavioral Biology* 20: 1–16.
943. Deboer, T., Vansteensel, M. J., Détári, L., and Meijer, J. H. (2003). Sleep states alter activity of suprachiasmatic nucleus neurons. *Nature Neuroscience* 6: 1086–1090.
944. Ray, B., Mallick, H. N., and Kumar, V. M. (2005). Changes in sleep-wakefulness in medial preoptic area lesioned rats: Role of thermal preference. *Behavioural Brain Research* 158: 43–52.

945. Tzischinsky, O. and Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.
946. Hayashi, M., Morikawa, T., and Hori, T. (2002). Circasemidian 12 h cycle of slow wave sleep under constant darkness. *Clinical Neurophysiology* 113: 1505–1516.
947. Tan, X., Uchiyama, M., Shibui, K., Tagaya, H., Suzuki, H., Kamei, Y., Kim, K., Aritaka, S., Ozaki, A., and Takahashi, K. (2003). Circadian rhythms in human's delta sleep electroencephalogram. *Neuroscience Letters* 344: 205–208.
948. Szymczak, J. T. (1987). Distribution of sleep and wakefulness in 24-h light–dark cycles in the juvenile and adult magpie, *Pica pica*. *Chronobiologia* 14: 277–287.
949. Lancel, M., van Riezen, H., and Glatt, A. (1991). Effects of circadian phase and duration of sleep deprivation on sleep and EEG power spectra in the cat. *Brain Research* 548: 206–214.
950. Marks, G. A. and Shaffery, J. P. (1996). A preliminary study of sleep in the ferret, *Mustela putorius furo*: A carnivore with an extremely high proportion of REM sleep. *Sleep* 19: 83–93.
951. Baranczuk, R. and Greenwald, G. S. (1973). Peripheral levels of estrogen in the cyclic hamster. *Endocrinology* 92: 805–812.
952. Seegal, R. F. and Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. *Biology of Reproduction* 12: 223–231.
953. Stetson, M. H. and Gibson, J. T. (1977). The estrous cycle in golden hamsters: A circadian pacemaker times preovulatory gonadotropin release. *Journal of Experimental Zoology* 201: 289–294.
954. Duvilanski, B. H., Alvarez, M. P., Castrillón, P. O., Cano, P., and Esquifino, A. I. (2003). Daily changes of GABA and taurine concentrations in various hypothalamic areas are affected by chronic hyperprolactinemia. *Chronobiology International* 20: 271–284.
955. Stern, J. M. and Reichlin, S. (1990). Prolactin circadian rhythm persists throughout lactation in women. *Neuroendocrinology* 51: 31–37.
956. Simoni, M., Montanini, V., Fustini, M. F., Del Rio, G., Cioni, K., and Marrama, P. (1992). Circadian rhythm of plasma testosterone in men with idiopathic hypogonadotrophic hypogonadism before and during pulsatile administration of gonadotrophin-releasing hormone. *Clinical Endocrinology* 36: 29–34.
957. Gupta, S. K., Lindemulder, E. A., and Sathyan, G. (2000). Modeling of circadian testosterone in healthy men and hypogonadal men. *Journal of Clinical Pharmacology* 40: 731–738.
958. Inoue, S., Satoh, S., Saito, M., Naitoh, M., Suzuki, H., and Egawa, M. (1995). Effects of selective vagotomy on circadian rhythms of plasma glucose, insulin and food intake in control and ventromedial hypothalamic (VMH) lesioned rats. *Obesity Research* 3(S): 747S–752S.
959. Wasan, K. M., Brunner, L. J., Berens, K. L., Meltzer, A. A., and Luke, D. R. (1989). Circadian assessment of lipids in the hyperphagic obese rat compared with lean litter-mates. *Chronobiology International* 6: 223–228.
960. Randeva, H. S., Karteris, E., Lewandowski, K. C., Sailesh, S., O'Hare, P., and Hillhouse, E. W. (2003). Circadian rhythmicity of salivary leptin in healthy subjects. *Molecular Genetics and Metabolism* 78: 229–235.
961. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2004). Daily and annual variations of free fatty acid, glycerol and leptin plasma concentrations in goats (*Capra hircus*) under different photoperiods. *Comparative Biochemistry and Physiology A* 138: 119–131.
962. Natalucci, G., Riedl, S., Gleiss, A., Zidek, T., and Frisch, H. (2005). Spontaneous 24-h ghrelin secretion pattern in fasting subjects: Maintenance of a meal-related pattern. *European Journal of Endocrinology* 152: 845–850.
963. Blum, J. W., Bruckmaier, R. M., Vacher, P. Y., Münger, A., and Jans, F. (2000). Twenty-four-hour patterns of hormones and metabolites in week 9 and 19 of lactation in high-yielding dairy cows fed triglycerides and free fatty acids. *Journal of Veterinary Medicine A* 47: 43–60.
964. Stephenson, R., Mohan, R. M., Duffin, J., and Jarsky, T. M. (2000). Circadian rhythms in the chemoreflex control of breathing. *American Journal of Physiology* 278: R282–R286.
965. Terman, M. and Terman, J. (1985). A circadian pacemaker for visual sensitivity? *Annals of the New York Academy of Sciences* 453: 147–161.
966. Cellier-Michel, S. and Berthon, J. L. (2003). Rhythmicity of the pigments in the compound eye of *Daphnia longispina* (Cladocera). *Journal of Freshwater Ecology* 18: 445–450.
967. Hankins, M. W., Jones, R. J. M., and Ruddock, K. H. (1998). Diurnal variation in the *b*-wave implicit time of the human electroretinogram. *Visual Neuroscience* 15: 55–67.
968. Hankins, M. W., Jones, S. R., Jenkins, A., and Morland, A. B. (2001). Diurnal daylight phase affects the temporal properties of both the b-wave and d-wave of the human electroretinogram. *Brain Research* 889: 339–343.
969. Wang, Y. and Mangel, S. C. (1996). A circadian clock regulates rod and cone input to fish retinal cone horizontal cells. *Proceedings of the National Academy of Sciences United States of America* 93: 4655–4660.
970. Pardo, B. F. and Sáenz, E. M. (1988). Action of deuterium oxide upon the ERG circadian rhythm in crayfish, *Procambarus bouvieri*. *Comparative Biochemistry and Physiology A* 90: 435–440.
971. Miranda-Anaya, M., Bartell, P. A., Yamazaki, S., and Menaker, M. (2000). Circadian rhythm of ERG in *Iguana iguana*: Role of the pineal. *Journal of Biological Rhythms* 15: 163–171.
972. Doyle, S. E., Grace, M. S., McIvor, W., and Menaker, M. (2002). Circadian rhythms of dopamine in mouse retina: The role of melatonin. *Visual Neuroscience* 19: 593–601.
973. Grace, M. S., Wang, L. A., Pickard, G. E., Besharse, J. C., and Menaker, M. (1996). The *tau* mutation shortens the period of rhythmic photoreceptor outer segment disk shedding in the hamster. *Brain Research* 735: 93–100.
974. Watson, W. H., Bedford, L., and Chabot, C. C. (2008). Rhythms of locomotion expressed by *Limulus polyphemus*, the American horseshoe crab: II. Relationship to circadian rhythms of visual sensitivity. *Biological Bulletin* 215: 46–56.
975. Halberg, F. and Conner, R. L. (1961). Circadian organization and microbiology: Variance spectra and periodogram on behavior of *Escherichia coli* growing in fluid culture. *Proceedings of the Minnesota Academy of Science* 29: 227–239.
976. Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S., and Johnson, C. H. (1993). Circadian rhythms in prokaryotes: Luciferase as a reporter of circadian gene expression in cyanobacteria. *Proceedings of the National Academy of Sciences United States of America* 90: 5672–5676.
977. Mihalcescu, I., Hsing, W., and Leibler, S. (2004). Resilient circadian oscillator revealed in individual cyanobacteria. *Nature* 430: 81–85.
978. Miller, R. P., Martinson, K. B., Sothern, R. B., Durgan, B. R., and Gunsolus, J. L. (2003). Circadian response of annual weeds in a natural setting to high and low application rates of four herbicides with different modes of action. *Chronobiology International* 20: 299–324.

979. Yen, U. C., Huang, T. C., and Yen, T. C. (2004). Observation of the circadian photosynthetic rhythm in cyanobacteria with a dissolved-oxygen meter. *Plant Science* 166: 949–952.
980. Fredeen, A. L., Hennessey, T. L., and Field, C. B. (1991). Biochemical correlates of the circadian rhythm in photosynthesis in *Phaseolus vulgaris*. *Plant Physiology* 97: 415–419.
981. Hennessey, T. L. and Field, C. B. (1992). Evidence of multiple circadian oscillators in bean plants. *Journal of Biological Rhythms* 7: 105–113.
982. Dodd, A. N., Salathia, N., Hall, A., Kévei, E., Tóth, R., Nagy, F., Hibberd, J. M., Millar, A. J., and Webb, A. A. R. (2005). Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science* 309: 630–633.
983. Vaadia, Y. (1960). Autonomic diurnal fluctuations in rate of exudation and root pressure of decapitated sunflower plants. *Physiologia Plantarum* 13: 701–717.
984. Urbanczyk-Wochniak, E., Baxter, C., Kolbe, A., Kopka, J., Sweetlove, L. J., and Fernie, A. R. (2005). Profiling of diurnal patterns of metabolite and transcript abundance in potato (*Solanum tuberosum*) leaves. *Planta* 221: 891–903.
985. Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-ike, N., and Ishiura, M. (2004). Large-scale screening of *Arabidopsis* circadian clock mutants by a high-throughput real-time bioluminescence monitoring system. *Plant Journal* 40: 1–11.
986. Feldman, J. F. and Hoyle, M. N. (1973). Isolation of circadian clock mutants of *Neurospora crassa*. *Genetics* 75: 605–613.
987. Vitalini, M. W., Morgan, L. W., March, I. J., and Bell-Pedersen, D. (2004). A genetic selection for circadian output pathway mutations in *Neurospora crassa*. *Genetics* 167: 119–129.
988. Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F., and Okamura, H. (2003). Control mechanism of the circadian clock for timing of cell division in vivo. *Science* 302: 255–259.
989. Von der Heide, F., Wilkens, A., and Rensing, L. (1992). The effects of temperature on the circadian rhythms of flashing and glow in *Gonyaulax polyedra*: Are the two rhythms controlled by two oscillators? *Journal of Biological Rhythms* 7: 115–123.
990. Perkins, M. (1931). Light of glow-worms. *Nature* 128: 905.
991. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences United States of America* 40: 1018–1029.
992. Winfree, A. T. (1971). Corkscrews and singularities in fruitflies: Resetting behavior of the circadian eclosion rhythm. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 81–106.
993. Myers, E. M., Yu, J., and Sehgal, A. (2003). Circadian control of eclosion: Interaction between a central and peripheral clock in *Drosophila melanogaster*. *Current Biology* 13: 526–533.
994. Paranjpe, D. A., Anitha, D., Joshi, A., and Sharma, V. K. (2004). Multi-oscillatory control of eclosion and oviposition rhythms in *Drosophila melanogaster*: Evidence from limits of entrainment studies. *Chronobiology International* 21: 539–552.
995. Kannan, N. N., Reveendran, R., Dass, S. H., Manjunatha, T., and Sharma, V. K. (2012). Temperature can entrain egg laying rhythm of *Drosophila* but may not be a stronger zeitgeber than light. *Journal of Insect Physiology* 58: 245–255.
996. Wu, S., Refinetti, R., Kok, L. T., Youngman, R. R., Reddy, G. V. P., and Xue, F. S. (2014). Photoperiod and temperature effects on the adult eclosion and mating rhythms in *Pseudopidorus fasciata* (Lepidoptera: Zygaenidae). *Environmental Entomology* 43: 1650–1655.
997. Johnson, C. R. (1971). Daily variation in the thermal tolerance of *Litoria caerulea* (Anura: Hylidae). *Comparative Biochemistry and Physiology A* 40: 1109–1111.
998. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., and Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *Journal-Lancet* 71: 312–319.
999. Halberg, F., Visscher, M. B., and Bittner, J. J. (1953). Eosinophil rhythm in mice: Range of occurrence, effects of illumination, feeding and adrenalectomy. *American Journal of Physiology* 174: 109–122.
1000. Dawes, C. (1972). Circadian rhythms in human salivary flow rate and composition. *Journal of Physiology* 220: 529–545.
1001. Fukui, Y., Yaegaki, K., Murata, T., Sato, T., Tanaka, T., Imai, T., Kamoda, T., and Herai, M. (2008). Diurnal changes in oral malodour among dental-office workers. *International Dental Journal* 58: 159–166.
1002. Piccione, G., Fazio, F., Caola, G., and Refinetti, R. (2008). Daily rhythmicity in nutrient content of asinine milk. *Livestock Science* 116: 323–327.
1003. Tillmann, V. and Clayton, P. E. (2001). Diurnal variation in height and the reliability of height measurements using stretched and unstretched techniques in the evaluation of short-term growth. *Annals of Human Biology* 28: 195–206.
1004. Siklar, Z., Sanli, E., Dallar, Y., and Tanyer, G. (2005). Diurnal variation of height in children. *Pediatrics International* 47: 645–648.
1005. Rodríguez, G., Moreno, L. A., Sarría, A., Fleta, J., and Bueno, M. (2000). Assessment of nutritional status and body composition in children using physical anthropometry and bioelectrical impedance: Influence of diurnal variations. *Journal of Pediatric Gastroenterology and Nutrition* 30: 305–309.
1006. Cugini, P., Salandri, A., Celli, V., Luparini, R. L., De Rosa, R., and Marigliano, V. (2002). Circadian rhythm of some parameters of body composition in the elderly investigated by means of bioelectrical impedance analysis. *Eating and Weight Disorders* 7: 182–189.
1007. Rao, S. S. C., Sadeghi, P., Beaty, J., Kavlock, R., and Ackerson, K. (2001). Ambulatory 24-h colonic manometry in healthy humans. *American Journal of Physiology* 280: G629–G639.
1008. Nelson, W., Scheving, L., and Halberg, F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *Journal of Nutrition* 105: 171–184.
1009. Héroux, O., Bhasin, J., Jobin, M., and Normand, M. (1979). The effect of diets on different factors involved in the duration of resistance to low temperature in rats. *International Journal of Biometeorolgy* 23: 31–49.
1010. Ho, K. J. (1979). Circadian rhythm of cholesterol biosynthesis: Dietary regulation in the liver and small intestine of hamsters. *International Journal of Chronobiology* 6: 39–50.
1011. Edwards, P. A., Muroya, H., and Gould, R. G. (1972). In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. *Journal of Lipid Research* 13: 396–401.
1012. Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C., and Porter, J. W. (1972). The effects of fasting, refeeding, and time of day on the levels of enzymes effecting the conversion of β-hydroxy-β-methylglutaryl coenzime A to squalene. *Journal of Biological Chemistry* 247: 3014–3022.
1013. Dugan, R. E., Slakey, L. L., Briedis, A. V., and Porter, J. W. (1972). Factors affecting the diurnal variation in the level of β-hydroxy-β-methylglutaryl coenzime A reductase and cholesterol-synthesizing activity in rat liver. *Archives of Biochemistry and Biophysics* 152: 21–27.

1014. Lanza-Jacoby, S., Stevenson, N. R., and Kaplan, M. L. (1986). Circadian changes in serum and liver metabolites and liver lipogenic enzymes in ad libitum- and meal-fed, lean and obese Zucker rats. *Journal of Nutrition* 116: 1798–1809.
1015. Jurevics, H., Hostettler, J., Barrett, C., Morell, P., and Toews, A. D. (2000). Diurnal and dietary-induced changes in cholesterol synthesis correlate with levels of mRNA for HMG-CoA reductase. *Journal of Lipid Research* 41: 1048–1053.
1016. Gälman, C., Angelin, B., and Rudling M. (2005). Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. *Gastroenterology* 129: 1445–1453.
1017. Liu, J. H. K., Kripke, D. F., Hoffman, R. E., Twa, M. D., Loving, R. T., Rex, K. M., Gupta, N., and Weinreb, R. N. (1998). Nocturnal elevation of intraocular pressure in young adults. *Investigative Ophthalmology and Visual Science* 39: 2707–2712.
1018. Liu, J. H. K. and Dacus, A. C. (1991). Endogenous hormonal changes and circadian elevation of intraocular pressure. *Investigative Ophthalmology and Visual Science* 32: 496–500.
1019. Aihara, M., Lindsey, J. D., and Weinreb, R. N. (2003). Twenty-four-hour pattern of mouse intraocular pressure. *Experimental Eye Research* 77: 681–686.
1020. Sit, A. J., Nau, C. B., McLaren, J. W., Johnson, D. H., and Hodge, D. (2008). Circadian variation of aqueous dynamics in young healthy adults. *Investigative Ophthalmology and Visual Science* 49: 1473–1479.
1021. Mansouri, K., Liu, J. H. K., Weinreb, R. N., Tafreshi, A., and Medeiros, F. A. (2012). Analysis of continuous 24-hour intraocular pressure patterns in glaucoma. *Investigative Ophthalmology and Visual Science* 53: 8050–8056.
1022. Scheving, L. E., Vedral, D. F., and Pauly, J. E. (1968). A circadian susceptibility rhythm in rats to pentobarbital sodium. *Anatomical Record* 160: 741–750.
1023. Rebuelto, M., Ambros, L., Waxman, S., and Montoya, L. (2004). Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. *Chronobiology International* 21: 591–600.
1024. Rebuelto, M., Ambros, L., Montoya, L., and Bonafine, R. (2002). Treatment-time-dependent difference of ketamine pharmacological response and toxicity in rats. *Chronobiology International* 19: 937–945.
1025. Bromage, T. G. (1991). Enamel incremental periodicity in the pig-tailed macaque: A polychrome fluorescent labeling study of dental hard tissues. *American Journal of Physical Anthropology* 86: 205–214.
1026. Ohtsuka-Isoya, M., Hayashi, H., and Shinoda, H. (2001). Effect of suprachiasmatic nucleus lesion on circadian dentin increment in rats. *American Journal of Physiology* 280: R1364–R1370.
1027. Jackson, B. F., Blumsohn, A., Goodship, A. E., Wilson, A. M., and Price, J. S. (2003). Circadian variation in biochemical markers of bone cell activity and insulin-like growth factor-I in two-year-old horses. *Journal of Animal Science* 81: 2804–2810.
1028. Williams, R. L., Soliman, K. F. A., and Mizinga, K. M. (1993). Circadian variation in tolerance to the hypothermic action of CNS drugs. *Pharmacology, Biochemistry and Behavior* 46: 283–288.
1029. Johnston, R. L. and Washeim, H. (1924). Studies in gastric secretion. II. Gastric secretion in sleep. *American Journal of Physiology* 70: 247–253.
1030. Saitoh, T., Watanabe, Y., Kubo, Y., Shinagawa, M., Otsuka, K., Ohkawa, S. I., and Watanabe, T. (2002). Effect of H2 blockers on the circadian rhythm of intragastric acidity. *Biomedicine and Pharmacotherapy* 56: 349s–352s.
1031. Piccione, G., Assenza, A., Grasso, F., and Caola, G. (2004). Daily rhythm of circulating fat soluble vitamin concentration (A, D, E, and K) in the horse. *Journal of Circadian Rhythms* 2: art. 3.
1032. La Fleur, S. E., Kalsbeek, A., Wortel, J., Fekkes, M. L., and Buijs, R. M. (2001). A daily rhythm in glucose tolerance: A role for the suprachiasmatic nucleus. *Diabetes* 50: 1237–1243.
1033. Armstrong, S., Clarke, J., and Coleman, G. (1978). Light-dark variation in laboratory rat stomach and small intestine content. *Physiology and Behavior* 21: 785–788.
1034. Bertolucci, C., Pinotti, M., Colognesi, I., Foà, A., Bernardi, F., and Portaluppi, F. (2005). Circadian rhythms in mouse blood coagulation. *Journal of Biological Rhythms* 20: 219–224.
1035. Khaldoun, M., Khaldoun, T., Mellado, M., Cambar, J., and Brudieux, R. (2002). Circadian rhythm in plasma aldosterone concentration and its seasonal modulation in the camel (*Camelus dromedarius*) living in the Algerian Sahara Desert. *Chronobiology International* 19: 683–693.
1036. Gay, F., Valiante, S., Sciarrillo, R., de Falco, M., Laforgia, V., and Capaldo, A. (2010). Annual and daily serum aldosterone and catecholamine patterns in males of the Italian crested newt, *Triturus carnifex* (Amphibia, Urodela). *Italian Journal of Zoology* 77: 384–390.
1037. Rappold, I. and Erkert, H. G. (1994). Re-entrainment, phase-response and range of entrainment of circadian rhythms in owl monkeys (*Aotus lemurinus g.*) of different age. *Biological Rhythm Research* 25: 133–152.
1038. Mahoney, M., Bult, A., and Smale, L. (2001). Phase response curve and light-induced Fos expression in suprachiasmatic nucleus and adjacent hypothalamus of *Arvicanthis niloticus*. *Journal of Biological Rhythms* 16: 149–162.
1039. Refinetti, R. (2007). Enhanced circadian photoresponsiveness after prolonged dark adaptation in seven species of diurnal and nocturnal rodents. *Physiology and Behavior* 90: 431–437.
1040. Glass, J. D., Tardif, S. D., Clements, R., and Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
1041. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S., and Tabata, M. (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *Journal of Comparative Physiology A* 181: 121–132.
1042. Sheeba, V., Chandrashekaran, M. K., Joshi, A., and Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster. Journal of Insect Physiology* 48: 25–32.
1043. Rajaratnam, S. M. W., and Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
1044. Aschoff, J. (1994). Naps as integral parts of the wake time within the human sleep-wake cycle. *Journal of Biological Rhythms* 9: 145–155.
1045. Czeisler, C. A., Duffy, J. F, Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M. et al. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177–2181.

1046. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A., and Dijk, D. J. (1999). Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *American Journal of Physiology* 277: R1152–R1163.
1047. Wever, R. A. (1989). Light effects on human circadian rhythms: A review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
1048. Honma, K., Honma, S., and Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
1049. Lockley, S. W., Skene, D. J., Butler, L. J., and Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 22: 616–623.
1050. Sack, R. L., Brandes, R. W., Kendall, A. R., and Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
1051. Page, T. L., Mans, C., and Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.
1052. Shimomura, K., Nelson, D. E., Ihara, N. L., and Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
1053. Ralph, M. R. and Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
1054. Refinetti, R. and Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
1055. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology* 106: 253–266.
1056. Rosenberg, R. S., Zee, P. C., and Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.
1057. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
1058. Refinetti, R., Nelson, D. E., and Menaker, M. (1992). Social stimuli fail to act as entraining agents of circadian rhythms in the golden hamster. *Journal of Comparative Physiology A* 170: 181–187.
1059. Davis, F. C. and Viswanathan, N. (1998). Stability of circadian timing with age in Syrian hamsters. *American Journal of Physiology* 275: R960–R968.
1060. Bittman, E. L., Costello, M. K., and Brewer, J. M. (2007). Circadian organization of *tau* mutant hamsters: Aftereffects and splitting. *Journal of Biological Rhythms* 22: 425–431.
1061. Bittman, E. L. (2012). Does the precision of a biological clock depend upon its period? Effects of the duper and tau mutations in Syrian hamsters. *PLOS ONE* 7: e36119.
1062. Von Gall, C., Duffield, G. E., Hastings, M. H., Kopp, M. D. A., Dehghani, F., Korf, H. W., and Stehle, J. H. (1998). CREB in the mouse SCN: A molecular interface coding the phase-adjusting stimuli light, glutamate, PACAP, and melatonin for clockwork access. *Journal of Neuroscience* 18: 10389–10397.
1063. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S., and Honma, K. (2001). Clock gene expressions in the suprachiasmatic nucleus and other areas of the brain during rhythm splitting in CS mice. *Molecular Brain Research* 87: 92–99.
1064. Benloucif, S. and Dubocovich, M. L. (1996). Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN mouse. *Journal of Biological Rhythms* 11: 113–125.
1065. Fuller, P. M. and Fuller, C. A. (2006). Genetic evidence for a neurovestibular influence on the mammalian circadian pacemaker. *Journal of Biological Rhythms* 21: 177–184.
1066. Kolker, D. E., Vitaterna, M. H., Fruechte, E. M., Takahashi, J. S., and Turek, F. W. (2004). Effects of age on circadian rhythms are similar in wild-type and heterozygous *Clock* mutant mice. *Neurobiology of Aging* 25: 517–523.
1067. Van der Leest, H. T., Rohling, J. H. T., Michel, S., and Meijer, J. H. (2009). Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLOS ONE* 4: e4976.
1068. Doi, M., Yujnovsky, I., Hirayama, J., Malerba, M., Tirotta, E., Sassone-Corsi, P., and Borrelli, E. (2006). Impaired light masking in dopamine D2 receptor-null mice. *Nature Neuroscience* 9: 732–734.
1069. Marchant, E. G. and Mistlberger, R. E. (1997). Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6J mice. *Brain Research* 765: 273–282.
1070. Dallmann, R., DeBruyne, J. P., and Weaver, D. R. (2011). Photic resetting and entrainment in CLOCK-deficient mice. *Journal of Biological Rhythms* 26: 390–401.
1071. Farjnia, S., Michel, S., Deboer, T., van der Leest, H. T., Houben, T., Rohling, J. H. T., Ramkisoensing, A., Yasenkov, R., and Meijer, J. H. (2012). Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. *Journal of Neuroscience* 32: 5891–5899.
1072. Basu, P., Singaravel, M., and Haldar, C. (2012). L-5-hydroxytryptophan resets the circadian locomotor activity rhythm of the nocturnal Indian pygmy field mouse, *Mus terricolor*. *Naturwissenschaften* 99: 233–239.
1073. Mohawk, J. A. and Lee, T. M. (2005). Restraint stress delays reentrainment in male and female diurnal and nocturnal rodents. *Journal of Biological Rhythms* 20: 245–256.
1074. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences United States of America* 59: 414–421.
1075. Puchalski, W. and Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: Effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.
1076. Weinert, D. and Schottner, K. (2007). An inbred lineage of Djungarian hamsters with a strongly attenuated ability to synchronize. *Chronobiology International* 24: 1065–1079.
1077. Hamasaka, Y., Watari, Y., Arai, T., Numata, H., and Shiga, S. (2001). Retinal and extraretinal pathways for entrainment of the circadian activity rhythm in the blow fly, *Protophormia terraenovae*. *Journal of Insect Physiology* 47: 867–875.
1078. Summer, T. L., Ferraro, J. S., and McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
1079. Canal-Corretger, M. M., Vilaplana, J., Cambras, T., and Díez-Noguera, A. (2001). Functioning of the rat circadian system is modified by light applied in critical postnatal days. *American Journal of Physiology* 280: R1023–R1030.
1080. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M., and Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.
1081. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M., and Daan, S. (1997). Stress-induced changes in circadian rhythms of body temperature and activity in rats are not caused by pacemaker changes. *Journal of Biological Rhythms* 12: 80–92.

1082. Mendoza, J., Angeles-Castellanos, M., and Escobar, C. (2005). A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *European Journal of Neuroscience* 22: 2855–2862.
1083. Jansen, H. T., Sergeeva, A., Stark, G., and Sorg, B. A. (2012). Circadian discrimination of reward: Evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiology International* 29: 454–468.
1084. Underwood, H. (1983). Circadian pacemakers in lizards: Phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
1085. Terai, Y., Asayama, M., Ogawa, T., Sugenoya, J., and Miyagawa, T. (1985). Circadian variation of preferred environmental temperature and body temperature. *Journal of Thermal Biology* 10: 151–156.
1086. Kattapong, K. R., Fogg, L. F., and Eastman, C. I. (1995). Effect of sex, menstrual phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International* 12: 257–266.
1087. Song, X., Körtner, G., and Geiser, F. (1998). Temperature selection and use of torpor by the marsupial *Sminthopsis macroura*. *Physiology and Behavior* 64: 675–682.

Section III

Mechanisms



Mechanical clockwork symbolizing the mechanism of circadian timing.
(Pixabay public domain image.)

This page intentionally left blank

6 Endogenous Mechanisms

6.1 ENDOGENOUS RHYTHMICITY

In Section II of this book, we looked at the phenomenology of biological rhythms. We examined ultradian and infradian rhythms in Chapter 4 and then focused on daily and circadian rhythms in Chapter 5. We saw that virtually every biological function ever measured was found to exhibit daily rhythmicity. We also saw that, whereas all daily rhythms are modulated by the alternation of day and night, many rhythms are generated endogenously. We saw that, under constant environmental conditions, the rhythms freerun with periods slightly different from 24.0 hours. For instance, we saw that the rhythm of locomotor activity can freerun in numerous species of invertebrates,[1–28] reptiles,[29–37] fishes,[38–44] birds,[45–59] and mammals,[60–117] including humans.[118–129]

Because geophysical cycles on Earth are expected to have 24.0-hour periods, the existence of circadian rhythms with periods different from 24.0 hours provides strong evidence in support of the hypothesis of endogenous rhythmicity. For the indulgence of skeptical readers, however, I should add that circadian rhythms have been recorded in humans kept in underground bunkers and caves,[126,130] as well as in space.[131–133] Also, free-running rhythms were recorded in people living in Arctic and Antarctic field camps, where Earth's influence is just as strong, but there is continuous sunlight throughout the summer and continuous darkness throughout the winter.[121,122] A little later, in Section 6.2, we will see that the period of circadian rhythms can be modified by genetic manipulation. I hope, therefore, that all readers accept the fact that circadian rhythms are endogenously generated.

6.1.1 Concept of a Pacemaker

If circadian rhythms are endogenously generated, there must exist a clock that generates them. Everyone knows what a clock is, but no one expects to find a clock similar to that shown in Figure 6.1 inside an organism. Evidently, we need to clarify what is that we mean by the word *clock*. Let us start by identifying three basic elements of time measurement, as represented by the three circles in Figure 6.2. The three elements are as follows: the ability to undergo a constant change of state over time, the ability to display absolute time, and the ability to generate a self-sustaining oscillation. Let us consider first the ability to undergo a constant change of state over time (lower circle). Many entities in the world do not undergo a change of state over time. Thus, for instance, water evaporates at 100°C today, it did so yesterday, and it will do so tomorrow. Other entities do change over time, and they do so at a constant rate (or at least in a predictable manner). This property is sufficient to define a *timer*. An hourglass and a mechanical countdown timer are examples of timers.

The circle on the right in Figure 6.2 represents the ability to display absolute time. We need not dwell on the meaning of "absolute" time. Time in relation to the Earth's position relative to the Sun will suffice. That is, the ability to display absolute time is the ability to tell what time of day it is. This property is sufficient to define a *clock*. A sundial is a perfect example of such a basic clock.

The circle on the left in Figure 6.2 represents the ability to generate a self-sustaining oscillation (i.e., the ability to repeat a process over and over without external input). This property is sufficient to define an *oscillator*. If the oscillator communicates the oscillations to other entities, we will call it a "pacemaker." A drummer in a rock-and-roll band is an example of a basic pacemaker. Notice that the drummer must be able to generate a self-sustaining oscillation (in this case, a rhythmic pattern of drum beats), but a bad drummer is still a drummer even if he or she does not provide a constant change of state (a constant tempo) or if the timing has no clear relationship with the time of day. That is, a timer, a pacemaker, and a clock are three distinct entities that perform three distinct functions.

Some confusion derives from the fact that the three functions of timing mechanisms need not be mutually exclusive, as shown by the overlap of the circles in Figure 6.2. Ancient Egyptians used *clepsydras* as clocks.[134,135] A clepsydra was a ceramic pot with a small hole at the bottom. Etchings on the inside of the pot allowed measurement of time as the water slowly flowed out of the pot. By exhibiting a constant change (or predictably variable change) of state over time, this instrument qualified as a timer. By appropriate calibration, it could also display absolute time and, therefore, it qualified as a clock (gray area number IV in Figure 6.2). Similarly, a metronome (used to set the beat in music) combines the properties of a timer and of a pacemaker (gray area number III). More impressively, virtually, any wristwatch that one can buy today is a sophisticated instrument that not only displays absolute time but also possesses a mechanism that undergoes a constant change of state over time as part of a self-sustained oscillatory process (gray area number II). That is, a wristwatch is a clock, a timer, *and* a pacemaker. In circadian physiology, the term *timer* (or *hourglass*) is usually reserved for pure timers—that is, mechanisms that do *not* possess the properties of either a pacemaker or a clock. The term *pacemaker* (or, sometimes, *oscillator*) is used for pure pacemakers just as often as for pacemaker–timer combinations. The term *clock* is often used in a nontechnical sense that includes timer, pacemaker, clock, and any of their combinations.[136] Thus, the "circadian pacemaker," which has the properties of a pacemaker as well as of a timer, is often referred to as the "biological clock." The *clock* designation in *biological clock* may also be justified by the fact that the

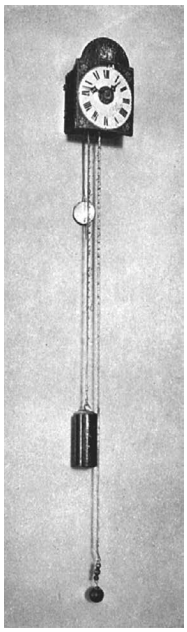


FIGURE 6.1 An old clock. When we think of devices that measure the passage of time, we often think of clocks such as this pendulum clock. (Copyright ArtToday, Tucson, AZ.)

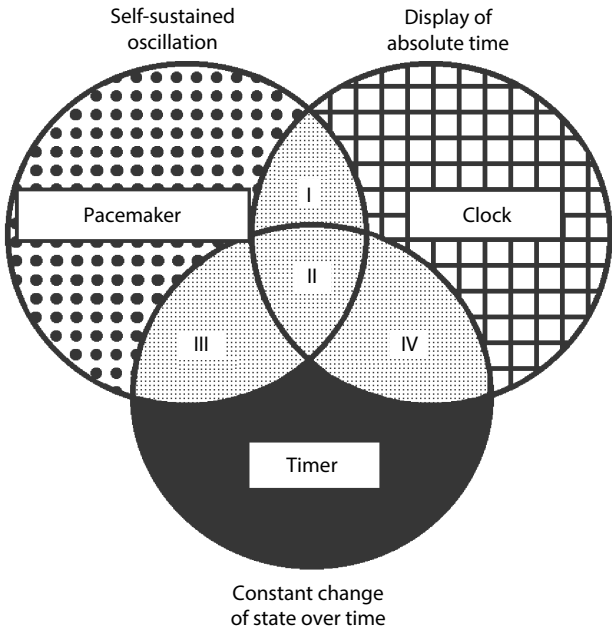


FIGURE 6.2 Timing instruments. Three terms are commonly used to designate timing instruments. A pacemaker is an entity capable of generating a self-sustained oscillation. A clock is an entity that can display absolute time. A timer is an entity that undergoes a constant change of state over time. Although these entities may overlap, no special names exist for the overlaps.

circadian pacemaker, when synchronized to the alternation of day and night, can provide a good estimate of time of day. In addition, a circadian pacemaker is a true clock for *internal* time, as it allows the organism to time different functions along the circadian cycle.

In Section IV of the book, we will examine the anatomical identity of the circadian pacemaker and will dissect its functional properties. Before we get there, however, we can examine the concept of a pacemaker in general functional terms. The central question is, of course, How does the circadian pacemaker *generate* time? The answer to this question can be both extremely simple and extremely complex. Because all biochemical reactions take place over time, time in itself is inherent to any biological process. All that is needed to produce a biological clock (pacemaker) is a biochemical loop—that is, a series of reactions that repeats itself at a constant rate. The cell division cycle (mitosis) is controlled by some sort of pacemaker, but we know that this pacemaker is distinct from the circadian pacemaker because cells that do not divide (such as nerve cells) exhibit circadian rhythmicity and because circadian rhythmicity is exhibited even by rapidly dividing bacteria whose life cycle is much shorter than that of a circadian cycle.[137]

Although the full ensemble of biochemical reactions responsible for the circadian pacemaker is still not known, great progress has been achieved recently, as will be discussed in Chapter 12. The fundamental requirement for the creation of a biological pacemaker is a negative feedback loop with delay.[138,139] If, for instance, structure A produces substance B, and substance B feeds back on structure A to inhibit its own production, then a biological clock will exist—and it will have a period equal to the time needed to produce enough substance B to reach the inhibitory threshold plus the time needed for substance B to be metabolized down to the threshold concentration. Thus, the concept of a biological pacemaker in itself is rather simple and requires no magical elements. Analogies based on water flow and electricity are usually helpful for understanding the concept. Think of a watermill (Figure 6.3). The constant flow of water falling

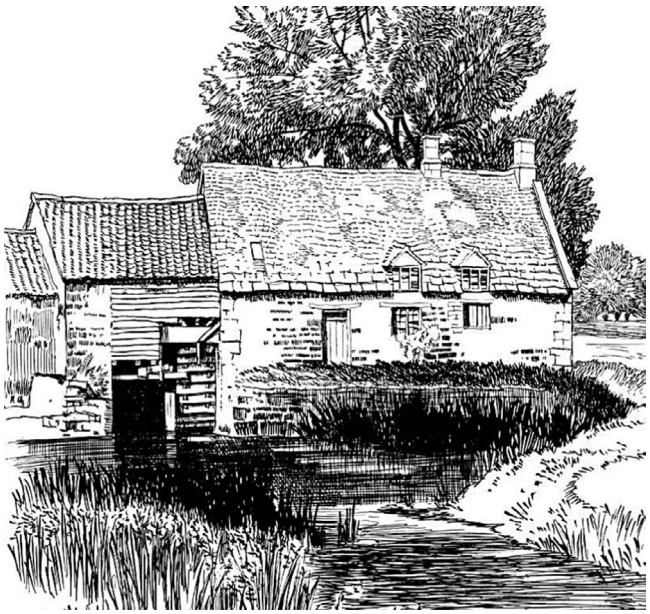


FIGURE 6.3 Moving slowly with the water. A watermill, such as the one attached to this cottage in the British countryside, is an example of a simple timer. (Copyright ArtToday, Tucson, AZ.)

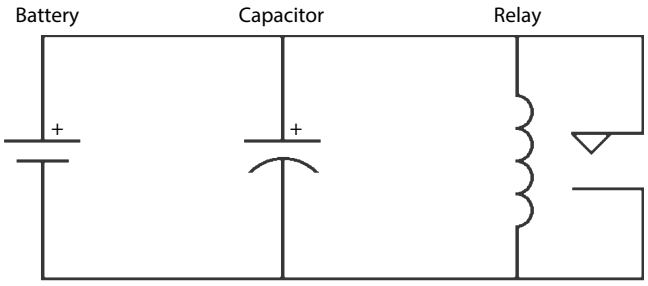


FIGURE 6.4 Let the current flow. An unpretentious electronic circuit, composed of a battery, a capacitor, and a relay, is an example of a simple pacemaker.

on the wooden wheel causes the wheel to spin, thus creating a circular movement that can run the mill. The repetitive movement of the wheel could be used as a timer or even as a clock (see Exercise 6.1). We cannot consider it a pacemaker, however, because it requires an external source (the flow of water). On the other hand, a very simple pacemaker can be built with a battery, a capacitor, and a relay (Figure 6.4). In this simple circuit, the battery activates the relay, and the relay closure shortens the battery (thus causing the relay's coil to be deactivated). Of course, nothing will happen if the actions are instantaneous (i.e., the relay will close and open at the same time, which is impossible). However, the capacitor will add the necessary delay in the feedback loop, thus creating a cycle of opening and closure of the relay that will repeat itself over and over until the battery dies (see Exercise 6.2).

In the early 1900s, a Dutch electrical engineer called Balthasar van der Pol experimented with a slightly more complex circuit than that shown in Figure 6.4. Employing vacuum tubes to create *negative resistance* across the battery poles, he developed and mathematically described what is known today as a *van der Pol oscillator*.[140,141] He used his mathematical model to study the cardiac pacemaker, and many biologists since then have used the model to study the circadian pacemaker.[142–148] We will return to this topic at the end of Chapter 7 after we have had a chance to examine environmental factors that can synchronize the pacemaker.

6.1.2 Free-Running Rhythms

Although the existence of free-running circadian rhythms was extensively documented in Chapter 5, we did not address the issue of how long the rhythms can freerun. We simply assumed that the rhythms would freerun for the whole life of the organism. Long freeruns under constant environmental conditions have indeed been documented in various species.[47,50,94,97,149–157] For example, records of running-wheel activity of a domestic mouse (*Mus musculus*) over many months are shown in Figure 6.5. The animal was maintained under a light–dark cycle for 6 weeks and then released into constant darkness for over 4 months. The pattern of activity remained robust throughout the study, even as the period of the rhythm underwent a slow shortening followed by slow lengthening. With an average of 6 km ran each day, the mouse ran more than

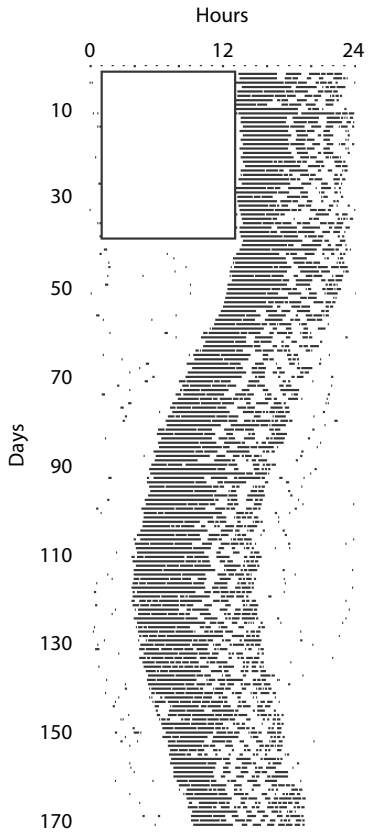


FIGURE 6.5 Runaway mouse. This actogram shows the records of running-wheel activity of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle for 6 weeks and in constant darkness for over 4 months. The rectangle indicates the light phase of the light–dark cycle. Notice that the period of the activity rhythm is different from 24 hours in the absence of the light–dark cycle. (If you are not familiar with actograms, look back at Figure 3.19 in Chapter 3.) (Data from the archives of the Refinetti lab.)

1000 km (600 miles) during the 170 days of the study. If the mouse had been released into the outside world—and had not been eaten by a cat—it could have traveled from Charleston, South Carolina, to Miami, Florida! For our European readers, this would be from Paris to Vienna on foot! I should point out that the shortening followed by lengthening of the free-running period seen in Figure 6.5 is not unusual, but it is not normative either. For instance, the activity records of a laboratory rat (*Rattus norvegicus*) shown in Figure 6.6 exhibit a very stable circadian period of 24.4 hours throughout the 4-month freerun. To be exact, I must acknowledge that right after being released from the 24.0-hour light–dark cycle, the rhythm freeran with a period slightly shorter (i.e., closer to 24.0 hours) than the *real* period of 24.4 hours. Thus, during the first week of freerun, the period was 24.3 hours. This *aftereffect* of the light–dark cycle will be discussed in detail in Chapter 7.

The average value of the free-running period, as well as its interindividual variability, depends on the species under study. Figure 6.7 shows the distributions of free-running periods (measured for 10 days, starting a week after release into constant darkness) for 46 individuals of three different



FIGURE 6.6 Runaway rat. This actogram shows the records of running-wheel activity of a laboratory rat (*Rattus norvegicus*) maintained under a light–dark cycle for 2 weeks and in constant darkness for 4 months. The rectangle indicates the light phase of the light–dark cycle. Notice that the period of the activity rhythm is consistently longer than 24 hours in the absence of the light–dark cycle. (Data from the archives of the Refinetti lab.)

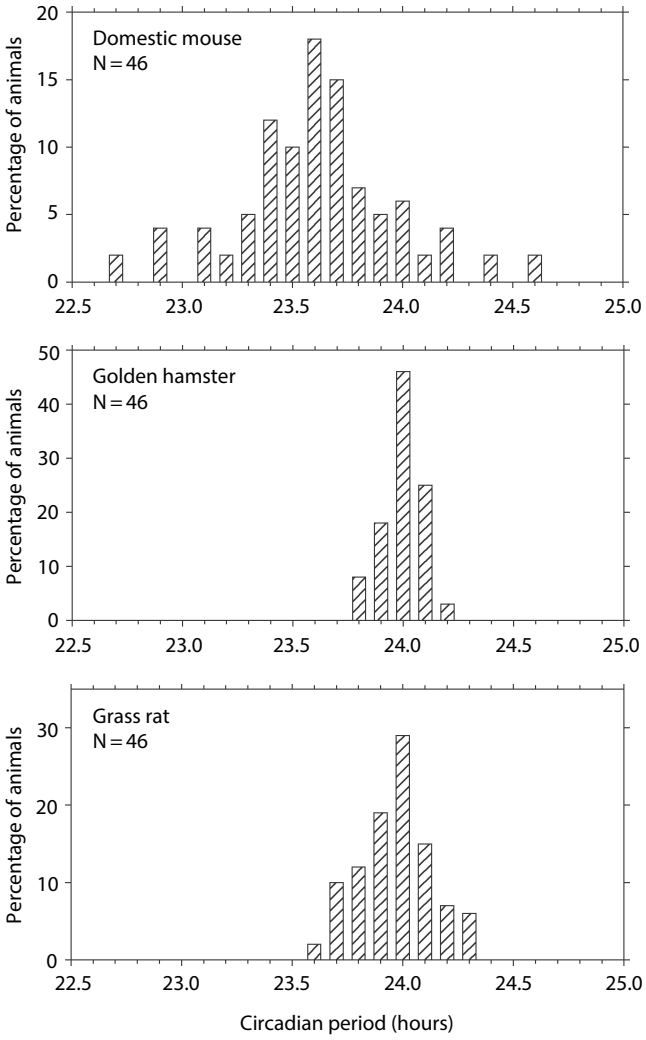


FIGURE 6.7 How long is your period? The graphs show the distributions of free-running period of 46 individuals of three species of small rodents. Notice that the species differ not only in their average periods but also in the spread of the distribution of periods. (Data from Refinetti, R., *Physiol. Behav.*, 74, 101, 2001; Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004.)

species of rodents: the domestic mouse (*M. musculus*), the golden hamster (*Mesocricetus auratus*), and the Nile grass rat (*Arvicanthis niloticus*). Notice, for instance, that golden hamsters not only have a longer free-running period than mice do (24.04 hours vs. 23.62 hours) but also that their interindividual variability is much smaller. The modes of the distributions are identical for golden hamsters and Nile grass rats (24.0 hours), but the spread is narrower for the former than for the latter. Free-running periods exhibit interindividual variation in humans too, with a range of more than an hour from the shortest to the longest period, and with a small effect of race: whites (τ = 24.4 hours) have a period slightly longer than blacks (τ = 24.2 hours).[129]

Table 6.1 shows the mean free-running periods of 77 animal species, including invertebrates, fishes, reptiles, birds, and mammals. The species are listed in alphabetical order of Latin names, and you may consult the list of "Organisms Used" (at the end of the book), or the program Organism in the circadian software package, if you need help identifying the common names. The mean free-running period for all species combined is 23.89 hours with a standard deviation of 0.65 hours. Thus, the mean free-running period is only 7 minutes shorter than the duration of a day, and the mean deviation from this mean is 39 minutes. The accuracy and precision of the circadian system are remarkable, particularly if one considers that the duration of a day was several hours shorter than 24 hours when the first animals evolved, as will be discussed in Chapter 9.

Long freeruns like those shown in Figures 6.5 and 6.6 are not unusual, but they are not normative either. It is true that some species continue to exhibit circadian rhythmicity in conditions of constant darkness or constant light for the whole life of the organism. As a matter of fact, 77% of fruit flies from a population maintained in constant light for 600 generations (the equivalent of 18,000 years for humans) exhibited circadian rhythmicity in constant darkness.[158] Nonetheless, a gradual, or even abrupt, loss of rhythmicity has been reported for various species maintained in conditions of constant darkness or constant light for a few days or weeks. An example of gradual loss of rhythmicity is shown in Figure 6.8. Generally, the rhythm of running-wheel activity of golden hamsters

TABLE 6.1
Circadian Period in Constant Darkness[a]

Species[b]	Period (hours)	Sources
Acomys cahirinus	24.2	[442]
Acomys russatus	23.7	[196]
Anopheles gambiae	23	[16]
Aotus azarai	23.8	[157]
Aotus lemurinus	24.3	[189]
Aotus trivirgatus	23.6	[169]
Apis cerana	22.8	[11,17]
Apis mellifera	23.3	[18–20,443]
Apteronemobius asahinai	25.5	[22]
Armadillidium vulgare	24.5	[5]
Arvicanthis ansorgei	24.1	[21,444–446]
Arvicanthis niloticus	23.8	[219,220,447,448]
Bombus terrestris	23.7	[23,24]
Caenorhabditis elegans	23.6	[14]
Callithrix jacchus	23.3	[449]
Camelus dromedarius	24.5	[215]
Carassius auratus	25.0	[39,450]
Cavia porcellus	23.6	[177,451]
Clethrionomys rutilus	23.9	[170]
Columba livia	23.1	[48]
Coturnix coturnix	23.1	[166,177,359,452]
Cryptomys hottentotus	24.1	[453]
Ctenomys knighti	24.3	[112]
Danio rerio	25.4	[38,42,43]
Dasyuroides byrnei	23.7	[111]
Dimorphostylis asiatica	24.9	[2]
Dipodomys merriami	24.0	[32]
Drosophila melanogaster	24.4	[12,25,454]
Eulemur fulvus	25.2	[157]
Eutamias sibiricus	23.9	[386]
Felis catus	23.9	[99]
Funambulus pennanti	23.4	[113,455]
Gekko gecko	23.1	[34]
Georychus capensis	24.3	[388,456]
Glaucomys volans	23.8	[91]
Glyphiulus cavernicolus	25.7	[6]
Hipposideros speoris	23.6	[109,457]
Homo sapiens[c]	24.5	[118–122,127–129,223,413,414, 416,425,427,428, 458–463]
Iguana iguana	24.0	[29,30,163]
Lemniscomys barbarus	23.9	[114]
Leucophaea maderae	23.3	[464]
Limulus polyphemus	23.9	[465]
Lycosa tarentula	24.1	[1]
Macaca mulatta	24.0	[466,467]
Macaca nemestrina	23.0	[107]
Meriones unguiculatus	24.2	[448,468]
Mesocricetus auratus	24.0	[87,90,151,152,312,316,323, 338,379,448,469–471]
Microcebus murinus	23.0	[103,195,472–474]
Microtus arvalis	23.5	[173]
Molossus molossus	23.2	[475]
Mus booduga	23.4	[476,477]
Mus musculus	23.6	[72,74,75,77,79,80,154,175, 204–207,258,356,448,469, 471,478–497]
Octodon degus	23.6	[218,419,448,468,498–500]
Oryctolagus cuniculus	23.9	[96]
Otolemur garnettii	22.6	[501]
Ovis aries	23.8	[190]
Passer domesticus	24.8	[50,52,502]
Perognathus longimembris	23.6	[503]
Peromyscus leucopus	24.0	[469]
Peromyscus maniculatus	22.9	[469]
Phodopus sungorus	24.0	[358,448,504]
Platynereis dumerilii	23.6	[15]
Podarcis sicula	23.3	[505]
Protophormia terraenovae	25.0	[506]
Rattus norvegicus	24.3	[21,61,64,68,69,153,180,197, 209,448,507–515]
Saimiri sciureus	24.8	[221,222]
Sceloporus occidentalis	23.6	[216]
Sigmodon hispidus	23.2	[117]
Sminthopsis macroura	23.2	[211]
Spalacopus cyanus	23.7	[516]
Sturnus vulgaris	23.7	[46,47]
Sylvia borin	23.8	[59]
Tamias striatus	24.9	[517]
Tinca tinca	22.9	[40]
Tupaia belangeri	23.7	[214]
Urocitellus parryii	24.5	[518]
Ursus arctos	24.3	[519]

[a] In a few cases, values measured in constant darkness were not available, and the values shown were obtained in constant dim light. The values shown are the means for each species, and it should be pointed out that some species exhibit greater interindividual variability than others.

[b] For common English equivalents of scientific names of the species, see the table of "Organisms Used" at the end of this book.

[c] Conditions of constant illumination were not standard in studies with humans. Differences in illumination conditions may be responsible for the great variability in reported values of free-running period (with a range of 24.2–26.0 hours).

maintained in constant darkness loses robustness after a month or two. It had already been shown, as early as 1875, that an *Acacia*-like plant, the crested wattle (*Paraserianthes lophantha*), loses rhythmicity within a few days after being released into constant darkness[159] (Figure 6.9).

Not surprisingly, different individuals of the same species may be affected differently by constant environmental conditions. Figure 6.10 shows running-wheel activity data for four Nile grass rats released into constant darkness. The animals whose records appear in Panels A and B exhibited robust rhythmicity both under a light–dark cycle and in constant darkness. However, rhythm robustness was drastically

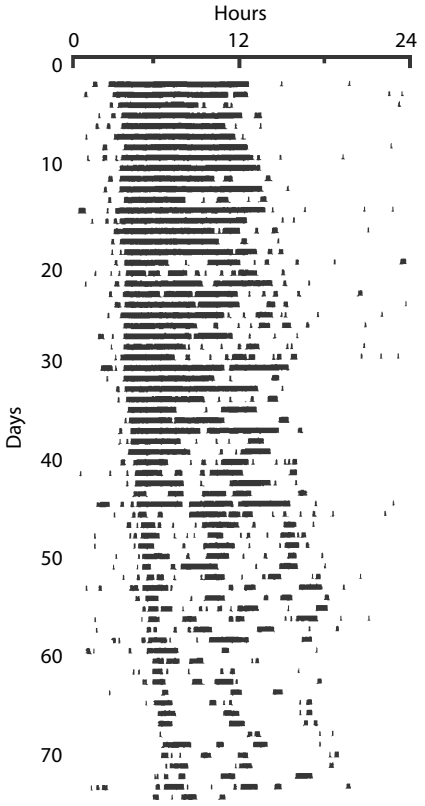


FIGURE 6.8 Nocturnal animal bothered by prolonged darkness. Although golden hamsters (*Mesocricetus auratus*) are fond of darkness and are more active during the dark phase than during the light phase of a light–dark cycle, their activity rhythms usually deteriorate if they are maintained in constant darkness for many weeks. This actogram shows the rhythm of running-wheel activity of a typical male golden hamster maintained in constant darkness. Activity becomes sparse approximately 50 days after the light–dark cycle is discontinued. (Adapted from Refinetti, R. et al., *J. Comp. Physiol. A*, 170, 181, 1992.)

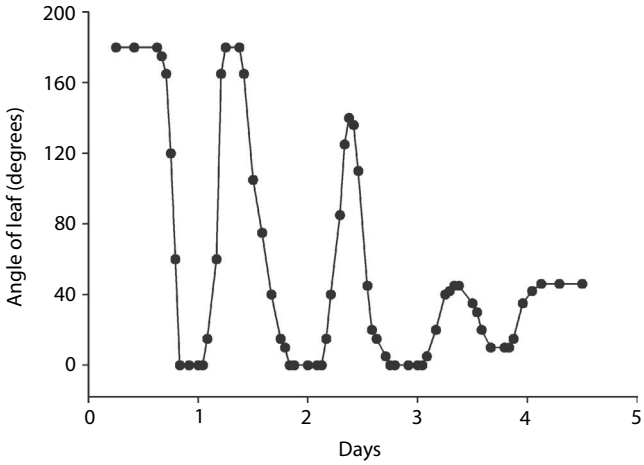


FIGURE 6.9 Plant bothered by darkness. The *sleep* movement of many plants persists in constant darkness, but only for a few days. The graph shows the daily variation in the position of a leaf of *Paraserianthes (Acacia) lophantha* in continuous darkness. The amplitude of the rhythm is greatly reduced after 2 or 3 days. (Data from Pfeffer, W., *Die periodischen Bewegungen der Blattorgane*, Wilhelm Engelmann, Leipzig, Germany, 1875.)

reduced in the animals whose records appear in Panels C and D. It should be pointed out that Nile grass rats do *not* lose rhythm robustness when maintained in constant *light*, as exemplified in Figure 6.11. In contrast, golden hamsters (which are nocturnal) often exhibit weak activity rhythms in constant light (Figure 6.12). When abrupt loss of rhythmicity occurs, it is generally observed only in constant light in nocturnal organisms and only in constant darkness in diurnal organisms, although gradual loss of rhythmicity may occur both in constant darkness and in constant light. Abrupt or gradual loss of rhythmicity has been observed in bacteria,[160] plants,[159,161] invertebrate animals,[1,3–5,12,20] lower vertebrates,[162,163] birds,[51,149,164–167] and mammals.[61,71,88,153,155,168–190]

In principle, loss of rhythmicity may result either from deterioration of the circadian pacemaker or from impairment of the overt rhythm (despite the presence of a fully functional pacemaker). That the latter possibility is correct in at least some cases is indicated by the data shown in Figure 6.13. Two laboratory rats were kept initially under a light–dark cycle (LD), then transferred to constant light (LL), and later transferred to constant darkness (DD). Both animals exhibited clear free-running rhythms in LL for the first 3 weeks. Afterward, the rhythms started to lose robustness, more so for the rat whose records are shown in Panel A than for the other rat. Both animals exhibited robust rhythmicity in DD. The smooth transition from LL to DD in Panel B indicates clearly that the clock never stopped running and did not experience any noticeable phase shift. The data in Panel A cannot be easily interpreted, but the rapid resumption of rhythmicity upon transfer to DD also suggests that the clock remained functional. Thus, it is reasonable to conclude that the reduction of rhythm robustness in LL in both animals was due to impairment of the overt rhythm and not to deterioration of the circadian pacemaker. Results from a study in mice support this conclusion and provide a potential explanatory mechanism. Although not as often as rats, some mice maintained in constant light become behaviorally arrhythmic. Researchers selected five of these mice, removed their brains, and recorded activity of the master circadian pacemaker *in vitro*. They found that the global activity pattern of the brain tissue was arrhythmic (just like the behavior of the live animal) but that activity of individual brain cells was rhythmic. That is, the brain cells remained rhythmic and were simply out of synchrony with each other when the animals were kept in constant light for long.[191]

A curiosity about the data in Figure 6.13 is that the free-running periods were much longer in LL than in DD. During the first 2 weeks in LL, both rats exhibited a free-running period of 24.9 hours; during the first 2 weeks in DD, they exhibited a free-running period of 24.4 hours. Thus, constant light caused a half-hour lengthening of the circadian period. This phenomenon was noticed many years ago by two of the three forefathers of circadian physiology (who were presented in Chapter 1). While reviewing numerous studies in various species, Aschoff noticed that different intensities of constant

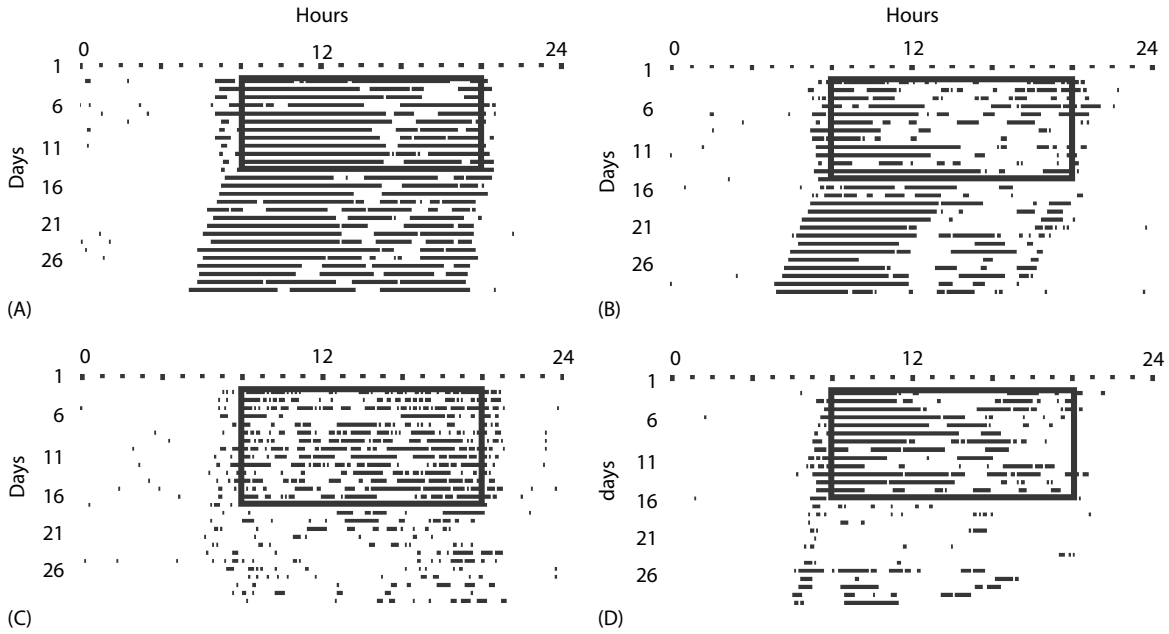


FIGURE 6.10 Diurnal animal bothered by darkness. Nile grass rats (*Arvicanthis niloticus*) are diurnal and, consequently, are more active during the light phase than during the dark phase of a light–dark cycle. When placed in constant darkness, some individuals remain rhythmic but others show great deterioration in the activity pattern. These actograms show the rhythms of running-wheel activity of four grass rats (Panels A through D) transferred from a light–dark cycle (as indicated by the rectangles) to constant darkness. (Data from the archives of the Refinetti lab.)

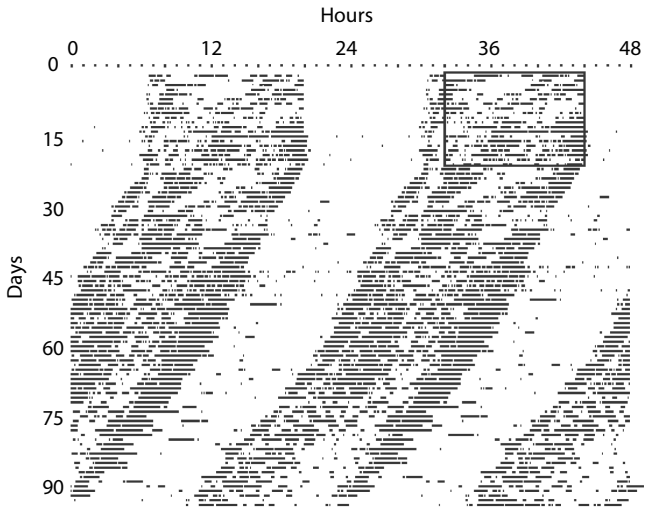


FIGURE 6.11 Diurnal animal not bothered by light. This double-plotted actogram shows the running-wheel activity pattern of a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle for 3 weeks (as indicated by the single-plotted rectangle) and in constant light (300 lux) for 10 weeks. Robust rhythmicity with a period of 23.8 hours was maintained throughout the exposure to constant light. (Data from the archives of the Refinetti lab.)

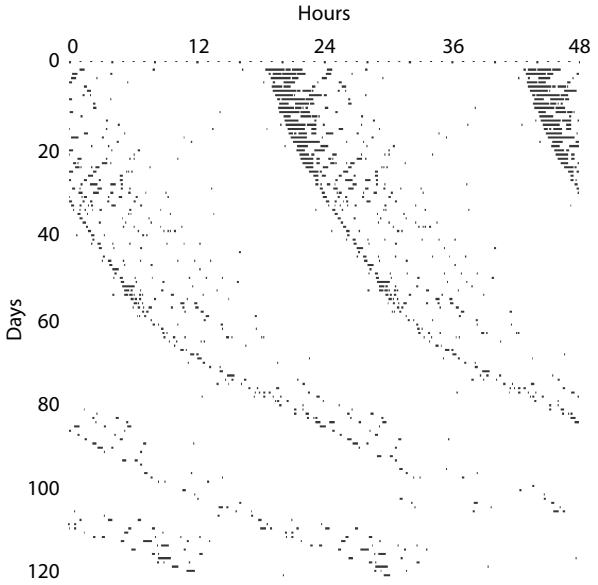


FIGURE 6.12 Hamster bothered by light. The activity rhythms of golden hamsters (*Mesocricetus auratus*) deteriorate not only under constant darkness (as seen in Figure 6.8) but also under constant light, as seen in this double-plotted actogram of the running-wheel activity of a male golden hamster maintained in constant light (300 lux) for 4 months. (Data from the archives of the Refinetti lab.)

light had different effects on the free-running period.[192,193] As a general rule (subject to many exceptions), he postulated that increased light intensity lengthens period in nocturnal animals but shortens period in diurnal animals. Pittendrigh referred to this dependence of the free-running period on the intensity of constant illumination as "Aschoff's rule."[194]

We will discuss the meaning of Aschoff's rule in Chapter 7 (after we have examined the mechanism by which light affects the circadian pacemaker), but we must characterize it a little further here. We may start by examining the activity records shown in Figure 6.14. As is evident in

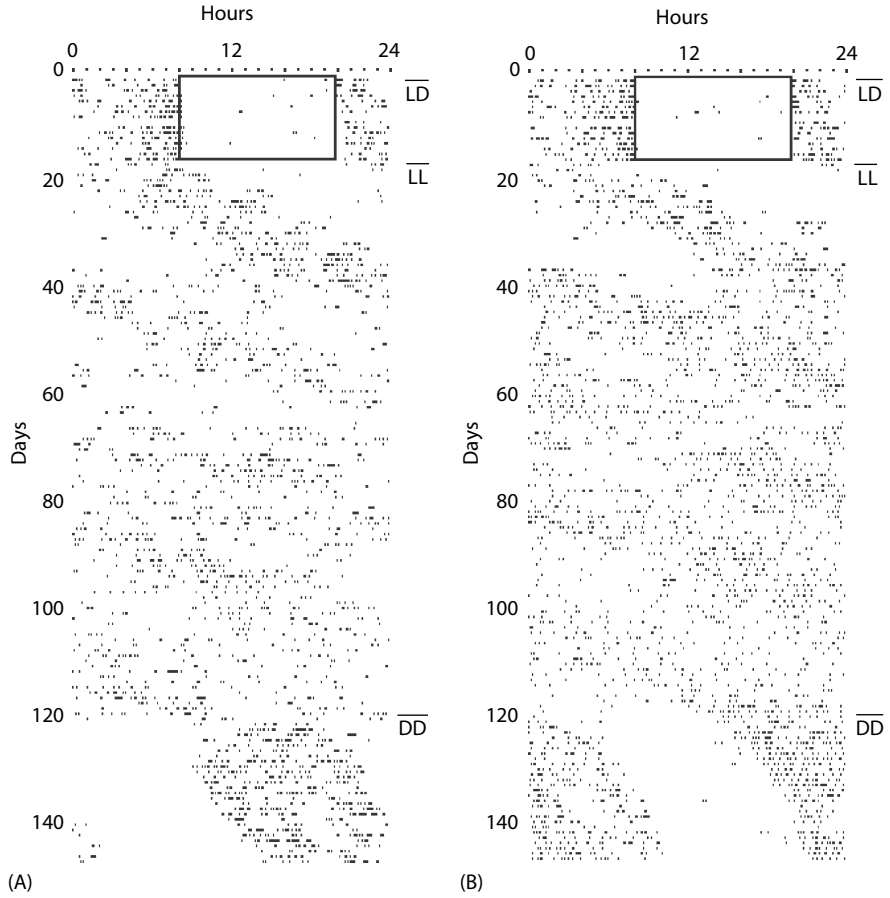


FIGURE 6.13 Rats greatly bothered by light. The activity rhythms of laboratory rats (*Rattus norvegicus*) are not disturbed by exposure to constant darkness, but they exhibit great deterioration in constant light. The rats whose running-wheel activity rhythms are shown in these two actograms were exposed to a light–dark cycle for 2 weeks (LD), to constant light (300 lux) for 15 weeks (LL), and to constant darkness for 3 weeks (DD). Although both rats (Panels A and B) exhibited free-running rhythms (with periods approaching 25 hours) in the first weeks under constant light, the rhythms deteriorated later on. Exposure to constant darkness rapidly restored rhythmicity. (Data from the archives of the Refinetti lab.)

Panel A, domestic mice are nocturnal, whereas Nile grass rats are diurnal. When kept in constant darkness, both species exhibit free-running rhythmicity with periods shorter than 24 hours (Panel B). When kept in constant light (360 lux), both species still exhibit free-running rhythmicity, but now the period is longer than 24 hours in both cases (Panel C). Evidently, domestic mice follow Aschoff's rule, whereas Nile grass rats do not. Nevertheless, it is evident that constant light affects the free-running period of both species, even if not in the way predicted by Aschoff. As a matter of fact, Aschoff explicitly admitted that his rule had as many exceptions as positive cases in the subgroup of diurnal mammals.[193] Inspection of the results obtained in 47 studies published after Aschoff's last review indicates much agreement regarding nocturnal mammals,[103,117,155,175,177,180,195–210] with a single exception of a marsupial species,[211] but many exceptions among diurnal animals of various phyla and classes. Although free-running periods have been found to be shorter in constant light than in constant darkness in some diurnal animals,[24,38,55,177,212–215] they have been found to be *longer* in constant light than in constant darkness in diurnal honey bees,[11,17,19,20] goldfish,[39] lizards,[216,217] quail,[166] rodents,[114,218–220] and primates.[107,221–223] It would seem, therefore, that the rule is useless for diurnal organisms. In order to honor Aschoff, it might be wise to state his rule as "the free-running period is affected by the intensity of constant illumination," and to discard the claim about the difference between diurnal and nocturnal organisms.

More data from Nile grass rats are shown in Figure 6.15. Notice that, for the particular animal whose records are shown in the figure, the free-running period is longer than 24.0 hours for light intensities of 10 lux and above. At 1 lux (a twilight level of illumination), the period is slightly shorter than 24.0 hours. This animal had an atypically short free-running period of 23.3 hours in constant darkness (0 lux). The mean values of circadian period for 16 Nile grass rats are shown in Figure 6.16. Period clearly lengthens as the intensity of illumination increases. Although intensities higher than 100 lux were not tested, the slight loss of slope of the curve suggests an asymptotic level of about 24.6 hours at 1000 lux in this species.

If you paid attention to the data in Figure 6.15, you must have noticed that the duration of the active phase of the

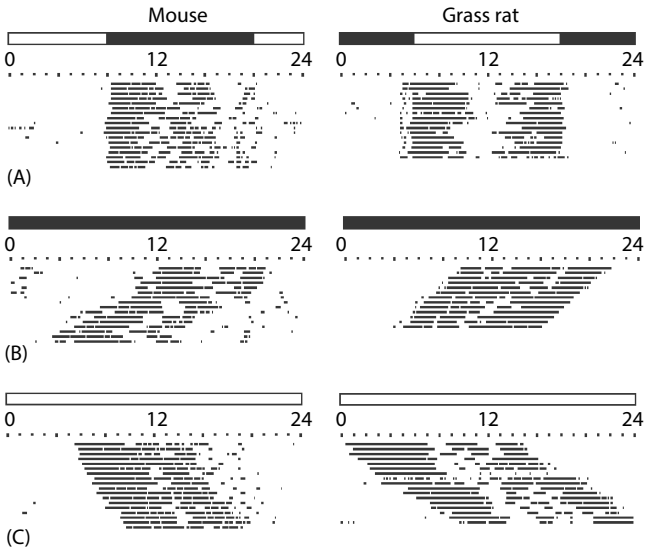


FIGURE 6.14 Effects of constant illumination on circadian period. The actograms show the rhythms of running-wheel activity of a domestic mouse (*Mus musculus*) and a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle, in constant darkness, and in constant light (as indicated by the horizontal bars above each actogram in Panels A through C). For both species, circadian period is 24.0 hours under the light–dark cycle, shorter than 24.0 hours in constant darkness, and longer than 24.0 hours in constant light (300 lux). (Data from the archives of the Refinetti lab.)

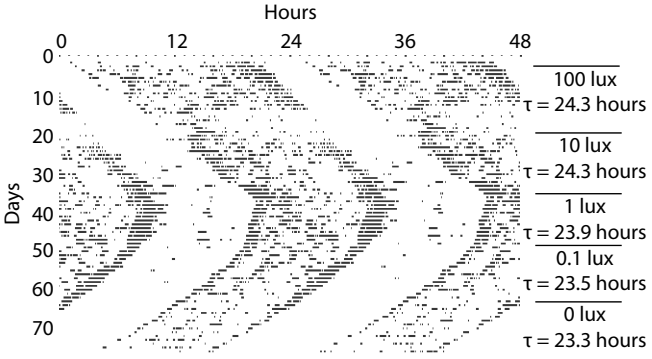


FIGURE 6.15 Constant light and circadian period. This double-plotted actogram shows the rhythm of running-wheel activity of a Nile grass rat (*Arvicanthis niloticus*) maintained under constant light of varying intensities (as indicated on the right margin). Although the circadian period is longer than 24.0 hours under bright light, it is shorter than 24.0 hours under dim light or in darkness. (Data from Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004.)

circadian cycle (α) seemed to shrink when the period became shorter than 24 hours. As will be discussed in Chapter 7, this may be a side effect of the action of light on the circadian pacemaker. However, it is important to point out that actograms normally give the impression of a compression of α even when there is no actual compression. Consider Figure 6.17. The same data set (running-wheel activity data from a golden hamster) is plotted in three different formats: Modulo 24 (the usual format), Modulo 23 (to simulate a 1-hour lengthening of period), and Modulo 25 (to simulate

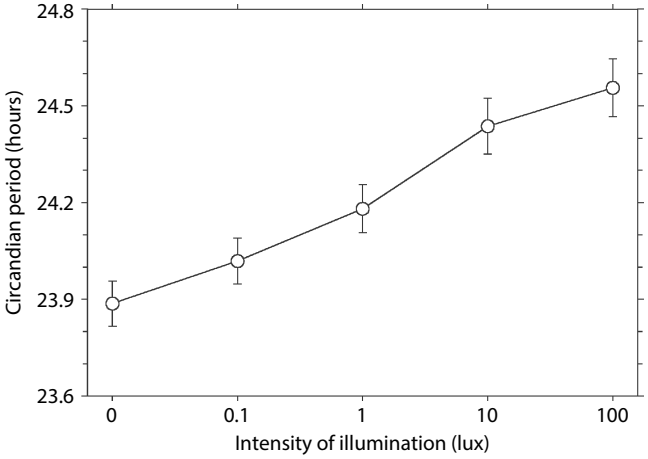


FIGURE 6.16 Intensity of illumination and circadian period. The graph shows the mean (±SE) circadian period of 16 Nile grass rats (*Arvicanthis niloticus*) maintained under constant illumination of varying intensities. Notice that the abscissa is logarithmic and that, for convenience, 0 lux is shown at the position of 0.01 lux. It can be clearly seen that circadian period lengthens as the intensity of illumination increases. (Data from Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004.)

a 1-hour shortening of period). Notice the apparent reduction of α when the period is shorter or longer than 24 hours. Of course, α has not actually changed, as the data set is the same in the three formats. The slope of the actogram gives the *impression* that α is shorter. In other words, the shrinking of α during freeruns is often just an illusion. If you want to verify it yourself, you can do it easily. Figure 6.17 was built using the program **Plot** from the circadian physiology software package and the sample data set **A03**. The take-home message here is that actograms are not a reliable tool for the study of α. Based on comments made in several published articles (which I will not identify lest I embarrass the authors), I believe that many circadian physiologists are unaware of this limitation of actograms.

The level of illumination is not the only factor that has been shown to affect circadian period. Administration of various drugs (Figure 6.18) has also been shown to alter the free-running period. The effects of *heavy* drugs on the circadian system have not been thoroughly investigated, but a few studies have been conducted on the action of cocaine,[224,225] morphine,[226] and heroin.[227] Probably because of their clear and reproducible effects on circadian period, two drugs have received special attention: methamphetamine and deuterium oxide.

Methamphetamine mixed in the drinking water, or chronically infused intravenously, lengthens the free-running period of circadian rhythms in rats and mice. In normal animals, methamphetamine adds up to 5 hours to the duration of the circadian cycle.[228–232] As if this were not impressive enough, methamphetamine *induces* circadian rhythmicity in animals previously rendered arrhythmic by surgical destruction of the master circadian clock in the brain[150,233–235] or by mutagenic ablation of a gene essential

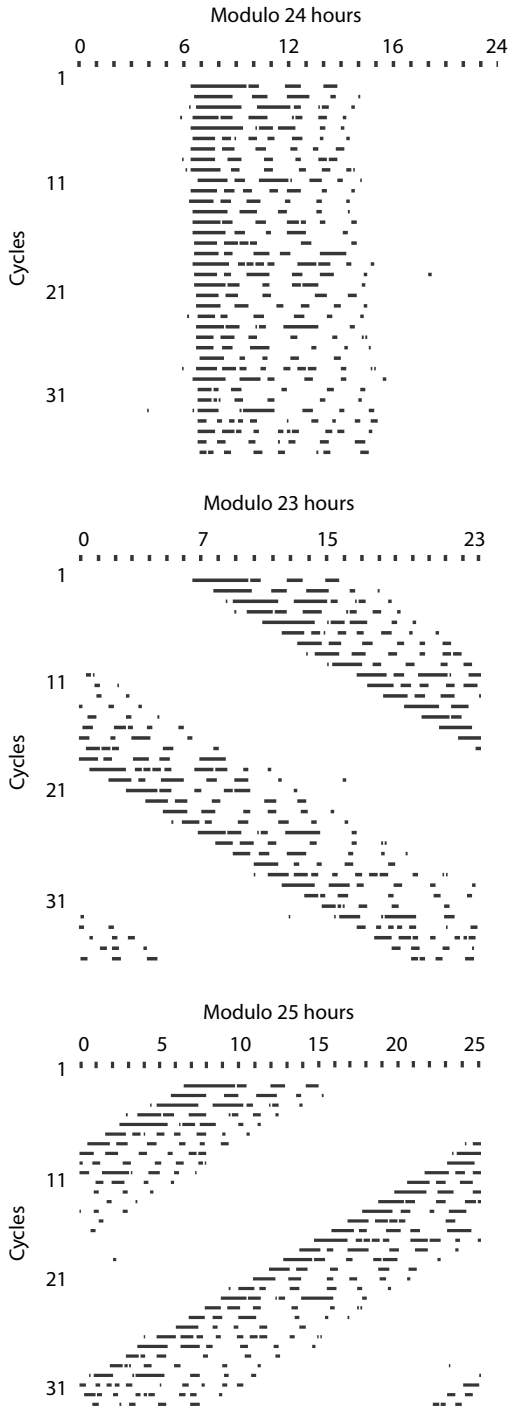


FIGURE 6.17 Optical illusion. The same data (running-wheel activity of a golden hamster with a free-running period of 24.0 hours) are plotted in 24-, 23-, and 25-hour actograms (Modulo 24, Modulo 23, and Modulo 25, respectively). The duration of the activity phase (α) seems to be shorter when the data are plotted in Modulo 23 and Modulo 25 than in Modulo 24.

for rhythmicity.[229,236] Representative records from two mice are shown in Figure 6.19. The records in Panel A correspond to a normal mouse that was maintained in constant darkness and received methamphetamine in the drinking water during the days between *Start* and *Stop*. Notice that a free-running



FIGURE 6.18 Snorting cocaine. Many drugs are known to affect the operation of the circadian pacemaker. (Copyright ArtToday, Tucson, AZ.)

rhythm with period slightly shorter than 24 hours was present initially and persisted throughout the study. Notice also that methamphetamine administration added a 28-hour rhythm on top of the shorter rhythm. It is very difficult *not* to infer that methamphetamine activated a second clock, distinct from the normal circadian pacemaker. The records in Panel B reinforce this inference. A genetically mutated mouse that did not exhibit circadian rhythmicity in constant darkness started to exhibit 28-hour rhythmicity when given methamphetamine in the drinking water. In the case of this particular mouse (and, actually, in the case of 6 out of 10 mice tested), 24-hour rhythmicity persisted for about a month after the administration of methamphetamine was discontinued. The reason for this is not known.

Deuterium oxide (heavy water) mixed with regular drinking water also lengthens the free-running period of circadian rhythms. In crayfish, deuterium oxide adds up to 10 hours to the duration of the circadian cycle,[237] but period lengthening of "only" 1 or 2 hours has been observed in the fruit fly[238] and in rodents.[194,239–241] Average data for the fruit fly are shown in Figure 6.20. Flies were kept in constant darkness and were given drinking water with varying percentages of deuterium oxide. Notice that the average period lengthened from 24.2 hours at 0% to 26.8 hours at 50% concentration. Unlike methamphetamine, deuterium oxide seems to act directly on the master clock, as it does not add a second component to the original rhythm[240,241] and does not seem to induce rhythmicity in rodents rendered arrhythmic by surgical destruction of the circadian clock.[239]

Research has also been conducted on the effects of many other drugs on properties of the circadian system. Drugs that naturally serve as neurotransmitters in the nervous system will be discussed in Chapters 12 and 13, and the circadian effects of antidepressants will be discussed in Chapter 16. Two drugs that should be mentioned here are alcohol (ethanol) and the benzodiazepines. Ingestion of *alcohol* has many effects on the body, some of which interfere with circadian rhythms, but direct effects of alcohol on the circadian system seem to

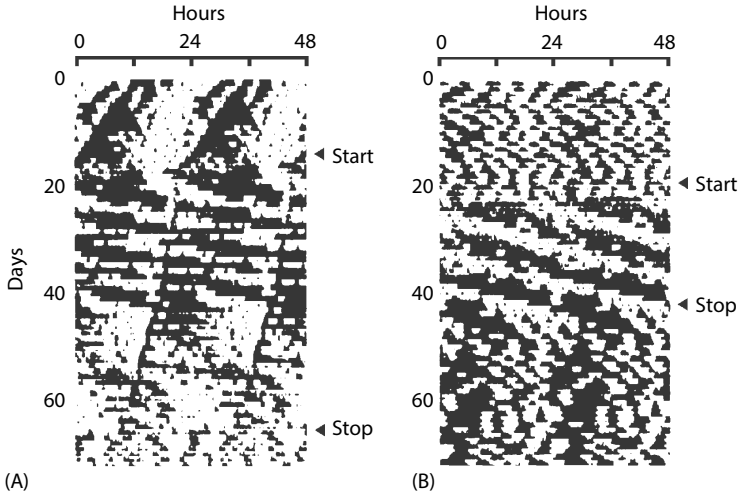


FIGURE 6.19 Methamphetamine induces circadian rhythmicity. These double-plotted actograms show the running-wheel activity rhythms of two mice (*Mus musculus*) maintained in constant darkness. Both mice were treated with methamphetamine hydrochloride dissolved in their drinking water, starting and stopping on the days indicated on the right margins. In a normal mouse (A), administration of methamphetamine added a 28-hour component to the existing 24-hour rhythm. In a genetically arrhythmic mouse (B), administration of methamphetamine induced 28-hour rhythmicity. (Adapted from Masubuchi, S. et al., *Eur. J. Neurosci.*, 14, 1177, 2001.)

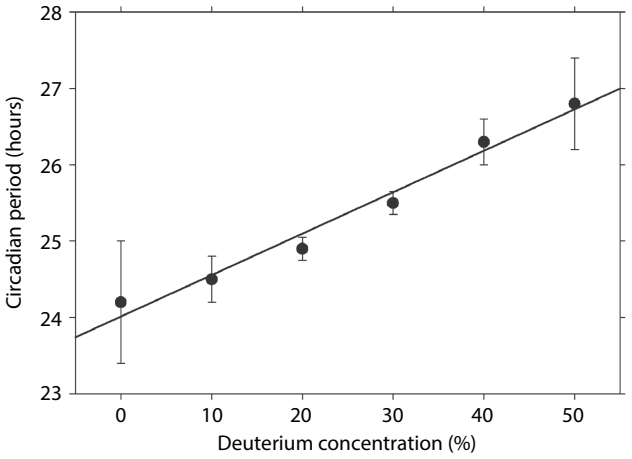


FIGURE 6.20 Heavy water lengthens circadian period. The graph shows the mean circadian periods of the activity rhythm of fruit flies (*Drosophila melanogaster*) maintained in constant darkness and provided with drinking water containing different amounts of deuterium oxide (D_2O). Each point corresponds to the mean (±SE) of approximately 20 flies. The higher the concentration of deuterium, the longer is the circadian period. (Data from White, L. et al., *Chronobiol. Int.*, 9, 250, 1992.)

be minor.[73,156,242–247] *Benzodiazepines* (a class of drugs widely used in the treatment of anxiety and insomnia) have a well-documented effect on the circadian system: in single doses, they can reset the circadian clock[248–252] and, if administered chronically, they have a small effect on circadian period.[253] The effects of benzodiazepines on the circadian system are believed to be mediated by their primary effect on motivation and physical activity.[254] Finally, a few studies have suggested that vitamin B_{12}[255–257] and a high-fat diet[258,259] may also have a small effect on the circadian system.

6.1.3 Temperature Compensation

An important aspect of endogenous rhythmicity that we have not discussed yet is that of *temperature compensation* of the circadian clock. Despite the complexity of behavioral and autonomic functions, the basic process of life comes down to chemical reactions catalyzed by enzymes. The rate of the reactions is temperature dependent, so that an elevation of 10°C causes approximately a doubling of the reaction rate.[260,261] This temperature dependence is numerically expressed as a Q_{10} (the quotient of reaction rates 10°C apart), the usual value of Q_{10} being about 2. Thus, a temperature fall of 10°C slows down the functioning of the body to half of what it was earlier. In cold-blooded organisms, this is evident in the dependence of metabolic rate of the whole organism on the temperature of the environment, as has been extensively documented in plants,[262–265] arachnids,[266,267] crustaceans,[268–275] insects,[276–279] and fishes.[280–286]

Birds and mammals have freed themselves from the consequences of temperature dependence by evolving warm-bloodedness—that is, by maintaining the temperature of the body constant at all times. However, cold-blooded animals (as well as plants, fungi, bacteria, and other life forms) do not have this luxury. Their body temperature (and, therefore, their metabolic rate) varies along with ambient temperature. As a matter of fact, as we saw in Chapter 5, even warm-blooded animals may experience fluctuations of body temperature of up to 5°C or more as part of their normal circadian rhythmicity. This could render the circadian clock useless. If the circadian clock were not temperature compensated, it would be speeded up by high temperatures and slowed down by low temperatures. As indicated by the exponential curve in Figure 6.21, an organism with a circadian period of 20 hours at a body temperature of 30°C would have a period of more

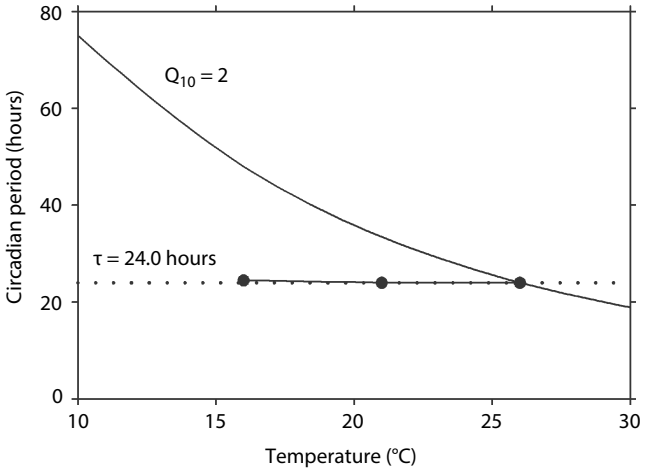


FIGURE 6.21 Temperature compensation in the circadian system of an ectotherm. The graph shows that the period of the rhythm of eclosion of the fruit fly (*Drosophila pseudoobscura*) is independent of environmental temperature, at least within the tested range. The sloped line indicates the normal temperature dependence of cellular processes ($Q_{10} = 2$). The dotted line indicates full temperature independence ($\tau = 24.0$ hours). Clearly, the circadian system of the fruit fly is temperature compensated. (Data points from Pittendrigh, C.S., *Proc. Natl. Acad. Sci. USA*, 40, 1018, 1954.)

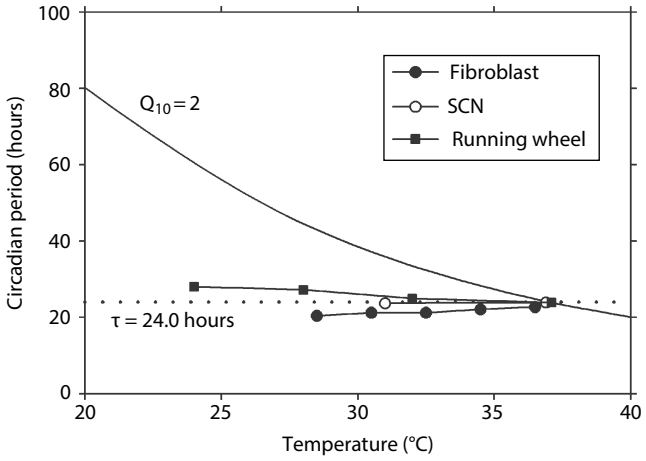


FIGURE 6.22 Temperature compensation in the circadian system of an endotherm. The graph shows that the period of circadian rhythms in the laboratory rat (*Rattus norvegicus*) is independent of tissue temperature. Data are shown for cultured fibroblasts exhibiting circadian rhythmicity *in vitro*, cultured brain tissue containing the master circadian clock (SCN) *in vitro*, and the rhythm of running-wheel activity of rats whose body temperature was temporarily lowered by immobilization and immersion in freezing water. The sloped line indicates the normal temperature dependence of cellular processes ($Q_{10} = 2$). The dotted line indicates full temperature independence ($\tau = 24.0$ hours). Clearly, the circadian system of the rat is temperature compensated. (Data points from Izumo, M. et al., *Proc. Natl. Acad. Sci. USA*, 100, 16089, 2003; Ruby, N.F. et al., *J. Neurosci.*, 19, 8630, 1999; Gibbs, F.P., *Am. J. Physiol.*, 241, R17, 1981.)

than 70 hours at a body temperature of 10°C. In contrast, the experimentally determined circadian period of eclosion of fruit flies is indicated by the line connecting the closed circles. Notice that the line essentially overlaps the dotted line that indicates a temperature-independent period of 24 hours. That is, the circadian clock that controls eclosion in fruit flies is temperature compensated ($Q_{10} = 1$, not 2).

Temperature compensation of circadian period has been documented in a variety of organisms,[193,287] including bacteria,[288–290] mold,[291] bean plant,[292] roundworm,[14,293] fruit fly,[294] zebrafish,[38,42] ruin lizard,[295,296] bat,[297] and various species of rodents.[95,104,298–305] Examples of data collected from laboratory rats are shown in Figure 6.22. Measurements of circadian period were made from fibroblast cultures, brain slices containing the master circadian pacemaker (SCN), and running-wheel activity. Notice that, although none of the variables was absolutely temperature independent, all three of them exhibited a Q_{10} very close to 1. Whatever process is responsible for temperature compensation of period, it is evidently active in tissues of warm-blooded animals to the same extent as in tissues of cold-blooded organisms. This property ensures that the clock runs at its natural speed regardless of changes in the temperature of the body or of the environment.

6.2 INHERITANCE MECHANISMS

The endogenous nature of the circadian clock necessitates some form of genetic inheritance. Since different species have clearly distinct circadian periods, even though they live in similar environments, genetic influences must be stronger than environmental influences. However, additional information is necessary to ascertain how strong the genetic influence is. Valuable information has been provided by various studies dealing with mutations of the genes that control circadian rhythmicity. In the early 1970s, specific single-gene mutations were identified in the fruit fly, *Drosophila melanogaster*,[306] and in bread mold, *Neurospora crassa*.[307] In the fruit fly (Figure 6.23), the gene—called "per," for period—is located in the X chromosome and exhibits multiple mutant alleles. The first three alleles that were identified shortened (per^S, $\tau = 19$ hours), lengthened (per^L, $\tau = 28$ hours), or eliminated (per^0) circadian rhythmicity of activity.[306] Thus, a homozygous fly with the per^S alleles maintained in a constant environment lived 19-hour days rather than 24-hour days! "Flies from Mars!" could have been the newspaper headline (Figure 6.24), except that the Martian day is actually very close to 24 hours.

Examples of eclosion rhythms of fruit flies maintained in constant darkness are shown in Figure 6.25. As is true in many arthropods, adult fruit flies eclode from the pupal stage at a predictable time during development, and the fine-tuning of eclosion time is controlled by the circadian system. Notice that there is one eclosion cycle per day for wild types ($\tau = 24$ hours), but four cycles in 3 days for per^S mutants ($\tau = 19$ hours) and not quite three cycles in 3 days for per^L mutants ($\tau = 28$ hours). Of course, the fact that a single-gene mutation can have such a drastic effect on free-running rhythms indicates that the period of the circadian pacemaker has a very strong genetic component. Several other *per* alleles were identified later,[308] and,



FIGURE 6.23 Fruit fly up close. The fruit fly (*Drosophila melanogaster*) is widely used in biological research, including research on circadian rhythms. (Image courtesy of Jim Ehrman, Digital Microscopy Facility, Mount Allison University, Sackville, New Brunswick, Canada.)

in the early 2000s, three other mutant genes were created: *tim* (for timeless),[309] *tik* (for timekeeper),[309] and *andante*.[310] Of six mutant alleles of *tim*, two shorten the circadian period (to about 21 hours) and four lengthen it (to about 27 hours). When a long *tim* is combined with a long *per*, the circadian period reaches 41 hours[309]—a duration so long that it would not receive the designation of *circadian* were it not for the fact that we know that we are actually fiddling with the circadian system!

In bread mold (Figure 6.26), three mutations of the gene *frq* (for frequency) were originally identified.[307] Whereas wild types have a period of 21.6 hours, *frq-1* mutants have a period of 16.5 hours, *frq-2* mutants have a period of 19.3 hours, and *frq-3* mutants have a period of 24.0 hours. Evidently, the *frq-3* mutants are more *normal* than the wild types. Additional clock mutations in bread mold were identified later.[311]

So, circadian period is genetically determined in flies and fungi. What about mammals? In 1989, a natural clock mutation was identified in the golden hamster,[312] and in 1994, a mutation was produced in the laboratory mouse by mutagenesis and subsequent screening.[313] Here is their story:

The discovery of the *tau* mutation in the golden hamster (Figure 6.27) was fortuitous. Martin Ralph, now a professor at the University of Toronto, was then a graduate student in Michael Menaker's laboratory at the University of Oregon. Menaker, as you remember from Chapter 1, is currently at the University of Virginia. As part of his experiments, Ralph had numerous hamsters free-running in constant darkness. One of the hamsters displayed an unusual pattern of activity, seeming to phase advance 2 hours each day. Knowing about the studies in fruit flies, Ralph restrained himself from the impulse to discard the *defective* hamster and replace it with a *good* one. Rather, he started in-breeding successive generations of the unusual hamster. He soon found out that the abnormal behavior was the result of a single-gene mutation with a single mutant allele, *tau*. While normal hamsters (+/+) have a circadian period very close to 24 hours, heterozygous mutants (+/*tau*) have a period of 22 hours, and homozygotes (*tau*/*tau*) have a period of 20 hours.[312,314,315] Headline news: "Hamsters from Mars!"

Figure 6.28 shows how dramatic the effects of the mutation are. The actogram on the left shows the running-wheel activity rhythm of a wild-type hamster (+/+) kept in constant darkness. As is typical of golden hamsters, the rhythm is very robust, and the free-running period is very close to 24.0 hours. The actogram on the right shows the running-wheel activity rhythm of a homozygous mutant (*tau*/*tau*). The rhythm is also quite robust, but the free-running period is so short (20.1 hours) that it is impossible not to notice the difference. A very interesting feature of the mutation is that it shortens the activity cycle proportionally, so that, if the actograms are plotted on time scales proportional to circadian period, there is no obvious difference between the rhythms of wild types and homozygous mutants (Figure 6.29). The proportional shortening of the rhythm occurs not only for the running-wheel activity rhythm[316,317] but also for the body temperature rhythm,[84] the feeding rhythm,[318] the rhythm of energy expenditure,[319] rhythms of hormone secretion,[320,321] the rhythm of photoreceptor regeneration in the eye,[322] and the circadian modulation of the reproductive system's photoperiodic response.[323,324] This demonstrates that the effect of the *tau* gene is general to the circadian pacemaker rather than restricted to a particular effector mechanism. Furthermore, there is good evidence that the mutation is specific to the circadian system and does not alter other processes of biological timing. Thus, the mutation does not affect the frequency of the pacemaker of the heart,[89] the duration of the estrous cycle[325] (the equivalent of the menstrual cycle in rodents), or the maturation of the photoperiodic response.[326] Although ultradian oscillations in body temperature were found to be shorter in the mutants than in wild types,[81] the difference was probably an artifact of Fourier analysis, as previously discussed in Chapter 3. On the other hand, the finding of a lengthening of the ultradian period of hormone pulsatile secretion in mutants[327] seems to be legitimate.

Of course, we do not live in an ideal world, and it would be very surprising if the *tau* gene were as *pure* as I made it look so far. Additional studies on the effects of the mutation have identified several physiological changes not directly related to the shortening of circadian period. For example, the responsiveness of the circadian pacemaker to photic stimulation is enhanced in *tau*-mutant hamsters,[328–332] and the mutation may also affect the property of temperature compensation[305] and increase the longevity of the animals.[333] Homozygous *tau*-mutant hamsters bred in various laboratories around the world tend to be about 20% smaller in body size than wild-type littermates.[324,326,330,334,335] Efforts to understand these secondary effects of the mutation were deterred for many years by the



The New World Times

June 1, 1971 50 cents

FLIES FROM MARS!

They look like normal flies, but don't let their appearance fool you!

Their day is only 19 hours long!

By R.O. Dent

Knopka and Benzer announced today the the discovery of mutant flies whose day is only 19 hours long. It seems that their biological clock runs much faster than that of normal flies. It all results from the mutation of a single gene. Different mutations produce flies with long days or with no days at all (arrhythmic flies).

See FLIES, Page 2

They could be from Neptune, not Mars

By I.M. Dumb

The duration of a Martian day is not substantially different from a terrestrial day. However, days in Neptune are much shorter than days on Earth. Thus, the newly discovered flies may come from Neptune, not Mars. No evidence of a spaceship has been provided.

We have been unable to determine why these flies have come to Earth at this particular time, and the idea of a planned invasion cannot be excluded. A joint meeting of the Armed Forces has been called to evaluate potential preventive

FIGURE 6.24 Flies from Mars! This fictitious newspaper announces the discovery of the first genetic mutation found to specifically affect the circadian system.

lack of knowledge of the molecular aspects of the mutation. The situation started to improve in the year 2000, when the molecular identity of the *tau* gene was discovered. The *tau* allele is an allele of the casein kinase 1 epsilon (*ck1ε*) gene,[336] which codes for an enzyme (kinase) used for the phosphorylation of proteins in animals.

Before we move on to other single-gene mutations that affect circadian period in mammals, I should add a few comments about the heterozygous *tau*-mutant hamster (+/*tau*). As shown in Figure 6.30, the behavioral phenotype of +/*tau* hamsters is intermediary between those of +/+ and *tau*/*tau* hamsters. The offspring of heterozygous parents exhibits the phenotypic distribution expected of a semidominant allele. As exemplified in Figure 6.31 for a group of 78 hamsters with heterozygous parents, the distribution of phenotypes follows the 1:2:1 proportion. I should also mention a second fortuitous discovery of a mutation that affects circadian period in the golden hamster. In 2011, a mutation called "duper" was identified.[337] It also shortens the free-running period. A double mutant, homozygous for both *duper* and *tau*, has a free-running period of 18 hours.[338]

As for the *clock* mutation in the mouse, it was identified in 1994 by a team of researchers led by Joseph Takahashi, who was then at Northwestern University, in Illinois. Many years earlier, Takahashi had been a graduate student of Menaker's at the University of Oregon. When Menaker identified the *tau* mutation in 1989, Takahashi (who graduated in 1981) was very excited about it, but, of course, he wished that *he* had been the one to identify the first mammalian gene involved in circadian rhythmicity. More importantly, the *tau* gene was not very useful to molecular biologists, as the molecular biology of the hamster was not (and still is not) well known. A few years later, however, Takahashi, Menaker, and various other researchers received a multimillion dollar grant from the National Science

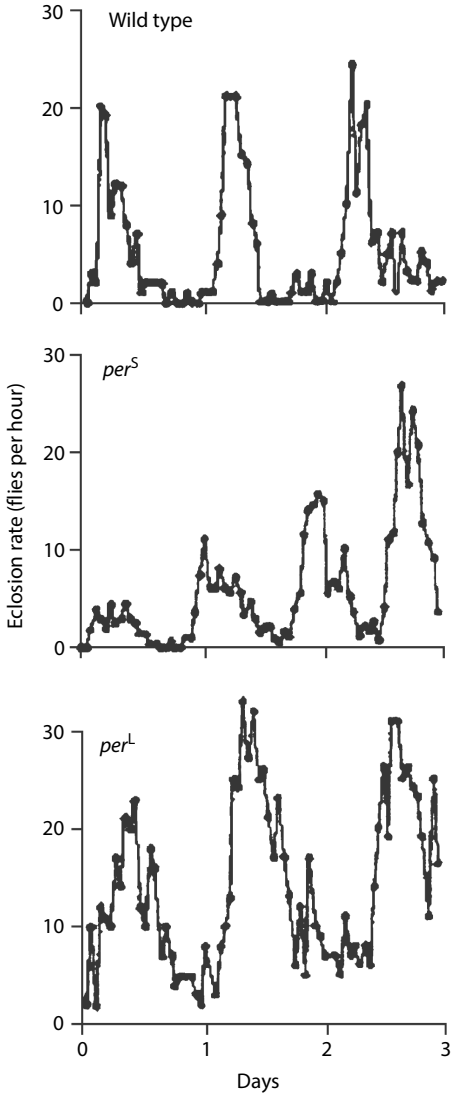


FIGURE 6.25 The mutant flies. The graphs show the variation in eclosion time of fruit flies (*Drosophila melanogaster*) kept in constant darkness. Wild-type flies exhibit one cycle per day (period ≈ 24 hours), per^S mutants exhibit four cycles in 3 days (period ≈ 19 hours), and per^L mutants exhibit fewer than three cycles in 3 days (period ≈ 28 hours). (Adapted from Konopka, R.J. and Benzer, S., *Proc. Natl. Acad. Sci. USA*, 68, 2112, 1971).

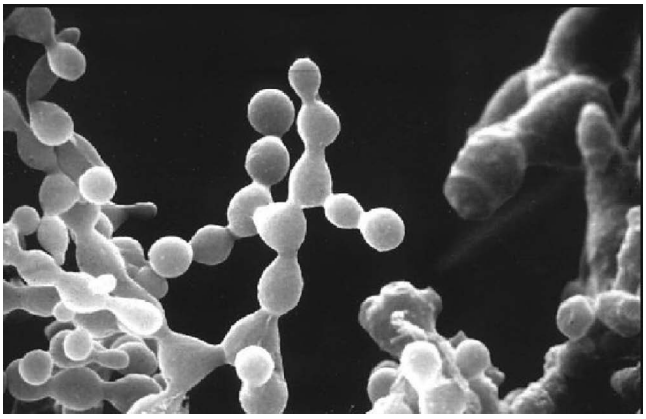


FIGURE 6.26 Mold up close. Bread mold (*Neurospora crassa*) has been widely used in the study of the genetics of circadian rhythmicity. (From Springer, M. and Yanofsky, C., *Genes Dev.*, 6, 1052, Copyright 1992 Cold Spring Harbor Laboratory Press. Reproduced with permission from the publisher and the authors.)

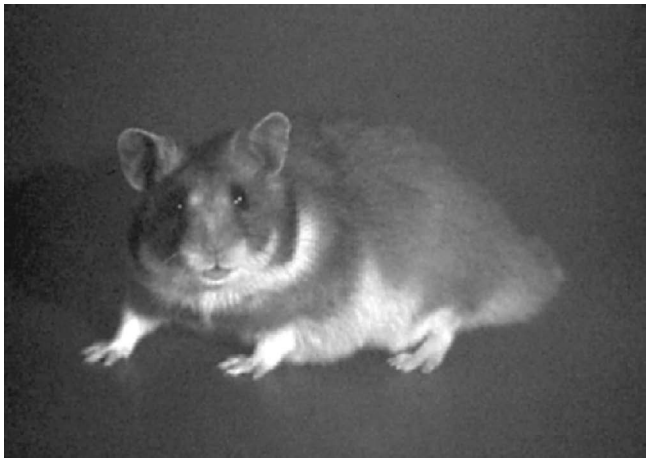


FIGURE 6.27 Mutant hamster up close. The golden hamster (*Mesocricetus auratus*) was the first mammal in which a genetic mutation that specifically affects the circadian system was found (the *tau* mutation). (Photo by R. Refinetti.)

Foundation to create a Center for Biological Timing, with headquarters at the University of Virginia. Emboldened by the financial and intellectual support of the federal government, Takahashi and several colleagues embarked on a very ambitious project to overdo nature and to create a circadian mutation of their own in the mouse. And their ambition paid off. After an extensive program of mutagenesis and screening of mutants, a *clock* mutant was created. The *clock* gene is a single-locus, semidominant gene located in chromosome 5. The mutation lengthens the circadian period of mice: unaffected mice (+/+) have a circadian period of 23.3 hours, heterozygous mice (+/*clock*) have a period of 24.4 hours, and homozygotes (*clock*/*clock*) have a period of 27.3 hours.[313,339,340] The *clock* mutant is an excellent animal for research at the molecular level, but it has not been studied at the organismal level as extensively as the *tau* mutant, partially because the homozygotes (*clock*/*clock*) lose circadian rhythmicity a few weeks after being placed in constant darkness. Representative actograms of the running-wheel activity rhythms of the three phenotypes are shown in Figure 6.32. Notice that the three animals were properly synchronized to the light–dark cycle (LD) at first and that all of them initially freeran in constant darkness (DD), but that the homozygous mutant lost circadian rhythmicity after a couple of weeks. Curiously, *clock*/*clock* mice do not lose rhythmicity in constant light, a condition under which some wild-type mice lose rhythmicity.[155] Other nonperiod-specific effects of the *clock* mutation include a disruption of reproductive cyclicity in females,[341] reduced fecundity in males,[342] impaired responsiveness to photic stimulation,[343] abnormal responsiveness to nonphotic stimulation,[344] *manic-like* behavior,[345] and obesity.[346]

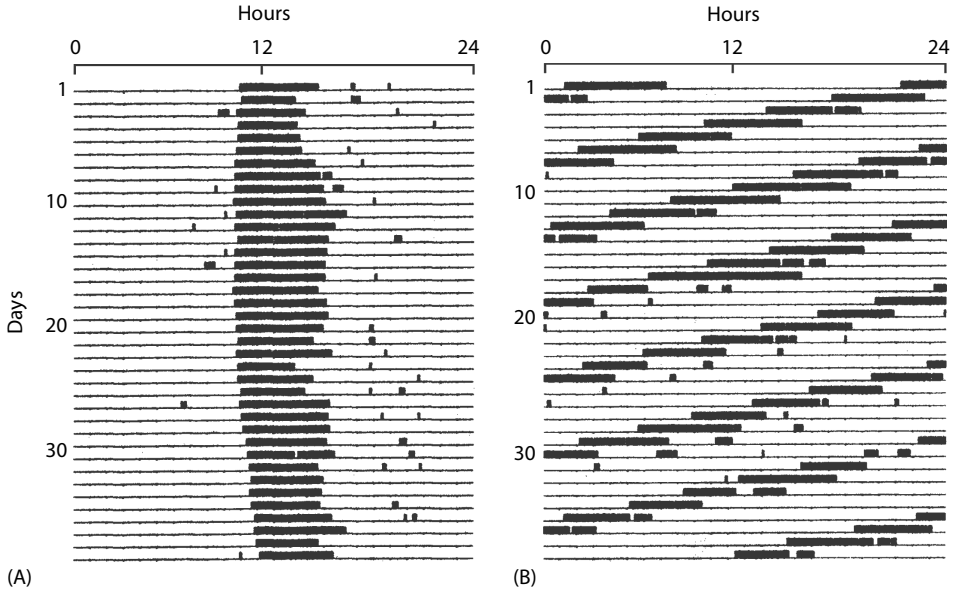


FIGURE 6.28 Hamster in a hurry. The actograms show the rhythms of running-wheel activity of a wild-type golden hamster (A) and of a homozygous *tau*-mutant hamster (B) maintained in constant darkness. The wild-type hamster completes a circadian cycle in almost exactly 24 hours, whereas the mutant hamster completes each circadian cycle in approximately 20 hours. (From Menaker, M. and Refinetti, R., The *tau* mutation in golden hamsters, in: Young, M.W., *Molecular Genetics of Biological Rhythms*, Marcel Dekker, New York, 1992, pp. 255–269. Reprinted by courtesy of Marcel Dekker, Inc.)

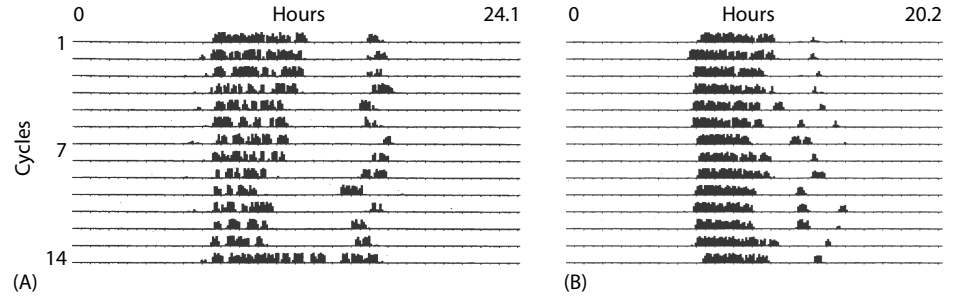


FIGURE 6.29 Proportionally short. When the running-wheel activity rhythms of a wild-type golden hamster (A) and of a homozygous *tau*-mutant hamster (B) are plotted in actograms with hour axes proportional to the circadian periods of the animals, it is evident that the patterns of activity of the two phenotypes are very similar to each other. (Adapted from Refinetti, R. and Menaker, M., *J. Physiol.*, 501, 449, 1997.)

Another team of researchers, led by Gary Pickard from the University of Pennsylvania, conducted a mutagenesis program similar to Takahashi's but was not as fortunate. Whereas Takahashi came out with an excellent mutant that eventually gained him induction into the US National Academy of Sciences, the mutant gene that Pickard's team ended up with (*wheels*, located in chromosome 4) lengthened circadian period in heterozygous mice but was lethal in homozygotes and produced various unrelated phenotypical abnormalities in heterozygotes, such as hyperactive bidirectional circling motion, diminished balance, abnormalities in the inner ear, and reduced body size.[347] Headline news: "Sick Mice from Mars!"

A third team, this one at the Medical Research Council in Harwell, United Kingdom, conducted a large mutagenesis program in mice that yielded five lines with abnormal free-running periods, although the lines were never fully characterized.[348] After the discovery of several genes that are at the core of the circadian pacemaker (see Chapter 12), various research groups have generated knockout mice (i.e., mice with targeted deletions of particular genes) or mutant mice that either are arrhythmic or have abnormal free-running periods.[349–356] Several selective-breeding studies have also been conducted,[357–359] although this approach is too crude to allow single-gene selection. A mutagenesis program in the mustard plant (*Arabidopsis thaliana*) yielded six lines of arrhythmic mutants and 29 lines of period mutants with periods ranging from 16 to 27 hours.[360] A mutagenesis program in zebra fish (*Danio rerio*) yielded a mutation (*lager*) that shortens the free-running period by 1.3 hours in homozygotes.[42] Finally, a study of human twins provided evidence in support of the heritability of the daily pattern of feeding of men and women.[361]

Following up on the early work with the *tau*-mutant hamster, a research team led by Andrew Loudon at the University of Manchester (in the United Kingdom) recreated the *tau* mutation in the mouse.[362] Because of the technical advantages

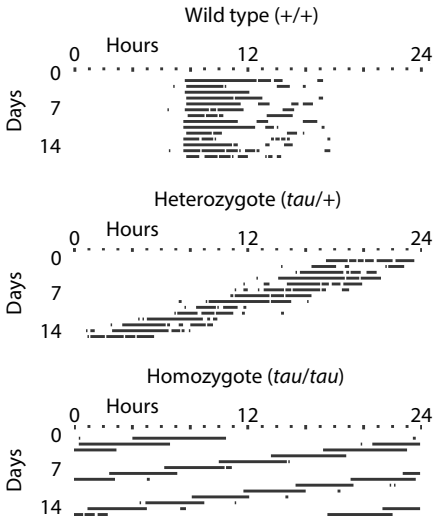


FIGURE 6.30 Three genotypes and three phenotypes. These actograms show the running-wheel activity rhythms of representative members of the three genotypes of the *tau* gene maintained in constant darkness. Wild-type hamsters have circadian periods very close to 24 hours, heterozygous mutants have periods of approximately 22 hours, and homozygous mutants have circadian periods of approximately 20 hours. (Data from the archives of the Refinetti lab.)

of working with mice, the team was also able to create mice with the *ck1ε* gene knocked out. Examples of the activity rhythms of individual mice bearing the various combinations of *ck1ε* genes and maintained in constant darkness are shown in Figure 6.33. The wild-type mouse (+/+) has a period slightly shorter than 24 hours, as indicated by the slope of the activity onsets. The heterozygous mutant (+/*tau*) has a period close to 22 hours, whereas the homozygous mutant (*tau*/*tau*) has a period close to 20 hours. The mice with one allele knocked out (+/–) or with the two alleles knocked out

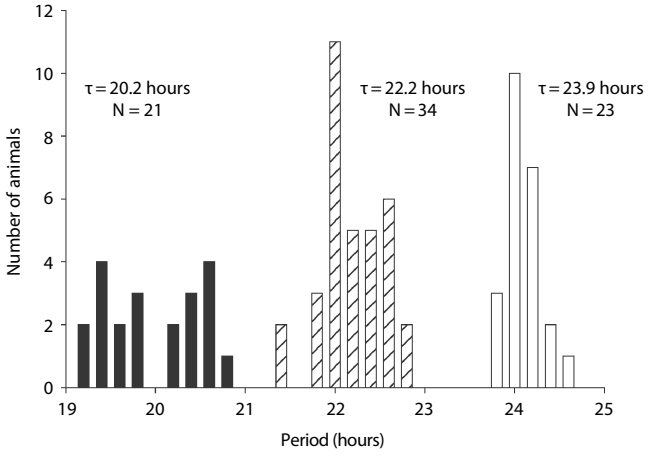


FIGURE 6.31 Hamsters and peas. The frequency distribution of free-running periods of the progenies of pairs of heterozygous *tau*-mutant hamsters delineates three groups with frequencies similar to the 1:2:1 ratio expected from the crossing of heterozygotes as originally described for peas by Mendel. (Data from Ralph, M.R. and Menaker, M., *Science*, 241, 1225, 1988.)

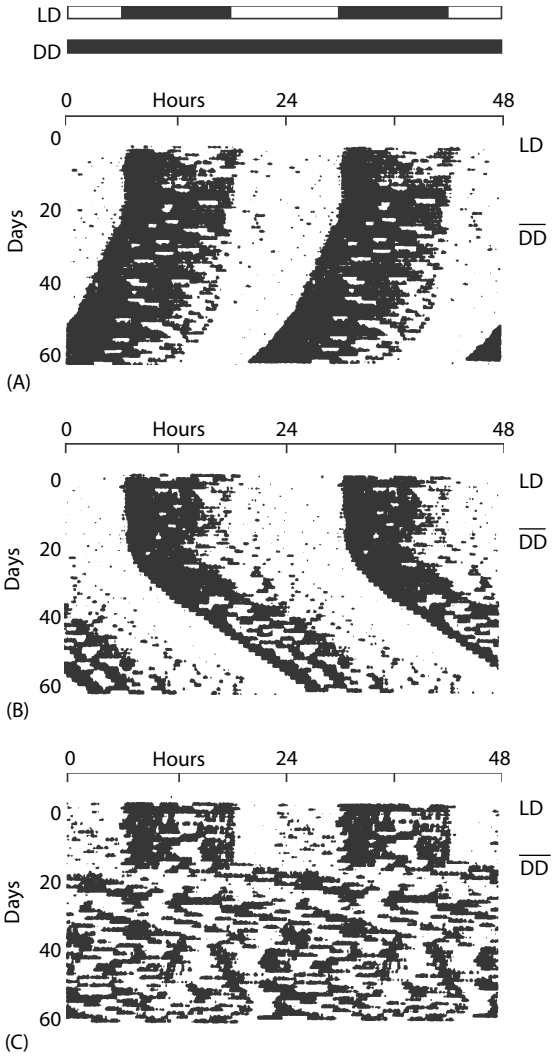


FIGURE 6.32 Mutant mice. These double-plotted actograms show the running-wheel activity rhythms of three representative mice bearing different genotypes of the *clock* gene. The animals were maintained under a light–dark cycle for 2 weeks (as indicated by the top horizontal bar above the actograms) and in constant darkness afterward (as indicated by the bottom horizontal bar above the actograms). The wild-type mouse (A) exhibited a free-running period of 23.6 hours. The heterozygous mutant (B) exhibited a period of 24.8 hours. The homozygous mutant (C) exhibited a period of 27.1 hours at first but became arrhythmic later on. (Adapted from Vitaterna, M.H. et al., *Science*, 264, 719, 1994.)

(–/–) resemble the wild-type mouse, whereas the mouse with only one *tau* allele (*tau*/–) resembles the heterozygous mutant. These phenotypes clearly indicate that the *tau* mutation is a gain-of-function mutation. The full knockout mouse has the phenotype of a normal mouse (perhaps because of substitution by an alternate gene); the *tau* mutation speeds up the circadian clock. We will look into this matter in greater detail in Chapter 12.

The information provided so far indicates that circadian period has a very strong genetic component. But does the early environment—during development of the adult organism—have any effect at all? As previously mentioned in Section 6.1,

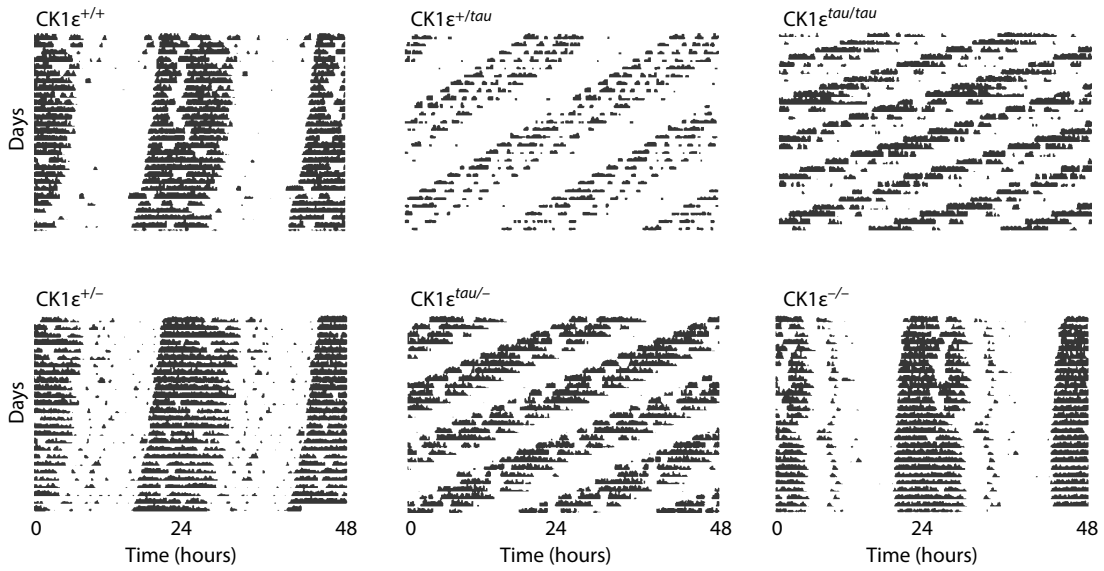


FIGURE 6.33 More mutant mice. These double-plotted actograms show the running-wheel activity rhythms of six representative mice bearing different alleles of the casein kinase 1 epsilon (*ck1ε*) gene, as indicated. (Adapted from Meng, Q.J. et al., *Neuron*, 58, 78, 2008.)

the period of free-running rhythms in adults can be affected by the previous exposure of the animals to light–dark cycles with different periods. For instance, animals previously maintained under a light–dark cycle with a 22-hour period have a shorter circadian period (when allowed to freerun in constant darkness) than animals previously maintained under a regular 24-hour day. This effect may last for months but eventually disappears. Would these *aftereffects* become permanent if the animals were raised from birth (or, even better, from conception) under different light–dark cycles? As far as vertebrates are concerned, this question has not been addressed experimentally often enough to allow a definitive answer, but two studies—one in mice[76] and one in hamsters[363]—have revealed absolutely no effect of the early environment beyond that expected as an aftereffect of the light–dark cycle. Let us start by inspecting Figure 6.34. The graphs show the frequency distributions of free-running periods of the progenies of +/*tau* hamsters. Some litters were conceived and raised under 24-hour light–dark cycles, whereas other litters were conceived and raised under 20-hour light–dark cycles. At 2 months of age, the animals were placed in individual cages in constant darkness, and their free-running periods were determined. Inspection of the graphs suggests that the period of the LD cycle prevailing during gestation and development had a strong effect on the free-running period exhibited by the animals after they grew up. One gets the impression that the heterozygous hamsters were transformed into homozygous mutants by the 20-hour LD cycle. Yet, a very similar distribution of free-running periods is obtained if adult hamsters (raised under a 24-hour LD cycle) are maintained under a 20-hour LD cycle for 2 months and then released into constant darkness.[363] That is, the shortening of the free-running period of heterozygous hamsters seen in Figure 6.34 (Panel B) is not a permanent effect of the early environment but simply a temporary aftereffect of the light–dark cycle to which the hamsters were previously exposed. It seems, therefore, that the old nature–nurture controversy has been decided in favor of nature as far as the period of the circadian pacemaker in vertebrates is concerned. The situation may be different in invertebrates.[364]

A few years ago, a group of researchers at Vanderbilt University (in Tennessee) claimed to have documented an effect of the early environment on the circadian period of adult mice.[365] Aside from the fact that the observation has not been confirmed by other research teams, a major problem with this finding is that there is no reasonable explanation for it. The authors found that mice raised under a long photoperiod (LD 16:8) had shorter free-running periods as adults. That is, both groups of animals (LD 16:8 and LD 8:16) were raised under 24-hour light–dark cycles, but one group ended up with an adult period of 23.3 hours, whereas the other group ended up with an adult period of 23.9 hours.

Another research team, this one at the University of Manchester (in the United Kingdom), raised mice under three different lighting schedules: LL, DD, or LD. When the adults were tested, there was not much difference between them, except that, when tested under LL (but not under DD), the mice raised under LL had shorter circadian period than the mice raised under DD or LD.[366] So, mice raised under LL followed Aschoff's rule but to a lesser extent than the other mice, which suggests that they were less sensitive to light. Because the authors examined the eyes of the mice and found no evidence of retinal damage in mice raised under LL, they assumed that the effect of the early environment must have been registered (*imprinted*) in the brain.[366] Whether in the eye or in the brain, the effect of LL during early environment does not seem to be related to the speed of the circadian clock but to its responsiveness to light.

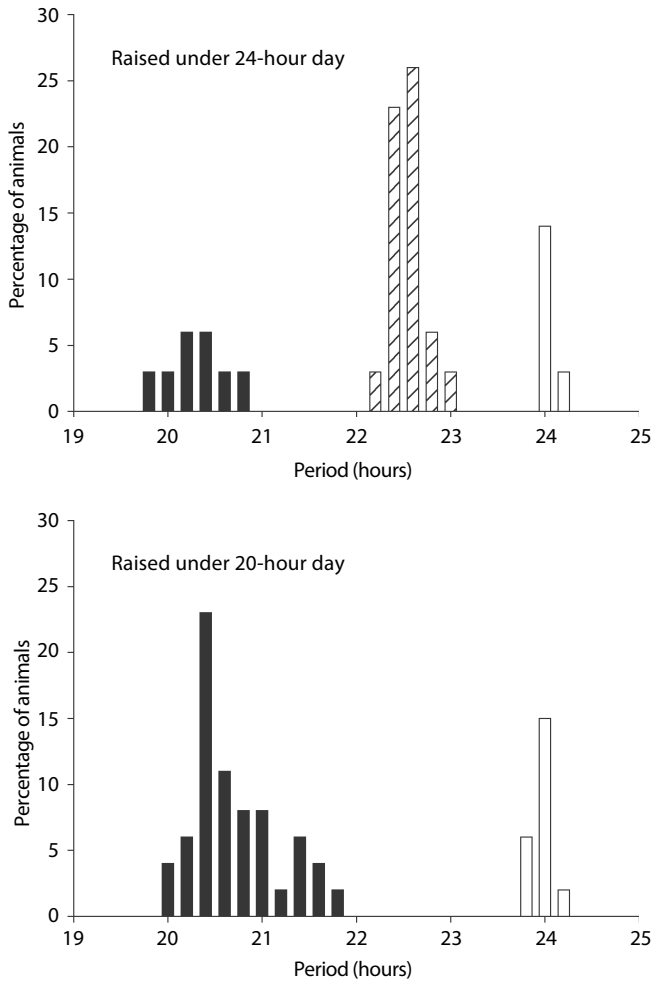


FIGURE 6.34 Nature or nurture? The graphs show the frequency distributions of free-running periods of the progenies of pairs of heterozygous *tau*-mutant hamsters conceived and raised under a 24-hour or a 20-hour light–dark cycle. The data suggest that hamsters raised under 20-hour days tend to have shorter free-running periods later in life. However, the measurements of free-running period were started only a week after the animals were transferred to constant darkness, so that lingering effects of the previous photic environment cannot be excluded as an explanation of the phenomenon. (Data from Refinetti, R., *Behav. Genet.*, 28, 153, 1998.)

6.3 SINGLE OR MULTIPLE OSCILLATORS

Because many biological variables exhibit circadian rhythmicity, it is not unreasonable to wonder whether there are many circadian clocks. Is there one circadian pacemaker or many pacemakers? Since the existence of multiple clocks would create a serious problem of synchronization, it is reasonable to expect the existence of a single clock. But, of course, what is reasonable is not always true. At the cellular level, it is certainly wrong to say that there is only one circadian clock. As we will see in Chapter 12, individual cells are autonomous pacemakers, so that the human brain contains many thousands of circadian clocks. The important question, however, is whether these cells make up a functional unit that can be considered to be a single clock. There is plenty of evidence for multiple clocks in protists[367,368] and lower vertebrates,[36,56] but, in my opinion, the master circadian clock in mammals is a single clock. On the other hand, many authors over the years have proposed that there are actually two clocks. There have been two distinct versions of the two-clock model: one of the versions was initially proposed as an explanation for the phenomenon of *splitting* and was later extended to other phenomena, whereas the other version was proposed as an explanation of the phenomenon of *spontaneous internal desynchronization* in humans. I will discuss each version separately.

6.3.1 SPLITTING

Splitting refers to the separation of a free-running rhythm into two separate components 180° apart, as exemplified in Figure 6.35. This bizarre phenomenon has been consistently observed in many laboratories when golden hamsters are maintained in constant light for extended periods of time.[172,198,369–379] Because the two split components exhibit different circadian periods during the initial stage of splitting, some researchers suggested that two separate clocks might be involved. According to this view, the circadian pacemaker would be not a single oscillator but the combined output of two distinct yet coupled oscillators, often called the "evening oscillator" and the "morning oscillator."[194,380–382] Of course, good skeptics are never willing to accept multiple elements when the facts can be explained by a single element. As an alternative, the phenomenon of splitting could be seen as a minor anomaly yet to be explained rather than as a fundamental piece of evidence requiring a revision of the concept of the circadian pacemaker.

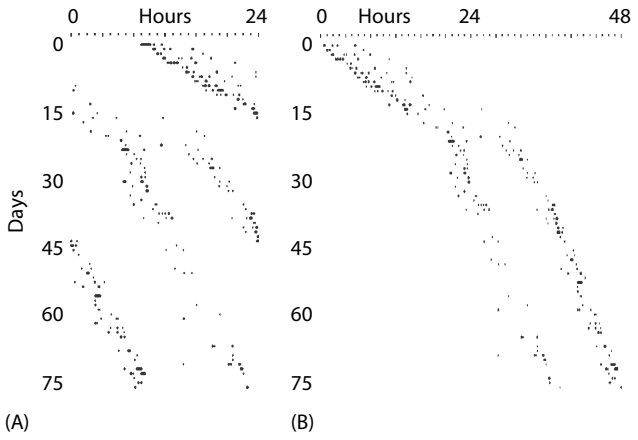


FIGURE 6.35 Splitting in constant light. When golden hamsters (*Mesocricetus auratus*) are maintained in constant light, their running-wheel activity rhythms are much less robust than when the animals are maintained under a light–dark cycle or in constant darkness and, in 50% of the animals, the activity pattern splits into two separate components. The activity records of a typical hamster are shown here both in standard actogram format (A) and in double-plot format with omission of repeated segments (B). The animal had been in constant light for 2 weeks before the beginning of the records, so that splitting occurred 4 weeks after the transfer to constant light (360 lux). (Data from the archives of the Refinetti lab.)

For a variety of reasons, I favor the *anomaly* alternative for the explanation of splitting, and I like the fact that a recent model of the circadian clock can explain splitting as the natural output of a single oscillator interacting with sleep-regulatory systems.[148] Here are the reasons why I favor the *anomaly* alternative for the explanation of splitting:

First, the two split components have identical periods during the split state. The difference in periods occurs only during the very initial stage of splitting and, therefore, could be simply a transient disruption in the expressed rhythms. *Second*, typically only half of the hamsters kept in constant light develop split rhythms.[172,198,369–374,376–378] If the circadian pacemaker were indeed made up of two separate oscillators, one would expect splitting in a larger proportion of animals (or in *all* animals if the phenomenon were truly fundamental). *Third*, although splitting in constant light has been consistently observed in golden hamsters, it has only occasionally been observed in other species, and usually in only a few individuals under special conditions.[12,191,383–386] Of course, one would expect a fundamental principle of circadian organization to be expressed in at least the majority of species.

Fourth, the idea that constant light causes the two putative oscillators to uncouple is inconsistent with the fact that some species exhibit splitting when maintained in constant darkness or dim light.[56,77,189,213,387,388] *Fifth*, if the activity rhythm is induced to split and reunite multiple times, the evening and morning components trade places, indicating that the two components are not generated by two oscillators with distinct properties.[198] *Sixth*, although the coupled oscillators model assumes that the two oscillators respond differently to photic stimulation, empirical investigation of the photic sensitivity of the putative oscillators failed to demonstrate differential responsiveness.[213]

Seventh, as discussed in Chapter 12, research on the neural substrates of the circadian pacemaker has not supported the concept of two distinct oscillators. It is true that, as early as 1982, a research team showed that splitting of the activity rhythm could be eliminated by unilateral surgical destruction of the master circadian clock in the brain,[370] which could be interpreted as evidence that the two oscillators are housed in the opposite sides of the brain. However, another research team showed later that almost *any* brain lesion could eliminate splitting,[389] thus refuting the idea that the two sides of the brain contain two distinct oscillators. More recently, recording of neural activity by markers of gene expression in brain slices from hamsters in the split state provided renewed evidence that the evening and morning oscillators may be located on opposite sides of the brain.[390] However, another group of researchers, working with a special breed of mice that show splitting in constant darkness, found no indication that the two sides of the brain are in antiphase during splitting.[391] Another group working with hamsters did find splitting between left and right brain hemispheres in behaviorally split animals but also found a dorsoventral split in the brain that complicates the concept of morning and evening oscillators.[392]

Additional support for the two-oscillator hypothesis might be sought in electrophysiological recordings from brain slices of hamsters that were not split.[393] These recordings revealed two daily peaks of activity, presumably corresponding to the evening and morning components. However, the bimodal electrophysiological pattern can be observed only in hamsters (not in rats or mice) and only if the brain is sliced in a particular fashion (horizontally rather than coronally).[394] Also, of course, a bimodal pattern of activity in the brain is no more significant than a bimodal pattern of behavioral activity (which, as we saw in Chapter 5, is typical of most animals). Both patterns may simply reflect the bimodal waveform of rhythms generated by a single pacemaker. Studies in fruit flies showed that the bimodal pattern of locomotor activity can be transformed into a unimodal pattern without the evening peak if a specific group of nerve cells (LN_d) is genetically deactivated.[395] Although this might constitute evidence that the LN_d is the evening oscillator (the adjacent LN_v being the morning oscillator), there is no indication that the LN_d is a functional oscillator capable of driving the activity rhythm in constant darkness. A more recent study seems to suggest that the circadian pacemaker responsible for locomotor activity in the fruit fly is made up of many cellular oscillators, not just two.[396]

For the seven reasons just listed, I do not believe that the phenomenon of splitting justifies the assumption of two independent oscillators. Nonetheless, a series of recent observations has raised the issue of splitting in a different context that requires further elaboration. By equating rhythm bimodality with the coupling of morning and evening oscillators, some authors have referred to experimenter-induced alterations in rhythm waveform as "splitting."

The story began with a rather convoluted experimental design in which "splitting" in golden hamsters was attained by the induction of daily bouts of activity in the middle of the light phase of the light–dark cycle.[397,398] Because hamsters do not normally exercise in illuminated environments, the lights were turned off during the induction of activity (attained by the presentation of a novel running wheel). Consequently, the animals were actually exposed to two light–dark cycles each day and, not quite surprisingly, exhibited two daily episodes of running activity (during the two dark phases). This "split" rhythm persisted for a few days in constant darkness.

Michael Gorman, a researcher at the University of California at San Diego, adapted the induced-splitting protocol[399,400] and noticed that splitting could be obtained simply by the use of two daily light–dark cycles, without the need for the novel running wheel.[401] A sample of his data is shown in Figure 6.36. Golden hamsters originally maintained under a light–dark cycle with 14 hours of light and 10 hours of darkness per day (LD 14:10) were transferred to a double light–dark cycle of the type LDLD 9:5:5:5. Under LD 14:10 (Section A), animal activity was concentrated in the single dark phase, but under LDLD 9:5:5:5 (Section B), the activity split into two daily bouts. As will be discussed in Chapter 7, synchronization to two daily light–dark cycles is not an unusual phenomenon (see "entrainment by *frequency demultiplication*"), but splitting of activity, as observed by Gorman, is a novel and curious finding.

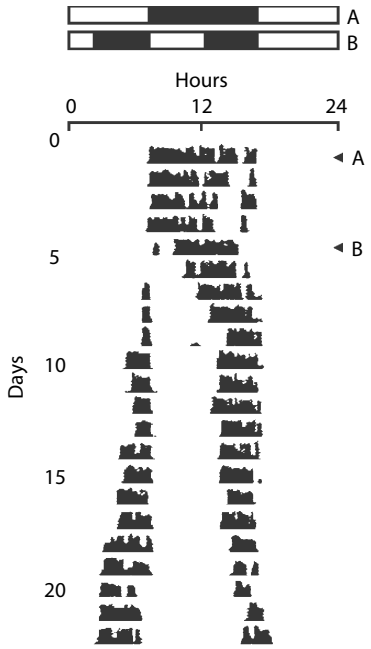


FIGURE 6.36 Splitting induced by fragmentation of the light–dark cycle. The unimodal daily pattern of activity exhibited by golden hamsters under a regular 24-hour light–dark cycle can be altered into a bimodal pattern if the light–dark cycle is split into two daily cycles. This actogram shows the rhythm of running-wheel activity of a golden hamster maintained under an LD 14:10 for 4 days (A) and under an LDLD 9:5:5:5 afterward (B). Splitting of the activity rhythm into two daily components can be clearly seen. (Adapted from Gorman, M.R., *J. Comp. Physiol. A*, 187, 793, 2001.)

I tried to replicate Gorman's study in hamsters, mice, and Nile grass rats, but found no evidence of splitting (Figure 6.37). Evidently, the splitting phenomenon was not robust and required specific experimental conditions that I failed to replicate. Soon afterward, however, Gorman developed a much simpler and more effective protocol that involved an LD 7:5 with normal bright light during the L phase and very dim light during the D phase.[402,403] Splitting under this protocol is quite robust, and I had no problem replicating it in mice in my laboratory, as shown in Figure 6.38. The requirement of dim light during the dark phase is quite interesting and will hopefully be explained by further research. At this time, the physiological significance of splitting induced by LD 7:5 is not clear. I see it as a curious modulation of the waveform of the oscillation generated by *the* circadian pacemaker, and not as evidence in support of the two-oscillator hypothesis.

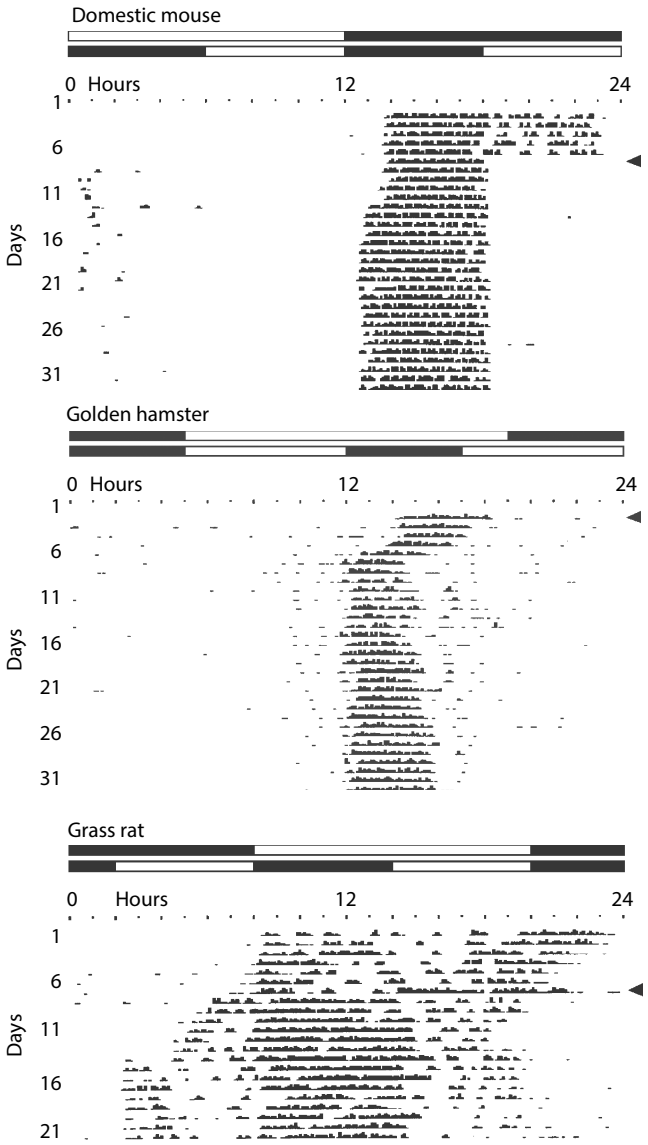


FIGURE 6.37 Refusing to split. Domestic mice (*Mus musculus*), golden hamsters (*Mesocricetus auratus*), and Nile grass rats (*Arvicanthis niloticus*) did not develop split rhythms when subjected to exotic light–dark cycles in the author's laboratory, as exemplified in these actograms of running-wheel activity. The mouse and the grass rat were transferred from LD 12:12 to LD 6:6, whereas the hamster was transferred from LD 14:10 to LD 7:5, on the days indicated by the arrowheads. Although the change in light–dark cycle clearly affected the daily patterns of activity, no splitting of the activity rhythms occurred. (Data from the archives of the Refinetti lab.)

6.3.2 Spontaneous Internal Desynchronization

In most studies in circadian physiology, only one variable is studied at a time. As we will see in Chapter 9, a lot can be learned from the simultaneous study of two or more variables. The interesting question is, of course, whether the various variables are mutually synchronized. They normally are, although transient desynchronization can be induced by various environmental manipulations.[404–416] In studies of free-running rhythms in which two or more variables are recorded simultaneously, the rhythms are generally found to freerun together with the same period. There is always some amount of "biological noise" that can lead a hasty observer to see a transient episode of "spontaneous" internal desynchronization,[47,417] but, in the long run, no evidence of *spontaneous internal desynchronization* has been found in numerous studies of nonhuman vertebrates maintained in constant darkness

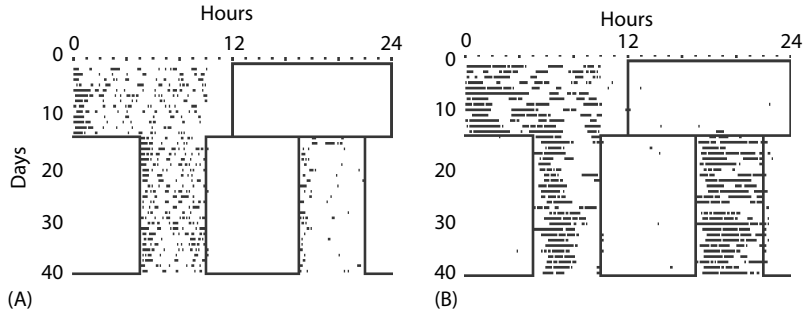


FIGURE 6.38 Splitting under 12-hour light–dark cycle with background of dim constant light. These actograms show the running-wheel activity rhythms of two mice (*Mus musculus*) maintained first under a 24-hour light–dark cycle (LD 12:12) and then under a 12-hour light–dark cycle (LD 7:5). The rectangles indicate the duration of the light phases (100 lux). To encourage splitting, dim white light (0.1 lux) was present in the background at all times. The records in (A) show very little splitting, whereas the records in (B) show a robust partition of activity into two daily blocks. (Data from the archives of the Refinetti lab.)

or constant light for extended periods of time.[48,56,70,85,106,169,179,214,221,418,419] One research group described what seemed to be desynchronization of the rhythm of body temperature from the rhythm of sleep stages in rats, but if the desynchronization was real it was not spontaneous; instead, it was induced by exposure of the animals to a light–dark cycle with a period of 22 hours, which is too short to produce effective entrainment of the circadian system of the rat.[420]

Spontaneous internal desynchronization has *not* been observed in many studies even in humans,[121,125,127,128,130,421,422] but various investigators, in different laboratories, have found evidence of spontaneous internal desynchronization in human subjects maintained in constant conditions. Some have detected what they believed to be evidence for three distinct clocks,[423,424] but their results were never replicated and have been rightfully ignored. More commonly, spontaneous internal desynchronization has been found between the sleep–wake rhythm, on one side, and all other rhythms (although the body temperature rhythm is usually the only other rhythm measured), on the other side.[118,120,123,126,223,425–429] Typically, the period of the sleep–wake rhythm becomes very long (up to 30 or even 50 hours) after a week or so, whereas the period of the body temperature rhythm (and other rhythms) remains between 24 and 25 hours. This happens in 15%–60% of the subjects, depending on the study.

An example of spontaneous internal desynchronization is shown in Figure 6.39. Notice that, although the body temperature rhythm maintained a free-running period slightly longer than 25 hours throughout the study, the period of the sleep–wake rhythm lengthened to 33 hours starting on day 13. The data are certainly impressive! In order to explain the existence of rhythms with two different periods in the same individual, it was proposed that humans have two distinct circadian pacemakers, one controlling the sleep–wake rhythm and the other controlling the temperature rhythm as well as other rhythms.[143,430]

Before we move on with the discussion of the implications of the phenomenon of spontaneous internal desynchronization, we must agree on what *synchronization* means. Synchronization is a relatively common word in everyday language. However, we often do not realize that there is a subtle difference between two meanings of synchronization. Consider Figure 6.40. In Panel A, the faces of two clocks are shown on four successive time points. The two clocks are clearly *not* synchronized, as the clock on the right advances 6 hours per frame, while the clock on the left advances only 3 hours. Panel B shows what you most likely would call a pair of synchronized clocks. These two clocks show exactly the same

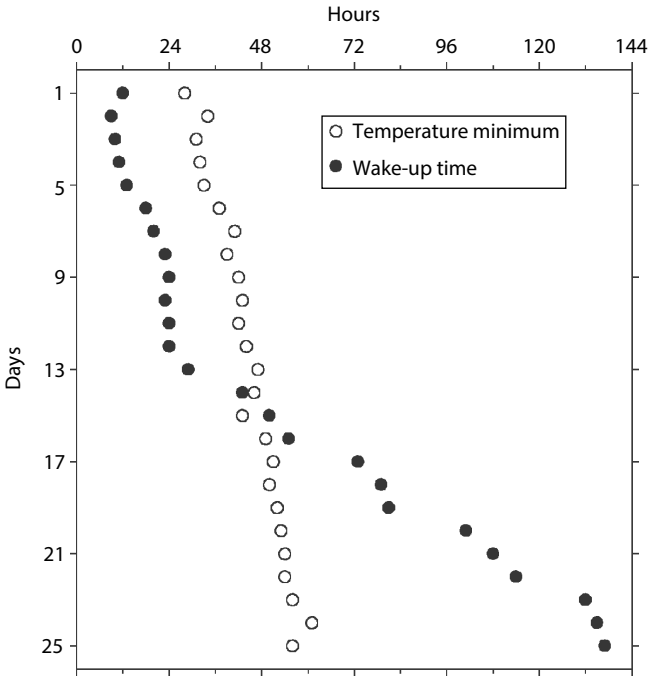


FIGURE 6.39 Spontaneous internal desynchronization. This graph shows the daily time of the trough of the body temperature rhythm and the daily time of awakening of a human subject kept under constant environmental conditions for a month. The hour axis is sextuple plotted and repeated segments are omitted. In such a plot, clear dissociation of the rhythms of body temperature and wake–sleep can be observed starting on day 14. (Data from Aschoff, J. and Wever, R., The circadian system of man, in Aschoff, J., ed., *Biological Rhythms*, Plenum, New York, 1981, pp. 311–331.)

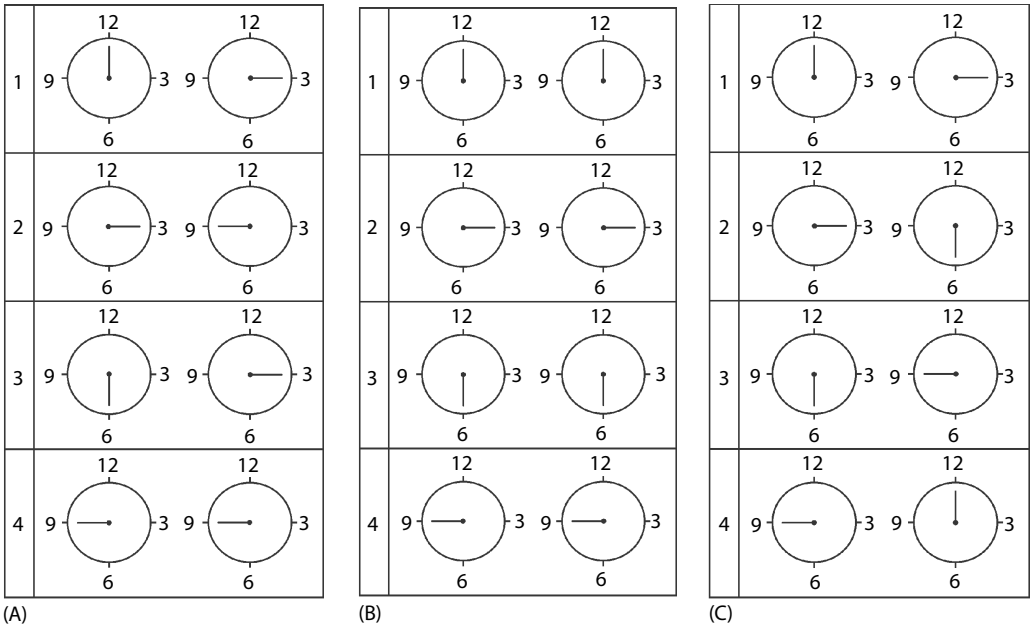


FIGURE 6.40 Too many clocks? Do not allow the apparent complexity of these diagrams to scare you off. Look closely and notice that the two clocks in (A) are not synchronized but those in (B) and (C) are. See text for details.

time every time. However, Panel C also shows synchronized clocks, even though this may not be evident at first. Look carefully and notice that the two clocks move together through the four frames. The clock on the right is 3 hours ahead of the clock on the left, but the difference between the two clocks remains constant throughout. Now, in circadian physiology, we are concerned with both types of synchronization, not just the one in Panel B. As a matter of fact, the pattern in Panel C is much more common than that in Panel B. For instance, the rhythms of body temperature and locomotor activity of a tree shrew that are shown in Figure 6.41 are synchronized. The daily troughs of the temperature rhythm (open circles) and the daily onsets of activity (closed circles) do not occur at the same time, but the time difference between the two is relatively constant throughout the 100 days of the study.

Now that we agree on what synchronization means, let us make sure that we understand what it means to be internally desynchronized. Suppose that you go into a cave where you have no way to tell what time of the day it is. If you stay there for several weeks, the only clock you can rely on is your own internal clock. So, although you do not realize it, you will start going to bed later and later each day. Assuming a circadian period of 25 hours, you will go to bed at 10 p.m. today, 11 p.m. tomorrow, midnight the day after tomorrow, and so on. Of course, you do not realize that this is happening because you have no way of telling what time of the day it is. As a matter of fact, some male subjects have the impression that their beard is growing faster in the cave, as the interval between daily shaves increases without their awareness. In any event, if you do not develop internal desynchronization, you will be going to bed at noon after 2 weeks in the cave. This will also be the time of your day when you

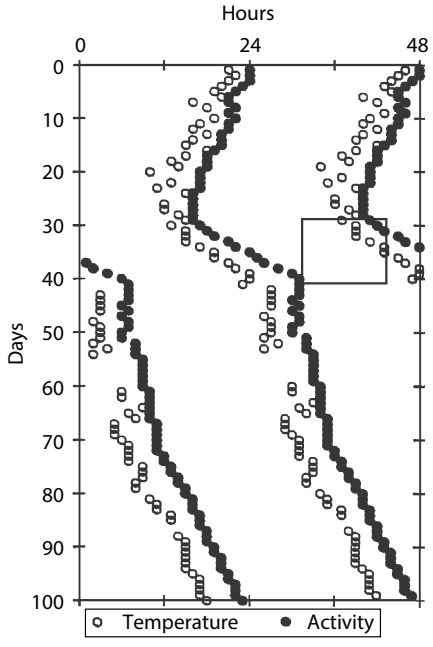


FIGURE 6.41 No internal desynchronization in tree shrews. This graph shows the daily time of the trough of the body temperature rhythm and the daily time of initiation of activity of a tree shrew (*Tupaia belangeri*) as recorded by telemetry for over 3 consecutive months. The figure is double plotted to facilitate visual inspection. The animal was maintained in constant light (400 lux) for a month, then under a light–dark cycle for a few days (with the duration of the light phase indicated by the single-plotted rectangle), and then in constant light (30 lux) for 2 months. Notice the persistent synchronization of the rhythms of body temperature and activity. (Data from Refinetti, R. and Menaker, M., *J. Exp. Zool.*, 263, 453, 1992.)

feel sleepy. However, if you do develop internal desynchronization, your life will be a mess. You will be going to sleep in 30-hour intervals (or more), while your concentration, your hunger, your need to go to the bathroom, and so on will be cycling in 25-hour intervals. Because of the oddity of such an arrangement, several researchers have suggested that internal desynchronization is unlikely to reflect a true dissociation of rhythms resulting from the dissociation of two biological clocks and, instead, may be due simply to a lot of *noise* in the expression of the sleep–wake rhythm.[408,422,431] Indeed, look again at Figure 6.39. Notice that the hour axis is different from that of typical actograms. The axis is based on an extended scale that includes many days on each line but does not include repeated segments on repeated days. This format was used by the authors of the study to emphasize what they felt was the true nature of the data. If we replot Figure 6.39 using a standard actogram format, we obtain a more neutral view of the actual relationship between temperature and sleep–wake. In the standard format (Figure 6.42), differences in period are not as obvious, and similarities between the rhythms are much more evident. Simply put, the data may look *noisier* from day 20 onward, but internal desynchronization is no longer obvious. For further comparison, Figure 6.43 is a fictitious actogram that was built using the periods that the authors claimed to have identified in the data. Although there are some similarities between Figures 6.43 and 6.42, particularly for the open circles (which correspond to the noncontroversial temperature data), there are also many differences, particularly for the closed circles (which correspond to the sleep–wake data). My interpretation is that the sleep–wake rhythm in Figure 6.42 is certainly *noisy* but does not exhibit a period of 33 hours from day 20 onward. In other words, the only reliable period is the well-established circadian period (in this case, 25 hours).

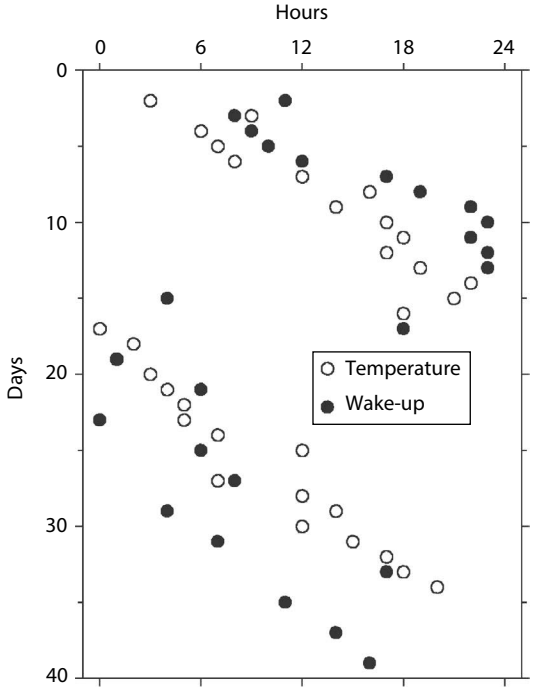


FIGURE 6.42 It depends on how you slice it. The data shown in Figure 6.39 are replotted here in the usual actogram format. Inspection of this plot, unlike inspection of the other plot, suggests that the rhythms of body temperature and wake–sleep are *noisy* but provides no clear indication of internal desynchronization.

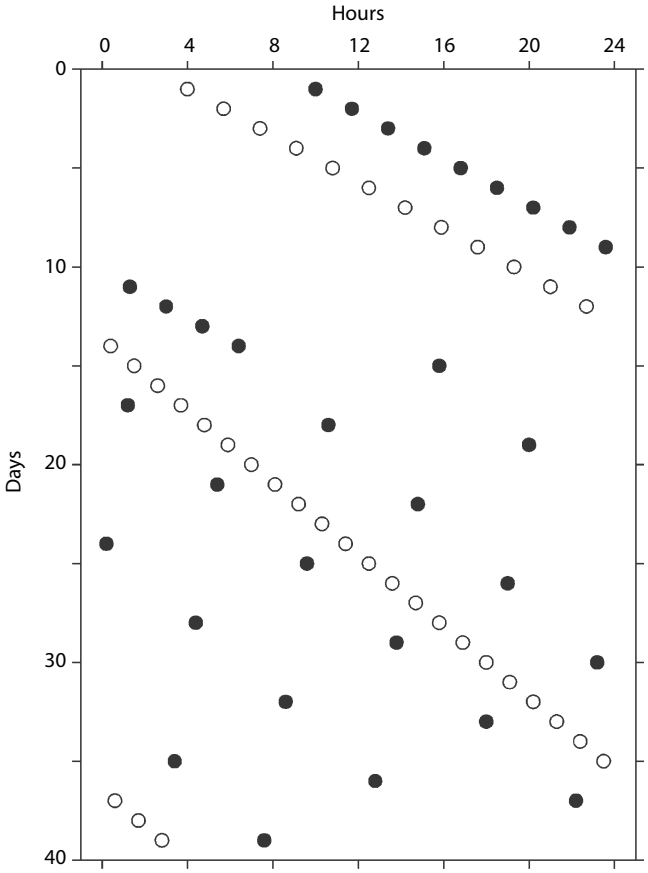


FIGURE 6.43 What would it look like? This graph, which should be compared to that of Figure 6.42, shows the expected outcome of actual desynchronization of the rhythms of body temperature and wake–sleep starting on day 14. See text for detailed discussion.

The conclusion that internal desynchronization is *not* a result of the dissociation of two biological clocks is supported by the observation that, in internally desynchronized subjects—just as in normal subjects—sleepiness is strongly associated with the trough of the body temperature rhythm.[120,124,422,432,433] This means that people feel like sleeping (and often do sleep) at the particular time of the circadian day when the body temperature rhythm is at its low point. Now, if the sleep–wake rhythm and the body temperature rhythm are connected in such way, then they are obviously not independent of each other. In other words, they do not freerun separately. And, if they do not freerun separately, they need not be generated by separate circadian clocks.

The absence of the requirement for separate pacemakers does not rule out the possibility of the existence of separate pacemakers. Mathematical models have been developed to show that the data obtained in internal desynchronization studies can be explained by the action of two separate but

coupled oscillators.[143,430] However, models have also been developed to show that the data can be explained by the action of a single pacemaker.[434,435] In the absence of additional evidence, parsimony leads us to conclude that there is only one circadian pacemaker.

Additional experimental evidence in support of the two-pacemaker hypothesis seemed to surface when some animal studies indicated that destruction of the brain area believed to control circadian rhythmicity of activity did not eliminate circadian rhythmicity of body temperature.[436–440] However, the apparent evidence was later shown to result from an artifact in the analysis of rhythmicity.[441] But let us not put the cart before the horse. Before we address the issue of the physical location of the circadian pacemaker (Chapter 12), we must complete our analysis of the functional properties of the pacemaker as revealed by the study of circadian rhythms. This will be our task in the next four chapters.

SUMMARY

1. Under constant environmental conditions, circadian rhythms freerun with periods slightly different from 24.0 hours. However, in some species, rhythmicity may be inhibited by constant light and, in other species, by constant darkness. In those species that exhibit free-running rhythms in constant light, the period of the rhythm is affected by the intensity of the light (Aschoff's rule). Some drugs, such as methamphetamine and deuterium oxide, can also affect circadian period.
2. Although circadian period can be transiently affected by environmental factors, the base value of period is genetically determined. Different species tend to have different circadian periods, and single-gene mutations that affect circadian period have been described in various species. Four well-known genes that affect circadian period are *per* in fruit flies, *frq* in bread mold, *tau* in golden hamsters, and *clock* in domestic mice.
3. *Splitting* and *spontaneous internal desynchronization* are two phenomena that have given rise to the hypothesis that the master circadian pacemaker in mammals is composed of two distinct oscillators. The available evidence in support of this hypothesis is not strong enough to justify disposal of the more parsimonious hypothesis of a single clock.

EXERCISES

6.1 A very simple timing device. In Section 6.1, various models of pacemakers were described. To understand the concept of a pacemaker, it is helpful to first understand how a simple timing device can be built. In this exercise, we will look at a very simple device that uses a steady flow of water to generate a cyclic process. In essence, we will set up a miniature watermill. All you will need is a sink with a faucet and a rodent running-wheel. Place the wheel under the faucet and, before turning on the water, notice the obvious: that the wheel is not spinning by itself. Then, turn on the faucet. You may have to adjust the placement of the wheel, but you should soon be able to cause it to spin. Notice not only that the wheel is spinning now, but also that it is spinning at a regular rate. Every so many seconds, the wheel completes a full revolution. And it does it over and over again, at the same speed. Should you care to calibrate it, you could use it as a clock. Next, increase or decrease the flow of water and notice that you can speed up or down the clock. That's it! You have built your own clock!

6.2 A slightly more sophisticated timing device. A simple electronic circuit was mentioned as a model of a pacemaker in Section 6.1. In this exercise, we will actually assemble a circuit. The required parts are shown in Figure 6.44. They include a battery (or power supply, B_1), an adjustable resistor (a trimmer or potentiometer, R_1), a capacitor (C_1), and a relay (RY_1, which should not be a solid-state one, because you must be able to hear the clicking of the solenoid). As soon as you connect the parts as shown in the figure, you should hear a clicking sound at a constant rate. The electric current from the battery is shortened by the capacitor while it charges. When the capacitor is charged, current flows to the relay's coil, which causes the switch to close. The switch closure allows the capacitor to discharge, which brings the circuit back to its initial state. The sound you hear is the closing of the relay's switch. By varying the resistance in the variable resistor (R_1), you can vary the speed of charging/discharging of the capacitor and, consequently, you can increase or decrease the frequency of the clicking sound. As in the case of the watermill, you could use the circuit as a clock if you cared to calibrate it and to synchronize it to solar time. For more sophisticated electronic circuits, see the "Websites to Explore" section for this chapter. The following are the suggested specifications of the circuit components: B_1 9 VDC, R_1 1 kΩ, C_1 2000 μF electrolytic, and RY_1 9 VDC NO.

6.3 Using a computer model of the circadian pacemaker. In this exercise, we will use the program

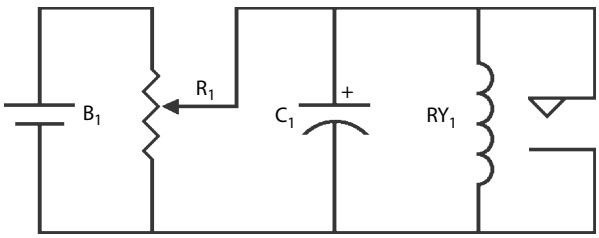


FIGURE 6.44 Building a simple clock. This schematic drawing of an electronic circuit will help you build a simple pacemaker. B_1, 9 V battery or DC power supply; R_1, 1 kΩ adjustable resistor; C_1, 2000 μF electrolytic capacitor; RY_1, 9 VDC mechanical relay.

Model (the fifth icon from the right in the Circadian banner) to simulate a circadian pacemaker. Details about the operation of this program will be provided in Section 7.3, and further exercises using **Model** will appear in Chapter 7. For now, it is sufficient to realize that whatever we accomplish with this program is the result of a computer simulation and not of the operation of a real clock.

1. Start the program **Model**.
2. At the top of the window, notice that you can stipulate the "endogenous" period of the "organism" and can choose to have one to four blocks of simulations. In this exercise, we will need only one block. We will use more blocks in future exercises.
3. Set the period to **24.1** hours (which is approximately the free-running period of a golden hamster) and move down to *Block 1*. Set the *Days* to **60** (2 months) and the *Mode* to **DD** (constant darkness). Click on *Run*.
4. You will see an actogram with a smooth drift to the right, which is consistent with a circadian period longer than 24.0 hours. Click on *Close* to return to the main window, and then change the period to **23.9** hours and click on *Run* again. You will see an actogram with a smooth drift to the left, which is consistent with a circadian period shorter than 24.0 hours.
5. Go back to the main window and try a period of **23.6** hours (the typical free-running period of a mouse). Then try a period of **20.2** hours (the typical free-running period of a homozygous tau-mutant hamster). Notice how difficult it is to discern the activity pattern in the actogram when the period is so short.
6. Now, set the period to **24.0** hours. *Run* the simulation again to see a perfectly vertical actogram, as you would expect. Then switch the *Mode* to **LL** (constant light) and run another simulation. Notice that, although the endogenous period is still 24.0 hours, the expressed period is actually a little longer. As mentioned in Section 6.1, organisms do not truly freerun in constant light. They do freerun in the sense that they do not receive temporal cues from the environment, but their expressed circadian period is not exactly the same as their endogenous period. This issue will be discussed in further detail in Section 7.2.

6.4 Recording a free-running rhythm. If you assembled the data acquisition system in Exercise 2.3, or if you have access to a data acquisition system for rodent activity in a researcher's laboratory, or if you have access to any system for the measurement of circadian rhythms, you can conduct this exercise. The exercise is quite simple: you will keep your experimental subjects under a light–dark cycle for a week or two, transfer them to constant darkness for two or more weeks, and then inspect the records. If you are using the data acquisition system from Exercise 2.3 and the **Collect** program, you can inspect the records using **Plot** (in actogram mode) as soon as you finish collecting the data. Regardless of how you inspect the data, you should be able to observe that the period of the rhythm is 24.0 hours under a light–dark cycle but is either shorter or longer in constant darkness (unless you have the bad luck of getting an animal with an endogenous period of exactly 24.0 hours). Observing free-running rhythms in your own experimental subjects will help you appreciate the functional value of the circadian clock. *Note: Depending on where you live, you may need a permit to conduct this exercise because it involves a vertebrate species (even though no invasive procedures are involved). Check with your local authorities first. If you are a university student, ask your professor about it.*

SUGGESTIONS FOR FURTHER READING

Rensing, L., Meyer-Grahle, U., and Ruoff, P. (2001). Biological timing and the clock metaphor: Oscillatory and hourglass mechanisms. *Chronobiology International* 18: 329–369.

A review article that discusses the distinction between oscillatory and hourglass mechanisms of biological timing.

Pittendrigh, C. S. (1993). Temporal organization: Reflections of a Darwinian clock-watcher. *Annual Review of Physiology* 55: 17–54.

In this *legacy* article, published 3 years before his death, Pittendrigh explores the theme of endogenous timing as he recollects his 40-year career as a "clock watcher."

Winfree, A. T. (2001). *The Geometry of Biological Time*, 2nd edn. New York: Springer.

If you are interested in mathematical modeling of biological timing, this book is for you. It covers the topic in more than 800 pages.

McEachron, D. (2012). *Chronobioengineering: Introduction to Biological Rhythms with Applications*. San Rafael, CA: Morgan & Claypool.

An engineer's view of circadian rhythms in 290 pages.

WEBSITES TO EXPLORE

Center for Neurodynamics at the University of Missouri: http://www.umsl.edu/~neurodyn/.

Circadian Clocks Web Course (on Coursera): https://www.coursera.org/course/circaclock.

Circadian Modelling (Millar Research Group): http://millar.bio.ed.ac.uk/PEBrown/CircadianModelling/CircadianModelling.htm.

Electronic Oscillators: http://www.electronics-tutorials.com/oscillators/oscillators.htm.

Steve Kay's Laboratory (University of Southern California): https://dornsife.usc.edu/labs/kay-laboratory.

Van der Pol Oscillator: http://www.iro.umontreal.ca/~eckdoug/vibe/Relaxation/VanDerPol.html.

REFERENCES

1. Ortega-Escobar, J. (2002). Circadian rhythms of locomotor activity in *Lycosa tarantula* (Araneae, Lycosidae) and the pathways of ocular entrainment. *Biological Rhythm Research* 33: 561–576.
2. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
3. Cloudsley-Thompson, J. L. (1952). Studies in diurnal rhythms. II. Changes in the physiological responses of the woodlouse *Oniscus asellus* to environmental stimuli. *Journal of Experimental Biology* 29: 295–303.
4. Dainton, B. H. (1954). The activity of slugs. I. The induction of activity by changing temperatures. *Journal of Experimental Biology* 31: 165–187.
5. Refinetti, R. (2000). Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 73: 575–583.
6. Koilraj, A. J., Sharma, V. K., Marimuthu, G., and Chandrashekaran, M. K. (2000). Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae: Spirostreptida). *Chronobiology International* 17: 757–765.
7. Dreisig, H. (1980). Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia* 44: 376–389.
8. Page, T. L. (1985). Circadian organization in cockroaches: Effects of temperature cycles on locomotor activity. *Journal of Insect Physiology* 31: 235–242.
9. Sharma, V. K., Lone, S. R., Mathew, D., Goel, A., and Chandrashekaran, M. K. (2004). Possible evidence for shift work schedules in the media workers of the ant species *Camponotus compressus*. *Chronobiology International* 21: 297–308.
10. Toma, D. P., Bloch, G., Moore, D., and Robinson, G. E. (2000). Changes in *period* mRNA levels in the brain and division of labor in honey bee colonies. *Proceedings of the National Academy of Sciences United States of America* 97: 6914–6919.
11. Shimizu, I., Kawai, Y., Taniguchi, M., and Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
12. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., and Tomioka, K. (2004). *Drosophila cry*^{b} mutation reveals two circadian clocks that drive locomotor rhythm and have different responsiveness to light. *Journal of Insect Physiology* 50: 479–488.
13. North, R. D. (1993). Entrainment of the circadian rhythm of locomotor activity in wood ants by temperature. *Animal Behaviour* 45: 393–397.
14. Saigusa, T., Ishizaki, S., Watabiki, S., Ishii, N., Tanakadate, A., Tamai, Y., and Hasegawa, K. (2002). Circadian behavioural rhythm in *Caenorhabditis elegans*. *Current Biology* 12: R46–RR47.
15. Zantke, J., Ishikawa-Fujiwara, T., Arboleda, E., Lohs, C., Schipany, K., Hallay, N., Straw, A. D., Todo, T., and Tessmar-Raible, K. (2013). Circadian and circalunar clock interactions in a marine annelid. *Cell Reports* 5: 99–113.
16. Jones, M. D. R., Hill, M., and Hope, A. M. (1967). The circadian flight activity of the mosquito *Anopheles gambiae*: Phase setting by the light regime. *Journal of Experimental Biology* 47: 503–511.
17. Fuchikawa, T. and Shimizu, I. (2008). Parametric and non-parametric entrainment of circadian locomotor rhythm in the Japanese honeybee *Apis cerana japonica*. *Biological Rhythm Research* 39: 57–67.
18. Bloch, G., Shemesh, Y., and Robinson, G. E. (2006). Seasonal and task-related variation in free running activity rhythms in honey bees (*Apis mellifera*). *Insectes Sociaux* 53: 115–118.
19. Frisch, B. and Koeniger, N. (1994). Social synchronization of the activity rhythms of honeybees within a colony. *Behavioral Ecology and Sociobiology* 35: 91–98.
20. Moore, D. and Rankin, M. A. (1985). Circadian locomotor rhythms in individual honeybees. *Physiological Entomology* 10: 191–197.
21. Cuesta, M., Clesse, D., Pévet, P., and Challet, E. (2009). From daily behavior to hormonal and neurotransmitter rhythms: Comparison between diurnal and nocturnal rat species. *Hormones and Behavior* 55: 338–347.
22. Takekata, H., Matsuura, Y., Goto, S. G., Satoh, A., and Numata, H. (2012). RNAi of the circadian clock gene *period* disrupts the circadian rhythm but not the circatidal rhythm in the mangrove cricket. *Biology Letters* 8: 488–491.
23. Yerushalmi, S., Bodenhaimer, S., and Bloch, G. (2006). Developmentally determined attenuation in circadian rhythms links chronobiology to social organization in bees. *Journal of Experimental Biology* 209: 1044–1051.
24. Stelzer, R. J., Stanewsky, R., and Chittka, L. (2010). Circadian foraging rhythms of bumblebees monitored by radio-frequency identification. *Journal of Biological Rhythms* 25: 257–267.
25. Rieger, D., Wülbeck, C., Rouyer, F., and Helfrich-Förster, C. (2009). *Period* gene expression in four neurons is sufficient for rhythmic activity of *Drosophila melanogaster* under dim light conditions. *Journal of Biological Rhythms* 24: 271–282.
26. Edgar, A. L. and Yuan, H. A. (1968). Daily locomotory activity in *Phalangium opilio* and seven species of *Leiobunum* (Arthropoda: Phalangida). *Bios* 39: 167–176.
27. Williams, B. G. and Naylor, E. (1967). Spontaneously induced rhythm of tidal periodicity in laboratory-reared *Carcinus*. *Journal of Experimental Biology* 47: 229–234.
28. Chiesa, J. J., Aguzzi, J., García, J. A., Sardà, F., and de la Iglesia, H. O. (2010). Light intensity determines temporal niche switching of behavioral activity in deep-water *Nephrops norvegicus* (Crustacea: Decapoda). *Journal of Biological Rhythms* 25: 277–287.
29. Tosini, G. and Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *Journal of Biological Rhythms* 10: 248–255.
30. Bartell, P. A., Miranda-Anaya, M., and Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
31. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
32. Lowe, C. H., Hinds, D. S., Lardner, P. J., and Justice, K. E. (1967). Natural free-running period in vertebrate animal populations. *Science* 156: 531–534.
33. Bertolucci, C., Sovrano, V. A., Magnone, M. C., and Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
34. Refinetti, R. and Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.

35. Janik, D. S., Pickard, G. E., and Menaker, M. (1990). Circadian locomotor rhythms in the desert iguana. II. Effects of electrolytic lesions to the hypothalamus. *Journal of Comparative Physiology A* 166: 811–816.
36. Tosini, G. and Menaker, M. (1998). Multioscillatory circadian organization in a vertebrate, *Iguana iguana*. *Journal of Neuroscience* 18: 1105–1114.
37. Ellis, D. J., Firth, B. T., and Belan, I. (2009). Thermocyclic and photocyclic entrainment of circadian locomotor activity rhythms in sleepy lizards, *Tiliqua rugosa*. *Chronobiology International* 26: 1369–1388.
38. Hurd, M. W., Debruyne, J., Straume, M., and Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiology and Behavior* 65: 465–472.
39. Iigo, M. and Tabata, M. (1996). Circadian rhythms of locomotor activity in the goldfish *Carassius auratus*. *Physiology and Behavior* 60: 775–781.
40. Herrero, M. J., Madrid, J. A., and Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
41. Chen, W. M. and Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
42. Debruyne, J., Hurd, M. W., Gutiérrez, L., Kaneko, M., Tan, Y., Wells, D. E., and Cahill, G. M. (2004). Isolation and phenogenetics of a novel circadian rhythm mutant in zebrafish. *Journal of Neurogenetics* 18: 403–428.
43. Gandhi, A. V., Mosser, E. A., Oikonomou, G., and Prober, D. A. (2015). Melatonin is required for the circadian regulation of sleep. *Neuron* 85: 1193–1199.
44. Kasai, M. and Kiyohara, S. (2010). Food- and light-entrainable oscillators control feeding and locomotor activity rhythms, respectively, in the Japanese catfish, *Plotosus japonicus*. *Journal of Comparative Physiology A* 196: 901–912.
45. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W., and Bacon, W. L. (2000). Circadian rhythm of the preovulatory surge of luteinizing hormone and its relationships to rhythms of body temperature and locomotor activity in turkey hens. *Biology of Reproduction* 62: 1452–1458.
46. King, V. M., Bentley, G. E., and Follett, B. K. (1997). A direct comparison of photoperiodic time measurement and the circadian system in European starlings and Japanese quail. *Journal of Biological Rhythms* 12: 431–442.
47. Ebihara, S. and Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
48. Oshima, I. and Ebihara, S. (1988). The measurement and analysis of circadian locomotor activity and body temperature rhythms by a computer-based system. *Physiology and Behavior* 43: 115–119.
49. Oshima, I., Yamada, H., Goto, M., Sato, K., and Ebihara, S. (1989). Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. *Journal of Comparative Physiology A* 166: 217–226.
50. Eskin, A. (1971). Some properties of the system controlling the circadian activity rhythm of sparrows. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 55–80.
51. Berger, R. J. and Phillips, N. H. (1994). Constant light suppresses sleep and circadian rhythms in pigeons without consequent sleep rebound in darkness. *American Journal of Physiology* 267: R945–R952.
52. Hau, M. and Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
53. Heigl, S. and Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
54. Aschoff, J. and von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
55. Pohl, H. (1993). Does aging affect the period of the circadian pacemaker in vertebrates? *Naturwissenschaften* 80: 478–481.
56. Underwood, H. and Edmonds, K. (1995). The circadian rhythm of thermoregulation in Japanese quail. II. Multioscillator control. *Journal of Biological Rhythms* 10: 234–247.
57. Kumar, V., Gwinner, E., and Van't Hof, T. J. (2000). Circadian rhythms of melatonin in European starlings exposed to different lighting conditions: Relationship with locomotor and feeding rhythms. *Journal of Comparative Physiology A* 186: 205–215.
58. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
59. Bartell, P. A. and Gwinner, E. (2005). A separate circadian oscillator controls nocturnal migratory restlessness in the songbird *Sylvia borin*. *Journal of Biological Rhythms* 20: 538–549.
60. Mistlberger, R. E., Lukman, H., and Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: Assessment and intervention. *Appetite* 30: 255–267.
61. Borbély, A. A. and Neuhaus, H. U. (1978). Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. *Journal of Comparative Physiology* 128: 37–46.
62. Zucker, I., Fitzgerald, K. M., and Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.
63. Albers, H. E., Gerall, A. A., and Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
64. Sridaran, R. and McCormack, C. E. (1980). Parallel effects of light signals on the circadian rhythms of running activity and ovulation in rats. *Journal of Endocrinology* 85: 111–120.
65. Weber, A. L. and Adler, N. T. (1978). Dissociation of persistent estrus and a free-running activity cycle in different light cycles. *Biology of Reproduction* 19: 425–430.
66. Heusner, A. (1959). Variation nycthémérale de la température centrale chez le rat adapté à la neutralité thermique. *Comptes Rendus de Séances de la Société de Biologie de Strasbourg* 153: 1258–1261.
67. Isobe, Y., Aoki, K., Furuyama, F., and Hashitani, T. (1992). Effect of warm or cold on circadian rhythm of running wheel activity and body temperature in spontaneously hypertensive rats. *Medical Principles and Practice* 3: 63–71.
68. Ikeda, M. and Inoué, S. (1998). Simultaneous recording of circadian rhythms of brain and intraperitoneal temperatures and locomotor and drinking activities in the rat. *Biological Rhythm Research* 29: 142–150.
69. Francis, A. J. P. and Coleman, G. J. (1988). The effect of ambient temperature cycles upon circadian running and drinking activity in male and female laboratory rats. *Physiology and Behavior* 43: 471–477.
70. Honma, K. and Hiroshige, T. (1978). Internal synchronization among several circadian rhythms in rats under constant light. *American Journal of Physiology* 235: R243–R249.

71. Honma, K. and Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
72. Edgar, D. M., Kilduff, T. S., Martin, C. E., and Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior* 50: 373–378.
73. Hofstetter, J. R., Grahame, N. J., and Mayeda, A. R. (2003). Circadian activity rhythms in high-alcohol-preferring and low-alcohol-preferring mice. *Alcohol* 30: 81–85.
74. Edgar, D. M., Martin, C. E., and Dement, W. C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *Journal of Biological Rhythms* 6: 185–199.
75. Possidente, B., McEldowney, S., and Pabon, A. (1995). Aging lengthens circadian period for wheel-running activity in C57BL mice. *Physiology and Behavior* 57: 575–579.
76. Davis, F. C. and Menaker, M. (1981). Development of the mouse circadian pacemaker: Independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
77. Abe, H., Honma, S., Honma, K., Suzuki, T., and Ebihara, S. (1999). Functional diversities of two activity components of circadian rhythm in genetical splitting mice (CS strain). *Journal of Comparative Physiology A* 184: 243–251.
78. Yoshimura, T., Nishio, M., Goto, M., and Ebihara, S. (1994). Differences in circadian photosensitivity between retinally degenerate CBA/J mice (*rd/rd*) and normal CBA/M mice (+/+). *Journal of Biological Rhythms* 9: 51–60.
79. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
80. Edgar, D. M. and Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
81. Refinetti, R. (1996). Ultradian rhythms of body temperature and locomotor activity in wild-type and tau-mutant hamsters. *Animal Biology* 5: 111–115.
82. Fitzgerald, K. M. and Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences United States of America* 73: 2923–2927.
83. Davis, F. C., Stice, S., and Menaker, M. (1987). Activity and reproductive state in the hamster: Independent control by social stimuli and a circadian pacemaker. *Physiology and Behavior* 40: 583–590.
84. Refinetti, R. and Menaker, M. (1992). The circadian rhythm of body temperature of normal and tau-mutant golden hamsters. *Journal of Thermal Biology* 17: 129–133.
85. DeCoursey, P. J., Pius, S., Sandlin, C., Wethey, D., and Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiology and Behavior* 65: 457–463.
86. Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology A* 162: 35–46.
87. Weinert, D., Fritzche, P., and Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
88. Oklejewicz, M., Hut, R. A., Daan, S., Loudon, A. S. I., and Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.
89. Refinetti, R. and Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
90. Boulos, Z., Macchi, M., Houpt, T. A., and Terman, M. (1996). Photic entrainment in hamsters: Effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
91. DeCoursey, P. (1960). Phase control of activity in a rodent. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 49–55.
92. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
93. DeCoursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science* 131: 33–35.
94. Mrosovsky, N., Boshes, M., Hallonquist, J. D., and Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.
95. Lee, T. M., Holmes, W. G., and Zucker, I. (1990). Temperature dependence of circadian rhythms in golden-mantled ground squirrels. *Journal of Biological Rhythms* 5: 25–34.
96. Kennedy, G. A., Hudson, R., and Armstrong, S. M. (1994). Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiology and Behavior* 55: 385–389.
97. Jilge, B. (1991). Restricted feeding: A nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
98. Johnson, R. F. and Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
99. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., and Johnson, R. F. (1987). A two-peak circadian system in body temperature and activity in the domestic cat, *Felis catus*. *Journal of Thermal Biology* 12: 27–37.
100. Hawking, F., Lobban, M. C., Gammage, K., and Worms, M. J. (1971). Circadian rhythms (activity, temperature, urine and microfilariae) in dog, cat, hen, duck, Thamnomys and Gerbillus. *Journal of Interdisciplinary Cycle Research* 2: 455–473.
101. Siwak, C. T., Tapp, P. D., Zicker, S. C., Murphey, H. L., Muggenburg, B. A., Head, E., and Cotman, C. W. (2003). Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience* 117: 813–824.
102. Rauth-Widmann, B., Fuchs, E., and Erkert, H. G. (1996). Infradian alteration of circadian rhythms in owl monkeys (*Aotus lemurinus griseimembra*): An effect of estrous? *Physiology and Behavior* 59: 11–18.
103. Perret, M. and Aujard, F. (2001). Daily hypothermia and torpor in a tropical primate: Synchronization by 24-h light–dark cycle. *American Journal of Physiology* 281: R1925–R1933.
104. Robinson, E. L. and Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
105. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
106. Boulos, Z., Frim, D. M., Dewey, L. K., and Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
107. Tokura, H. and Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.

108. Ingram, D. L. and Mount, L. E. (1973). The effects of food intake and fasting on 24-hourly variations in body temperature in the young pig. *Pflügers Archiv* 339: 299–304.
109. Marimuthu, G., Rajan, S., and Chandrashekaran, M. K. (1981). Social entrainment of the circadian rhythm in the flight activity of the microchiropteran bat *Hipposideros speoris*. *Behavioral Ecology and Sociobiology* 8: 147–150.
110. Harlow, H. J., Phillips, J. A., and Ralph, C. L. (1982). Circadian rhythms and the effects of exogenous melatonin in the nine-banded armadillo, *Dasypus novemcinctus*: A mammal lacking a distinct pineal gland. *Physiology and Behavior* 29: 307–313.
111. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
112. Flôres, D. E. F., Tomotani, B. M., Tachinardi, P., Oda, G. A., and Valentinuzzi, V. S. (2013). Modeling natural photic entrainment in a subterranean rodent (*Ctenomys knighti*), the tuco-tuco. *PLOS ONE* 8: e68243.
113. Kumar, D. and Singaravel, M. (2014). Phase and period responses to short light pulses in a wild diurnal rodent, *Funambulus pennanti*. *Chronobiology International* 31: 320–327.
114. Lahmam, M., El M'rabet, A., Ouarour, A., Pévet, P., Challet, E., and Vuillez, P. (2008). Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent *Lemniscomys barbarus*. *Chronobiology International* 25: 882–904.
115. Ocampo-Garcés, A., Mena, W., Hernández, F., Cortés, N., and Palacios, A. G. (2006). Circadian chronotypes among wild-captured west Andean octodontids. *Biological Research* 39: 209–220.
116. Basu, P. and Singaravel, M. (2013). Accurate and precise circadian locomotor activity rhythms in male and female Indian pygmy field mice, *Mus terricolor*. *Biological Rhythm Research* 44: 531–539.
117. Johnston, P. G. and Zucker, I. (1983). Lability and diversity of circadian rhythms of cotton rats, *Sigmodon hispidus*. *American Journal of Physiology* 244: R338–RR346.
118. Chandrashekaran, M. K., Marimuthu, G., and Geetha, L. (1997). Correlations between sleep and wake in internally synchronized and desynchronized circadian rhythms in humans under prolonged isolation. *Journal of Biological Rhythms* 12: 26–33.
119. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
120. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C., and Knauer, R. S. (1980). Human sleep: Its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
121. Kennaway, D. J. and Van Dorp, C. F. (1991). Free-running rhythms of melatonin, cortisol, electrolytes, and sleep in humans in Antarctica. *American Journal of Physiology* 260: R1137–R1144.
122. Steel, G. D., Callaway, M., Suedfeld, P., and Palinkas, L. (1995). Human sleep–wake cycles in the high Arctic: Effects of unusual photoperiodicity in a natural setting. *Biological Rhythm Research* 26: 582–592.
123. Aschoff, J., Gerecke, U., and Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
124. Zulley, J., Wever, R., and Aschoff, J. (1981). The dependence of onset and duration of sleep on the circadian rhythm of rectal temperature. *Pflügers Archiv* 391: 314–318.
125. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F., and Pauly, J. E. (Eds.), *Chronobiology*. Tokyo, Japan: Igaku Shoin, pp. 451–459.
126. Lund, R. (1974). Personality factors and desynchronization of circadian rhythms. *Psychosomatic Medicine* 36: 224–228.
127. Campbell, S. S., Dawson, D., and Zulley, J. (1993). When the human circadian system is caught napping: Evidence for endogenous rhythms close to 24 hours. *Sleep* 16: 638–640.
128. Endo, T., Honma, S., Hashimoto, S., and Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
129. Eastman, C. I., Molina, T. A., Dziepak, M. E., and Smith, M. R. (2012). Blacks (African Americans) have shorter free-running circadian periods than Whites (Caucasian Americans). *Chronobiology International* 29: 1072–1077.
130. Colin, J., Timbal, J., Boutelier, C., Houdas, Y., and Siffre, M. (1968). Rhythm of the rectal temperature during a 6-month free-running experiment. *Journal of Applied Physiology* 25: 170–176.
131. Gundel, A., Polyakov, V. V., and Zulley, J. (1997). The alteration of human sleep and circadian rhythms during spaceflight. *Journal of Sleep Research* 6: 1–8.
132. Monk, T. H., Buysse, D. J., Billy, B. D., Kennedy, K. S., and Willrich, L. M. (1998). Sleep and circadian rhythms in four orbiting astronauts. *Journal of Biological Rhythms* 13: 188–201.
133. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J. et al. (2001). Sleep, performance, circadian rhythms, and light–dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
134. Jespersen, J. and Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency*, 2nd edn. Mineola, NY: Dover.
135. Barnett, J. E. (1998). *Time's Pendulum: From Sundials to Atomic Clocks*. New York: Plenum.
136. Rensing, L., Meyer-Grahle, U., and Ruoff, P. (2001). Biological timing and the clock metaphor: Oscillatory and hourglass mechanisms. *Chronobiology International* 18: 329–369.
137. Kondo, T., Mori, T., Lebedeva, N. V., Aoki, S., Ishiura, M., and Golden, S. S. (1997). Circadian rhythms in rapidly dividing cyanobacteria. *Science* 275: 224–227.
138. Friesen, W. O., Block, G. D., and Hocker, C. G. (1993). Formal approaches to understanding biological oscillators. *Annual Review of Physiology* 55: 661–681.
139. Tsai, T. Y., Choi, Y. S., Ma, W., Pomerening, J. R., Tang, C., and Ferrell, J. E. (2008). Robust, tunable biological oscillations from interlinked positive and negative feedback loops. *Science* 321: 126–129.
140. van der Pol, B. (1960). *Selected Scientific Papers*. Amsterdam, the Netherlands: North Holland.
141. Hilborn, R. C. (2000). *Chaos and Nonlinear Dynamics*, 2nd edn. New York: Oxford University Press.
142. Pavlidis, T. (1981). Mathematical models. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 41–54.
143. Kronauer, R. E., Czeisler, C. A., Pilato, S. F., Moore-Ede, M. C., and Weitzman, E. D. (1982). Mathematical model of the human circadian system with two interacting oscillators. *American Journal of Physiology* 242: R3–R17.

144. De Goede, J., Olofson, E., and Rietveld, W. J. (1992). A mathematical model of the circadian system with a delayed feedback loop. *Journal of Interdisciplinary Cycle Research* 23: 142–143.
145. Pedersen, M. and Johnsson, A. (1994). A study of the singularities in a mathematical model for circadian rhythms. *BioSystems* 33: 193–201.
146. Barrio, R. A., Zhang, L., and Maini, P. K. (1997). Hierarchically coupled ultradian oscillators generating robust circadian rhythms. *Bulletin of Mathematical Biology* 59: 517–532.
147. Kunz, H. and Achermann, P. (2003). Simulation of circadian rhythm generation in the suprachiasmatic nucleus with locally coupled self-sustained oscillators. *Journal of Theoretical Biology* 224: 63–78.
148. Phillips, A. J. K., Fulcher, B. D., Robinson, P. A., and Klerman, E. B. (2013). Mammalian test/activity patterns explained by physiologically based modeling. *PLoS Computational Biology* 9: e1003213.
149. Binkley, S., Kluth, E., and Menaker, M. (1971). Pineal function in sparrows: Circadian rhythms and body temperature. *Science* 174: 311–314.
150. Honma, S., Honma, K., Shirakawa, T., and Hiroshige, T. (1988). Rhythms in behavior, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. *Physiology and Behavior* 44: 247–255.
151. Refinetti, R., Nelson, D. E., and Menaker, M. (1992). Social stimuli fail to act as entraining agents of circadian rhythms in the golden hamster. *Journal of Comparative Physiology A* 170: 181–187.
152. Davis, F. C. and Viswanathan, N. (1998). Stability of circadian timing with age in Syrian hamsters. *American Journal of Physiology* 275: R960–R968.
153. Canal-Corretger, M. M., Vilaplana, J., Cambras, T., and Díez-Noguera, A. (2001). Functioning of the rat circadian system is modified by light applied in critical postnatal days. *American Journal of Physiology* 280: R1023–R1030.
154. Marchant, E. G. and Mistlberger, R. E. (1996). Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. *Physiology and Behavior* 60: 657–663.
155. Spoelstra, K., Oklejewicz, M., and Daan, S. (2002). Restoration of self-sustained circadian rhythmicity by the mutant *Clock* allele in mice in constant illumination. *Journal of Biological Rhythms* 17: 520–525.
156. Seggio, J. A., Fixaris, M. C., Redd, J. D., Logan, R. W., and Rosenwasser, A. M. (2009). Chronic ethanol intake alters circadian phase shifting and free-running period in mice. *Journal of Biological Rhythms* 24: 304–312.
157. Erkert, H. G. and Cramer, B. (2006). Chronobiological background to cathemerality: Circadian rhythms in *Eulemur fulvus albifrons* (Prosimii) and *Aotus azarai boliviensis* (Anthropoidea). *Folia Primatologica* 77: 87–103.
158. Sheeba, V., Chandrashekaran, M. K., Joshi, A., and Sharma, V. K. (2002). Locomotor activity rhythm in *Drosophila melanogaster* after 600 generations in an aperiodic environment. *Naturwissenschaften* 89: 512–514.
159. Pfeffer, W. (1875). *Die periodischen Bewegungen der Blattorgane*. Leipzig, Germany: Wilhelm Engelmann.
160. Yen, U. C., Huang, T. C., and Yen, T. C. (2004). Observation of the circadian photosynthetic rhythm in cyanobacteria with a dissolved-oxygen meter. *Plant Science* 166: 949–952.
161. Hillman, W. S. (1970). Carbon dioxide output as an index of circadian timing in *Lemna* photoperiodism. *Plant Physiology* 45: 273–279.
162. Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science* 284: 2124–2128.
163. Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. *Journal of Comparative Physiology A* 179: 135–142.
164. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
165. Winget, C. M. and Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
166. Lumineau, S. and Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
167. Reierth, E. and Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
168. Kuwabara, N., Seki, K., and Aoki, K. (1986). Circadian, sleep and brain temperature rhythms in cats under sustained daily light–dark cycles and constant darkness. *Physiology and Behavior* 38: 283–289.
169. Hoban, T. M., Levine, A. H., Shane, R. B., and Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
170. Tavernier, R. J., Largen, A. L., and Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
171. Mrosovsky, N., Melnyk, R. B., Lang, K., Hallonquist, J. D., Boshes, M., and Joy, J. E. (1980). Infradian cycles in dormice (*Glis glis*). *Journal of Comparative Physiology A* 137: 315–339.
172. Boulos, Z. and Rusak, B. (1982). Circadian phase response curves for dark pulses in the hamster. *Journal of Comparative Physiology A* 146: 411–417.
173. Gerkema, M. P., Groos, G. A., and Daan, S. (1990). Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. *Journal of Biological Rhythms* 5: 81–95.
174. Halberg, F., Visscher, M. B., and Bittner, J. J. (1953). Eosinophil rhythm in mice: Range of occurrence, effects of illumination, feeding and adrenalectomy. *American Journal of Physiology* 174: 109–122.
175. Muñoz, M., Peirson, S. N., Hankins, M. W., and Foster, R. G. (2005). Long-term constant light induces constitutive elevated expression of mPER2 protein in the murine SCN: A molecular basis for Aschoff's rule? *Journal of Biological Rhythms* 20: 3–14.
176. Chen, R., Seo, D., Bell, E., von Gall, C., and Lee, C. (2008). Strong resetting of the mammalian clock by constant light followed by constant darkness. *Journal of Neuroscience* 28: 11839–11847.
177. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G., and Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
178. Witte, K., Grebmer, W., Scalbert, E., Delagrande, P., Guardiola-Lemaître, B., and Lemmer, B. (1998). Effects of melatoninergic agonists on light-suppressed circadian rhythms in rats. *Physiology and Behavior* 65: 219–224.

179. Eastman, C. and Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
180. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
181. Edelstein, K., Pfaus, J. G., Rusak, B., and Amir, S. (1995). Neonatal monosodium glutamate treatment prevents effects of constant light on circadian temperature rhythms of adults rats. *Brain Research* 675: 135–142.
182. Deprés-Brummer, P., Metzger, G., and Lévi, F. (1996). Analyses des rythmes de la température corporelle du rat en libre cours. *Pathologie Biologie* 44: 150–156.
183. Deprés-Brummer, P., Lévi, F., Metzger, G., and Touitou, Y. (1995). Light-induced suppression of the rat circadian system. *American Journal of Physiology* 268: R1111–R1116.
184. Usui, S., Okazaki, T., and Honda, Y. (2003). Interruption of the rat circadian clock by short light–dark cycles. *American Journal of Physiology* 284: R1255–R1259.
185. Granados-Fuentes, D., Prolo, L. M., Abraham, U., and Herzog, E. D. (2004). The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *Journal of Neuroscience* 24: 615–619.
186. Yoshikawa, T., Yamazaki, S., and Menaker, M (2005). Effects of preparation time on phase of cultured tissues reveal complexity of circadian organization. *Journal of Biological Rhythms* 20: 500–512.
187. Chiesa, J. J., Cambras, T., Carpentieri, A. R., and Díez-Noguera, A. (2010). Arrhythmic rats after SCN lesions and constant light differ in short time scale regulation of locomotor activity. *Journal of Biological Rhythms* 25: 37–46.
188. Tapia-Osorio, A., Salgado-Delgado, R., Angeles-Castellanos, M., and Escobar, C. (2013). Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behavioural Brain Research* 252: 1–9.
189. Rappold, I. and Erkert, H. G. (1994). Re-entrainment, phase-response and range of entrainment of circadian rhythms in owl monkeys (*Aotus lemurinus g.*) of different age. *Biological Rhythm Research* 25: 133–152.
190. Ebling, F. J. P., Lincoln, G. A., Wollnik, F., and Anderson, N. (1988). Effects of constant darkness and constant light on circadian organization and reproductive responses in the ram. *Journal of Biological Rhythms* 3: 365–384.
191. Ohta, H., Yamazaki, S., and McMahon, D. G. (2005). Constant light desynchronizes mammalian clock neurons. *Nature Neuroscience* 8: 267–269.
192. Aschoff, J. (1958). Tierische Periodik unter dem Einfluß von Zeitgebern. *Zeitschrift für Tierpsychologie* 15: 1–30.
193. Aschoff, J. (1979). Circadian rhythms: Influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
194. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. III. Heavy water and constant light: Homeostasis of frequency? *Journal of Comparative Physiology* 106: 267–290.
195. Schilling, A., Richard, J. P., and Servière, J. (1999). Duration of activity and period of circadian activity-rest rhythm in a photoperiod-dependent primate, *Microcebus murinus*. *Comptes Rendus de l'Académie des Sciences* 322: 759–770.
196. Cohen, R. and Kronfeld-Schor, N. (2006). Individual variability and photic entrainment of circadian rhythms in golden spiny mice. *Physiology and Behavior* 87: 563–574.
197. Summer, T. L., Ferraro, J. S., and McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
198. Earnest, D. J. and Turek, F. W. (1982). Splitting of the circadian rhythm of activity in hamsters: Effects of exposure to constant darkness and subsequent re-exposure to constant light. *Journal of Comparative Physiology* 145: 405–411.
199. Pohl, H. (1985). The circadian system of the Turkish hamster, *Mesocricetus brandti*: Responses to light. *Comparative Biochemistry and Physiology A* 81: 613–618.
200. Witting, W., Boerma, D., Koster-Van-Hoffen, G. C., Swaab, D. F., and Mirmiran, M. (1995). Light suppresses frequency and endogenous amplitude of the circadian system in nocturnal animals. *Biological Rhythm Research* 26: 477–485.
201. Morin, L. P. and Pace, L. (2002). The intergeniculate leaflet, but not the visual midbrain, mediates hamster circadian rhythm response to constant light. *Journal of Biological Rhythms* 17: 217–226.
202. Mrosovsky, N. (2003). Aschoff's rule in retinally degenerate mice. *Journal of Comparative Physiology A* 189: 75–78.
203. Koteja, P., Swallow, J. G., Carter, P. A., and Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.
204. Fuller, P. M. and Fuller, C. A. (2006). Genetic evidence for a neurovestibular influence on the mammalian circadian pacemaker. *Journal of Biological Rhythms* 21: 177–184.
205. van der Veen, D. R. and Archer, S. N. (2010). Light-dependent behavioral phenotypes in PER3-deficient mice. *Journal of Biological Rhythms* 25: 3–8.
206. Dallmann, R., DeBruyne, J. P., and Weaver, D. R. (2011). Photic resetting and entrainment in CLOCK-deficient mice. *Journal of Biological Rhythms* 26: 390–401.
207. Studholme, K. M., Gompf, H. S., and Morin, L. P. (2013). Brief light stimulation during the mouse nocturnal activity phase simultaneously induces a decline in core temperature and locomotor activity followed by EEG-determined sleep. *American Journal of Physiology* 304: R459–R471.
208. Ferraro, J. S. and McCormack, C. E. (1986). Minimum duration of light signals capable of producing the Aschoff effect. *Physiology and Behavior* 38: 139–144.
209. Murakami, D. M., Horwitz, B. A., and Fuller, C. A. (1995). Circadian rhythms of temperature and activity in obese and lean Zucker rats. *American Journal of Physiology* 269: R1038–R1043.
210. Canal-Corretger, M. M., Cambras, T., and Díez-Noguera, A. (2003). Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiology International* 20: 21–35.
211. Francis, A. J. P. and Coleman, G. J. (1990). Ambient temperature cycles entrain the free-running circadian rhythms of the stripe-faced dunnart, *Sminthopsis macroura*. *Journal of Comparative Physiology A* 167: 357–362.
212. DeCoursey, P. J., Krulas, J. R., Mele, G., and Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior* 62: 1099–1108.
213. Meijer, J. H., Daan, S., Overkamp, G. J. F., and Hermann, P. M. (1990). The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *Journal of Biological Rhythms* 5: 1–16.
214. Refinetti, R. and Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.

215. El Allali, K., Achaâban, M. R., Bothorel, B., Piro, M., Bouâouda, H., El Allouchi, M., Ouassat, M., Malan, A., and Pévet, P. (2013). Entrainment of the circadian clock by daily ambient temperature cycles in the camel (*Camelus dromedarius*). *American Journal of Physiology* 304: R1044–R1052.
216. Underwood, H. (1983). Circadian pacemakers in lizards: Phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
217. Miranda-Anaya, M., Bartell, P. A., Yamazaki, S., and Menaker, M. (2000). Circadian rhythm of ERG in *Iguana iguana*: Role of the pineal. *Journal of Biological Rhythms* 15: 163–171.
218. Lee, T. M. and Labyak, S. E. (1997). Free-running rhythms and light- and dark-pulse phase response curves for diurnal *Octodon degus* (Rodentia). *American Journal of Physiology* 273: R278–R286.
219. Refinetti, R. (2004). Parameters of photic resetting of the circadian system of a diurnal rodent, the Nile grass rat. *Acta Scientiae Veterinariae* 32: 1–6.
220. Gall, A. J., Smale, L., Yan, L., and Nunez, A. A. (2013). Lesions of the intergeniculate leaflet lead to a reorganization in circadian regulation and a reversal in masking responses to photic stimuli in the Nile grass rat. *PLOS ONE* 8: e67387.
221. Fuller, C. A. and Edgar, D. M. (1986). Effects of light intensity on the circadian temperature and feeding rhythms in the squirrel monkey. *Physiology and Behavior* 36: 687–691.
222. Aschoff, J. and Tokura, H. (1986). Circadian activity rhythms in squirrel monkeys: Entrainment by temperature cycles. *Journal of Biological Rhythms* 1: 91–99.
223. Wever, R. A. (1989). Light effects on human circadian rhythms: A review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
224. Giorgetti, M. and Zhdanova, I. V. (2000). Chronic cocaine treatment induces dysregulation in the circadian pattern of rats' feeding behavior. *Brain Research* 877: 170–175.
225. Glass, J. D., Brager, A. J., Stowie, A. C., and Prosser, R. A. (2012). Cocaine modulates pathways for photic and nonphotic entrainment of the mammalian SCN circadian clock. *American Journal of Physiology* 302: R740–R750.
226. Marchant, E. G. and Mistlberger, R. E. (1995). Morphine phase-shifts circadian rhythms in mice: Role of behavioural activation. *NeuroReport* 7: 209–212.
227. Park, R. N., Hirst, M., and Gowdey, C. W. (1980). The influence of single daily heroin injections on drinking patterns in the rat. *Canadian Journal of Physiology and Pharmacology* 58: 1295–1299.
228. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M., and Honma, K. (2000). Clock genes outside the suprachiasmatic nucleus involved in manifestation of locomotor activity rhythm in rats. *European Journal of Neuroscience* 12: 4206–4214.
229. Masubuchi, S., Honma, S., Abe, H., Nakamura, W., and Honma, K. (2001). Circadian activity rhythm in methamphetamine-treated *Clock* mutant mice. *European Journal of Neuroscience* 14: 1177–1180.
230. Tataroglu, Ö., Davidson, A. J., Benvenuto, L. J., and Menaker, M. (2006). The methamphetamine-sensitive circadian oscillator (MASCO) in mice. *Journal of Biological Rhythms* 21: 185–194.
231. Pezuk, P., Mohawk, J. A., Yoshikawa, T., Sellix, M. T., and Menaker, M. (2010). Circadian organization is governed by extra-SCN pacemakers. *Journal of Biological Rhythms* 25: 432–441.
232. Pendergast, J. S., Oda, G. A., Niswender, K. D., and Yamazaki, S. (2012). *Period* determination in the food-entrainable and methamphetamine-sensitive circadian oscillator(s). *Proceedings of the National Academy of Sciences United States of America* 109: 14218–14223.
233. Honma, K., Honma, S., and Hiroshige, T. (1987). Activity rhythms in the circadian domain appear in suprachiasmatic nuclei lesioned rats given methamphetamine. *Physiology and Behavior* 40: 767–774.
234. Honma, S., Honma, K., and Hiroshige, T. (1989). Methamphetamine induced locomotor rhythm entrains to restricted daily feeding in SCN lesioned rats. *Physiology and Behavior* 45: 1057–1065.
235. Honma, S. and Honma, K. (1995). Phase-dependent phase shift of methamphetamine-induced circadian rhythm by haloperidol in SCN-lesioned rats. *Brain Research* 674: 283–290.
236. Coward, D. J., Cain, S. W., and Ralph, M. R. (2001). A circadian rhythm in mice that is unaffected by the period mutation at *Clock*. *Biological Rhythm Research* 32: 233–242.
237. Pardo, B. F. and Sáenz, E. M. (1988). Action of deuterium oxide upon the ERG circadian rhythm in crayfish, *Procambarus bouvieri*. *Comparative Biochemistry and Physiology A* 90: 435–440.
238. White, L., Ringo, J., and Dowse, H. (1992). A circadian clock of *Drosophila*: Effects of deuterium oxide and mutations at the *period* locus. *Chronobiology International* 9: 250–259.
239. Pickard, G. E. and Zucker, I. (1986). Influence of deuterium oxide on circadian activity rhythms of hamsters: Role of the suprachiasmatic nuclei. *Brain Research* 376: 149–154.
240. Oklejewicz, M., Hut, R. A., and Daan, S. (2000). Effect of deuterium on the circadian period and metabolism in wild-type and *tau* mutant Syrian hamsters. *Physiology and Behavior* 71: 69–74.
241. Mistlberger, R. E., Marchant, E. G., and Kippin, T. E. (2001). Food-entrained circadian rhythms in rats are insensitive to deuterium oxide. *Brain Research* 919: 283–291.
242. Mistlberger, R. E. and Nadeau, J. (1992). Ethanol and circadian rhythms in the Syrian hamster: Effects on entrained phase, reentrainment rate, and period. *Pharmacology, Biochemistry and Behavior* 43: 159–165.
243. Eastman, C. I., Stewart, K. T., and Weed, M. R. (1994). Evening alcohol consumption alters the circadian rhythm of body temperature. *Chronobiology International* 11: 141–142.
244. Danel, T., Libersa, C., and Touitou, Y. (2001). The effect of alcohol consumption on the circadian control of human core body temperature is time dependent. *American Journal of Physiology* 281: R52–R55.
245. Sei, H., Sakata-Haga, H., Ohta, K., Sawada, K., Morita, Y., and Fukui, Y. (2003). Prenatal exposure to alcohol alters the light response in postnatal circadian rhythm. *Brain Research* 987: 131–134.
246. Rosenwasser, A. M., Logan, R. W., and Fecteau, M. E. (2005). Chronic ethanol intake alters circadian period-responses to brief light pulses. *Chronobiology International* 22: 227–236.
247. Nascimento, N. F., Carlson, K. N., Amaral, D. N., Logan, R. W., and Seggio, J. A. (2015). Alcohol and lithium have opposing effects on the period and phase of the behavioral free-running activity rhythm. *Alcohol* 49: 367–376.
248. Van Reeth, O. and Turek, F. W. (1987). Adaptation of circadian rhythmicity to shift in light-dark cycle accelerated by a benzodiazepine. *American Journal of Physiology* 253: R204–R207.

249. Wickland, C. and Turek, F. W. (1991). Phase-shifting effect of triazolam on the hamster's circadian rhythm of activity is not mediated by a change in body temperature. *Brain Research* 560: 12–16.
250. Van Reeth, O. and Turek, F. W. (1992). Changes in the phase response curve of the circadian clock to a phase-shifting stimulus. *Journal of Biological Rhythms* 7: 137–147.
251. Biello, S. M. and Mrosovsky, N. (1993). Circadian phase-shifts induced by chlordiazepoxide without increased locomotor activity. *Brain Research* 622: 58–62.
252. Vansteensel, M. J., Deboer, T., Dahan, A., and Meijer, J. H. (2003). Differential responses of circadian activity onset and offset following GABA-ergic and opioid receptor activation. *Journal of Biological Rhythms* 18: 297–306.
253. Van Reeth, O. and Turek, F. W. (1990). Daily injections of triazolam induce long-term changes in hamster circadian period. *American Journal of Physiology* 259: R514–R520.
254. Penev, P. D., Zee, P. C., and Turek, F. W. (1994). Monoamine depletion blocks triazolam-induced phase advances of the circadian clock in hamsters. *Brain Research* 637: 255–261.
255. Takahashi, Y., Usui, S., and Honda, Y. (1992). Effect of vitamin B_{12} (mecobalamin) on the free-running period of rat circadian behavioral rhythm. *Japanese Journal of Psychiatry and Neurology* 46: 222–224.
256. Tsujimaru, S., Egami, H., Honma, G., Ida, Y., Mukasa, H., and Nakazawa, Y. (1992). Effects of vitamin B_{12} on the period of free-running rhythm in rats. *Japanese Journal of Psychiatry and Neurology* 46: 225–226.
257. Ikeda, M., Honda, K., and Inoué, S. (1996). Vitamin B_{12} amplifies circadian phase shifts induced by a light pulse in rats. *Experientia* 52: 691–694.
258. Kohsaka, A., Laposky, A. D., Ramsey, K. M., Estrada, C., Joshu, C., Kobayashi, Y., Turek, F. W., and Bass, J. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabolism* 6: 414–421.
259. Mendoza, J., Pévet, P., and Challet, E. (2008). High-fat feeding alters the clock synchronization to light. *Journal of Physiology* 586: 5901–5910.
260. Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. London, U.K.: Chapman and Hall.
261. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th edn. New York: Cambridge University Press.
262. Priddle, J. (1980). The production ecology of benthic plants in some Arctic lakes. II. Laboratory physiology studies. *Journal of Ecology* 68: 155–166.
263. Jones, M. B., Leafe, E. L., Stiles, W., and Collett, B. (1978). Pattern of respiration of a perennial ryegrass crop in the field. *Annals of Botany* 42: 693–703.
264. Scholander, P. F., Flagg, W., Walters, V., and Irving, L. (1952). Respiration in some Arctic and tropical lichens in relation to temperature. *American Journal of Botany* 39: 707–713.
265. Wager, H. G. (1941). On the respiration and carbon assimilation rates of some arctic plants as related to temperature. *New Phytologist* 40: 1–19.
266. Humphreys, W. F. (1975). The respiration of *Geolycosa godeffroyi* (Araneae: Lycosidae) under conditions of constant and cyclic temperature. *Physiological Zoology* 48: 269–281.
267. Withers, P. C. and Smith, G. T (1993). Effect of temperature on the metabolic rate and evaporative water loss of the scorpion *Urodacus armatus*. *Journal of Thermal Biology* 18: 13–18.
268. Edwards, G. A. and Irving, L. (1943). The influence of temperature and season upon the oxygen consumption of the sand crab, *Emerita talpoida* Say. *Journal of Cellular and Comparative Physiology* 21: 169–182.
269. Husain, M. Z. and Alikhan, M. A. (1979). A comparative study of the respiratory metabolism in *Porcellio laevis* and *Armadillidium vulgare*: Response to temperature, photoperiod and oxygen concentration. *Archives Internationales de Physiologie et de Biochimie* 87: 697–710.
270. Gromysz-Kalkowska, K. and Szubartowska, E. (1984). Oxygen consumption in two terrestrial species of crustaceans (Isopoda). *Bulletin of the Polish Academy of Sciences, Biological Sciences* 32: 47–56.
271. Newell, R. C., Wieser, W., and Pye, V. I. (1974). Factors affecting oxygen consumption in the woodlouse *Porcellio scaber*. *Oecologia* 16: 31–51.
272. Gromysz-Kalkowska, K., Oder, M., and Szubartowska, E. (1984). Oxygen consumption in *Armadillidium nasutum* Buddle-Lund (Isopoda). *Folia Biologica Kraków* 32: 89–108.
273. Halcrow, K. and Boyd, C. M. (1967). The oxygen consumption and swimming activity of the amphipod *Gammarus oceanicus* at different temperatures. *Comparative Biochemistry and Physiology* 23: 233–242.
274. Moreira, G. S. and Nelson, S. G. (1990). The effects of temperature on the respiratory metabolism of *Calcinus laevimanus* (Crustacea: Anomura) in different salinities. *Journal of Thermal Biology* 15: 37–39.
275. Reiber, C. L. and Birchard, G. F. (1993). Effect of temperature on metabolism and hemolymph pH in the crab *Stoliczia abbotti*. *Journal of Thermal Biology* 18: 49–52.
276. Harrison, J. F., Fewell, J. H., Roberts, S. P., and Hall, H. G. (1996). Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science* 274: 88–90.
277. Schmolz, E., Lamprecht, I., and Schricker, B, (1993). Calorimetric investigations on social thermogenesis in the hornet *Vespa crabo* L. (Hymenoptera: Vespinae). *Thermochimica Acta* 229: 173–180.
278. Singh, S. P. and Das, A. B. (1977). Seasonal variation and acclimatization of respiratory metabolism of *Periplaneta americana* (Linn.). *Comparative Physiology and Ecology* 2: 133–138.
279. Frears, S. L., Chown, S. L., and Webb, P. I. (1997). Behavioural thermoregulation in the mopane worm (Lepidoptera). *Journal of Thermal Biology* 22: 325–330.
280. Haugaard, N. and Irving, L. (1943). The influence of temperature upon the oxygen consumption of the cunner (*Tautogolabrus adspersus* Walbaum) in summer and in winter. *Journal of Cellular and Comparative Physiology* 21: 19–26.
281. Fry, F. E. J. and Hart, J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. *Biological Bulletin* 94: 66–77.
282. Henry, J. A. C. and Houston, A. H. (1984). Absence of respiratory acclimation to diurnally-cycling temperature conditions in rainbow trout. *Comparative Biochemistry and Physiology A* 77: 727–734.
283. Vondracek, B., Cech, J. J., and Longanecker, D. (1982). Effect of cycling and constant temperatures on the respiratory metabolism of the Tahoe sucker, *Catostomus tahoensis* (Pisces: Catostomidae). *Comparative Biochemistry and Physiology A* 73: 11–14.
284. Duthie, G. G. and Houlihan, D. F. (1982). The effect of single step and fluctuating temperature changes on the oxygen consumption of flounders, *Platichthys flesus* (L.): Lack of temperature adaptation. *Journal of Fish Biology* 21: 215–226.
285. Refinetti, R. and Refinetti, K. Z. (1988). Absence of metabolic acclimation to cycling temperature conditions in the goldfish. *Physiology and Behavior* 43: 121–122.

286. Crawshaw, L. I., Ackerman, R. A., White, F. N., and Heath, M. E. (1982). Metabolic and acid–base changes during selection of warmer water by cold-acclimated fish. *American Journal of Physiology* 242: R157–R161.
287. Sweeney, B. M. and Hastings, J. W. (1960). Effects of temperature upon diurnal rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 87–103.
288. Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S., and Johnson, C. H. (1993). Circadian rhythms in prokaryotes: Luciferase as a reporter of circadian gene expression in cyanobacteria. *Proceedings of the National Academy of Sciences United States of America* 90: 5672–5676.
289. Tomita, J., Nakajima, M., Kondo, T., and Iwasaki, H. (2005). No transcription–translation feedback in circadian rhythm of KaiC phosphorylation. *Science* 307: 251–254.
290. Nakajima, M., Imai, K., Ito, H., Nishiwaki, T., Murayama, Y., Iwasaki, H., Oyama, T., and Kondo, T. (2005). Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* 308: 414–415.
291. Liu, Y., Merrow, M., Loros, J. J., and Dunlap, J. C. (1998). How temperature changes reset a circadian oscillator. *Science* 281: 825–829.
292. Hennessey, T. L. and Field, C. B. (1992). Evidence of multiple circadian oscillators in bean plants. *Journal of Biological Rhythms* 7: 105–113.
293. Kippert, F., Saunders, D. S., and Blaxter, M. L. (2002). *Caenorhabditis elegans* has a circadian clock. *Current Biology* 12: R47–R49.
294. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences United States of America* 40: 1018–1029.
295. Hoffmann, K. (1957). Über den Einfluß der Temperatur auf die Tagesperiodik bei einem Poikilothermen. *Naturwissenschaften* 44: 358.
296. Magnone, M. C., Jacobmeier, B., Bertolucci, C., Foà, A., and Albrecht, U. (2005). Circadian expression of the clock gene *Per2* is altered in the ruin lizard (*Podarcis sicula*) when temperature changes. *Molecular Brain Research* 133: 281–285.
297. Menaker, M. (1959). Endogenous rhythms of body temperature in hibernating bats. *Nature* 184: 1251–1252.
298. Gibbs, F. P. (1981). Temperature dependence of rat circadian pacemaker. *American Journal of Physiology* 241: R17–R20.
299. Ruby, N. F., Burns, D. E., and Heller, H. C. (1999). Circadian rhythms in the suprachiasmatic nucleus are temperature-compensated and phase-shifted by heat pulses *in vitro*. *Journal of Neuroscience* 19: 8630–8636.
300. Herzog, E. D. and Huckfeldt, R. M. (2003). Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 763–770.
301. Izumo, M., Johnson, C. H., and Yamazaki, S. (2003). Circadian gene expression in mammalian fibroblasts revealed by real-time luminescence reporting: Temperature compensation and damping. *Proceedings of the National Academy of Sciences United States of America* 100: 16089–16094.
302. Isojima, Y., Nakajima, M., Ukai, H., Fujishima, H., Yamada, R. G., Masumoto, K. H., Kiuchi, R. et al. (2009). CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proceedings of the National Academy of Sciences United States of America* 106: 15744–15749.
303. Buhr, E. D., Yoo, S. H., and Takahashi, J. S. (2010). Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* 330: 379–385.
304. Gibbs, F. P. (1983). Temperature dependence of the hamster circadian pacemaker. *American Journal of Physiology* 244: R607–R610.
305. Tosini, G. and Menaker, M. (1998). The *tau* mutation affects temperature compensation of hamster retinal circadian oscillators. *NeuroReport* 9: 1001–1005.
306. Konopka, R. J. and Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences United States of America* 68: 2112–2116.
307. Feldman, J. F. and Hoyle, M. N. (1973). Isolation of circadian clock mutants of *Neurospora crassa*. *Genetics* 75: 605–613.
308. Hall, J. C. (1990). Genetics of circadian rhythms. *Annual Review of Genetics* 24: 659–697.
309. Rothenfluh, A., Abodeely, M., Price, J. L., and Young, M. W. (2000). Isolation and analysis of six *timeless* alleles that cause short- or long-period circadian rhythms in Drosophila. *Genetics* 156: 665–675.
310. Akten, B., Jauch, E., Genova, G. K., Kim, E. Y., Edery, I., Raabe, T., and Jackson, F. R. (2003). A role for CK2 in the *Drosophila* circadian oscillator. *Nature Neuroscience* 6: 251–257.
311. Dunlap, J. C. (1996). Genetic and molecular analysis of circadian rhythms. *Annual Review of Genetics* 30: 579–601.
312. Ralph, M. R. and Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
313. Vitaterna, M. H., King, D. P., Chang, A. M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., Dove, W. F., Pinto, L. H., Turek, F. W., and Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264: 719–725.
314. Mrosovsky, N. (1989). Mutant hamster in a hurry. *Nature* 337: 213.
315. Menaker, M. and Refinetti, R. (1992). The *tau* mutation in golden hamsters. In: Young, M. W. (Ed.), *Molecular Genetics of Biological Rhythms*. New York: Marcel Dekker, pp. 255–269.
316. Refinetti, R. and Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
317. Osiel, S., Golombek, D. A., and Ralph, M. R. (1998). Conservation of locomotor behavior in the golden hamster: Effects of light cycle and a circadian period mutation. *Physiology and Behavior* 65: 123–131.
318. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A., and Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
319. Refinetti, R. (2007). Absence of circadian and photoperiodic conservation of energy expenditure in three rodent species. *Journal of Comparative Physiology B* 177: 309–318.
320. Tosini, G. and Menaker, M. (1996). Circadian rhythms in cultured mammalian retina. *Science* 272: 419–421.
321. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M., and Loudon, A. S. I. (1999). Free running circadian rhythms of melatonin, luteinizing hormone, and cortisol in Syrian hamsters bearing the circadian *tau* mutation. *Endocrinology* 140: 758–764.
322. Grace, M. S., Wang, L. A., Pickard, G. E., Besharse, J. C., and Menaker, M. (1996). The *tau* mutation shortens the period of rhythmic photoreceptor outer segment disk shedding in the hamster. *Brain Research* 735: 93–100.
323. Shimomura, K., Nelson, D. E., Ihara, N. L., and Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.

324. Loudon, A. S. I., Ihara, N., and Menaker, M. (1998). Effects of a circadian mutation on seasonality in Syrian hamsters (*Mesocricetus auratus*). *Proceedings of the Royal Society of London B* 265: 517–521.
325. Refinetti, R. and Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.
326. Lucas, R. J., Stirland, J. A., Mohammad, Y. N., and Loudon, A. S. I. (2000). Postnatal growth rate and gonadal development in circadian *tau* mutant hamsters reared in constant dim red light. *Journal of Reproduction and Fertility* 118: 327–330.
327. Loudon, A. S. I., Wayne, N. L., Krieg, R., Iranmanesh, A., Veldhuis, J. D., and Menaker, M. (1994). Ultradian endocrine rhythms are altered by a circadian mutation in the Syrian hamster. *Endocrinology* 135: 712–718.
328. Shimomura, K. and Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
329. Grosse, J., Loudon, A. S. I., and Hastings, M. H. (1995). Behavioural and cellular responses to light of the circadian system of *tau* mutant and wild-type Syrian hamsters. *Neuroscience* 65: 587–597.
330. Scarbrough, K. and Turek, F. W. (1996). Quantitative differences in the circadian rhythm of locomotor activity and vasopressin and vasoactive intestinal peptide gene expression in the suprachiasmatic nucleus of *tau* mutant compared to wild-type hamsters. *Brain Research* 736: 251–259.
331. Shimomura, K., Kornhauser, J. M., Wisor, J. P., Umezu, T., Yamazaki, S., Ihara, N. L., Takahashi, J. S., and Menaker, M. (1998). Circadian behavior and plasticity of light-induced *c-fos* expression in SCN of *tau* mutant hamsters. *Journal of Biological Rhythms* 13: 305–314.
332. Agostino, P. V., Harrington, M. E., Ralph, M. R., and Golombek, D. A. (2009). Casein kinase 1 ε and circadian photic responses in hamsters. *Chronobiology International* 26: 126–133.
333. Oklejewicz, M. and Daan, S. (2002). Enhanced longevity in *tau* mutant Syrian hamsters, *Mesocricetus auratus*. *Journal of Biological Rhythms* 17: 210–216.
334. Oklejewicz, M., Pen, I., Durieux, G. C. R., and Daan, S. (2001). Maternal and pup genotype contribution to growth in wild-type and *tau* mutant Syrian hamsters. *Behavior Genetics* 31: 383–390.
335. Refinetti, R. (2014). Relationship between circadian period and body size in the *tau*-mutant golden hamster. *Canadian Journal of Physiology and Pharmacology* 92: 27–33.
336. Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamazaki, S., Zemenides, P. D., Ralph, M. R., Menaker, M., and Takahashi, J. S. (2000). Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* 288: 483–491.
337. Monecke, S., Brewer, J. M., Krug, S., and Bittman, E. L. (2011). Duper: A mutation that shortens hamster circadian period. *Journal of Biological Rhythms* 26: 283–292.
338. Bittman, E. L. (2012). Does the precision of a biological clock depend upon its period? Effects of the duper and tau mutations in Syrian hamsters. *PLOS ONE* 7: e36119.
339. King, D. P., Zhao, Y., Sangoram, A. M., Wilsbacher, L. D., Tanaka, M., Antoch, M. P., Steeves, T. D. L. et al. (1997). Positional cloning of the mouse circadian *Clock* gene. *Cell* 89: 641–653.
340. Antoch, M. P., Song, E. J., Chang, A. M., Vitaterna, M. H., Zhao, Y., Wilsbacher, L. D., Sangoram, A. M., King, D. P., Pinto, L. H., and Takahashi, J. S. (1997). Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. *Cell* 89: 655–667.
341. Miller, B. H., Olson, S. L., Turek, F. W., Levine, J. E., Horton, T. H., and Takahashi, J. S. (2004). Circadian *Clock* mutation disrupts estrous cyclicity and maintenance of pregnancy. *Current Biology* 14: 1367–1373.
342. Dolatshad, H., Campbell, E. A., O'Hara, L., Maywood, E. S., Hastings, M. H., and Johnson, M. H. (2006). Developmental and reproductive performance in circadian mutant mice. *Human Reproduction* 21: 68–79.
343. Shearman, L. P. and Weaver, D. R. (1999). Photic induction of *Period* gene expression is reduced in *Clock* mutant mice. *NeuroReport* 10: 613–618.
344. Challet, E., Takahashi, J. S., and Turek, F. W. (2000). Nonphotic phase-shifting in *Clock* mutant mice. *Brain Research* 859: 398–403.
345. Arey, R. and McClung, C. A. (2012). An inhibitor of casein kinase 1 ε/δ partially normalizes the manic-like behaviors of the Clock-Δ19 mouse. *Behavioral Pharmacology* 23: 392–396.
346. Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A. et al. (2005). Obesity and metabolic syndrome in circadian *Clock* mutant mice. *Science* 308: 1043–1045.
347. Pickard, G. E., Sollars, P. J., Rinchik, E. M., Nolan, P. M., and Bucan, M. (1995). Mutagenesis and behavioral screening for altered circadian activity identifies the mouse mutant, *Wheels*. *Brain Research* 705: 255–266.
348. Bacon, Y., Ooi, A., Kerr, S., Shaw-Andrews, L., Winchester, L., Breeds, S., Tymoska-Lalanne, Z., Clay, J., Greenfield, A. G., and Nolan, P. M. (2004). Screening for novel ENU-induced rhythm, entrainment and activity mutants. *Genes, Brain and Behavior* 3: 196–205.
349. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D., and Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
350. Bunger, M. K., Wilsbacher, L. D., Moran, S. M., Clendenin, C., Radcliffe, L. A., Hogenesch, J. B., Simon, M. C., Takahashi, J. S., and Bradfield, C. A. (2000). *Mop3* is an essential component of the master circadian pacemaker in mammals. *Cell* 103: 1009–1017.
351. Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P. et al. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 282: 1490–1494.
352. Abraham, D., Dallmann, R., Steinlechner, S., Albrecht, U., Eichele, G., and Oster, H. (2006). Restoration of circadian rhythmicity in circadian clock-deficient mice in constant light. *Journal of Biological Rhythms* 21: 169–176.
353. McDearmon, E. L., Patel, K. N., Ko, C. H., Walisser, J. A., Schook, A. C., Chong, J. L., Wilsbacher, L. D. et al. (2006). Dissecting the functions of the mammalian clock protein BMAL1 by tissue-specific rescue in mice. *Science* 314: 1304–1308.
354. Godinho, S. I., Maywood, E. S., Shaw, L., Tucci, V., Barnard, A. R., Busino, L., Pagano, M. et al. (2007). The After-hours mutant reveals a role for Fbxl3 in determining mammalian circadian period. *Science* 316: 897–900.
355. Xu, Y., Toh, K. L., Jones, C. R., Shin, J. Y., Fu, Y. H., and Ptácek, L. J. (2007). Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell* 128: 59–70.
356. Power, A., Hughes, A. T. L., Samuels, R. E., and Piggins, H. D. (2010). Rhythm-promoting actions of exercise in mice with deficient neuropeptide signaling. *Journal of Biological Rhythms* 25: 235–246.

357. Connolly, M. S. and Lynch, C. B. (1983). Classical genetic analysis of circadian body temperature rhythms in mice. *Behavior Genetics* 13: 491–500.
358. Puchalski, W. and Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: Effects of photoperiodic pretreatment and artificial selection. *American Journal of Physiology* 261: R670–R676.
359. Guyomarc'h, C., Lumineau, S., and Richard, J. P. (1998). Circadian rhythm of activity in Japanese quail in constant darkness: Variability of clarity and possibility of selection. *Chronobiology International* 15: 219–230.
360. Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-ike, N., and Ishiura, M. (2004). Large-scale screening of *Arabidopsis* circadian clock mutants by a high-throughput real-time bioluminescence monitoring system. *Plant Journal* 40: 1–11.
361. De Castro, J. M. (2001). Heritability of diurnal changes in food intake in free-living humans. *Nutrition* 17: 713–720.
362. Meng, Q. J., Logunova, L., Maywood, E. S., Gallego, M., Yoo, S. H., Takahashi, J. S., Virshup, D. M., Boot-Handford, R. P., Hastings, M. H. & Loudon, A. S. I. (2008). Setting clock speed in mammals: The CK1ε *tau* mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. *Neuron* 58: 78–88.
363. Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.
364. Barrett, R. K. and Page, T. L. (1989). Effects of light on circadian pacemaker development. I. The freerunning period. *Journal of Comparative Physiology A* 165: 41–49.
365. Ciarleglio, C. M., Axley, J. C., Strauss, B. R., Gamble, K. L., and McMahon, D. G. (2011). Perinatal photoperiod imprints the circadian clock. *Nature Neuroscience* 14: 25–27.
366. Brooks, E., Patel, D., and Canal, M. M. (2014). Programming of mice circadian photic responses by postnatal light environment. *PLOS ONE* 9: e97160.
367. Roenneberg, T. and Morse, D. (1993). Two circadian oscillators in one cell. *Nature* 362: 362–364.
368. Morse, D., Hastings, J. W., and Roenneberg, T. (1994). Different phase responses of the two circadian oscillators in *Gonyaulax*. *Journal of Biological Rhythms* 9: 263–274.
369. Swann, J. and Turek, F. W. (1982). Cycle of lordosis behavior in female hamsters whose circadian activity rhythm has split into two components. *American Journal of Physiology* 243: R112–R118.
370. Pickard, G. E. and Turek, F. W. (1982). Splitting of the circadian rhythm of activity is abolished by unilateral lesions of the suprachiasmatic nuclei. *Science* 215: 1119–1121.
371. Pickard, G. E., Kahn, R., and Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiology and Behavior* 32: 763–766.
372. Swann, J. M. and Turek, F. W. (1985). Multiple circadian oscillators regulate the timing of behavioral and endocrine rhythms in female golden hamsters. *Science* 228: 898–900.
373. Morin, L. P. (1988). Age-related changes in hamster circadian period, entrainment, and rhythm splitting. *Journal of Biological Rhythms* 3: 237–248.
374. Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *Journal of Biological Rhythms* 8: 189–197.
375. Pickard, G. E., Turek, F. W., and Sollars, P. J. (1993). Light intensity and splitting in the golden hamster. *Physiology and Behavior* 54: 1–5.
376. Hallonquist, J., Lindegger, M., and Mrosovsky, N. (1994). Rubidium chloride fuses split circadian activity rhythms in hamsters housed in bright constant light. *Chronobiology International* 11: 65–71.
377. De la Iglesia, H. O., Meyer, J., and Schwartz, W. J. (2003). Lateralization of circadian pacemaker output: Activation of left- and right-sided luteinizing hormone-releasing hormone neurons involves a neural rather than a humoral pathway. *Journal of Neuroscience* 23: 7412–7414.
378. Mendoza, J. Y., Dardente, H., Escobar, C., Pevét, P., and Challet, E. (2004). Dark pulse resetting of the suprachiasmatic clock in Syrian hamsters: Behavioral phase-shifts and clock gene expression. *Neuroscience* 127: 529–537.
379. Bittman, E. L., Costello, M. K., and Brewer, J. M. (2007). Circadian organization of <I<tau mutant hamsters: Aftereffects and splitting. *Journal of Biological Rhythms* 22: 425–431.
380. Daan, S. and Berde, C. (1978). Two coupled oscillators: Simulations of the circadian pacemaker in mammalian activity rhythms. *Journal of Theoretical Biology* 70: 297–313.
381. Díez-Noguera, A. (1994). A functional model of the circadian system based on the degree of intercommunication in a complex system. *American Journal of Physiology* 267: R1118–R1135.
382. Oda, G. A. and Friesen, W. O. (2002). A model of "splitting" of running-wheel activity in hamsters. *Journal of Biological Rhythms* 17: 76–88.
383. Cheung, P. W. and McCormack, C. E. (1983). Splitting of the locomotor activity rhythm in rats by exposure to continuous light. *American Journal of Physiology* 244: H573–H576.
384. Schardt, U., Wilhelm, I., and Erkert, H. G. (1989). Splitting of the circadian activity rhythm in common marmosets (*Callithrix j. jacchus*; Primates). *Experientia* 45: 1112–1115.
385. Zlomanczuk, P., Margraf, R. R., and Lynch, G. R. (1991). In vitro electrical activity in the suprachiasmatic nucleus following splitting and masking of wheel-running behavior. *Brain Research* 559: 94–99.
386. Honma, S. and Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
387. Pohl, H. (1983). Light pulses entrain the circadian activity rhythm of a diurnal rodent (*Ammospermophilus leucurus*). *Comparative Biochemistry and Physiology B* 76: 723–729.
388. Lovegrove, B. G. and Papenfus, M. E. (1995). Circadian activity rhythms in the solitary Cape mole rat (*Georychus capensis*: Bathyergidae) with some evidence of splitting. *Physiology and Behavior* 58: 679–685.
389. Harrington, M. E., Eskes, G. A., Dickson, P., and Rusak, B. (1990). Lesions dorsal to the suprachiasmatic nuclei abolish split activity rhythms of hamsters. *Brain Research Bulletin* 24: 593–597.
390. De la Iglesia, H. O., Meyer, J., Carpino, A., and Schwartz, W. J. (2000). Antiphase oscillation of the left and right suprachiasmatic nuclei. *Science* 290: 799–801.
391. Watanabe, T., Yoshimura, T., McMahon, D. G., and Ebihara, S. (2003). Unimodal circadian rhythm in the suprachiasmatic nucleus of behaviorally splitting mice. *Neuroscience Letters* 345: 49–52.
392. Yan, L., Foley, N. C., Bobula, J. M., Kriegsfeld, L. J., and Silver, R. (2005). Two antiphase oscillations occur in each suprachiasmatic nucleus of behaviorally split hamsters. *Journal of Neuroscience* 25: 9017–9026.

393. Jagota, A., de la Iglesia, H. O., and Schwartz, W. J. (2000). Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro*. *Nature Neuroscience* 3: 372–376.
394. Burgoon, P. W., Lindberg, P. T., and Gillette, M. U. (2004). Different patterns of circadian oscillation in the suprachiasmatic nucleus of hamster, mouse, and rat. *Journal of Comparative Physiology A* 190: 167–171.
395. Grima, B., Chélot, E., Xia, R., and Rouyer, F. (2004). Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. *Nature* 431: 869–873.
396. Yao, Z. and Shafer, O. T. (2014). The *Drosophila* circadian clock is a variably coupled network of multiple peptidergic units. *Science* 343: 1516–1520.
397. Mrosovsky, N. and Janik, D. (1993). Behavioral decoupling of circadian rhythms. *Journal of Biological Rhythms* 8: 57–65.
398. Edelstein, K., de la Iglesia, H. O., and Mrosovsky, N. (2003). Period gene expression in the suprachiasmatic nucleus of behaviorally decoupled hamsters. *Molecular Brain Research* 114: 40–45.
399. Gorman, M. R., Yellon, S. M., and Lee, T. M. (2001). Temporal reorganization of the suprachiasmatic nuclei in hamsters with split circadian rhythms. *Journal of Biological Rhythms* 16: 552–563.
400. Gorman, M. R. and Lee, T. M. (2001). Daily novel wheel running reorganizes and splits hamster circadian activity rhythms. *Journal of Biological Rhythms* 16: 541–551.
401. Gorman, M. R. (2001). Exotic photoperiods induce and entrain split circadian activity rhythms in hamsters. *Journal of Comparative Physiology A* 187: 793–800.
402. Gorman, M. R., Elliott, J. A., and Evans, J. A. (2003). Plasticity of hamster circadian entrainment patterns depends on light intensity. *Chronobiology International* 20: 233–248.
403. Gorman, M. R. and Elliott, J. A. (2003). Entrainment of 2 subjective nights by daily light:dark:light:dark cycles in 3 rodent species. *Journal of Biological Rhythms* 18: 502–512.
404. Kleitman, N. and Kleitman, E. (1953). Effect of non-twenty-four-hour routines of living on oral temperature and heart rate. *Journal of Applied Physiology* 6: 283–291.
405. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.
406. Moore-Ede, M. C., Kass, D. A., and Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
407. Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sánchez, R., Ríos, C. D., Freitag, W. O., Richardson, G. S., and Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep–wake cycle. *Science* 233: 667–671.
408. Minors, D. S., Waterhouse, J. M., Folkard, S., and Totterdell, P. (1993). Circadian rhythms of body temperature and urinary excretion in subjects on 30-h "days". *Journal of Interdisciplinary Cycle Research* 24: 277–283.
409. Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D., and Lalouette, A. (1994). Dissociation in body temperature, drinking and feeding rhythms following a light–dark cycle inversion in the rat. *Medical Science Research* 22: 53–55.
410. Dijk, D. J. and Czeisler, C. A. (1994). Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. *Neuroscience Letters* 166: 63–68.
411. Roberts, M. H. and Xie, X. (1996). Phase relationship between ocular and behavioral circadian rhythms in *Bulla gouldiana* exposed to different photoperiods. *Physiology and Behavior* 59: 703–708.
412. Waterhouse, J., Minors, D., Folkard, S., Owens, D., Atkinson, G., Macdonald, I., Reilly, T., Sytnik, N., and Tucker, P. (1998). Light of domestic intensity produces phase shifts of the circadian oscillator in humans. *Neuroscience Letters* 245: 97–100.
413. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A., and Dijk, D. J. (1999). Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *American Journal of Physiology* 277: R1152–R1163.
414. Lockley, S. W., Skene, D. J., Butler, L. J., and Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 22: 616–623.
415. Wright, K. P., Hull, J. T., and Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *American Journal of Physiology* 283: R1370–R1377.
416. Hack, L. M., Lockley, S. W., Arendt, J., and Skene, D. J. (2003). The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects. *Journal of Biological Rhythms* 18: 420–429.
417. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Spontaneous internal desynchronization of circadian rhythms in the squirrel monkey. *Comparative Biochemistry and Physiology A* 58: 63–67.
418. Ruis, J. F., Rietveld, W. J., and Buys, P. J. (1987). Effects of suprachiasmatic nuclei lesions on circadian and ultradian rhythms in body temperature in ocular enucleated rats. *Journal of Interdisciplinary Cycle Research* 18: 259–273.
419. Labyak, S. E. and Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
420. Cambras, T., Weller, J. R., Anglès-Pujoràs, M., Lee, M. L., Christopher, A., Diez-Noguera, A., Krueger, J. M., and de la Iglesia, H. O. (2007). Circadian desynchronization of core body temperature and sleep stages in the rat. *Proceedings of the National Academy of Sciences United States of America* 104: 7634–7639.
421. Miles, L. E. M., Raynal, D. M., and Wilson, M. A. (1977). Blind man living in normal society has circadian rhythms of 24.9 hours. *Science* 198: 421–423.
422. Zulley, J. and Campbell, S. S. (1985). Napping behavior during "spontaneous internal desynchronization": Sleep remains in synchrony with body temperature. *Human Neurobiology* 4: 123–126.
423. Folkard, S., Wever, R. A., and Wildgruber, C. M. (1983). Multi-oscillatory control of circadian rhythms in human performance. *Nature* 305: 223–226.
424. Folkard, S., Hume, K. I., Minors, D. S., Waterhouse, J. M., and Watson, F. L. (1985). Independence of the circadian rhythm in alertness from the sleep/wake cycle. *Nature* 313: 678–679.
425. Green, J., Pollak, C. P., and Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
426. Motohashi, Y. (1990). Desynchronization of oral temperature and grip strengths: Circadian rhythms in healthy subjects with irregular sleep–wake behavior. *Progress in Clinical and Biological Research* 341B: 57–63.
427. Aschoff, J. (1994). The timing of defecation within sleep–wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
428. Aschoff, J. (1994). Naps as integral parts of the wake time within the human sleep–wake cycle. *Journal of Biological Rhythms* 9: 145–155.
429. Aschoff, J. (1998). Human perception of short and long time intervals: Its correlation with body temperature and the duration of wake time. *Journal of Biological Rhythms* 13: 437–442.

430. Aschoff, J. and Wever, R. (1976). Human circadian rhythms: A multioscillatory system. *Federation Proceedings* 35: 2326–2332.
431. Mills, J. N. (1973). Transmission processes between clocks and manifestations. In: Mills, J. N. (Ed.), *Biological Aspects of Circadian Rhythms*. New York: Plenum, pp. 27–84.
432. Campbell, S. and Zulley, J. (1988). Napping as a biological rhythm: Disentrainment of the human sleep/wake system. In: Koella, W. P., Obál, F., Schultz, H., and Visser, P. (Eds.), *Sleep '86*. Stuttgart, Germany: Gustav Fischer, pp. 3–10.
433. Campbell, S. S. and Zulley, J. (1989). Evidence for circadian influence on human slow wave sleep during daytime sleep episodes. *Psychophysiology* 26: 580–585.
434. Daan, S., Beersma, D. G. M., and Borbély, A. A. (1984). Timing of human sleep: Recovery process gated by a circadian pacemaker. *American Journal of Physiology* 246: R161–R178.
435. Eastman, C. (1984). Are separate temperature and activity oscillators necessary to explain the phenomena of human circadian rhythms? In: Moore-Ede, M. C. and Czeisler, C. A. (Eds.), *Mathematical Models of the Circadian Sleep-Wake Cycle*. New York: Raven, pp. 81–101.
436. Dunn, J. D., Castro, A. J., and McNulty, J. A. (1977). Effect of suprachiasmatic ablation on the daily temperature rhythm. *Neuroscience Letters* 6: 345–348.
437. Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N., and Scheving, L. E. (1979). Nomifensine chronopharmacology, schedule-shifts and circadian temperature rhythms in di-suprachiasmatically lesioned rats: Modeling emotional chronopathology and chronotherapy. *Chronobiologia* 6: 405–424.
438. Powell, E. W., Halberg, F., Pasley, J. N., Lubanovic, W., Ernsberger, P., and Scheving, L. E. (1980). Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *Journal of Thermal Biology* 5: 189–196.
439. Fuller, C. A., Lydic, R., Sulzman, F. M., Albers, H. E., Tepper, B., and Moore-Ede, M. C. (1981). Circadian rhythm of body temperature persists after suprachiasmatic lesions in the squirrel monkey. *American Journal of Physiology* 241: R385–R391.
440. Satinoff, E. and Prosser, R. A. (1988). Suprachiasmatic nuclear lesions eliminate circadian rhythms of drinking and activity, but not of body temperature, in male rats. *Journal of Biological Rhythms* 3: 1–22.
441. Refinetti, R., Kaufman, C. M., and Menaker, M. (1994). Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters. *Journal of Comparative Physiology A* 175: 223–232.
442. Weber, E. T. and Hohn, V. M. (2005). Circadian activity rhythms in the spiny mouse, *Acomys cahirinus*. *Physiology and Behavior* 86: 427–433.
443. Moritz, R. F. A. and Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.
444. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P., and Challet, E. (2003). Circadian profile and photic regulation of clock genes in the suprachiasmatic nucleus of a diurnal mammal, *Arvicanthis ansorgei*. *Neuroscience* 116: 583–591.
445. Slotten, H. A., Krekling, S., and Pévet, P. (2005). Photic and nonphotic effects on the circadian activity rhythm in the diurnal rodent *Arvicanthis ansorgei*. *Behavioural Brain Research* 165: 91–97.
446. Mendoza, J., Gourmelen, S., Dumont, S., Sage-Ciocca, D., Pévet, P., and Challet, E. (2012). Setting the main circadian clock of a diurnal mammal by hypocaloric feeding. *Journal of Physiology* 590: 3155–3168.
447. Mahoney, M., Bult, A., and Smale, L. (2001). Phase response curve and light-induced Fos expression in suprachiasmatic nucleus and adjacent hypothalamus of *Arvicanthis niloticus*. *Journal of Biological Rhythms* 16: 149–162.
448. Refinetti, R. (2007). Enhanced circadian photoresponsiveness after prolonged dark adaptation in seven species of diurnal and nocturnal rodents. *Physiology and Behavior* 90: 431–437.
449. Glass, J. D., Tardif, S. D., Clements, R., and Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
450. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S., and Tabata, M. (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *Journal of Comparative Physiology A* 181: 121–132.
451. Büttner, D. (1992). Social influences on the circadian rhythm of locomotor activity and food intake of guinea pigs. *Journal of Interdisciplinary Cycle Research* 23: 100–112.
452. Houdelier, C., Guyomarc'h, C., Lumineau, S., and Richard, J. P. (2002). Circadian rhythms of oviposition and feeding activity in Japanese quail: Effects of cyclic administration of melatonin. *Chronobiology International* 19: 1107–1119.
453. Schöttner, K., Oosthuizen, M. K., Broekman, M., and Bennett, N. C. (2006). Circadian rhythms of locomotor activity in the Lesotho mole-rat, *Cryptomys hottentotus* subspecies from Sani Pass, South Africa. *Physiology and Behavior* 89: 205–212.
454. Sheeba, V., Chandrashekaran, M. K., Joshi, A., and Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *Journal of Insect Physiology* 48: 25–32.
455. Rajaratnam, S. M. W. and Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
456. Oosthuizen, M. K., Cooper, H. M., and Bennett, N. C. (2003). Circadian rhythms of locomotor activity in solitary and social species of African mole-rats (Family: Bathyergidae). *Journal of Biological Rhythms* 18: 481–490.
457. Joshi, D. S. and Vanlalnghaka, C. (2005). Non-parametric entrainment by natural twilight in the microchiropteran bat, *Hipposideros speoris*, inside a cave. *Chronobiology International* 22: 631–640.
458. Reinberg, A., Halberg, F., Ghata, J., and Siffre, M. (1966). Spectre thermique (rythmes de la température rectale) d'une femme adulte avant, pendant et après son isolement souterrain de trois mois. *Comptes Rendus de l'Académie des Sciences* 262: 782–785.
459. Pollak, C. P. and Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
460. Czeisler, C. A., Duffy, J. F, Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M. et al. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177–2181.
461. Honma, K., Honma, S., and Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
462. Sack, R. L., Brandes, R. W., Kendall, A. R., and Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
463. Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep–wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.

464. Page, T. L., Mans, C., and Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.
465. Barlow, R. B. (1983). Circadian rhythms in the *Limulus* visual system. *Journal of Neuroscience* 3: 856–870.
466. Honnebier, M. B. O. M., Jenkins, S. L., and Nathanielsz, P. W. (1992). Circadian timekeeping during pregnancy: Endogenous phase relationships between maternal plasma hormones and the maternal body temperature rhythm in pregnant rhesus monkeys. *Endocrinology* 131: 2051–2058.
467. Masuda, K. and Zhdanova, I. V. (2010). Intrinsic activity rhythms in *Macaca mulatta*: Their entrainment to light and melatonin. *Journal of Biological Rhythms* 25: 361–371.
468. Refinetti, R. (2006). Variability of diurnality in laboratory rodents. *Journal of Comparative Physiology A* 192: 701–714.
469. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology* 106: 253–266.
470. Rosenberg, R. S., Zee, P. C., and Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.
471. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
472. Perret, M., Aujard, F., Séguy, M., and Schilling, A. (2003). Olfactory bulbectomy modifies photic entrainment and circadian rhythms of body temperature and locomotor activity in a nocturnal primate. *Journal of Biological Rhythms* 18: 392–401.
473. Perret, M., Gomez, D., Barbosa, A., Aujard, F., and Théry, M. (2010). Increased late night response to light controls the circadian pacemaker in a nocturnal primate. *Journal of Biological Rhythms* 25: 186–196.
474. Pifferi, F., Dal-Pan, A., Menaker, M., and Aujard, F. (2011). Resveratrol dietary supplementation shortens the free-running circadian period and decreases body temperature in a prosimian primate. *Journal of Biological Rhythms* 26: 271–275.
475. Erkert, H. G. (2004). Extremely low threshold for photic entrainment of circadian activity rhythms in molossid bats (*Molossus molossus*; Chiroptera, Molossidae). *Mammalian Biology* 69: 361–374.
476. Sharma, V. K. (1996). Light-induced phase response curves of the circadian activity rhythm in individual field mice, *Mus booduga*. *Chronobiology International* 13: 401–409.
477. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
478. Von Gall, C., Duffield, G. E., Hastings, M. H., Kopp, M. D. A., Dehghani, F., Korf, H. W., and Stehle, J. H. (1998). CREB in the mouse SCN: A molecular interface coding the phase-adjusting stimuli light, glutamate, PACAP, and melatonin for clockwork access. *Journal of Neuroscience* 18: 10389–10397.
479. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S., and Honma, K. (2001). Clock gene expressions in the suprachiasmatic nucleus and other areas of the brain during rhythm splitting in CS mice. *Molecular Brain Research* 87: 92–99.
480. Benloucif, S. and Dubocovich, M. L. (1996). Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN mouse. *Journal of Biological Rhythms* 11: 113–125.
481. Kolker, D. E., Vitaterna, M. H., Fruechte, E. M., Takahashi, J. S., and Turek, F. W. (2004). Effects of age on circadian rhythms are similar in wild-type and heterozygous *Clock* mutant mice. *Neurobiology of Aging* 25: 517–523.
482. Liu, C., Li, S., Liu, T., Borjigin, J., and Lin, J. D. (2007). Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. *Nature* 447: 477–481.
483. Green, C. B., Douris, N., Kojima, S., Strayer, C. A., Fogerty, J., Lourim, D., Keller, S. R., and Besharse, J. C. (2007). Loss of nocturnin, a circadian deadenylase, confers resistance to hepatic steatosis and diet-induced obesity. *Proceedings of the National Academy of Sciences United States of America* 104: 9888–9893.
484. Van der Leest, H. T., Rohling, J. H. T., Michel, S., and Meijer, J. H. (2009). Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLOS ONE* 4: e4976.
485. Bullough, J. D., Figueiro, M. G., Possidente, B. P., Parsons, R. H., and Rea, M. S. (2005). Additivity in murine circadian phototransduction. *Zoological Science* 22: 223–227.
486. Castillo, M. R., Hochstetler, K. J., Greene, D. M., Firmin, S. I., Tavernier, R. J., Raap, D. K., and Bult-Ito, A. (2005). Circadian rhythm of core body temperature in two laboratory mouse lines. *Physiology and Behavior* 86: 538–545.
487. Kopp, C., Ressel, V., Wigger, E., and Tobler, I. (2006). Influence of estrous cycle and ageing on activity patterns in two inbred mouse strains. *Behavioural Brain Research* 167: 165–174.
488. Sans-Fuentes, M. A., Díez-Noguera, A., and Cambras, T. (2010). Light responses of the circadian system in leptin deficient mice. *Physiology and Behavior* 99: 487–494.
489. DeBruyne, J. P., Noton, E., Lambert, C. M., Maywood, E. S., Weaver, D. R., and Reppert, S. M. (2006). A clock shock: Mouse CLOCK is not required for circadian oscillator function. *Neuron* 50: 465–477.
490. Doi, M., Yujnovsky, I., Hirayama, J., Malerba, M., Tirotta, E., Sassone-Corsi, P., and Borrelli, E. (2006). Impaired light masking in dopamine D2 receptor-null mice. *Nature Neuroscience* 9: 732–734.
491. Doyle, S. E., Castrucci, A. M., McCall, M., Provencio, I., and Menaker, M. (2006). Nonvisual light responses in the *Rpe65* knockout mouse: Rod loss restores sensitivity to the melanopsin system. *Proceedings of the National Academy of Sciences United States of America* 103: 10432–10437.
492. Etchegaray, J. P., Machida, K. K., Noton, E., Constance, C. M., Dallmann, R., Di Napoli, M. N., DeBruyne, J. P. et al. (2009). Casein kinase 1 δ regulates the pace of the mammalian circadian clock. *Molecular and Cellular Biology* 29: 3853–3866.
493. Marchant, E. G. and Mistlberger, R. E. (1997). Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6J mice. *Brain Research* 765: 273–282.
494. Kuljis, D. A., Loh, D. H., Truong, D., Vosko, A. M., Ong, M. L., McClusky, R., Arnold, A. P., And Colwell, C. S. (2013). Gonadal- and sex-chromosome-dependent sex differences in the circadian system. *Endocrinology* 154: 1501–1512.
495. Wolff, G., Duncan, M. J., and Esser, K. A. (2013). Chronic phase advance alters circadian physiological rhythms and peripheral molecular clocks. *Journal of Applied Physiology* 115: 373–382.
496. Yang, Y., Duguay, D., Bédard, N., Rachalski, A., Baquiran, G., Na, C. H., Fahrenkrug, J. et al. (2012). Regulation of behavioral circadian rhythms and clock protein PER1 by the deubiquitinating enzyme USP2. *Biology Open* 1: 789–801.
497. Farjnia, S., Michel, S., Deboer, T., van der Leest, H. T., Houben, T., Rohling, J. H. T., Ramkisoensing, A., Yasenkov, R., and Meijer, J. H. (2012). Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. *Journal of Neuroscience* 32: 5891–5899.

498. Kas, M. J. H. and Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal–nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.
499. Mohawk, J. A. and Lee, T. M. (2005). Restraint stress delays reentrainment in male and female diurnal and nocturnal rodents. *Journal of Biological Rhythms* 20: 245–256.
500. Hummer, D. L., Jechura, T. J., Mahoney, M. M., and Lee, T. M. (2007). Gonadal hormone effects on entrained and free-running circadian activity rhythms in the developing diurnal rodent *Octodon degus*. *American Journal of Physiology* 292: R586–R597.
501. Erkert, H. G., Gburek, V., and Scheideler, A. (2006). Photic entrainment and masking of prosimian circadian rhythms (*Otolemur garnettii*, Primates). *Physiology and Behavior* 88: 39–46.
502. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences United States of America* 59: 414–421.
503. Lindberg, R. G. and Hayden, P. (1974). Thermoperiodic entrainment of arousal from torpor in the little pocket mouse, *Perognathus longimembris*. *Chronobiologia* 1: 356–361.
504. Weinert, D. and Schottner, K. (2007). An inbred lineage of Djungarian hamsters with a strongly attenuated ability to synchronize. *Chronobiology International* 24: 1065–1079.
505. Innicenti, A., Minutini, L., and Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
506. Hamasaka, Y., Watari, Y., Arai, T., Numata, H., and Shiga, S. (2001). Retinal and extraretinal pathways for entrainment of the circadian activity rhythm in the blow fly, *Protophormia terraenovae*. *Journal of Insect Physiology* 47: 867–875.
507. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S., and Takahashi, K. (1988). Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiology and Behavior* 42: 87–91.
508. Stokes, M. A., Kent, S., and Armstrong, S. M. (1999). The effect of multiple pulses of light on the circadian phase response of the rat. *Journal of Biological Rhythms* 14: 172–184.
509. Usui, S., Takahashi, Y., and Okazaki, T. (2000). Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. *American Journal of Physiology* 278: R1148–R1156.
510. Honma, K., von Goetz, C., and Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.
511. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M., and Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.
512. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M., and Daan, S. (1997). Stress-induced changes in circadian rhythms of body temperature and activity in rats are not caused by pacemaker changes. *Journal of Biological Rhythms* 12: 80–92.
513. Mendoza, J., Angeles-Castellanos, M., and Escobar, C. (2005). A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *European Journal of Neuroscience* 22: 2855–2862.
514. Numano, R., Yamazaki, S., Umeda, N., Samura, T., Sujino, M., Takahashi, R., Ueda, M. et al. (2006). Constitutive expression of the Period1 gene impairs behavioral and molecular circadian rhythms. *Proceedings of the National Academy of Sciences United States of America* 103: 3716–3721.
515. Jansen, H. T., Sergeeva, A., Stark, G., and Sorg, B. A. (2012). Circadian discrimination of reward: Evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiology International* 29: 454–468.
516. Begall, S., Daan, S., Burda, H., and Overkamp, G. J. F. (2002). Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83: 153–158.
517. Kramm, K. R. and Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.
518. Williams, C. T., Sheriff, M. J., Schmutz, J. A., Kohl, F., Øivind, T., Buck, C. L., and Barnes, B. M. (2011). Data logging of body temperatures provides precise information on phenology of reproductive events in a free-ling Arctic hibernator. *Journal of Comparative Physiology B* 181: 1101–1109.
519. Ware, J. V., Nelson, O. L., Robbins, C. T., and Jansen, H. T. (2012). Temporal organization of activity in the brown bear (*Ursus arctos*): Roles of circadian rhythms, light, and food entrainment. *American Journal of Physiology* 303: R890–R902.

This page intentionally left blank

7 Photic Environmental Mechanisms

7.1 NONPARAMETRIC THEORY OF ENTRAINMENT

In Chapters 5 and 6, it was repeatedly emphasized that, under constant environmental conditions, circadian rhythms freerun with periods slightly different from 24.0 hours. The fact that the periods are not exactly 24.0 hours was presented as a beneficial feature that allows us to be confident that the periodicity is truly endogenous and not merely induced by undetected environmental cycles. However, what is beneficial to the circadian physiologist is not necessarily beneficial to the organism. Indeed, the fact that the circadian period is not exactly 24.0 hours means that in the real world—where the Sun rises and sets every 24.0 hours—organisms can get into serious trouble. If a rat's internal clock tells it that it is midnight when it is actually midday, the rat may venture out of its burrow at the wrong time of day and end up being eaten by a hungry dog. Likewise, if your internal clock tells you that it is 8 a.m. when it is actually 6 p.m., your half-hour drive to work will take you only to a closed office. Evidently, the circadian pacemaker must be able to synchronize to the cycle of day and night if the organism is to thrive in the real world.

Fascinating as the endogenous nature of circadian rhythms may be, the truth is that most animals in natural environments are not free-running. The presence of daily cycles of light and darkness, ambient temperature, and food availability is the rule, not the exception. How does the circadian pacemaker adapt to these cycles? Before we attempt to answer this question, we need to agree on some terminology. Clearly, our interest is in understanding how the period of the circadian pacemaker (symbolized by the Greek lower case letter *tau*, τ) comes to match the period of an external clock (symbolized by the Roman upper case letter T). Because the external clock gives the time to the internal clock, it can be called a "time giver" (or—as originally named in German—a *Zeitgeber*). If the zeitgeber is effective, the internal clock will go along with it, as if it had boarded a train—thus, we will say that the pacemaker was *entrained* by the zeitgeber. And, in possession of this terminology, you can impress all your friends by casually stating that "the entrainment of the circadian pacemaker by a zeitgeber is attained by the modulation of τ toward T." In case you yourself need some time to make sense out of this sentence, we can reword it as "the biological clock synchronizes to the outside world by adjusting to a 24-hour day."

If the pacemaker is to be *entrained by* the zeitgeber (or—as it is sometimes said—if the pacemaker is to *entrain to* the zeitgeber), then its free-running period (τ) must be changed to match that of the zeitgeber (T). From extensive experimentation with man-made clocks, we know that there are two ways to change the period of a clock (Figure 7.1). We can actually change the speed of the clock, thus making it run faster or slower, or we can simulate a change in speed by resetting the clock each day. If you have one of those old winding watches (Figure 7.2), you must have employed the latter procedure recently. If the watch is slow (that is, if the small arm takes longer than 24 hours to go around the dial twice), you can fix it by advancing the arms a little bit each day. On the other hand, if the watch is fast, you can fix it by moving back (or *delaying*) the arms a little bit each day.

7.1.1 Phase Shifts

We saw in Chapter 6 that the intensity of constant light can change the period of circadian rhythms (Aschoff's rule), so we know that the speed of the circadian clock can be modified by environmental stimuli. What about the resetting mechanism of old winding watches? It can also be found in the circadian system. This was demonstrated by Pittendrigh in the early 1950s.[1]

Pittendrigh was studying the pattern of emergence (eclosion) of fruit flies from the pupal stage. He noticed that, if the flies were raised from eggs in constant darkness, no daily rhythmicity could be observed (Figure 7.3, Panel A). However, rhythmicity could be induced by exposing the pupae to a single, brief pulse of light (Panel B). Pittendrigh reasoned that this odd occurrence might be due to the fact that the light pulse synchronized the pupae, so that they started to eclode in daily bursts. He was unable to actually test this hypothesis because he was studying a populational rhythm (and, thus, could not identify processes occurring in individual pupae), but the idea was consistent with Bünning's work on the role of light in photoperiodism in the bean plant.[2] Of course, the concept that synchronization could be induced by light implied that light was capable of resetting the circadian clocks of the pupae. Pittendrigh himself and many other researchers later documented the resetting of the circadian clock of individual organisms by discrete light pulses in nonanimals and invertebrate animals,[1,3–9] reptiles and birds,[10–13] and many mammalian species, including golden hamsters,[14–27] domestic mice,[28–41] field mice,[42–47] laboratory rats,[48–52] humans,[53–74] and other mammals.[75–95] Comprehensive literature reviews of older studies were published in 1965[96] and 1999.[97] As an aside, I should mention that, at least in hamsters (and probably in other higher vertebrates as well), standard anesthetics block the resetting effect of light pulses.[98]

Two examples of resetting of circadian rhythms (*phase shifts*) are shown in Figure 7.4. Nile grass rats (*Arvicanthis niloticus*) were kept in constant darkness and received a single light pulse (1-hour duration) either 13 hours after the activity onset on the 16th day (Panel A) or 3 hours before the activity onset on the 17th day (Panel B). Notice that, in the top actogram, the activity onsets shifted 3 hours to the right (i.e., they

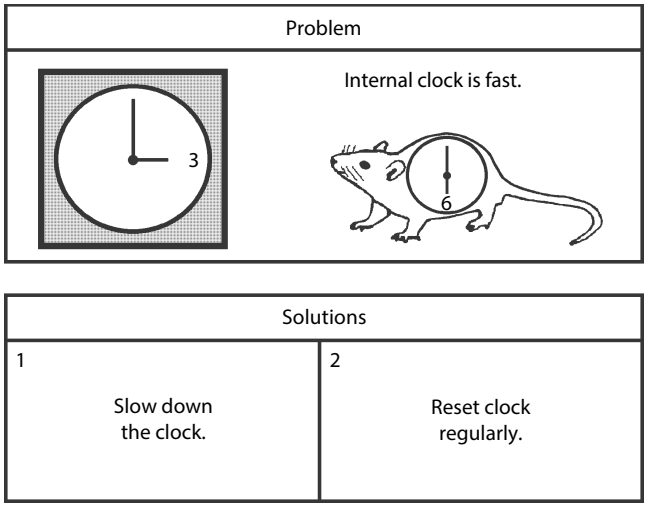


FIGURE 7.1 Two solutions for a problem. There are two possible solutions for the synchronization problem presented by the existence of endogenous periodicity with a period different from 24.0 hours: the speed of the internal clock may be corrected or the clock may be reset often.

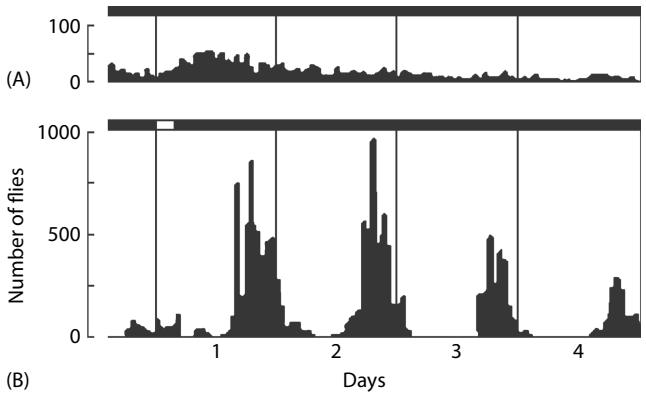


FIGURE 7.3 Flies are born. If eggs of the fruit fly (*Drosophila pseudoobscura*) are maintained in constant darkness, flies emerge from the pupal stage at random times of the day (Panel A). However, if the pupae are briefly exposed to light once, a circadian rhythm of eclosion ensues (Panel B). (Adapted from Pittendrigh, C.S., *Proc. Natl. Acad. Sci. USA*, 40, 1018, 1954.)



FIGURE 7.2 A pocket watch from the early 1900s. Old watches like this one needed to be reset almost daily to keep the correct time. (Photo by R. Refinetti.)

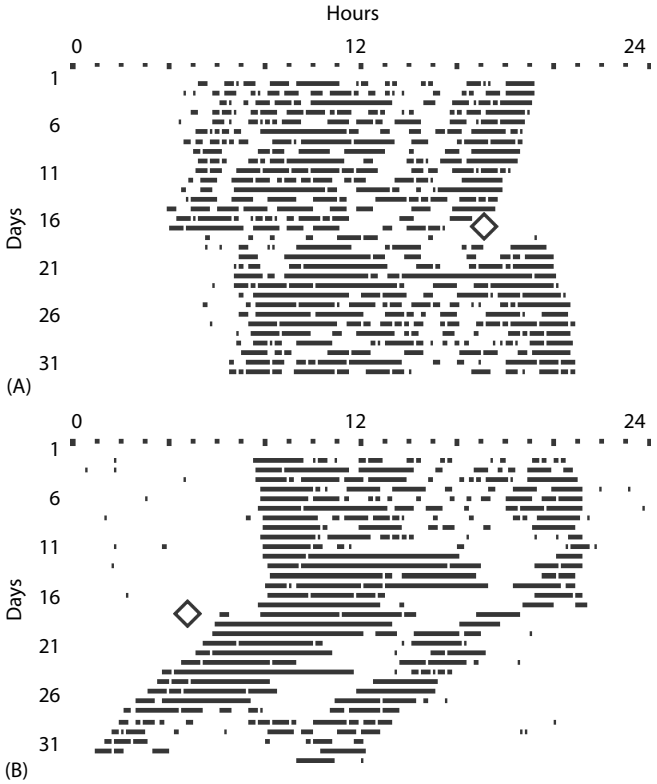


FIGURE 7.4 Shifted by light. The actograms show the running wheel activity rhythms of two Nile grass rats (*Arvicanthis niloticus*) maintained in constant darkness and presented with single brief (1 hour) pulses of light (400 lux). A light pulse presented 13 hours after the onset of activity (CT 13) evokes a phase delay of the activity rhythm (A). A light pulse presented 3 hours before the onset of activity (CT 21) evokes a phase advance of the activity rhythm (B). (Data from the archives of the Refinetti lab.)

were *delayed* by 3 hours) after the pulse. In Panel B, the onsets shifted about 2 hours to the left (i.e., they were *advanced* by 2 hours) after the pulse. Evidently, the direction of the shift (delay or advance) depends on the time when the light pulse is administered. You may have noticed that the light pulses also affected the periods of the rhythms, and we will get back to this issue momentarily. Prior to it, we should try to find

out how the direction of the phase shifts depends on the time when the stimulus is presented. Hopefully, you remember the concept of *circadian time* discussed in Chapter 5. If we define "circadian time zero" (CT 0) as the time at which the animal becomes active each day, then *subjective day* starts at CT 0, and *subjective night* starts at CT 12. To avoid an inconsistency in labeling, the onset of activity is defined as CT 12 in nocturnal animals, as they are active during subjective night.

Figure 7.5 shows some actograms depicting phase shifts (abbreviated as *delta phi*, $\Delta\phi$) of a fictitious animal evoked by light pulses presented at different circadian times. For instance, pulses presented at CT 2 or CT 8 cause no detectable shift ($\Delta\phi = 0$), a pulse presented at CT 14 causes a phase delay of about 2.5 hours, and a pulse presented at CT 20 causes a phase advance of 2 hours. Figure 7.5 also indicates how the phase shifts are plotted as a function of circadian time to generate a *phase–response curve* (PRC). Notice that, by convention, advances are plotted as positive shifts, whereas delays are plotted as negative shifts. This fictitious PRC is very similar to real mammalian PRCs in that there is a *dead zone* during most of subjective day (CT 0 to CT 10), there is a phase-delay region during early subjective night (CT 10 to CT 17), and there is a phase-advance region during late subjective night (CT 17 to CT 24). Exercise 7.1 allows you to practice the measurement of phase shifts, and Exercise 8.3 (in the next chapter) helps you go through the steps of building a PRC.

To prepare a detailed PRC, one needs to invest considerable time in obtaining several data points for each of the 24 circadian hours. Typically, the experimental subjects must spend at least 2 weeks under a light–dark cycle in order to attain stable entrainment before they are released into constant darkness. Then, another week is needed for determination of circadian phase before the light pulse, and a fourth week is needed for determination of circadian phase after the pulse (Figure 7.6A). Thus, each single data point for the PRC requires at least a full month of recording. If one needs as few as three data points for each of the 24 circadian hours, one must invest 72 months worth of recording (or have 72 subjects recorded simultaneously for a month). To reduce the duration of data collection, alternative protocols have been suggested. Aschoff listed six such protocols,[96] although only two have been regularly used by researchers. The *Type I* protocol is the one we have just discussed. The *Type II* protocol shortens the procedure by at least a week because it uses the circadian phase of the entrained state as the prepulse phase (Figure 7.6B). A potential problem of this protocol is—as suggested in the figure—that the phase of the entrained state may not reflect the phase of the circadian clock accurately. In some species (the laboratory mouse, for example), the activity onset on the first day in constant darkness is much earlier than on the days under the light–dark cycle. Thus, if we use the phase of the entrained state as our measure of prepulse onset time, we will have an incorrect estimate of circadian time. We could use the activity onset on the first day of constant darkness as our measure of prepulse onset time, but we would then be relying on a single day to estimate an essential parameter of the rhythm. Clearly, the Type II protocol should be used only under special circumstances, when the investigator can be confident that the circadian phase under the light–dark cycle would have been preserved in constant darkness if the light pulse had not been presented.

A PRC for the Nile grass rat is shown in Figure 7.7A. The animals were kept in constant darkness for 7 days before receiving 4-hour light pulses (360 lux) at different circadian times (Type I protocol). Notice the presence of a zone with rather small phase shifts (the dead zone, from CT 0 to CT 8), a phase-delay zone (from CT 8 to CT 18), and a phase-advance zone (from CT 18 to CT 24). Instead of plotting the phase shifts in the form of a PRC, we could plot them in the form of a phase–transition curve (PTC), as shown in Figure 7.7B. This type of curve shows the new phase (the phase after the

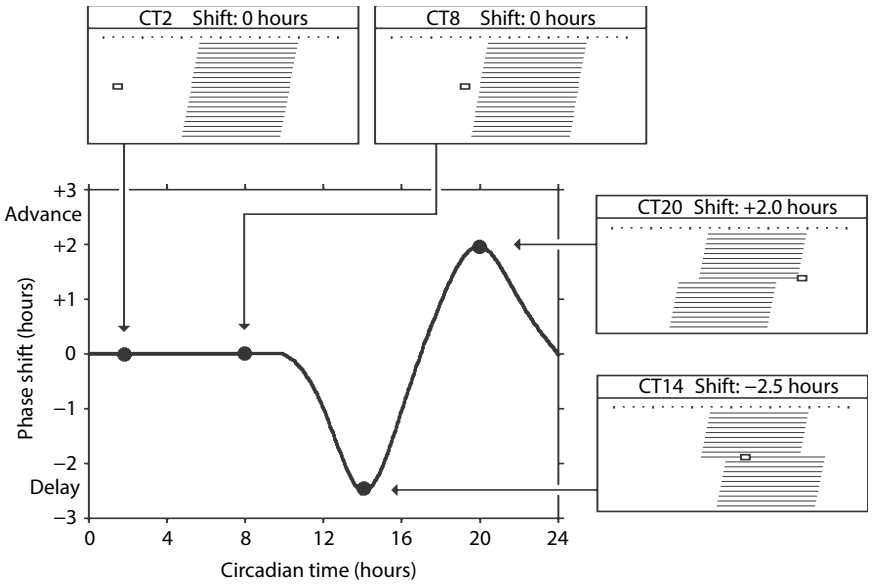


FIGURE 7.5 How to build a phase–response curve (PRC). By presenting light pulses at different circadian times and observing the evoked phase shifts, one can build a PRC. The small rectangles in the actograms indicate the times of the light pulses.

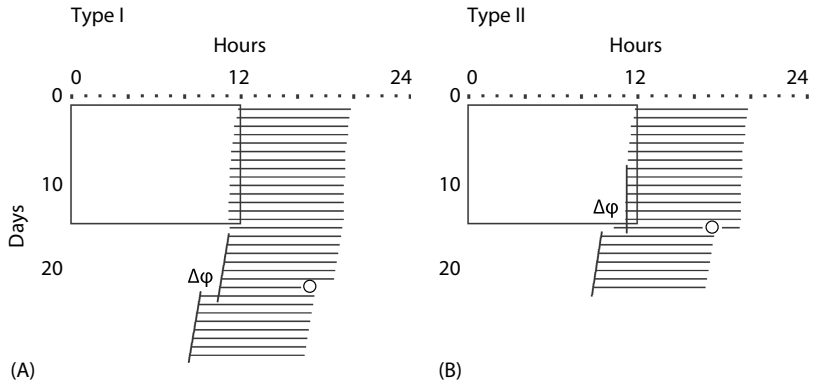


FIGURE 7.6 Protocols for phase shifting. Two protocols have been often used in the study of phase shifts evoked by single pulses. In the Type I protocol (A), the organism is allowed to freerun in constant darkness for several days before and after the pulse (which is indicated by the small open circle). In the Type II protocol (B), the pulse is presented on the first day after elimination of the light–dark cycle. In both diagrams, the large rectangles indicate the duration of the light phase of the light–dark cycle. The thin vertical lines across the activity onsets are drawn to facilitate the visual observation of the phase shifts (Δϕ).

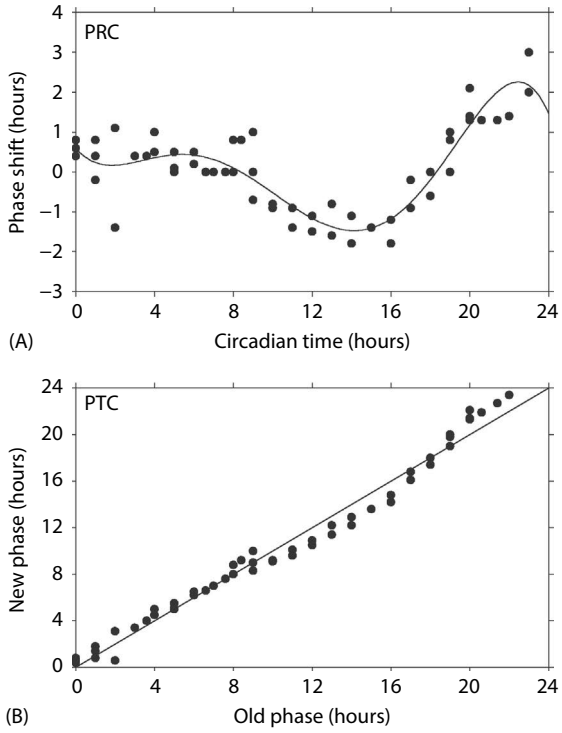


FIGURE 7.7 (A) Phase–response curve (PRC) and (B) phase–transition curve (PTC). The graphs show phase-shift data plotted as a PRC and as a PTC. The data points correspond to phase shifts of the running-wheel activity rhythms of Nile grass rats (*Arvicanthis niloticus*) evoked by 4-hour light pulses (360 lux) at different circadian times. In the PRC plot, phase advances are plotted as positive shifts and phase delays are plotted as negative shifts. (Data from Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004.)

light pulse) as a function of the old phase (the phase before the pulse) instead of showing phase shifts as a function of the old phase. In mammals, the PTC is usually very little informative, as the data points essentially lie on the line of equality between the new phase and the old phase (because the shifts are relatively small, often not exceeding 3 hours). However, as we will see later on, the PTC can be helpful in the analysis of phase shifts in organisms that exhibit large shifts.

To provide a sense of the differences and similarities in the PRCs of different species, five PRCs are shown in Figure 7.8. Notice that, although all PRCs have a phase-delay region during early subjective night, there are considerable interspecies differences in the shape of the curves. Notice also that the ordinates (Y-axes) of the graphs have been scaled to accommodate the various PRCs. Thus, for instance, the PRC of the domestic mouse has maximal phase advances slightly exceeding 1 hour and phase delays of less than 3 hours, whereas the PRC of the Asian chipmunk has maximal phase delays and advances exceeding 8 hours. PRCs for many other species can be viewed in the program **PRC**, which is part of the software package that accompanies this book (see Exercise 7.2).

In addition to interspecies differences in the PRC, it is instructive to examine differences in PRCs determined for a given species in different laboratories. Figure 7.9 shows the PRC for the golden hamster (*Mesocricetus auratus*) as determined in five different studies. There is great consistency in the delimitation of the dead zone and the phase-delay and phase-advance regions. The magnitude of the shifts varies perhaps more than one would expect from study to study, and this variance cannot be accounted for by differences in the duration or intensity of the light pulses used in the different studies. The duration and intensity of the light pulses were relatively similar in the five studies. As a matter of fact, most determinations of PRCs in rodents involve light pulses of 15–60 minutes at intensities (illuminance) of 150–400 lux.

Few investigators have examined the effects of large variations in stimulus duration and intensity on the magnitude of the evoked phase shifts. The results of one very good study conducted on golden hamsters are shown in Figure 7.10. Pulses of green light ($\lambda = 503$ nm) of various intensities and durations were presented at CT 19, a circadian time that corresponds to the peak of the phase-advance region in the hamster PRC. Notice that, regardless of stimulus duration, no phase shifts are evoked at intensities below 0.1 $mW \cdot m^{-2}$ (approximately

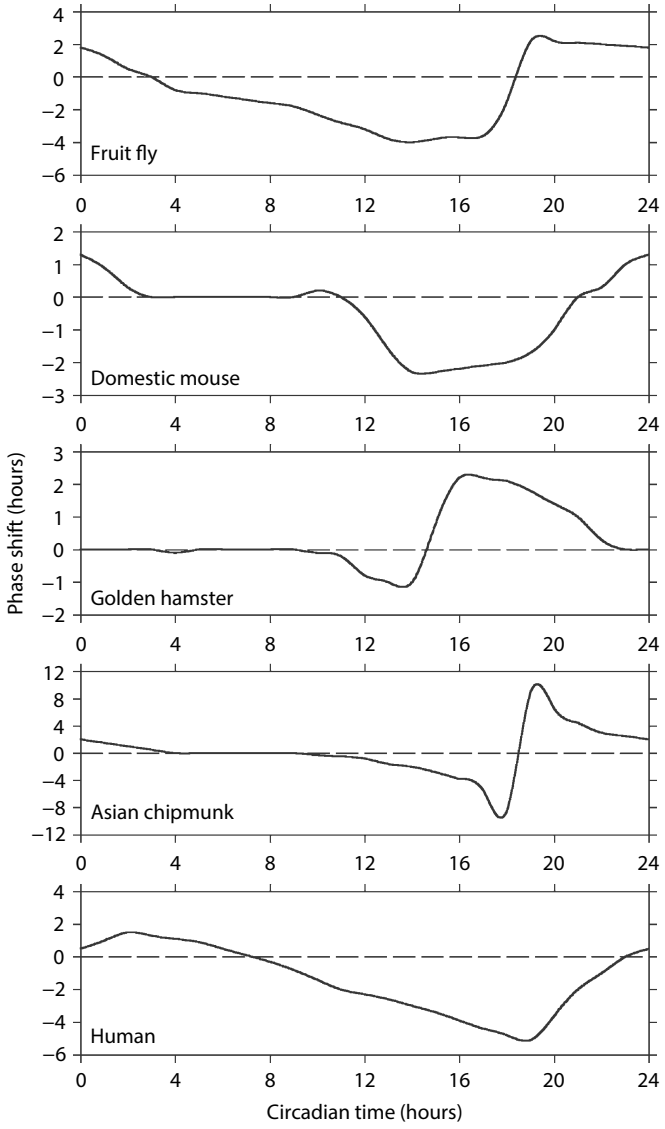


FIGURE 7.8 Interspecies differences in the phase–response curve (PRC). The graphs show the PRCs describing the phase-shifting effects of light pulses presented at different circadian times for five animal species. By convention, phase advances are plotted as positive shifts, whereas phase delays are plotted as negative shifts. (Data from Rothenfluh, A. et al., *Genetics*, 156, 665, 2000; Benloucif, S. and Dubocovich, M.L., *J. Biol. Rhythms*, 11, 113, 1996; Takahashi, J.S. et al., *Nature*, 308, 186, 1984; Honma, S. and Honma, K., *Am. J. Physiol.*, 276, R1390, 1999; Jewett, M.E. et al., *J. Biol. Rhythms*, 9, 295, 1994.)

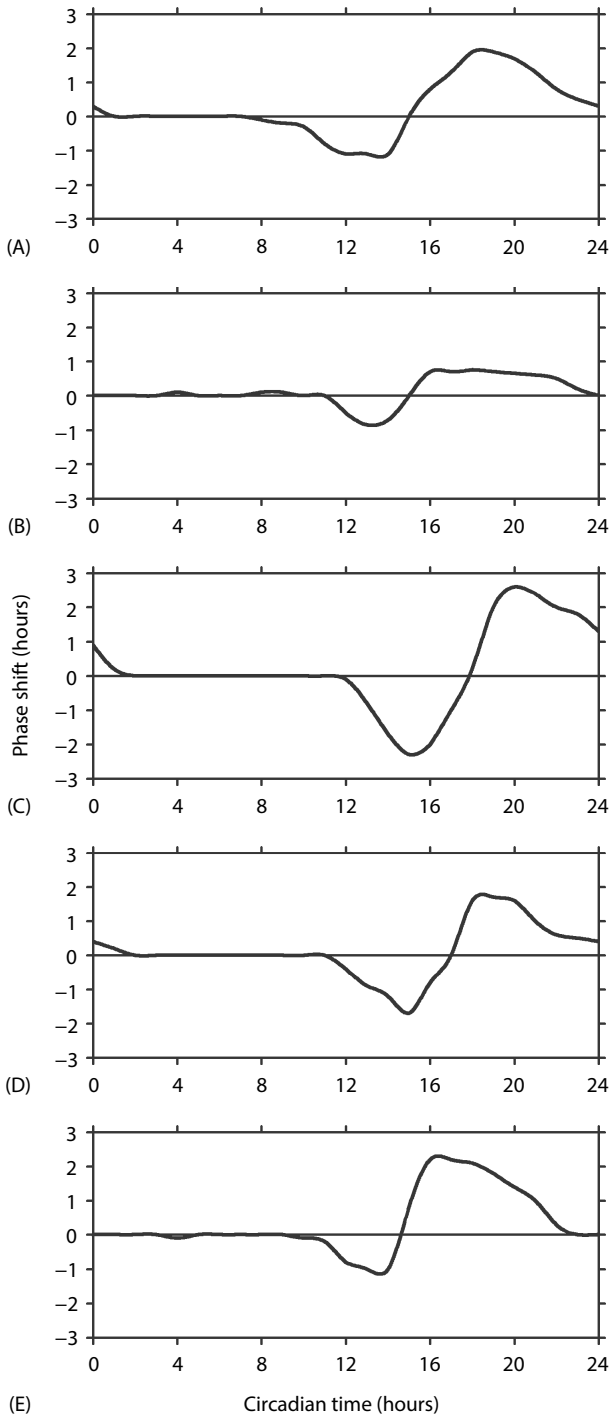


FIGURE 7.9 Intraspecies differences in the phase–response curve (PRC). The graphs show the PRCs describing the phase-shifting effects of light pulses presented at different circadian times to golden hamsters (*Mesocricetus auratus*) in five different laboratories (Panels A through E). Across the five studies, pulse duration varied from 15 to 60 minutes and pulse intensity varied from 150 to 400 lux. By convention, phase advances are plotted as positive shifts, whereas phase delays are plotted as negative shifts. (Data from Daan, S. and Pittendrigh, C.S., *J. Comp. Physiol.*, 106, 253, 1976; Grosse, J. et al., *Neuroscience*, 65, 587, 1995; Meijer, J.H. and De Vries, M.J., *J. Biol. Rhythms*, 10, 4, 1995; Rosenberg, R.S. et al., *Am. J. Physiol.*, 261, R491, 1991; Takahashi, J.S. et al., *Nature*, 308, 186, 1984.)

0.2 lux). Thus, 0.1 mW·m^{-2} (or 0.2 lux) is the threshold intensity for the elicitation of phase shifts.[17] Similar threshold values (0.1–0.3 lux) were found in other studies using pulses of green or white light in hamsters,[99–101] rats,[49] and mice.[28,36,45] A very low threshold (high sensitivity) was found in a bat (10^{-5} lux)[102] and a relatively high threshold (low sensitivity) in a nocturnal prosimian primate (10 lux).[103] Studies on humans must rely on less-than-ideal methods because subjects refuse to stay in constant darkness for several weeks,[62,66,104,105] but the results suggest that the threshold is at least two orders of magnitude higher (20–100 lux).[62,66] Of course, a higher threshold means

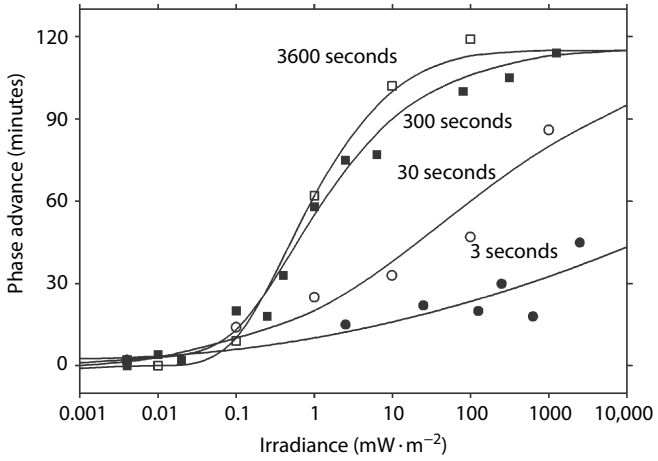


FIGURE 7.10 Temporal summation in the circadian system. In the circadian system of the golden hamster (*Mesocricetus auratus*), intensity and duration of the photic stimulus can be traded off to evoke the same phase shift. For instance, a 60-minute phase advance can be evoked either by a 1-hour pulse at 1 $mW \cdot m^{-2}$ or by a 30-second pulse at 100 $mW \cdot m^{-2}$. (Adapted from Nelson, D.E. and Takahashi, J.S., *J. Physiol.*, 439, 115, 1991.)

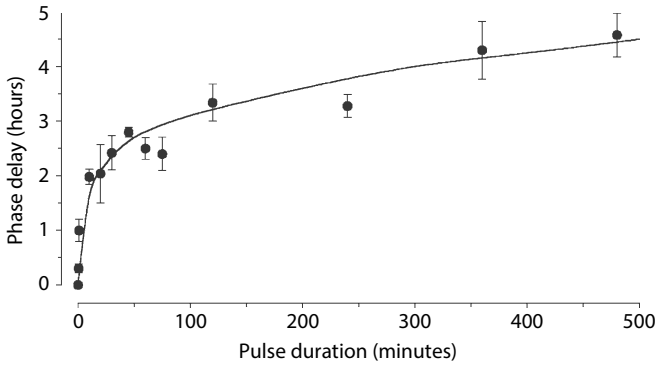


FIGURE 7.11 Endurance of temporal summation. In the circadian system of the domestic mouse (*Mus musculus*), the increase in the magnitude of phase delays resulting from the increase in the duration of photic stimulation levels off at durations of about 1 hour but persists through durations of at least 8 hours. In this graph, each point corresponds to the mean (±SE) of 5 mice. Light pulses of 360 lux were presented starting 4 hours after the onset of activity (CT 16). (Data from the archives of the Refinetti lab.)

lower sensitivity, and it is not surprising that diurnal humans should be less sensitive to light than nocturnal rodents.

Notice also in Figure 7.10 that the circadian system of the hamster exhibits the phenomenon of *temporal summation*, which is well known in the visual system.[106,107] *Temporal summation* means literally temporal summation—that is, the animal can sum stimuli over time, so that there is a trade-off between intensity and duration. For example, a 60-minute phase advance can be obtained either by a short bright pulse (30 seconds at 100 $mW \cdot m^{-2}$) or by a long weak pulse (3600 seconds at 1 $mW \cdot m^{-2}$). In the visual system, summation is restricted to durations of less than a second, but in the circadian system it seems to extend to over an hour or more. Because of this wide region of summation, it is difficult to determine the maximally effective (*saturating*) intensity. For a stimulus duration of 1 hour (3600 seconds), Figure 7.10 indicates a saturation level of about 100 $mW \cdot m^{-2}$ (30 lux). Similar saturation values (30–150 lux) were found in other studies using pulses of green or white light in hamsters,[99,101] rats,[49] and mice.[28,45] In humans, the saturation level seems to be much higher (1000 lux or more).[62,66]

One more feature of Figure 7.10 to be noticed is the distance between the curves for the different stimulus durations. The curve for 30 seconds rises much higher than that for 3 seconds, and the curve for 300 seconds rises higher than that for 30 seconds; however, the curve for 3600 seconds is very close to that for 300 seconds. This means that the initial seconds of stimulation are more crucial than the later seconds for the elicitation of phase shifts. Figure 7.11 shows it more clearly. Mice maintained in constant darkness were stimulated with light pulses of constant intensity (360 lux) but varying duration at CT 16, a circadian time that corresponds to the extreme of the phase-delay region in the mouse PRC. Notice that, although the curve is still not truly asymptotic by a pulse duration of 480 minutes (8 hours), half of the maximal phase shift is attained by a pulse of less than 30 minutes. The effects of very long stimuli cannot be easily studied because it is impossible to restrict the presentation of a multihour stimulus to any chosen circadian hour (that is, the experimental design necessarily confounds circadian time and stimulus duration). Nonetheless, it is clear that most of the phase shift caused by a light pulse is due to the first hour of stimulation.[17,23,72,99,108] Figure 7.12 shows a similar duration–response curve for the golden hamster, based on two independent studies. Here, the half-maximal response seems to be attained at a stimulus duration of 5 minutes, and response saturation seems to occur at a stimulus duration of about 40 minutes. Quite interestingly, other studies suggest that the duration of stimulation is more important than the stimulation itself. In mice, it has been shown that one-tenth of a second of

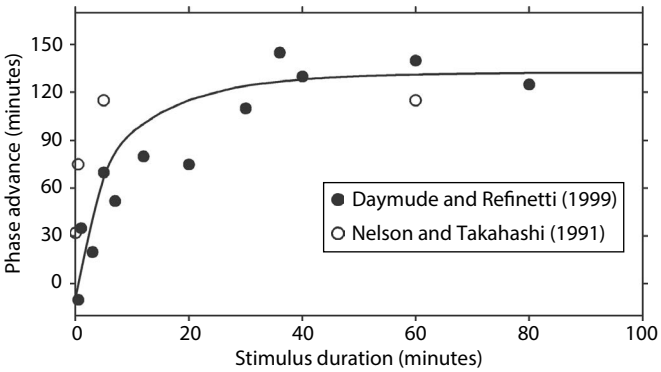


FIGURE 7.12 Ceiling of temporal summation. In the circadian system of the golden hamster (*Mesocricetus auratus*), the increase in the magnitude of phase advances resulting from the increase in the duration of photic stimulation levels off at durations of about half an hour. Data from two laboratories, using slightly different photic stimuli and times of stimulation, can be approximately described by the same function. (Data from Daymude, J.A. and Refinetti, R., *Biol. Rhythm Res.*, 30, 202, 1999; Nelson, D.E. and Takahashi, J.S., *J. Physiol.*, 439, 115, 1991.)

light is sufficient to evoke a full phase shift if this tiny bit of light is spread over a whole hour (that is, 60 pulses of 20 milliseconds presented at 1-minute intervals).[109] Although not as impressive, it has also been shown in hamsters that comparable phase shifts can be attained by a single 600-second pulse, ten 30-second pulses alternated with 30 seconds of darkness, one hundred 3-second pulses alternated with 3 seconds of darkness, or one thousand 0.3-second pulses alternated with 0.3 seconds of darkness.[110] In humans, it has been shown that five 15-minute pulses separated by 1 hour of darkness are just as effective as a single 6.5-hour pulse.[111] More research on this exciting topic is warranted.

Because it is impossible to restrict the presentation of a multihour stimulus to any chosen circadian hour, long light pulses stimulate the organism at more than one circadian time. We can predict the effects of long pulses, however, by referencing the PRC for brief pulses. We do not know what the smallest functional unit of time is, but let us assume that it is 30 minutes. Figure 7.13 shows a segment of the mouse PRC. According to this PRC, a single pulse presented at CT 16 causes a 2-hour phase delay. What magnitude of phase delay would be produced by a 3-hour pulse? A 3-hour pulse is 6 times as long as a 30-minute pulse. Will it evoke a phase shift of 12 hours (6 × 2 hours = 12 hours)? No, it will not. The computations are shown below the PRC in Figure 7.13. We start the pulse at CT 16, which may happen to be at 6:00 a.m. for us. The pulse will cause a 2-hour delay, thus moving the mouse's clock to CT 14. However, the mouse's clock is still ticking, so that at 6:30 it will be at CT 14.5 (a progression of 30 minutes in 30 minutes, assuming that $\tau = 24.0$ hours). At this time, the continuing light pulse will cause a 1.5-hour delay (because, as indicated by the PRC, a brief pulse at CT 14.5 evokes a 1.5-hour phase delay), which will move the mouse's clock to CT 13. The normal progression of the mouse's clock will take it to CT 13.5 at 7:00 a.m. The continuing light pulse will now evoke a 1-hour delay, thus moving the mouse's clock to CT 12.5. Notice that, from now on, the mouse's clock will lock onto a half-hour cycle of advances and delays, so that there will be no further progression of shifts. When the long light pulse finally ends at 9:00 a.m., the mouse's clock will be at CT 13. Because we started at CT 16, the total light-induced shift will be 3 circadian hours. Therefore, although a 3-hour pulse is 6 times as long as a 30-minute pulse, it evokes a phase shift that is only 1.5 times as large. As a matter of fact, for the PRC that we used, the maximal phase shift at CT 16 is attained by a 1.5-hour pulse. Any additional light has no phase-shifting effect.

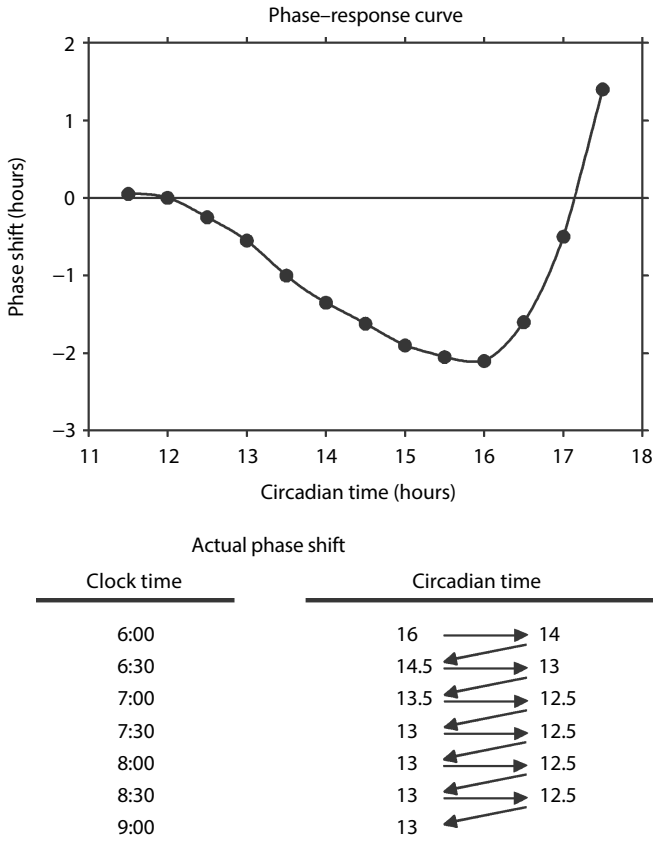


FIGURE 7.13 Temporal integration of the phase–response curve (PRC). Using the PRC for brief discrete light pulses, one can estimate the phase-shifting effect of longer light pulses. See text for details.

The preceding reasoning may partially explain why even very long light pulses do not evoke large phase shifts in most mammals. I must point out, however, that some organisms do exhibit large phase shifts when stimulated by long pulses. Consider Figure 7.14. It shows PRCs for the pupal eclosion rhythm of flesh flies (*Sarcophaga argyrostoma*). Notice that, as the pulse duration increases from 1 hour to 8 hours, the dead zone of the PRC is replaced by a wide phase-delay region and, more importantly, that the magnitudes of the maximal phase shifts increase. For 8-hour pulses, the PRC reaches the maximal possible amplitude (24 circadian hours, from –12 to +12 hours). This full resetting of the clock is often called a *Type 0* resetting and contrasts with the limited (*Type 1*) resetting observed in mammals.

The distinction between Type 0 and Type 1 resetting is easier to understand through an analogy with stopwatches and regular wristwatches. In a stopwatch, the hand of the clock can be fully and instantly reset to the starting position by activation of the reset button. Regardless of where the arm is on the dial, it always goes back to the point of origin. Thus, the "new phase" is always the same regardless of the "old phase," as illustrated in Panel A of Figure 7.15. If we convert this PTC into a PRC, we obtain the graph in Panel C of Figure 7.15. In contrast, resetting of a regular wristwatch requires the gradual adjustment of the clock's arms. If we move the hour arm by, say, a maximum of 2 hours in the direction of midnight, the relationship between "new phase" and "old phase" will be like the one described on Panel B, and the PRC will be like the one in Panel D. Because the stopwatch PTC has a slope of 0, its resetting mode is called Type 0. Similarly, because the wristwatch PTC has a slope of 1, its resetting mode is called Type 1.[112] I do realize that these concepts may be rather confusing, so I prepared a short computer tutorial that you can find in the program **Entrain** (see Exercise 7.3).

Whereas Type 0 PRCs have been described for invertebrates and simpler organisms,[97,113] only Type 1 PRCs have

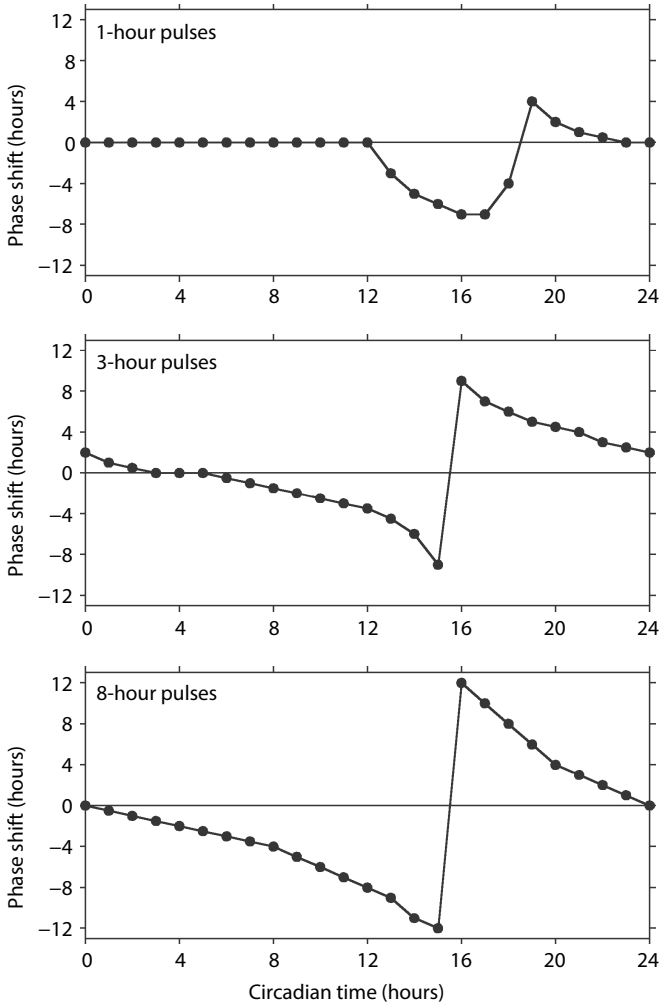


FIGURE 7.14 An awesome shift. In the flesh fly (*Sarcophaga argyrostoma*), increases in the duration of light pulses can lead to maximal phase shifts (full 12 hours), as shown in these three phase–response curves. The pulses consisted of white light at approximately 1000 lux. The variable studied was pupal eclosion. (Data from Saunders, D.S., *J. Comp. Physiol.*, 124, 75, 1978.)

been described in birds and mammals. Large phase shifts with a sharp breakpoint between the phase-delay region and the phase-advance region were documented in both birds and mammals,[11,80] but the shifts did not extend to full 12 circadian hours in both directions. One investigator reported full Type 0 PRCs in Turkish and Syrian (golden) hamsters,[83] but these results are inconsistent with the PRCs obtained in numerous other laboratories, as previously seen in Figure 7.9. Conceivably, large phase shifts could have been evoked by very strong stimuli, but the stimulus used in this study (1 hour, 150 lux) was not stronger than those used in the other studies. The unusually large phase shifts were probably caused by odd, unreplicable conditions in this particular laboratory. I myself observed once a 12-hour phase shift in a golden hamster but was never able to replicate the conditions.

Particularly controversial was the claim that Type 0 resetting could be achieved in humans by the use of a multipulse paradigm.[54,65] This unorthodox paradigm involved the presentation of three 5-hour pulses on three consecutive days, which led to large phase shifts resembling a Type 0 PRC. Similar paradigms did not evoke Type 0 resetting in hamsters or rats,[23,50] which suggested that the human circadian system may be peculiar.[114] However, it was cogently pointed out that the large shifts evoked by the 3-pulse paradigm can be explained by the summed effects of the 3 pulses in accordance with a Type 1 PRC.[115] Because of the current uncertainty about the exact shape of the human PRC for single pulses, it can be equally argued that the 3-pulse paradigm is *not* equivalent to the sum of 3 single pulses.[116] Resolution of this controversy must await further experimental results.

7.1.2 Entrainment

By now, you must have realized that I would not be talking so much about phase shifts if entrainment were due to the slowing down or speeding up of the circadian pacemaker (as mentioned in the beginning of the chapter). Indeed, according to Pittendrigh's so-called nonparametric theory of entrainment, entrainment is due to the daily resetting of the pacemaker by the zeitgeber.[117] In contrast, *parametric* theories of entrainment are based on the assumption that the magnitude of the zeitgeber has a proportional (parametric) effect on the speed of the clock.

Serge Daan (Figure 7.16), a leading Dutch researcher who worked closely with Pittendrigh in the development of the nonparametric theory of entrainment and who had frequent collaborations with Aschoff, says that Aschoff was a champion of parametric entrainment.[118] Daan knew Aschoff much better than I did, and I am sure that he described Aschoff's early views correctly. However, I must quote Aschoff in his last published article: "It seems likely that non-parametric entrainment plays a predominant role, [even though] contributions of parametric effects are probably underrated."[119] In other words, entrainment may be *modulated* by various photic parameters (see Section 7.2), but it is *established* by a nonparametric mechanism. *Aschoff's rule*, which was discussed in Chapter 6 and which states that the free-running period depends on the intensity of illumination,[120] might be seen as a contradiction to the nonparametric theory of entrainment. However, as Pittendrigh pointed out early on,[121] and Aschoff himself recognized,[122] the apparent parametric effect embodied in Aschoff's rule can be simply explained by nonparametric mechanisms. Indeed, continuous illumination will stimulate all points of the PRC and, unless the PRC is perfectly symmetrical, will result in a net daily phase shift leading to either a shortening of the circadian period (if the net shift is an advance) or a lengthening of the period (if the net shift is a delay). We will return to this issue in Section 7.3.

Once you understand that light pulses can cause phase shifts, it is very easy to understand nonparametric entrainment. Because most of the fundamental research on entrainment has been conducted using photic stimuli (light) as the zeitgeber, I will limit all discussions in this chapter to this class of stimuli. Nonphotic zeitgebers will be discussed in Chapter 8.

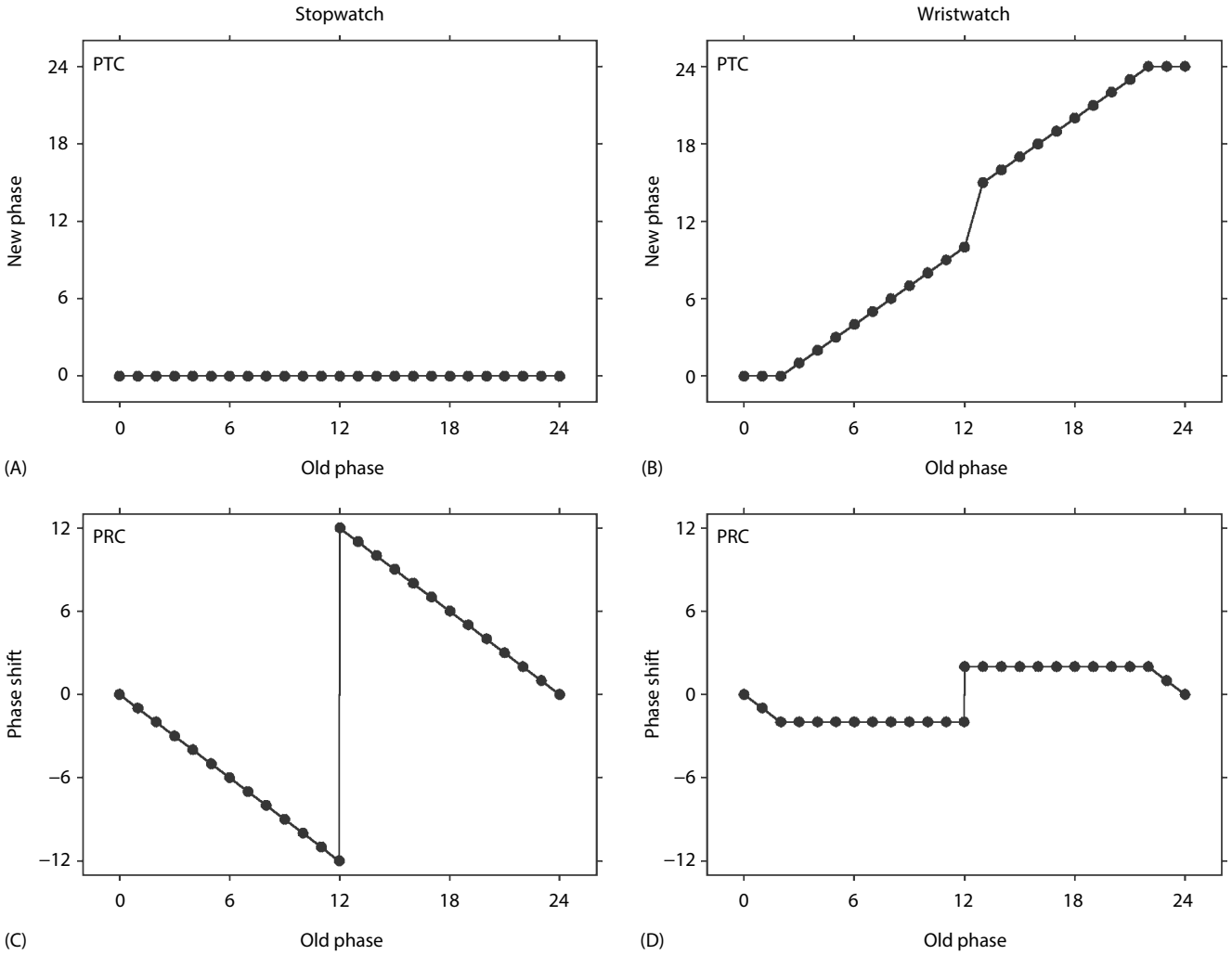


FIGURE 7.15 Type 0 and Type 1 resetting. The graphs show typical phase–transition curves and phase–response curves for resetting of a stopwatch (Type 0 resetting) and of a regular wristwatch (Type 1 resetting). See text for details.

Remember that, in order for entrainment to occur, the free-running period of the pacemaker (τ) must be changed to match that of the zeitgeber (T). The amount of time that needs to be adjusted each day (that is, the *shift* in the clock mechanism, $\Delta\phi$) is given by a simple equation: $\tau - T = \Delta\phi$ ("*tao* minus *tee* equals *delta fee*," Figure 7.17). Thus, if the period of the clock is 23.8 hours (a fast clock), you need to delay it by 12 minutes ($\Delta\phi = -0.2$ hour) each day; if, on the other hand, the period is 24.1 hours (a slow clock), you need to advance the clock by 6 minutes ($\Delta\phi = +0.1$ hour) each day. Let us say that an animal with a free-running period of 23.4 hours must entrain to a 24-hour cycle. If $\tau - T = \Delta\phi$, then the animal must delay 36 minutes each day (23.4 – 24.0 = –0.6 hour = –36 minutes). Using the PRC in Figure 7.13, we can see that a 36-minute (0.6-hour) delay is caused by a light pulse at CT 13 (CT 12 being the onset of activity in a nocturnal animal). Thus, if we present a light pulse at CT 13 each day, we will cause a 0.6-hour delay each day, and the observed period of the activity rhythm will be 24 hours (Figure 7.18).

I should point out that, continuing with our example, we do not even need to know when CT 13 is. If we present pulses every 24 hours, the animal's rhythm will be initially advanced or delayed (or not affected) haphazardly as light "hits" the pacemaker at different circadian times each cycle, until the point is reached at which the daily light pulse causes the exact 0.6-hour delay necessary for entrainment—at this point, the animal will be "locked" into the entrained position. Of course, if you are a detailed observer, you noticed that a 36-minute delay can be evoked not only at CT 13 but also at CT 17 (see Figure 7.13). However, the close proximity between CT 17 and the beginning of the advance zone of the PRC makes entrainment at this position rather unstable, so that CT 13 is favored for long-term entrainment. Regardless of this detail, notice that the nonparametric theory of entrainment provides a simple explanation for the latency of entrainment. If a light–dark cycle is introduced at the *wrong* time (for instance, at CT 22 in our example), it should take—as it does in real cases—many days until the circadian clock reaches the appropriate circadian phase to evoke the 0.6-hour delay necessary for entrainment. On the other hand, if we are lucky to introduce the light–dark cycle at the *right* time (CT 13), entrainment will occur right away.



FIGURE 7.16 Serge Daan. This circadian physiologist from the University of Groningen (in the Netherlands) worked closely with Pittendrigh in the development of the nonparametric theory of entrainment. (Photo courtesy of Serge Daan.)

Before we move on to look at specific experimental results that corroborate the nonparametric theory of entrainment, it will be instructive to briefly examine a potential generalization about the shape of the PRC. A simple way to compare the PRCs of different species is to compute the ratio of the largest phase delay and largest phase advance in the PRC of each species. This *D/A ratio* serves as an index of a species' potential for entrainment. For instance, it is reasonable to expect that organisms with relatively short endogenous periods should have large D/A ratios, because they require large delays in order to attain entrainment under a 24-hour zeitgeber. Conversely, organisms with relatively long endogenous periods should have small D/A ratios, because they require large advances in order to attain entrainment. In actuality, no such relationship is found across animal species (Figure 7.19). Evidently, this potential physiological advantage was not favored during evolution. As long as the PRC of a short-period organism has a sufficient phase-delay region, and the PRC of a long-period organism has a sufficient phase-advance region, the exact D/A ratio seems to be of little importance. That is, a short-period organism needs an ample phase-delay region, but it may or may not have a modest phase-advance region. Likewise, a long-period organism needs an ample phase-advance region, but it may or may not have a modest phase-delay region.

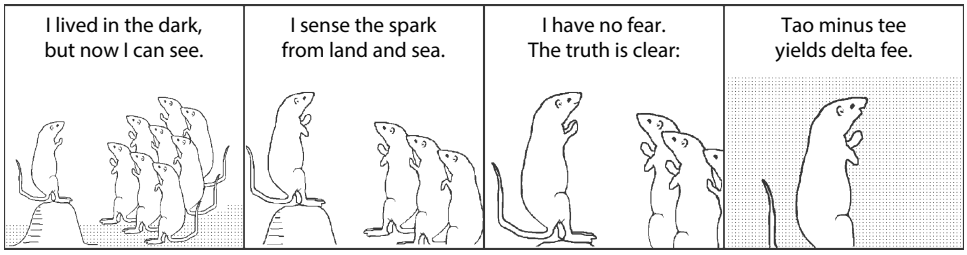


FIGURE 7.17 Entrainment rule: $\tau - T = \Delta\phi$. The wise rat intones rhymes about the basic rule of entrainment.

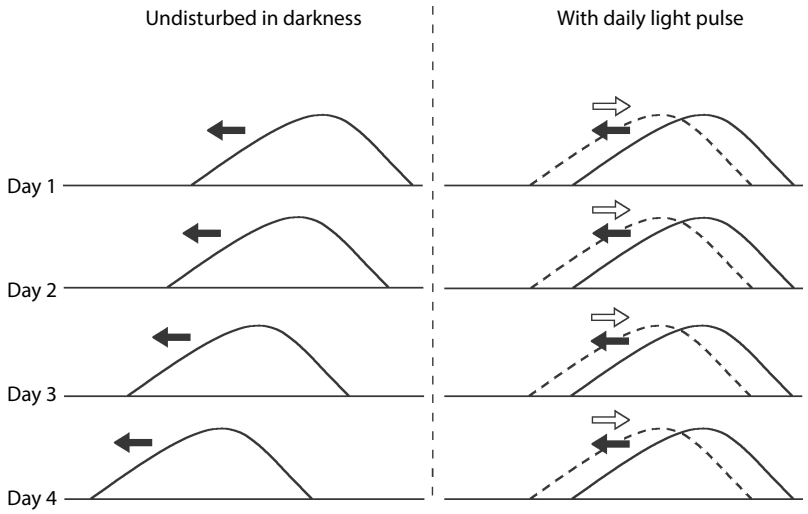


FIGURE 7.18 Entrainment rule: diagram. The free-running rhythm of an organism left undisturbed in darkness drifts slowly from day to day. A light pulse appropriately timed each day can cause a phase shift that offsets the natural drift of the rhythm, so that the expressed rhythm has a period of exactly 24 hours.

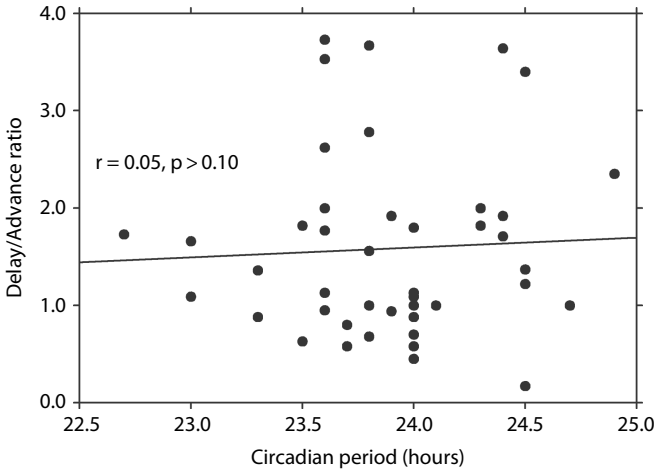


FIGURE 7.19 Relationship between free-running period and phase–response curve (PRC). This graph shows the relationship between free-running period (τ) and the ratio of maximal delay and maximal advance (D/A ratio) of the photic PRC, as determined by various investigators in a variety of vertebrate and invertebrate animal species. There is no statistically significant relationship between τ and D/A ratio. (Data from References 4, 7, 8, 10–16, 18, 20, 21, 25, 31, 33, 34, 43, 47–50, 56, 61, 67, 76–78, 80, 81, 84–90, 109, 116, and 309.)

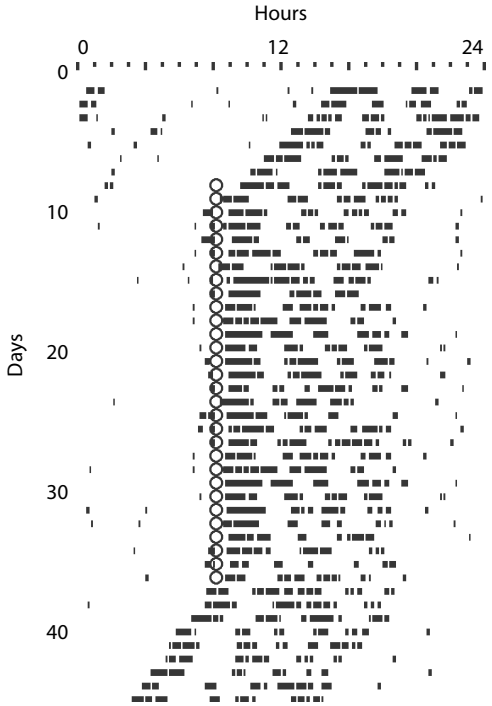


FIGURE 7.20 A pulse a day keeps the freerun away. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained in constant darkness except for a 15-minute light pulse (360 lux) each day, as indicated by the small circles. After a few days of freerun ($\tau < 24$ hours), the mouse reached a point where the phase-delaying effect of the light pulse exactly offsets the natural advance of the circadian clock—that is, entrainment was established ($\tau = 24.0$ hours). The freerun resumed when the daily pulses were discontinued. (Data from the archives of the Refinetti lab.)

So, the nonparametric theory of entrainment says that entrainment is attained by discrete daily phase shifts that amount to the difference between the endogenous period and the period of the zeitgeber ($\Delta\phi = \tau - T$, where T = 24 hours in most cases). We saw that light pulses can cause phase shifts and that phase shifts are theoretically sufficient to account for entrainment. But has this actually been tested? Yes it has. Laboratory studies using daily light pulses as entraining agents have been conducted and have confirmed that stable entrainment can be attained with the presentation of brief daily light pulses.[9,40,53,75,87,90,91,93,123–133] An example is shown in Figure 7.20. A domestic mouse (*Mus musculus*) was initially kept in constant darkness, and its running-wheel activity was continuously recorded. Starting on the 8th day, a single 15-minute light pulse was presented each day at 08:00 hours (indicated by the small open circles). The two hallmarks of entrainment are as follows: (1) the period and phase of the activity rhythm were consistently altered by the zeitgeber and (2) the rhythm freeran from the phase of entrainment after the zeitgeber was removed. These two points should be emphasized. If the period of the rhythm had not changed from $\tau < 24$ hours to $\tau = 24$ hours, we would not have been able to say that entrainment took place. However, if the animal had exhibited $\tau = 24$ hours from the start, we would not have been able to use this criterion. The fact that the rhythm assumed a particular *phase angle of entrainment* (ψ)—and maintained it for as long as the zeitgeber was present—is an important characteristic of the entrained state. Furthermore, the daily light pulses could conceivably have inhibited locomotor activity for several hours before their delivery, thus "masking" an actual freerun. The fact that, after the zeitgeber was discontinued, the rhythm freeran from the phase of entrainment (rather than from the point expected had the rhythm been free-running all along) confirms that the rhythm had actually been entrained. This will become more evident when we discuss *masking* in detail in Section 7.2.

If the nonparametric theory of entrainment is correct, we should be able to establish entrainment as long as we provide the necessary phase shift—even if we do not present light pulses every day. Indeed, as shown in Figure 7.21, a pulse every second day (but not every third day in this case) is sufficient to entrain the circadian system of the mouse. Inspection of the figure indicates clear entrainment during the once-daily-pulse segment. During the every-second-day segment, entrainment is still clear, although earlier onsets are "unmasked" on the days without the light pulse. During the every-third-day segment, entrainment was perhaps maintained (but with much earlier onsets) for a week or so, but was clearly lost afterward. For entrainment to be maintained by a single light pulse every 3 days, a phase delay of 1.2 hours is needed (because, with an endogenous period of 23.6 hours, the rhythm advances 3×0.4 hours every 3 days). This is still within the limits of the mouse PRC, but the phase-delay region is relatively narrow and may be missed by the single pulse. Whatever the true explanation is for the loss of entrainment in the every-third-day segment, the important aspect is that entrainment was maintained in the every-second-day segment.

Naturally, light–dark cycles in the real world are not limited to a single short pulse of light every day or every second day.

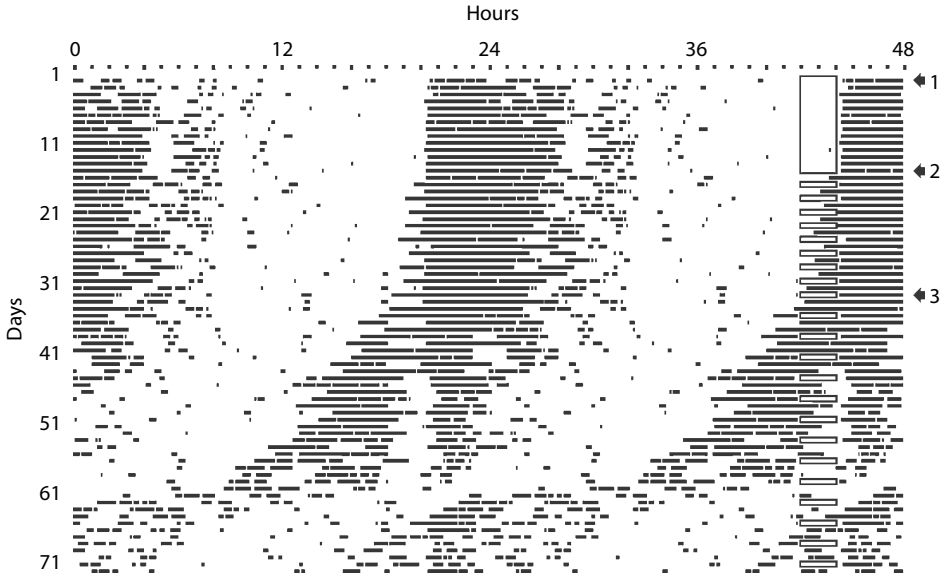


FIGURE 7.21 What if you skip a day or two? This double-plotted actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained in constant darkness and stimulated with a 2-hour light pulse (300 lux) under three different schedules: (1) every day, (2) every second day, and (3) every third day. The time of the pulses is single plotted as open rectangles. Pulses presented every third day were not capable of maintaining entrainment, as discussed in the text. (Data from the archives of the Refinetti lab.)

Light is present for many hours a day, or at least for brief periods at dawn and dusk. Light–dark cycles with only brief intervals of light at dawn and dusk are called "skeleton photoperiods." Many studies have shown that skeleton and complete photoperiods can entrain circadian rhythms,[33,79,81,87,90,126,134–145] even in species whose *visual* system is insensitive to light.[130,146–148] An example for a sighted animal (the golden hamster) is shown in Figure 7.22. The hamster was maintained initially under an LD 14:10 photoperiod and later under a skeleton photoperiod with half hour of light at dawn and half hour at dusk, prior to being released into constant darkness. Because this animal's endogenous period was very close to 24.0 hours, determination of entrainment by the change in circadian period is not reliable. However, small changes in the phase of the rhythm can be seen after both transitions. A second example is shown in Figure 7.23. In this case, a mouse was used. The pattern of entrainment was similar under a complete photoperiod and a skeleton photoperiod, but there was unmasking of early onsets in the latter condition. Pittendrigh placed considerable emphasis on skeleton photoperiods,[117] most likely because light pulses at dawn and dusk are necessary to modulate his two putative oscillators (which we discussed in Chapter 6). However, the effect of light pulses at dawn and dusk is generally not different from that of a single daily pulse (for instance, compare Figures 7.20 and 7.21 with Figures 7.22 and 7.23). Organisms with periods shorter than 24 hours need a light pulse at dusk (phase-delay region of the PRC), whereas organisms with periods longer than 24 hours need a light pulse at dawn (phase-advance region of the PRC). The second daily pulse is either irrelevant (if it falls on the dead zone) or merely a nuisance (if it falls on a phase-shifting section of the PRC).

When a full light–dark cycle (that is, a complete photoperiod, as opposed to a skeleton photoperiod) is present,

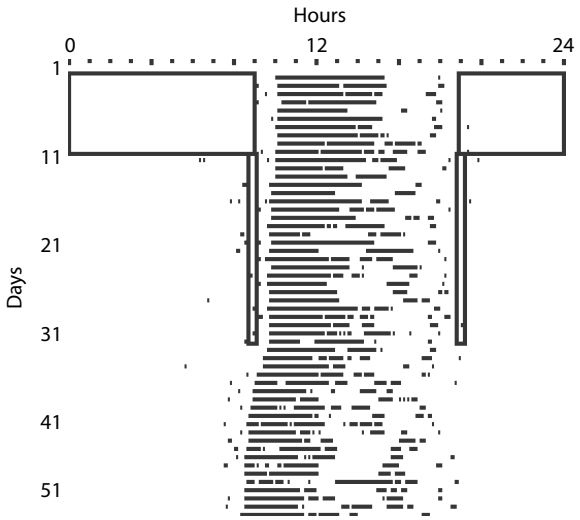


FIGURE 7.22 Dawn and dusk are enough for hamster. This actogram shows the running-wheel activity rhythm of a male golden hamster (*Mesocricetus auratus*) maintained first under a light–dark cycle (LD 14:10), then under a skeleton photoperiod with half hour of light at dawn and dusk, and then under constant darkness. Except for a minor difference in the phase angle of entrainment, the activity pattern is identical under the full light–dark cycle and the skeleton photoperiod. (Data from the archives of the Refinetti lab.)

entrainment is only slightly complicated by the presence of additional, "unnecessary" light. Most of the extra light is left in the insensitive zone of the PRC (to the left of the phase-delay region when τ is shorter than T, and to the right of the phase-advance region when τ is longer than T). However, more than a single point of the phase-delay or phase-advance regions is stimulated in this condition. The net effect presumably corresponds to the integration of the area under the curve

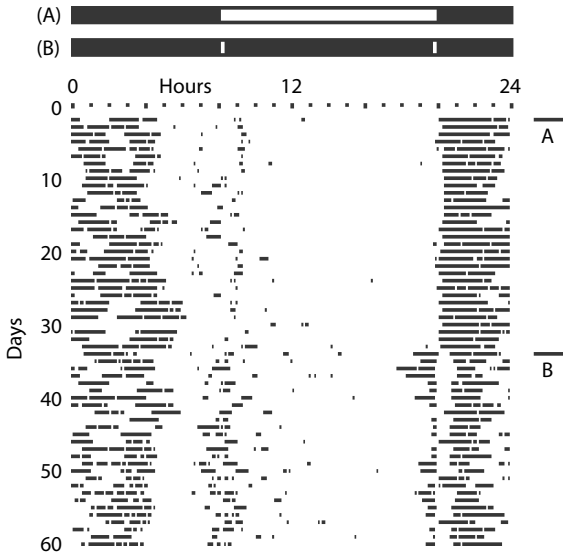


FIGURE 7.23 Dawn and dusk are enough for mouse. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained first under a light–dark cycle (A) and then under a skeleton photoperiod with half hour of light at dawn and dusk (B). The activity pattern is very similar under the full light–dark cycle and the skeleton photoperiod, but the onsets are slightly earlier and less regular under the skeleton photoperiod. (Data from the archives of the Refinetti lab.)

in the stimulated range, as will be discussed in Section 7.3. The persistence of entrainment under LD 12:12 for 8 consecutive months, which corresponds to about 1/3 of the life span of most laboratory rodents, is shown for a representative Siberian hamster (*Phodopus sungorus*) in Figure 7.24. There was a small drift of the onsets during the first few days, as the phase angle of entrainment was slowly established. After that, however, the activity pattern remained very stable, subject only to the usual irregularities associated with "biological noise."

It is important to point out that the phase angle of entrainment (ψ) is dependent on the free-running period of the organism, as predicted by the equation $\Delta\phi = \tau - T$. Look at Figure 7.25, which shows running-wheel activity records of two mice. Both animals were maintained in constant darkness before being subjected to LD 12:12 of low intensity (0.1 lux, to reduce photic masking of activity). Notice that, although both mice entrained to the LD cycle, the mouse with $\tau < 24$ hours started running before dusk each day, whereas the mouse with $\tau > 24$ hours started running after dusk. The mouse with $\tau < 24$ hours required photic stimulation during the phase-delay region of the PRC, which is located in early subjective night. Thus, its phase angle of entrainment was set to ensure stimulation at dusk. On the other hand, the mouse with $\tau > 24$ hours required photic stimulation during the phase-advance region of the PRC, which is located in late subjective night. Thus, its phase angle of entrainment was set to ensure stimulation at dawn. The dependence of ψ on τ is summarized in the diagram shown in Figure 7.26. Light at dawn (that is, during late subjective night or early subjective day) causes phase advances and, therefore, is needed by organisms with slow

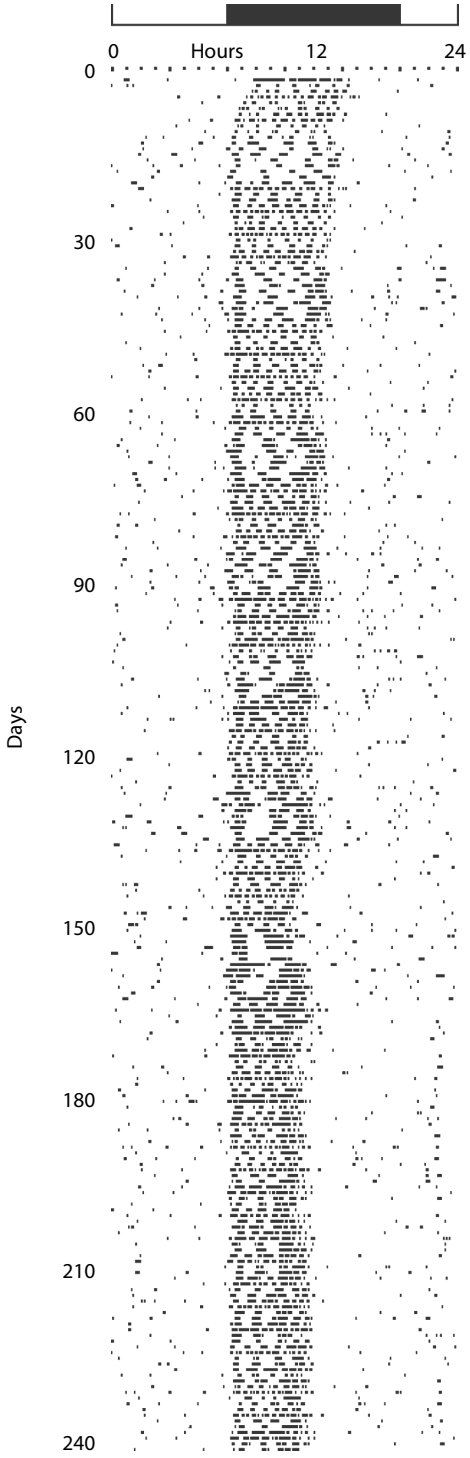


FIGURE 7.24 It keeps running, and running, and running. This actogram shows the running-wheel activity rhythm of a Siberian hamster (*Phodopus sungorus*) maintained under a light–dark cycle (LD 12:12) for 8 consecutive months (i.e., one-third of the animal's life). The rhythm drifted slightly during the first few days until the appropriate phase angle of entrainment was attained, but the activity pattern remained essentially constant through the recording period. (Data from the archives of the Refinetti lab.)

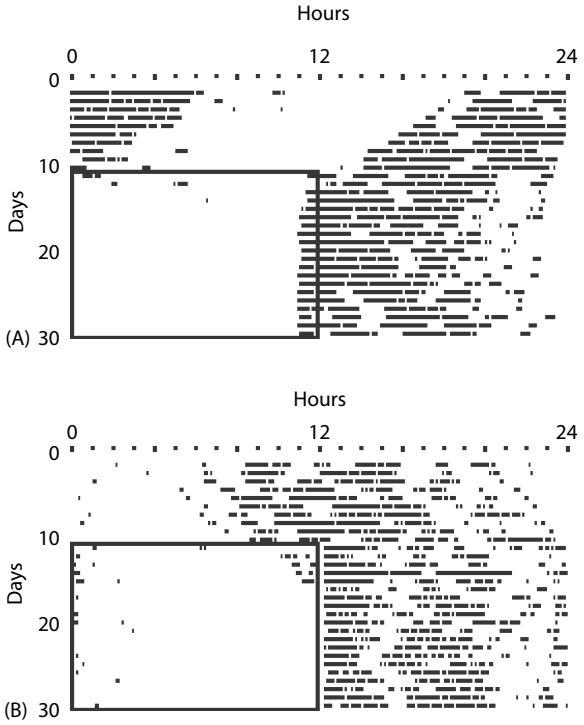


FIGURE 7.25 Different τ, different ψ. The actograms show the rhythms of running-wheel activity of two domestic mice (*Mus musculus*), one with an endogenous period shorter than 24 hours (A) and one with an endogenous period longer than 24 hours (B). The rectangles indicate the duration of the light phase of the light–dark cycle imposed on the 12th day (LD 12:12 with dim light of 0.1 lux during the light phase to prevent negative masking). Both animals exhibited entrainment, but their phase angles of entrainment differed. (Data from the archives of the Refinetti lab.)

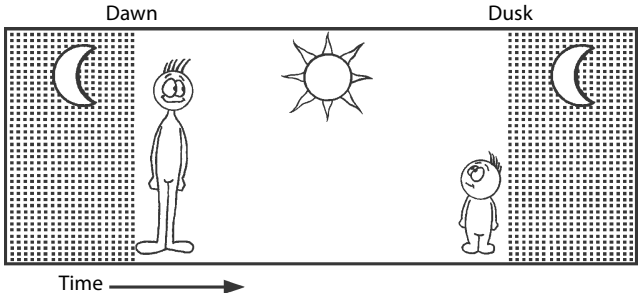


FIGURE 7.26 Long (tall) at dawn, short at dusk. Because of the characteristics of the photic PRC, organisms with a long circadian period need to be exposed to light at dawn, whereas organisms with a short circadian period need to be exposed to light at dusk, in order to be entrained.

clocks (that is, organisms with circadian periods longer than 24 hours). In contrast, light at dusk (that is, during late subjective day or early subjective night) causes phase delays and, therefore, is needed by organisms with fast clocks (that is, organisms with circadian periods shorter than 24 hours). Now, a natural way to facilitate light exposure at a given time of the day is to have the organism awake and active at that time. Thus, most organisms with long τ are active at dawn, whereas

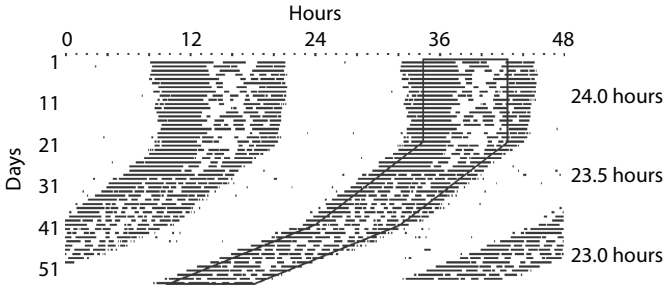


FIGURE 7.27 Moved by the light. This double-plotted actogram shows the running-wheel activity rhythm of a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle with 8 hours of light per cycle and a total duration of 24.0, 23.5, or 23.0 hours (as indicated by the single-plotted polygon and the numbers on the right margin). Notice how the activity pattern tracks the light–dark cycle. (Data from Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004.)

organisms with short τ are active at dusk (as well as during the day or the night, depending on whether they are diurnal or nocturnal, as discussed in greater detail in Chapter 9).

What if T is not 24 hours? This does not happen in nature, but the situation can be created in the laboratory. Light–dark cycles or skeleton photoperiods with periods different from 24.0 hours are called *T cycles*. Entrainment of circadian rhythms by T cycles has been documented in a variety of species.[12,13,19,35,130,149–175] An example is shown in Figure 7.27. A Nile grass rat was subjected to a T cycle of 24.0 hours, followed by a T cycle of 23.5 hours, and followed by a T cycle of 23.0 hours (with 8 hours of light per cycle in all three conditions). Notice that the activity pattern tracks the T cycles—that is, that the period of the rhythm follows the period of the T cycle. As mentioned in Chapter 6, the apparent shrinking of α (and of the width of the light phase of the T cycle) when τ deviates from 24 hours is an artifact of the actogram plot. Two additional examples of entrainment by T cycles are shown in Figure 7.28. Mice were used here, and the T cycle had a period of 23.6 hours with 2 hours of light per cycle. For one of the mice (Panel A), τ was shorter than T, so that entrainment required phase delays. Consequently, photic stimulation was needed during early subjective night, and the appropriate phase angle of entrainment (ψ) was attained by initiation of activity about one and a half hours after the light pulse. For the other mouse (Panel B), τ was longer than T so that entrainment required phase advances. Consequently, photic stimulation was needed during late subjective night, and the appropriate ψ was attained by termination of activity at about the time of lights on. In other words, the mouse with $\tau < T$ ran *after* the light, whereas the mouse with $\tau > T$ ran *before* the light.

When a T cycle has a long light phase, and light intensity is relatively high, masking may hide the true ψ. Actograms of the activity rhythms of mice maintained under T = 25 hours and T = 23 hours (illuminance = 300 lux) are shown in Figure 7.29. The actograms are plotted modulo T to facilitate visual inspection. Notice that both mice (which are nocturnal) seem to start running at the time of lights off, even though the beginning of subjective night for the mouse kept under T = 25 hours

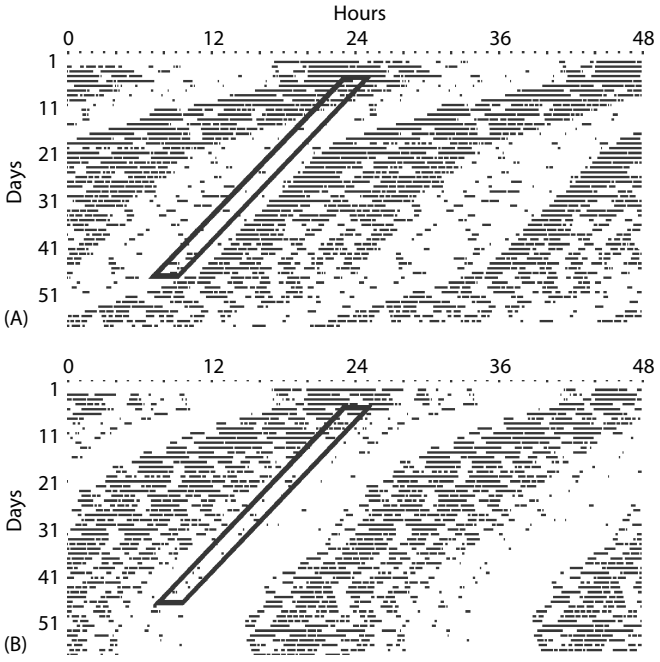


FIGURE 7.28 T cycles. Light–dark cycles with a period different from 24 hours are usually called T cycles. These two double-plotted actograms show the running-wheel activity rhythms of two domestic mice (*Mus musculus*) maintained under a T cycle (period = 23.6 hours, 2 hours of light per cycle), as indicated by the single-plotted parallelograms. The mouse with an endogenous period shorter than 23.6 hours started running after the light pulse each day (A), whereas the mouse with an endogenous period longer than 23.6 hours ran before the light pulse (B). (Data from Refinetti, R., *Chronobiol. Int.*, 20, 417, 2003.)

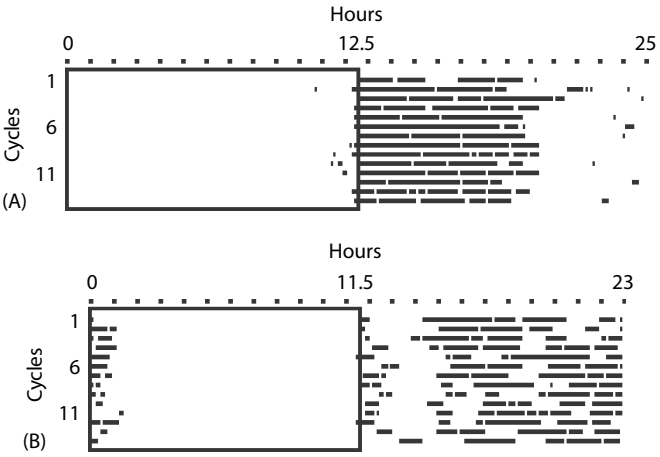


FIGURE 7.29 Different T, different ψ. These actograms show the running-wheel activity rhythms of domestic mice (*Mus musculus*) maintained under a T cycle with a period of 25 hours (A) and a T cycle with a period of 23 hours (B). In both cases, half of the cycle was illuminated (360 lux) and the other half was dark. The light phase is indicated by the rectangles. Under the 25-hour T cycle, activity started at or before lights off. Under the 23-hour T cycle, most activity occurred several hours after lights off and extended into the light phase. (Data from the archives of the Refinetti lab.)

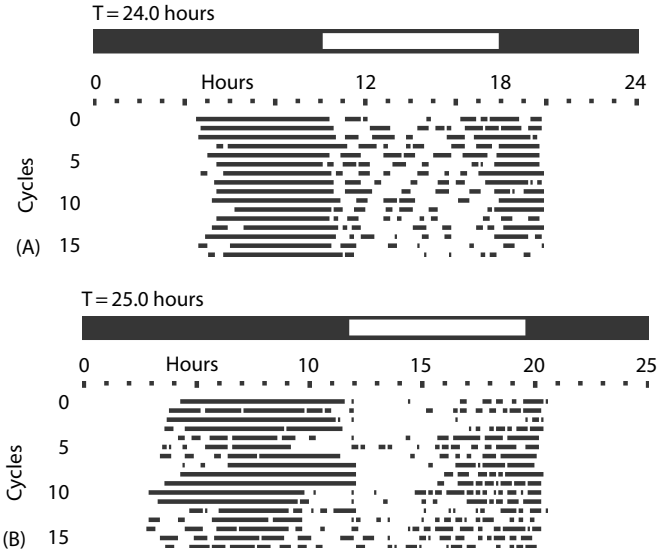


FIGURE 7.30 Different T, different ψ (again). These actograms show the running-wheel activity rhythms of a Nile grass rat (*Arvicanthis niloticus*) maintained under a T cycle with a period of 24 hours (A) and a T cycle with a period of 25 hours (B). In both cases, the light phase of the cycle (360 lux) lasted 8 hours, as indicated by the horizontal bars above the actograms. Activity started several hours earlier (in relation to the time of lights on) under the 25-hour T cycle than under the 24-hour cycle. (Data from the archives of the Refinetti lab.)

was most certainly several hours before lights off. The main difference between the two mice is that the one kept under T = 23 hours keeps running during the first hour of lights on. In contrast, differences in ψ can be clearly observed in animals that are less susceptible to masking, such as the Nile grass rat. As seen in Figure 7.30, a grass rat maintained under a T cycle of 24 hours (with 8 hours of light per cycle) starts running about 5 hours before lights on, whereas a grass rat maintained under a T cycle of 25 hours starts running earlier, about 8–9 hours before lights on. Actually, in this diurnal species that has an endogenous period shorter than 24 hours, entrainment to T > 24 hours requires phase delays of the clock—and, therefore, photic stimulation during early subjective night. Thus, the important segment of light is dusk, not dawn. Accordingly, notice that the termination of activity is about 2 hours after lights off for the grass rat under T = 24 hours but less than 1 hour after lights off for the grass rat under T = 25 hours.

7.1.3 Range of Entrainment

It will probably not surprise you to learn that there are limits to the period of an effective zeitgeber. Most organisms can be entrained by T = 23 hours or T = 25 hours, but circadian rhythms cannot be entrained by T = 5 hours or T = 39 hours, for example. The span of zeitgeber periods compatible with entrainment is called the *range of entrainment*. The range of entrainment of a species can be estimated by its PRC. For instance, the golden hamster PRC shown in Figure 7.9 (Panel A) indicates a maximal phase delay of 1 hour and a maximal phase advance of 2 hours. Because the endogenous period of the golden hamster is

TABLE 7.1
Predicted Ranges of Entrainment[a]

Species[b]	Lower End (hours)	Upper End (hours)	Source
Arvicanthis ansorgei	22.9	25.3	76
Arvicanthis niloticus	22.9	24.7	77
	21.6	25.3	78
Callithrix jacchus	21.6	24.8	90
Coturnix coturnix	16.5	33.8	11
	14.5	32.3	13
Drosophila melanogaster	22.3	28.3	7
	22.1	28.3	8
Eutamias sibiricus	14.9	32.4	80
Glaucomys volans	23.5	24.9	81
Gonyaulax polyedra	20.8	25.2	310
	12.5	26.0	3
Homo sapiens	23.0	29.6	116
	22.2	27.3	67
	21.5	28.6	61
	18.5	25.5	56
Leucophaea maderae	18.9	29.3	4
Mesocricetus auratus	23.2	24.9	21
	22.3	25.7	18
	22.1	25.1	15
	21.8	25.0	16
	21.7	25.6	25
	21.7	26.5	14
	21.4	26.3	20
Microcebus murinus	21.2	25.3	89
Mus booduga	22.9	26.3	43
	22.9	25.2	47
Mus musculus	22.8	26.5	15
	22.8	26.5	33
	22.8	25.7	109
	22.3	25.9	34
	22.2	26.4	31
	21.3	26.2	309
Octodon degus	22.9	24.5	84
	21.6	24.7	85
Passer domesticus	16.7	32.7	12
Peromyscus leucopus	23.5	24.9	15
Peromyscus maniculatus	22.5	25.1	15
Phodopus sungorus	22.7	26.2	86
Rattus norvegicus	23.1	26.9	49
	23.0	29.5	50
	23.0	26.8	48
Sarcophaga argyrostoma	11.8	35.8	5
Sceloporus occidentalis	21.6	25.5	10
Sturnus vulgaris	20.6	25.5	11
Tamias striatus	22.9	29.6	87
Tupaia belangeri	22.7	24.5	88

[a] In each case, the maximal delay and maximal advance in the PRC were obtained from the indicated sources. The free-running period was obtained from Table 6.1. The lower end of the range of entrainment was calculated as the free-running period minus the maximal advance, whereas the upper end was calculated as the free-running period plus the unsigned value of the maximal delay.

[b] For common English equivalents of scientific names of the species, see the table of "Organisms Used" at the end of this book.

24 hours, we can estimate its range of entrainment to comprise periods between 22 and 25 hours. Estimates of the range of entrainment for various species are listed in Table 7.1. Ranges of entrainment can be as narrow as 23.5–24.9 hours for the flying squirrel (*Glaucomys volans*) or as wide as 11.8–35.8 hours for the flesh fly (*Sarcophaga argyrostoma*). Of course, estimates of the range of entrainment based on the PRC can be only approximate. First of all, the choice of pulse duration (or the choice of stimulus intensity) can greatly affect the amplitude of the PRC. More critically, estimates based on the PRC assume that the organisms will receive a single light pulse per day, whereas typical light–dark cycles contain many hours of light per cycle.

Empirical studies of the range of entrainment have been carried out in various species,[78,140,149,162,163,166,170–172,176–178] although they have rarely examined in detail the relationship with the amplitude of the PRC. Results from a study on the range of entrainment of the Nile grass rat are shown in Figure 7.31. It can be seen that all animals exhibited entrainment from T = 23 hours to T = 25 hours. For zeitgeber periods outside this range, some animals still exhibited entrainment, but others did not, so that the group means started to regress toward the free-running period of 23.8 hours. If the criterion of entrainment in half of the animals is used, the range of entrainment of the Nile grass rat extends from 22.5 to 25.5 hours. Table 7.2 compares the ranges of entrainment obtained empirically and estimated by the PRC for this and three other mammalian species. Considering the margin of error involved in estimates based on the PRC, the two sets of data are in good agreement.

I must point out that the notion of range of entrainment may be misleading. Consider Figure 7.32. The two actograms depict running-wheel activity records of mice maintained under light–dark cycles. Entrainment is apparent in

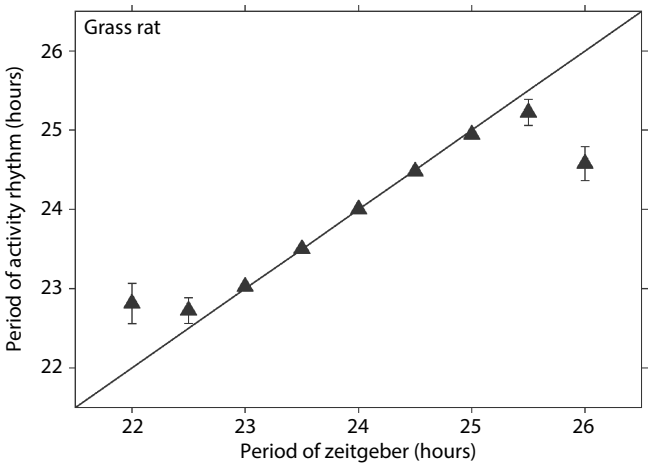


FIGURE 7.31 Range of entrainment. The graph shows the mean period of the rhythm of running-wheel activity of Nile grass rats (*Arvicanthis niloticus*) maintained under T cycles with 8 hours of light per cycle (360 lux). Each point corresponds to the mean (±SE) of 8 or 9 animals. The periods of the activity rhythms were identical to the periods of the zeitgeber (indicating entrainment) from 23.0 to 25.0 hours. For zeitgeber periods shorter than 23.0 hours or longer than 25.0 hours, fewer animals exhibited entrainment. (Data from Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004.)

TABLE 7.2
Comparison of Predicted and Empirical Ranges of Entrainment

	Predicted			Empirical		
Species	**Lower End (hours)**	**Upper End (hours)**	**Source**	**Lower End (hours)**	**Upper End (hours)**	**Source**
Arvicanthis niloticus	21.6	25.3	78	22.5	25.5	78
Homo sapiens	21.5	28.6	61	20.5	29.0	140
Mesocricetus auratus	22.3	25.7	18	22.5	25.5	176
Rattus norvegicus	23.0	29.5	50	23.5	28.5	172

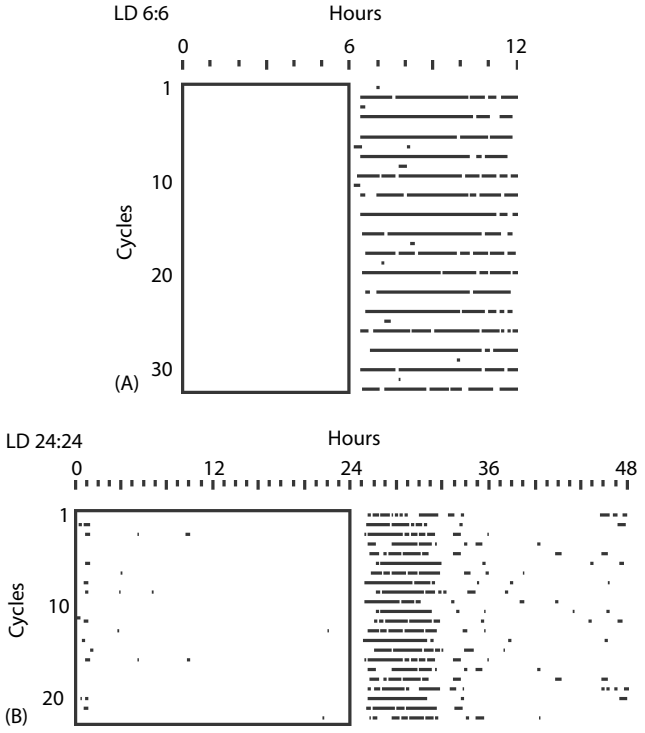


FIGURE 7.32 It looks like normal entrainment. These actograms show the running-wheel activity rhythms of a domestic mouse (*Mus musculus*) maintained under a 12-hour light–dark cycle (A) and a 48-hour cycle (B). Normal entrainment is apparent in both cases when the actograms are plotted with hour axes matching the period of the light–dark cycle (12 and 48 hours, respectively). (Data from the archives of the Refinetti lab.)

both cases. Yet, in one case T = 12 hours (LD 6:6) and in the other T = 48 hours (LD 24:24). Both of these zeitgeber periods are outside the range of entrainment for mice. What is happening here? The answer to this question will introduce us to the notion of entrainment by *frequency demultiplication*.

Let us start by noticing some subtle abnormalities in the actograms in Figure 7.32. Look closer and notice that, in the LD 6:6 condition, activity bands are missing every other cycle. That is, the animal ran for 6 hours on the first cycle, skipped the second cycle, run on the third cycle, and so on. In the LD 24:24 condition, the animal ran for a few hours after lights off each cycle, but then spent a long time inactive. To understand why this happened, we need to compare these activity patterns with the activity pattern exhibited under T = 24 hours (LD 12:12). This is possible in Figure 7.33. In Panel A, an LD 12:12 was followed by an LD 6:6. Under LD 12:12, the mouse ran during the night and rested during the day. The same was true under LD 6:6, except that there were two days and two nights every 24 hours. The animal ran during one of these two nights, but did not run during the other one because it took place during the mouse's subjective day. That is, the mouse's circadian rhythm remained circadian (one cycle every 24 hours). Strictly speaking, there was no entrainment to a 12-hour cycle. Yet, there is no doubt that the animal was entrained. What happened is that the zeitgeber (LD 6:6) had a period that was too short (i.e., had a high frequency), so that the mouse had to *demultiply* (divide) the frequency of the zeitgeber to bring it into the circadian range. The mouse essentially ignored every second 12-hour cycle, and this is why there were missing activity bands in alternate cycles in Figure 7.32.

In Panel B of Figure 7.33, an LD 12:12 was followed by an LD 24:24. Here, the mouse had to *multiply*, rather than to demultiply, the frequency of the zeitgeber. The situation was similar to that of a long light pulse every second day, as discussed earlier in this chapter. However, light masks the activity of the mouse, so that no activity is exhibited during the 24 hours of illumination (notice the missing bands in Panel B of Figure 7.33). Consequently, the animal runs only every other day. When plotted on a 48-hour axis (as in Figure 7.32), the duration of the activity phase looks rather short, but entrainment looks otherwise normal.

The foregoing comments should have made it clear that, although every species has a limited range of entrainment, this range may be expanded if the frequency of the zeitgeber is a multiple or submultiple of the circadian frequency. But what if the frequency of the zeitgeber is just slightly off the range of entrainment? In this case, one may observe the phenomenon of *relative coordination*.[96,103,179–181] As exemplified in Figure 7.34, the organism may *attempt to* entrain but ultimately fail. A hamster with an endogenous period of 24 hours needs a 1.5-hour delay to attain entrainment to T = 25.5 hours. Because the maximum phase delay in the hamster PRC is about 1 hour, the animal is unable to produce the shift necessary for entrainment. It will still shift when exposed to light in the phase-delay region, which will give the impression that the animal is trying to entrain, but eventually the freerun will be resumed (even though the light pulses are still being presented). An important lesson from this is that long records are necessary when one studies the phenomenon of entrainment. If analysis is based on short records, one may erroneously interpret relative coordination as evidence of entrainment.

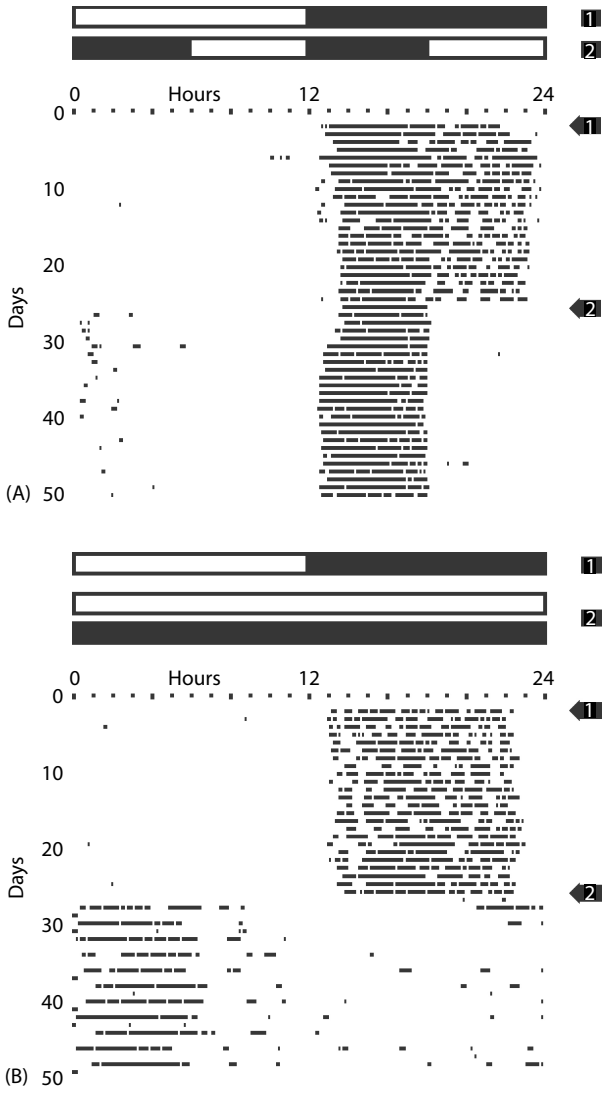


FIGURE 7.33 Entrainment by frequency demultiplication. These actograms show the same data shown in Figure 7.32, except that the hour axes are 24-hour long and additional data under a 24-hour light–dark cycle are included. The timing of the various light–dark cycles is indicated by the horizontal bars above the actograms. These plots clearly indicate that the entrainment patterns apparent in Figure 7.32 were misleading. Under an LD 12:12 (section 1 in both actograms here), normal entrainment takes place; however, the first of the two daily LD 6:6 is actually ignored by the mouse (section 2 in Panel A), and running activity is exhibited only on alternate days under the LD 24:24 (section 2 in Panel B). (Data from the archives of the Refinetti lab.)

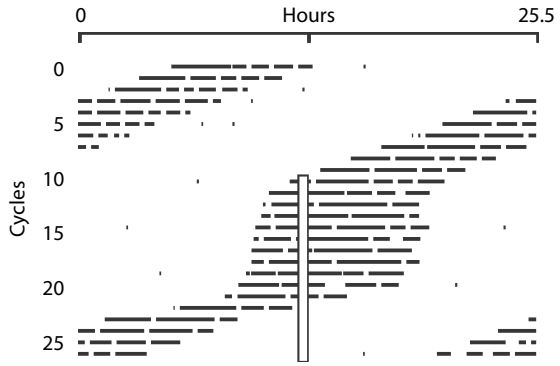


FIGURE 7.34 Relative coordination. A zeitgeber that is unable to entrain a rhythm may still be able to affect it, as shown in this actogram of the running-wheel activity rhythm of a golden hamster (*Mesocricetus auratus*) maintained in constant darkness at first and then exposed to a 25.5-hour T cycle with half hour of light per cycle (vertical rectangle). The activity rhythm is temporarily slowed down by the zeitgeber, but the effect is not strong enough to establish stable entrainment. (Data from the archives of the Refinetti lab.)

7.1.4 Transients

One aspect of phase shifts and entrainment that we have not addressed yet is how rapidly they occur. Various studies in mice and hamsters have provided strong evidence that the phase shifts evoked by single pulses are practically instantaneous, in the sense that the circadian clock is shifted within at least an hour of the presentation of the light pulse.[22,44,182–185] However, this is not always true regarding the expressed rhythms.[18–20,48,89,184] Look at Figure 7.35, which depicts phase advances and phase delays of the activity rhythms of representative hamsters and mice. The animals were maintained in constant darkness and received single light pulses at the times indicated by the stars. The phase delays were immediate for both hamster and mouse. The phase advance was also immediate for the mouse, but it took several days for the hamster. In this case, the new phase was attained through *transients* (that is, through progressive, transient daily shifts). It is not known why transients occur, but the latency between the phase shift of the circadian pacemaker and the full phase shift of the expressed rhythm must be a property of the coupling mechanism between the pacemaker and its output circuits.

Transients associated with entrainment to full light–dark cycles[186–189] are much more common and much less mysterious. Full light–dark cycles stimulate various parts of the PRC, so that they often evoke both phase delays and phase advances. Consequently, the net daily shifts of the pacemaker may be rather small, which explains the slow shift of the expressed rhythms. Direct your attention to Figure 7.36 now. The animal whose records are shown in Panel A needed only a small phase delay to attain entrainment and, consequently, entrainment was achieved with virtually no transients. In contrast, the animal whose records are shown in Panel C needed to advance many hours and, consequently, entrainment was achieved through many transients. The opposite (i.e., delay with transients) was true for the data in Panel D. The pattern in Panel B is a bit more complicated. It may seem that entrainment was achieved through phase advances, but the transients actually indicate phase-delay transition. This is easier to see in the double-plotted actogram redrawn in Figure 7.37.

7.1.5 Photoperiod

We saw in Chapter 4 that the relative duration of the light and dark phases of a light–dark cycle (*photoperiod*) has a strong effect on infradian rhythms. Photoperiod has also strong effects

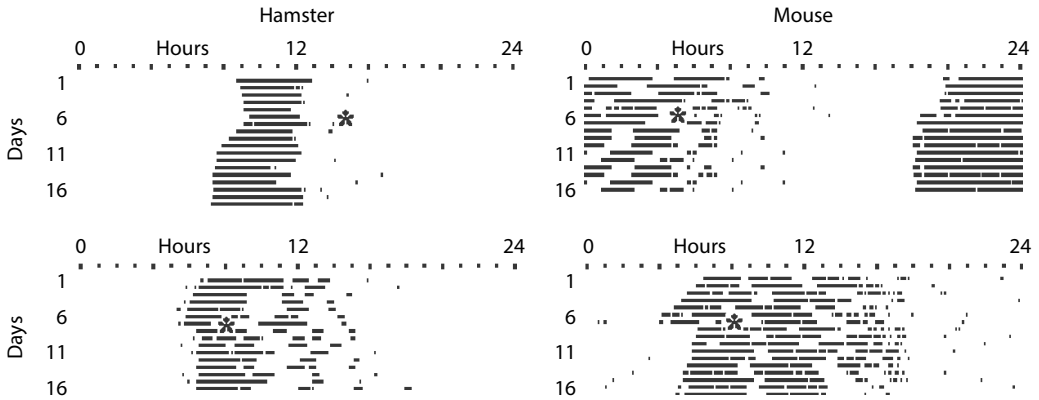


FIGURE 7.35 Transients after single pulses. The actograms show the running-wheel activity rhythms of two golden hamsters (*Mesocricetus auratus*) and two domestic mice (*Mus musculus*) maintained in constant darkness and presented with single light pulses (360 lux for 1 hour). The times of the light pulses are indicated by stars. The two top actograms show pulse-induced phase advances of the activity rhythms, whereas the two bottom actograms show pulse-induced phase delays. The phase advance of the golden hamster was achieved through several transient shifts (*transients*). (Adapted from Refinetti, R., *Physiol. Behav.*, 74, 101, 2001.)

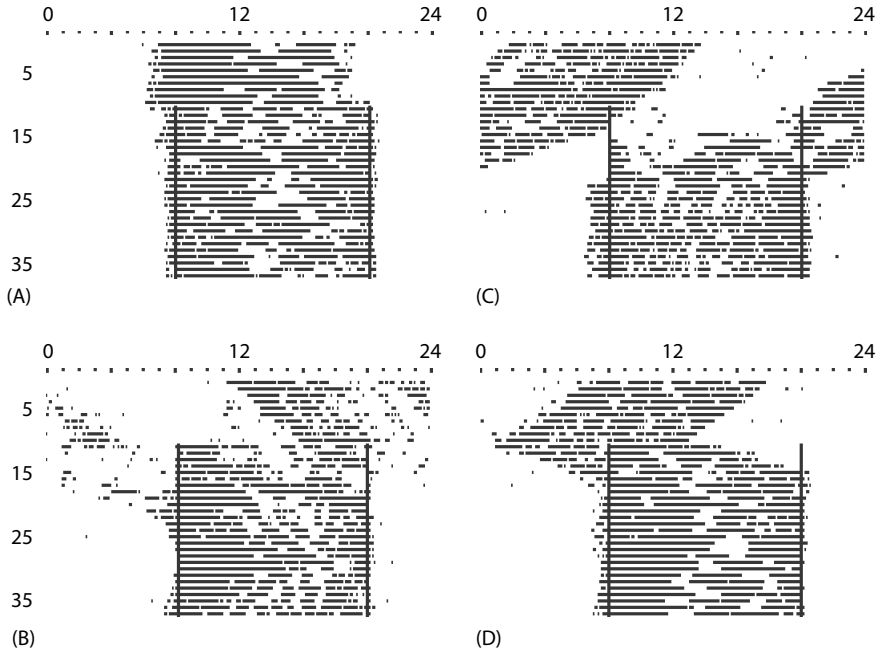


FIGURE 7.36 Transients prior to entrainment. The actograms show the running-wheel activity rhythms of Nile grass rats (*Arvicanthis niloticus*) maintained in constant darkness at first and then subjected to a 24-hour light–dark cycle (LD 12:12, 360 lux). The vertical lines indicate the beginning and end of the light phase of the light–dark cycle. Although all four animals (Panels A through D) eventually attained stable entrainment (with activity restricted to the light phase of the light–dark cycle), entrainment was achieved through transient shifts (transients), the quantity of which depended on the phase of the free-running period prior to the initiation of the light–dark cycle. (Data from the archives of the Refinetti lab.)

on circadian rhythms.[190] Such effects were first described in plants,[191] but they have also been documented in a variety of animals,[192–198] including rodents[24,27,32,39,86,87,92,148,199–225] and other mammals.[89,131,133,226–232] The most obvious effect of photoperiod on circadian rhythms in animals is the temporal compression or expansion of expressed rhythms. Examples for the running-wheel activity of mice are shown in Figure 7.38. Notice that the duration of the active phase (α) is much longer for animals maintained under a regimen of long scotophases (LD 8:16) than for animals maintained under a regimen of short scotophases (LD 16:8). Naturally, the opposite is true for diurnal animals. In sheep (Figure 7.39), α is longer when the animals are maintained under a regimen of long photophases (LD 16:8) than when they are maintained under a regimen of short photophases (LD 8:16). However, because secretion of the hormone melatonin is suppressed by photic stimulation, the temporal distribution of melatonin secretion is similar in diurnal and nocturnal animals. As shown in the figure, melatonin is secreted for a longer time under a regimen of short photophases in sheep (even though α is shorter under

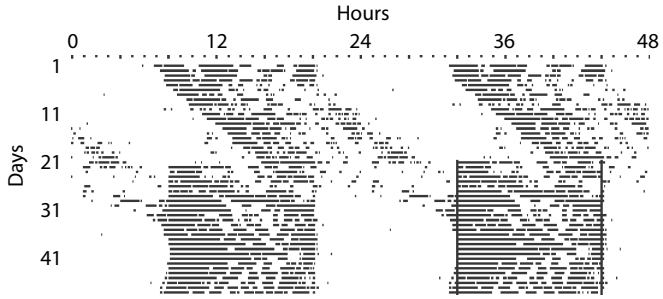


FIGURE 7.37 Advancing or delaying to entrain. The single-plotted actogram shown in Panel B of Figure 7.36 is presented here in double-plot format. In the single-plotted actogram, it seemed that entrainment had been achieved through a rapid advance shift. In the double-plotted actogram, it is evident that entrainment was achieved through several phase-delaying transients.

this regimen). The fact that melatonin secretion under LD 8:16 starts to decrease *before* the end of the scotophase (see Panel B in Figure 7.39) suggests that the effect of photoperiod is more than just negative masking.

Various studies have provided evidence that the circadian pacemaker has a "memory" of the photoperiod under which the organism is housed. Thus, the compression or expansion of rhythms caused by the photoperiodic history is preserved when animals are transferred to constant darkness.[32,227] Also, the waveform of the rhythm of neural activity of the master pacemaker in the mammalian brain is changed by the photoperiod to which the animal is exposed, as determined electrophysiologically[206] or through the analysis of gene expression.[207,213,216,217,226] Perhaps even more significantly, the light-responsive segment of the PRC is altered by the previous photoperiod, as determined at the level of brain cells[39,204,215] or of the whole organism.[32,86,205] An example of alterations of the PRC in mice is shown in Figure 7.40. Although the difference between the three PRCs may not be obvious at first, notice that the magnitude of phase delays evoked at CT 16 increases as the duration of the scotophase increases (that is, from LD 16:8 to LD 12:12 and to LD 8:16). Simultaneously, the magnitude of phase advances evoked at CT 19 decreases. Thus, there is a switch from capacity for phase advances to capacity for phase delays in winter photoperiods (photoperiods with long scotophases). This phenomenon has not been sufficiently studied, and it may be related to the more general phenomenon of *day length measurement*.

As we saw in Chapter 4, photoperiodic organisms utilize the seasonal variation in day length as a signal for seasonal changes in body mass, reproductive capability, and other physiological processes. In many animals, the shortening of day length in the winter is internally coded as a lengthening of the interval of melatonin secretion, but the nature of the coding process remains obscure. The process must involve more than just photic inhibition of melatonin synthesis (masking), but the exact nonparametric process has

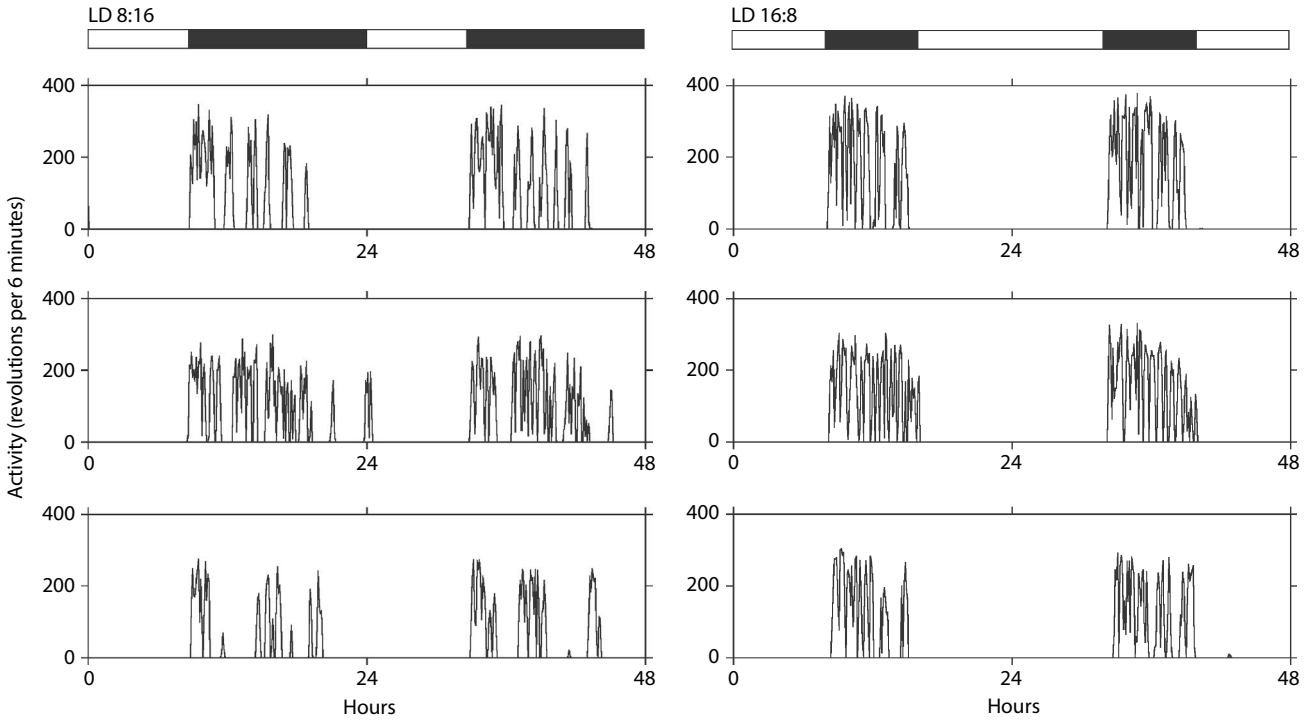


FIGURE 7.38 Photoperiod-induced compression of α in mice. The graphs show 2-day segments of the records of running-wheel activity of six domestic mice (*Mus musculus*) maintained under 24-hour light–dark cycles with 16 hours of darkness per day (LD 8:16) or 8 hours of darkness per day (LD 16:8). The duration of the active phase of the rhythm (α) is shorter under LD 16:8 than under LD 8:16, although the pattern of running is more compact in short nights (so that the total number of wheel revolutions per day is preserved). (Data from Refinetti, R., *Integr. Physiol. Behav. Sci.*, 37, 114, 2002.)

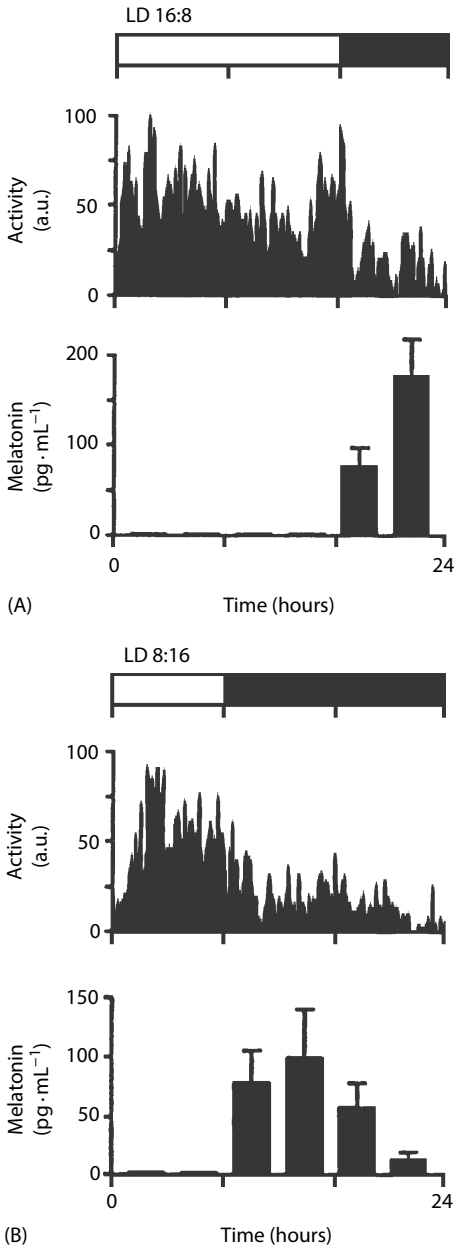


FIGURE 7.39 Photoperiod-induced compression of α in sheep. The graphs show the daily distributions of locomotor activity and melatonin secretion of sheep maintained under 24-hour light–dark cycles with 16 hours of light per day (A) or 8 hours of light per day (B). The activity data are based on 10 days of recording (with infrared motion sensors) from 28 sheep in each LD group. The melatonin data are means (±SE) of four animals per time point in each LD group. Notice the compression of α and expansion of melatonin secretion under short days (LD 8:16). (Data from Lincoln, G. et al., *Proc. Natl. Acad. Sci. USA*, 99, 13890, 2002.)

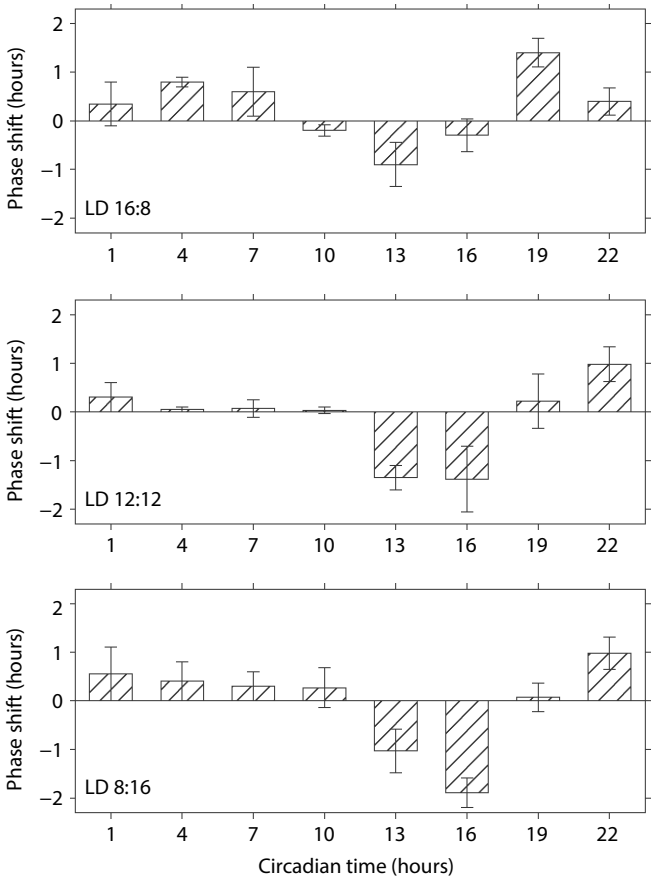


FIGURE 7.40 Previous photoperiod affects phase–response curve. The graphs show the mean phase shifts evoked by single light pulses (1 hour, 300 lux) presented at various circadian times to domestic mice (*Mus musculus*) maintained in constant darkness for 6 days after being exposed to light–dark cycles with different durations of light per day for 4 weeks. Each bar corresponds to the mean (±SE) of four mice. Notice an increase in the magnitude of phase delays (CT 16) and a decrease in the magnitude of phase advances (CT 19), as the nights become longer (that is, from LD 16:8 to LD 12:12 to LD 8:16). (Adapted from Refinetti, R., *Integr. Physiol. Behav. Sci.*, 37, 114, 2002.)

not been fully elucidated. Bünning's work on plants in the 1930s gave rise to the hypothesis of *external coincidence*—that is, that day length is measured by the coincidence of external photic stimulation and internal sensitivity to light.[191] The rival hypothesis (*internal coincidence*) derives from Pittendrigh's notion of morning and evening oscillators: day length would be determined by the phase relationship of the two oscillators.[117] Alterations of the PRC under short days (Figure 7.40) are more easily explained by the internal coincidence hypothesis than by the external coincidence hypothesis. However, it is also possible that the phenomenon derives from a parametric action of light on the pacemaker. Thus, we will turn now to the analysis of the parametric effects of photic stimulation.

7.2 PHOTIC PARAMETERS

At the beginning of this chapter, I pointed out that if a rat's internal clock tells it that it is midnight when it is actually midday, the rat may venture out of its burrow at the wrong time of day and end up being eaten by a hungry dog. This is true, and entrainment prevents it from happening. However, it is quite conceivable that a rat coming out of its burrow at

midday would quickly find out that it is the wrong time of day by noticing that it is quite bright outside. Evidently, light has parametric effects as well as nonparametric effects.

7.2.1 Masking

Perhaps the most ubiquitous parametric effect of light is *masking*.[119,233,234] As mentioned previously, masking refers to the inhibition of expressed rhythms (presumably without an effect on the pacemaker itself). Consider Figure 7.41. The top panel shows the records of running-wheel activity of a mouse maintained under LD 12:12. Initially, the intensity (illuminance) of the light was 300 lux. Under this condition, the mouse exhibited almost no activity during the light phase of the cycle. However, when the intensity of the light was reduced to 0.1 lux, a good deal of activity was unmasked at the beginning and especially at the end of the light phase. This unmasking indicates that the activity was previously being masked, although a change in the phase angle of entrainment may also have occurred. In contrast, in the grass rat (which is a diurnal animal), activity was unmasked during the *dark phase* when the intensity of the light was reduced to 0.1 lux. This unmasking was not as clear as that in the mouse, probably because there was no real change in illuminance during the dark phase. Presumably, the dark phase became subjectively "less dark" when the contrast between light and darkness was reduced, or perhaps the unmasking is convoluted with a change in the phase angle of entrainment resulting from the reduction in light intensity.

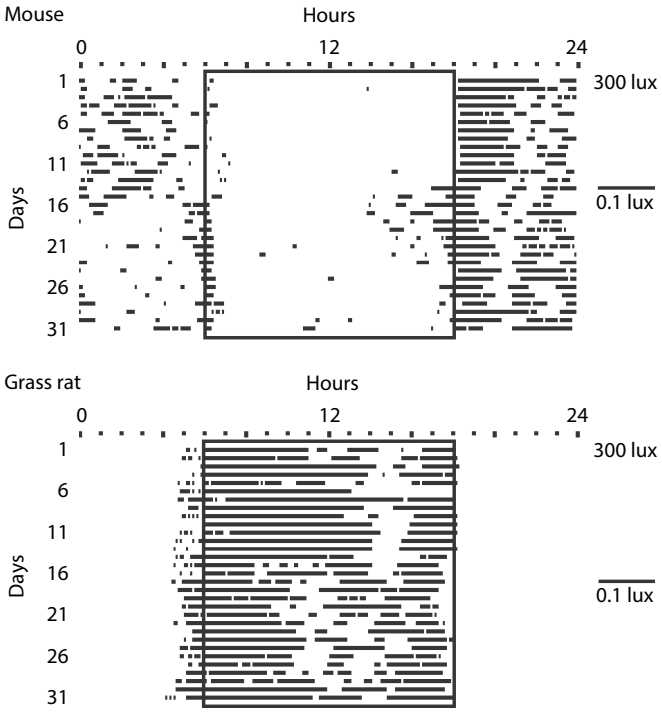


FIGURE 7.41 Brightness and masking. The actograms show the running-wheel activity rhythms of a domestic mouse (*Mus musculus*) and a Nile grass rat (*Arvicanthis niloticus*) maintained under light–dark cycles with 12 hours of light and 12 hours of darkness per day, as indicated by the rectangles. As a nocturnal animal, the mouse is active during the dark phase. As a diurnal animal, the grass rat is active during the light phase. A small amount of negative masking under the 300 lux LD cycle becomes apparent when the intensity of the LD cycle is reduced to 0.1 lux (as indicated on the right margin). (Data from the archives of the Refinetti lab.)

Generally, masking is produced by light in nocturnal animals and by the absence of light (i.e., darkness) in diurnal animals. Because it is awkward to speak of the effects of the *absence* of something, circadian physiologists use the expression *negative masking* to refer to masking caused by light (usually in nocturnal animals) and the expression *positive masking* to refer to the unmasking caused by light (usually in diurnal animals). Although masking has been documented in various species in various laboratories,[129,152,174,192,194,223,235–247] one researcher took the leading role in the study of this phenomenon. Nicholas Mrosovsky (Figure 7.42) was a professor of zoology at the University of Toronto (in Canada) until his death in 2015. He conducted numerous studies on masking[248–256] and published a good methodological article on it.[234] His interest in masking derived from the realization that, for an organism in the real world (which is exposed to the natural alternation of day and night), masking can be just as effective as entrainment in constraining the organism's physiology and behavior to a diurnal or nocturnal niche.

The acute masking effect of light can be much more easily demonstrated than its chronic effect. Look at Figure 7.43, which shows the mean body temperature of a group of eight rats recorded for 60 consecutive hours. The animals were kept in constant darkness. At the time indicated by the thin vertical rectangle, a 1-hour light pulse was presented. Notice the small but sharp fall in body temperature associated with the light pulse. Next, consider Figure 7.44, which shows the rhythms of

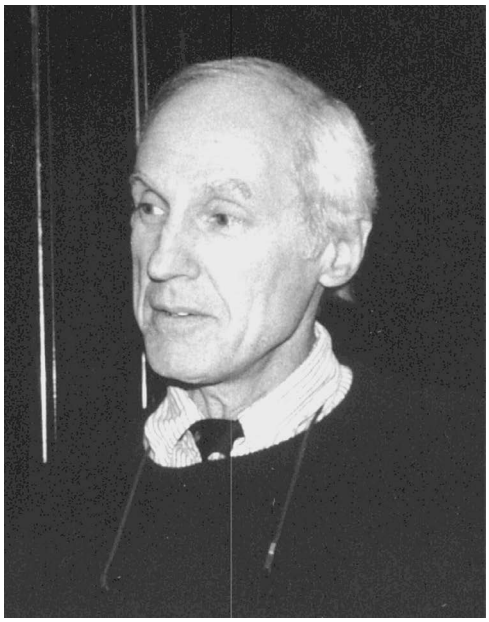


FIGURE 7.42 Nicholas Mrosovsky (1934–2015). This circadian physiologist from the University of Toronto (Canada) was the most active researcher of the mechanism of masking. (Photo courtesy of Nicholas Mrosovsky.)

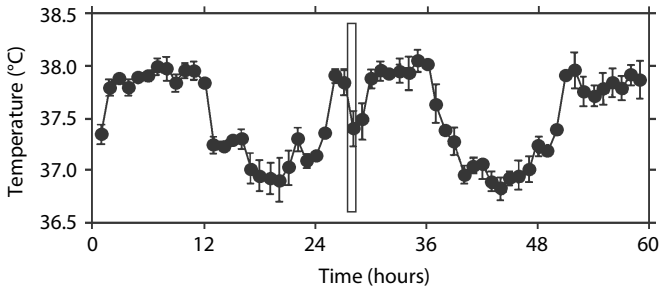


FIGURE 7.43 Masking by brief pulses in laboratory rats. The graph shows a 60-hour segment of the records of body temperature of a group of laboratory rats (*Rattus norvegicus*) maintained in constant darkness and exposed to a brief (1 hour) light pulse. The data points are the means (±SE) of 8 rats. A fall of approximately 0.5°C can be observed immediately after the light pulse. (Data from Sei, H. et al., *Brain Res.*, 987, 131, 2003.)

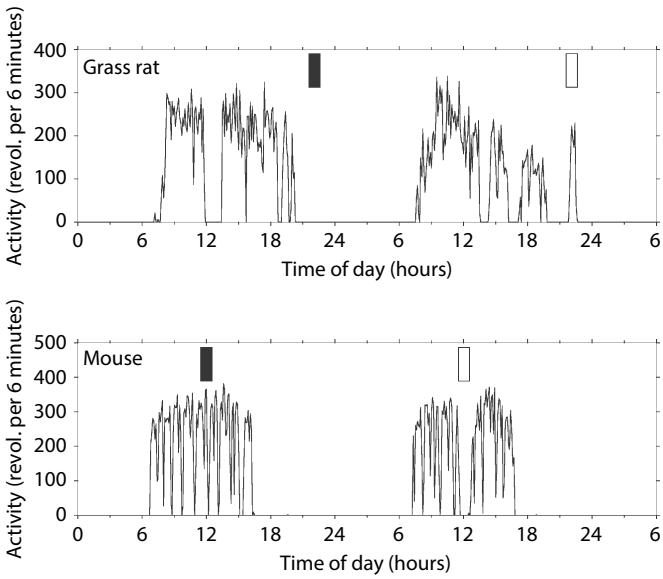


FIGURE 7.44 Masking by brief pulses in grass rats and mice. The graphs show 54-hour segments of the records of running-wheel activity of a Nile grass rat (*Arvicanthis niloticus*) and a domestic mouse (*Mus musculus*) maintained in constant darkness and exposed to a brief (1 hour) light pulse. The time of the light pulse is indicated by the white rectangles. The same time on the previous day (when no pulse was presented) is indicated by the dark rectangles. Notice positive masking by the light pulse in the records of the diurnal grass rat and negative masking in the records of the nocturnal mouse. (Data from the archives of the Refinetti lab.)

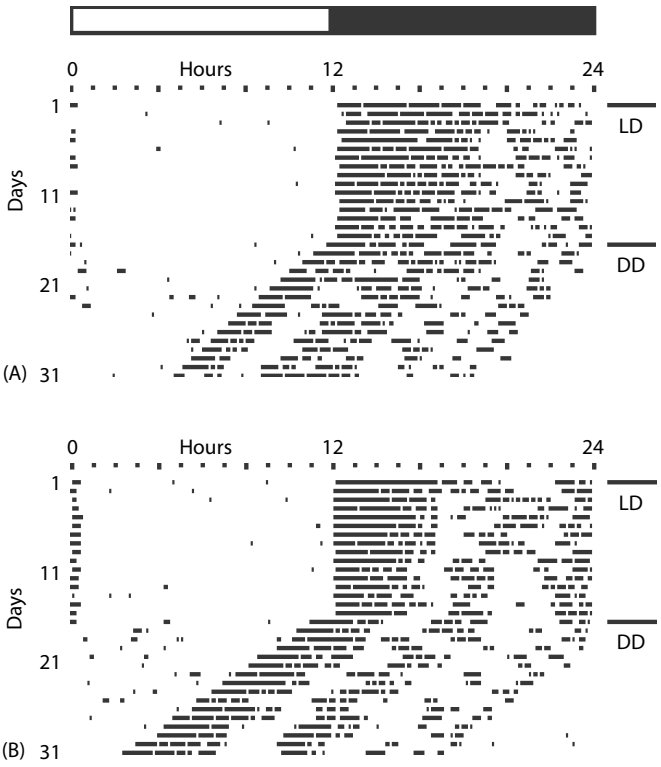


FIGURE 7.45 Masking by light–dark cycle in mice. The actograms show the running-wheel activity rhythms of two mice maintained under a light–dark cycle at first (as indicated by the horizontal bar at the top of the figure) and then in constant darkness (as indicated on the right margin). The animal whose records appear in Panel A freeran from the preceding phase angle of entrainment. The animal whose records appear in Panel B freeran from a point about an hour ahead of the preceding phase angle of entrainment, thus indicating the occurrence of negative masking under the light–dark cycle. (Data from the archives of the Refinetti lab.)

running-wheel activity of a Nile grass rat and a mouse, both of which were kept in constant darkness. The open rectangles indicate the times when 1-hour light pulses were presented, whereas the dark rectangles indicate the corresponding times on the previous day (when no light pulse was presented). It is evident that the light pulse produced positive masking in the grass rat and negative masking in the mouse.

It is important to reiterate that the presence of negative masking under a light–dark cycle can distort the analysis of the phase angle of entrainment. Figure 7.45 shows the activity records of two mice kept initially under LD 12:12 and later released into constant darkness (DD). The records in Panel A reveal no discontinuity between LD and DD, but the records in Panel B show a phase advance of more than an hour in the transition between LD and DD. This apparent phase advance is actually the unmasking of the true phase angle of entrainment under the LD cycle. During the LD segment, the initial hour of subjective night had been masked by the final hour of the light phase. Of course, whereas light may inhibit activity in nocturnal animals, darkness may inhibit activity in diurnal animals. Figure 7.46 shows activity records of four Nile grass rats. Under a 24-hour LD cycle with only 8 hours of light per day (LD 8:16), these animals cannot fit all of their activity time (α) into the light phase. The animal whose records are shown in Panel A solved the problem by initiating activity 3 hours before lights on and extending activity for 1 hour after lights off (for a total α of 12 hours). In Panel B, the animal initiated activity several hours before lights on but did not stay active for long and reinitiated activity when lights were on. A similar pattern is seen in Panel C. In Panel D, early activity was almost fully masked by darkness, so that α is only

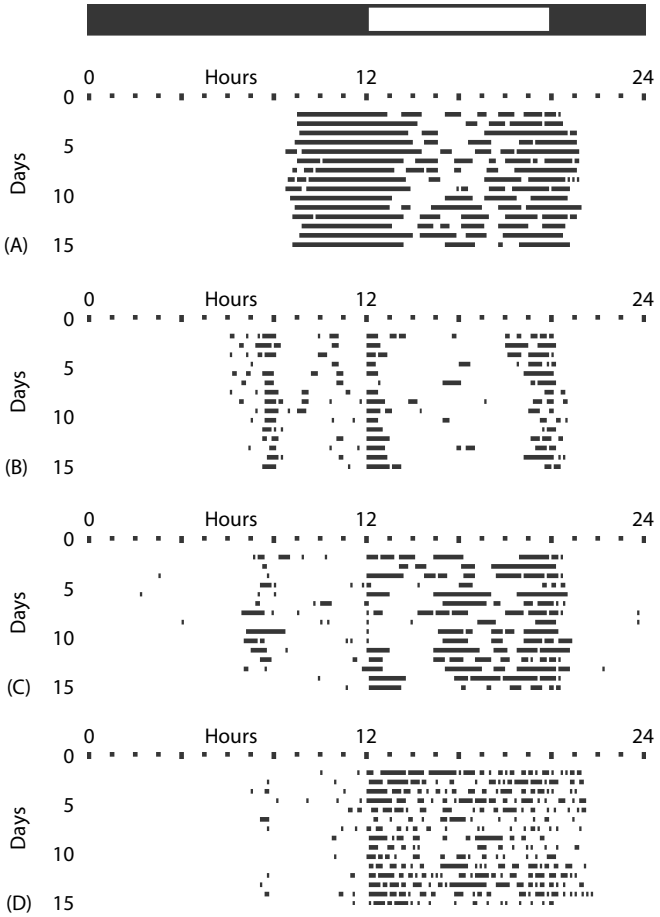


FIGURE 7.46 Masking by light–dark cycle in grass rats. The actograms show the running-wheel activity rhythms of 4 Nile grass rats (*Arvicanthis niloticus*) maintained under a 24-hour light–dark cycle with 8 hours of light per day (as indicated by the horizontal bar at the top of the figure). Although negative masking is not evident in the records in Panel A, it is present to different extents in the records in the other three panels (B–D). (Data from the archives of the Refinetti lab.)

9 hours long. Evidently, different individuals of the same species exhibit different degrees of masking when subjected to the same environmental conditions.

From the perspective of the laboratory researcher, the major problem of masking is the false impression of entrainment. Direct your attention to Figure 7.47. A mouse with an endogenous period of 23.6 hours was exposed to a 21-hour light–dark cycle (LD 10:11). This zeitgeber period is too short to allow entrainment. However, superficial inspection of the actogram suggests that entrainment took place (that is, the animal is active during the dark phase and inactive for most of the light phase). Closer inspection reveals the persistence of the 23-hour rhythm (drifting to the right in this actogram modulo 21) that is merely masked by the light. Notice that masking did restrict activity mostly to the dark phase, so that a naturalist might be justified in arguing that masking may be just as effective as entrainment in determining the temporal niche of a species. Masking *added to* entrainment would be the most effective form of niche selection.

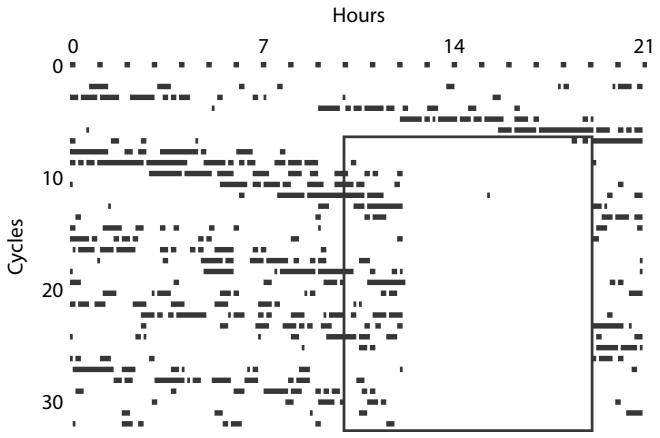


FIGURE 7.47 Masking, not entrainment. This actogram plotted in Modulo 21 shows the running-wheel activity rhythm of a domestic mouse (τ = 23.6 hours) exposed to a 21-hour light–dark cycle. The duration of the light phase of the light–dark cycle is indicated by the rectangle. Although the animal was mostly inactive during the light phase, and mostly active during the dark phase, the slope of the activity rhythm clearly indicates that entrainment was not achieved. The animal essentially freeran through the light–dark cycle, and the activity was negatively masked during the light phase. (Data from the archives of the Refinetti lab.)

Let us consider just one more case of masking. Figure 7.48 shows the activity rhythms of two pill bugs (*Armadillidium vulgare*). These small terrestrial crustaceans are predominantly nocturnal but show weak circadian rhythmicity in constant darkness.[257] The animal whose records appear in Panel A performed just as well under a skeleton photoperiod as under a full light–dark cycle. However, the animal whose records appear in Panel B became arrhythmic under the skeleton photoperiod. This suggests that the activity pattern exhibited under the full light–dark cycle had been the result of masking, not of entrainment, for this animal.

7.2.2 Aftereffects

When we discussed the phase-shifting effects of single light pulses in Section 7.1 (see Figure 7.4), we briefly noted that light pulses may cause not only phase shifts but also changes in the free-running period of the rhythm. The association between phase shifts ($\Delta\phi$) and changes in period ($\Delta\tau$) was noticed by various researchers.[12,20,29,46,51,80,87,258–261] However, the association was often found not to be systematic and to involve very small changes in period (under 0.2 hours). In a few studies, in both diurnal[75,80,87,94,258] and nocturnal[29,46,108,259,261,262] rodents, a systematic association between $\Delta\phi$ and $\Delta\tau$ was found: phase advances were associated with a shortening of period, whereas phase delays were associated with a lengthening of period. The actograms previously seen in Figure 7.4 exemplify this inverse relation between $\Delta\phi$ and $\Delta\tau$. The circadian period that was shorter than 24 hours (Panel A) lengthened to 24 hours after a phase delay, and the circadian period that was approximately 24 hours (Panel B) shortened considerably after a phase advance. Figure 7.49 shows correlation plots of $\Delta\phi$ and

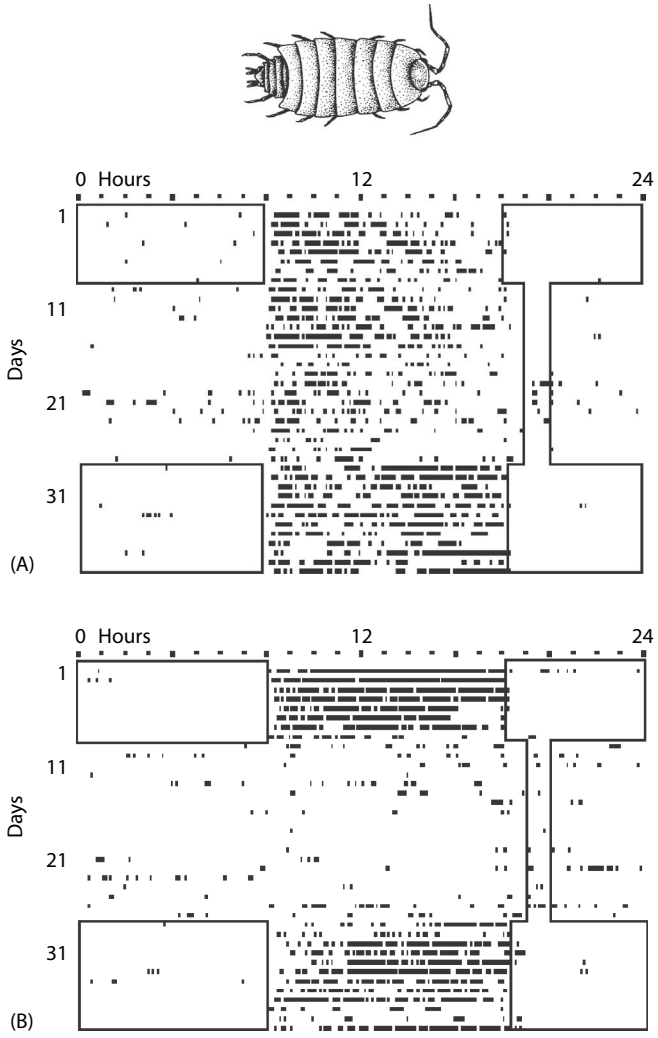


FIGURE 7.48 Bizarre masking in wood lice. The actograms show the rhythms of locomotor activity (measured by infrared motion sensors) of two pill bugs (*Armadillidium vulgare*) maintained under full and skeleton photoperiods (as indicated by the rectangles). The animal whose records appear in Panel A seems to have been entrained by both photoperiods (with activity concentrated in the dark phase). The animal whose records appear in Panel B seems to have been entrained by the full photoperiod but not by the skeleton photoperiod. However, absence of rhythmicity, rather than a free-running rhythm, is seen under the skeleton photoperiod. (Adapted from Refinetti, R., *Crustaceana*, 73, 575, 2000.)

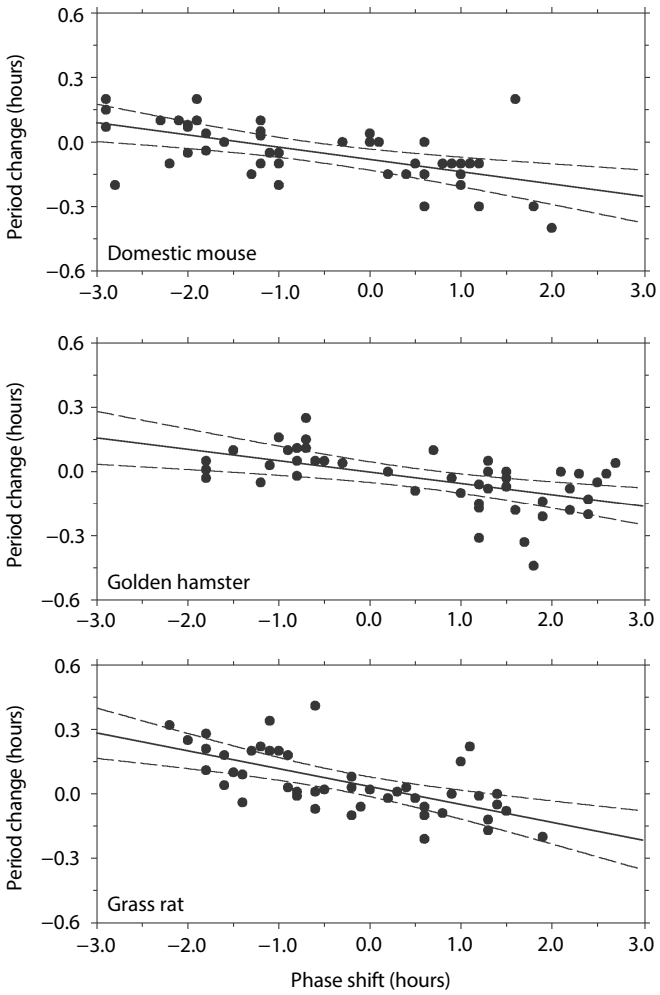


FIGURE 7.49 Phase shifts are accompanied by period changes. Phase shifts of circadian rhythms evoked by single light pulses are often accompanied by small changes in the period of the rhythm, as shown here for three rodent species. Phase shifts of different magnitudes were obtained by light pulses of different durations presented at two circadian times for each species (mouse, CT 16 and CT 0; hamster, CT 14 and CT 18; grass rat, CT 14 and CT 22). (Data from Refinetti, R., *Physiol. Behav.*, 74, 101, 2001; Archives of the Refinetti lab.)

$\Delta\tau$ for three different species. Notice, in all three plots, that a null shift is associated with a null change in period, that greater phase delays are associated with greater lengthening of τ, and that greater phase advances are associated with greater shortening of τ. The changes in period are relatively small, but may be large enough to modulate entrainment. The fact that phase shifts evoked by a nonphotic stimulus are also associated with changes in τ (although in the opposite direction)[263] suggests that $\Delta\tau$ derives from a property of the circadian pacemaker, and not from a specific effect of light.

Although the $\Delta\tau$ associated with $\Delta\phi$ may be considered an aftereffect of the light pulse (or of a phase-resetting stimulus in general), the term *aftereffect* is usually restricted to changes in period induced by previous exposure to a light–dark cycle. Thus, the expression *aftereffects of entrainment* refers to the observation that the free-running period of an organism can be affected by the previous exposure of the organism to light–dark cycles with different periods. For instance, animals previously maintained under a light–dark cycle with a 26-hour period have a longer circadian period (when allowed to freerun in constant conditions) than animals previously maintained under a regular 24-hour day. Aftereffects of entrainment have been documented in a variety of species,[4,12,19,103,163,165,171,173,176,264–269] and an example is provided in Figure 7.50. Heterozygous *tau*-mutant hamsters, which have an endogenous period of 22 hours (as discussed in Chapter 6), were placed under a 20-hour light–dark cycle for a month and then released into constant darkness. As expected, all animals entrained to the LD cycle

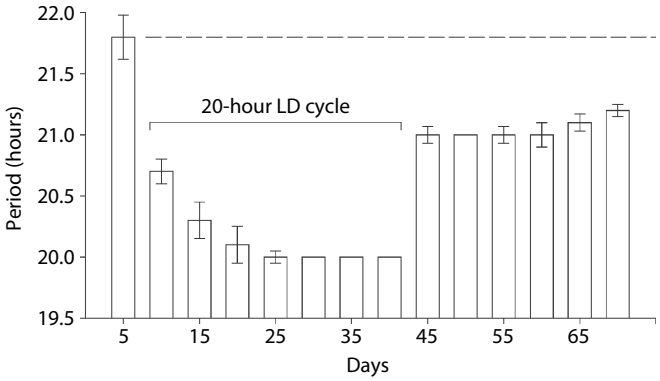


FIGURE 7.50 Aftereffects of entrainment. The period of a zeitgeber to which an organism is exposed prior to being released into constant darkness can have long-lasting effects on the free-running period, as exemplified by these data from a study on *tau*-mutant hamsters. Each bar corresponds to the mean (± SE) of 8 heterozygous mutant hamsters. The animals had been in constant darkness for 6 months and exhibited a mean free-running period of 21.8 hours (day 5). Subsequently, they were kept under a 20-hour light–dark cycle for a month and then returned to constant darkness. The period of the activity rhythms shortened to 20 hours in the presence of the 20-hour light–dark cycle, as expected, but remained shorter than the original 21.8 hours for at least a month after the animals were returned to constant darkness. (Data from Refinetti, R., *Behav. Genet.*, 28, 153, 1998.)

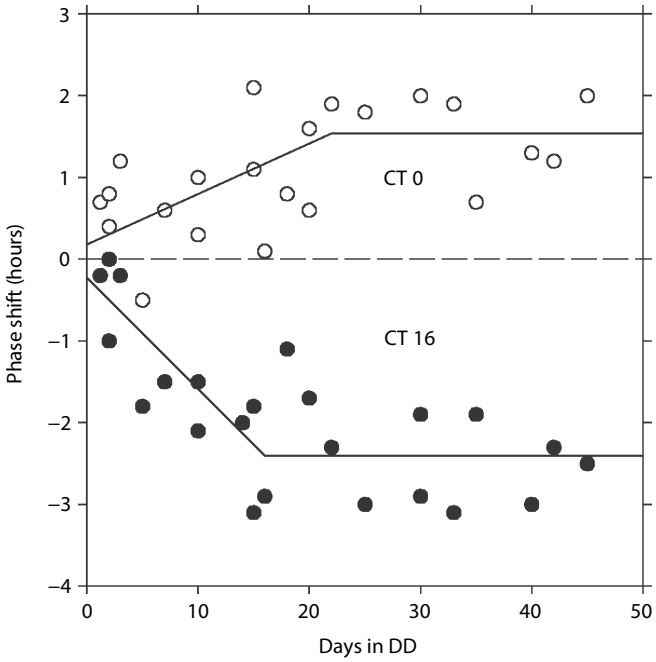


FIGURE 7.51 Dark adaptation in the circadian system of the mouse. The graph shows the phase shifts evoked by 1-hour light pulses presented to domestic mice (*Mus musculus*) maintained in constant darkness for different intervals of time. Both for phase advances (CT 0) and phase delays (CT 16), the magnitude of the shifts increased as the duration of exposure to constant darkness (DD) increased. Saturation was reached at 21 days for phase advances and at 17 days for phase delays. (Data from Refinetti, R., *Chronobiol. Int.*, 20, 417, 2003.)

within 2 weeks and exhibited a period of 20 hours. When the animals were returned to constant darkness, the free-running period lengthened rapidly to 21 hours but did not return to the original period (21.8 hours) for at least a month.[266] In a study in which the recovery of the free-running period was followed for longer, aftereffects were found to last for 3 months or more.[270] The cause of aftereffects is not known. Pittendrigh suggested that aftereffects could be due to changes in the phase relationship of his putative morning and evening oscillators,[270] but, at least in cockroaches, this was shown not to be correct.[4]

7.2.3 Dark Adaptation

One effect of light on the circadian system that has received very little attention is that involved in the process of *dark adaptation*. Dark adaptation is a very well-known process in the visual system.[106] As you know from your own experience, the visual system adapts for optimal performance at different levels of illuminance. When you enter a movie theatre in the middle of the afternoon, you are initially unable to see anything but the illuminated projection screen. However, an hour or so into the movie, you can easily see the teenager next to you trying to place his arm around his girlfriend. Evidently, your vision adapted to the darkness in the theatre. A similar process was described over 40 years ago in the circadian system of the fruit fly (*Drosophila melanogaster*).[271] It was reported that the responsiveness of the system to single light pulses (as indicated by shifts in the time of pupal eclosion) was greater the longer the pupae were maintained in darkness, up to about 2 days. Thus, the nonparametric response of the circadian system of the fruit fly seemed to be stronger in dark-adapted animals than in nonadapted animals. Only recently has interest in this phenomenon led to research in mammalian species. A few studies in golden hamsters[19,23] and mice[29,35,272,273] have shown that the magnitude of phase shifts induced by light pulses is greater the longer the animals are maintained in constant darkness before the pulse. Data for the mouse are shown in Figure 7.51. Both in the phase-delay region (CT 16) and the phase-advance region (CT 0), the magnitude of phase shifts evoked by single light pulses is greater when the animals are maintained in constant darkness longer, up to about 3 weeks. Full adaptation is achieved a few days earlier for phase delays than for phase advances. Notice that the effect of dark adaptation is substantial. The day after release into constant darkness, phase delays of only a few minutes are evoked; 3 weeks later, phase delays of 2–3 hours can be evoked. More detailed studies in mice have demonstrated that the increased responsiveness in dark-adapted animals results from the lack of exposure to light per se and not from collateral effects of exposure to constant darkness (such as the absence of entrainment).[35]

The limited number of studies conducted so far does not allow interspecies generalizations, but the data shown in Figure 7.52 indicate that there is considerable variability between mouse strains and different rodent species. For instance, although dark adaptation is robust in CD-1 mice, it

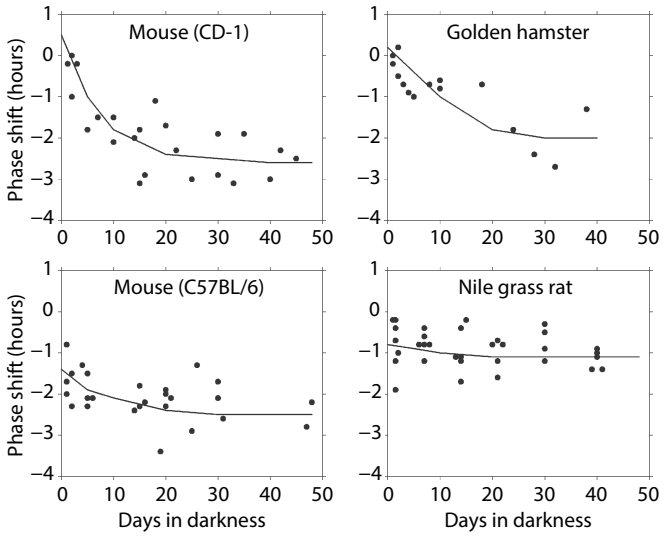


FIGURE 7.52 Dark adaptation in the circadian system of four rodents. The graphs show the phase shifts (delays) evoked by 1-hour light pulses presented to four groups of rodents maintained in constant darkness for different intervals of time. Each data point corresponds to one animal. The magnitude of the shifts clearly increased as the duration of exposure to constant darkness increased in CD-1 mice and golden hamsters but only marginally or not at all in C57BL/6 mice and Nile grass rats. (Data from Daymude, J.A. and Refinetti, R., *Biol. Rhythm Res.*, 30, 202, 1999; Refinetti, R., *Chronobiol. Int.*, 20, 417, 2003; Archives of the Refinetti lab.)

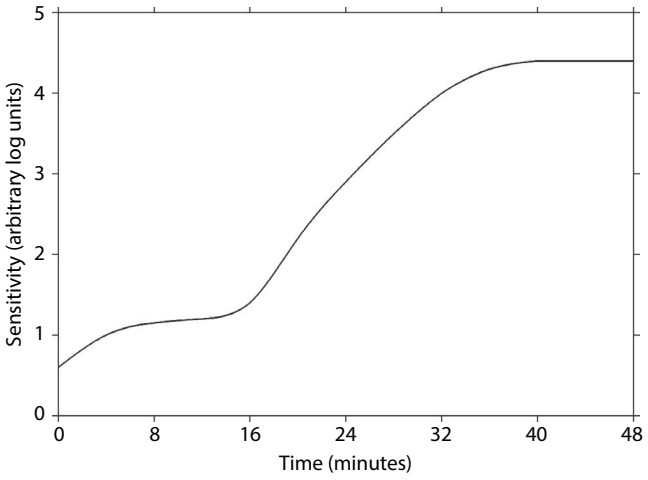


FIGURE 7.53 Dark adaptation in the human visual system. Visual sensitivity increases with time in darkness. Full adaptation is achieved in 40 minutes. (Adapted from Barlow, H.B. and Mollon, J.D., Psychophysical measurements of visual performance, in: H.B. Barlow and J.D. Mollon (eds.), *The Senses*, Cambridge University Press, New York, 1982, pp. 114–132.)

is modest in C57BL/6 mice and virtually nonexistent in Nile grass rats. CD-1 mice are albino, but circadian dark adaptation is also observed in pigmented hamsters.[19,23] In any event, when the phenomenon occurs, the effect of dark adaptation in the circadian system resembles that observed in the visual system—except that full adaptation in the visual system is achieved in less than 1 hour (Figure 7.53), whereas full adaptation in the circadian system of rodents takes up to 3 weeks.[23,29,35,274] Three studies in humans have also provided suggestive evidence that prolonged exposure to darkness or dim light may enhance the suppression of melatonin secretion by nocturnal photic stimulation,[275–277] and a study on rats has shown that prolonged exposure to darkness enhances the acute effects of light on sleep.[278] Furthermore, quantification of the photopigment melanopsin in the rat retina revealed increasing amounts for at least the first 5 days of exposure to constant darkness,[279] which could account for the increased photoresponsiveness of the circadian system.

Virtually nothing is known about the time course of the reverse process (i.e., *light* adaptation). Preliminary studies in golden hamsters[23] suggested that light adaptation of the circadian system is achieved after 15 hours of exposure to light. In mice, the time course must be shorter, as single 1-hour light pulses have been shown to fully reverse dark adaptation.[29] Studies in another laboratory also suggest that light adaptation proceeds rapidly in the circadian system of the mouse.[30] The precise temporal course of light adaptation must be investigated in future studies.

The mechanism of sensory adaptation responsible for the effects of circadian dark adaptation also needs to be further investigated. Although the experimental results available so far document an increase in responsiveness after prolonged exposure to darkness, very few attempts have been made to determine whether the increased responsiveness results from a gain in photic sensitivity or from an increase in maximal response. In case the distinction between sensitivity and responsiveness is not clear to you, Figure 7.54 should be able to help you. If we measure the response of an organism to various intensities of a stimulus, we routinely find that the response is related to stimulus intensity according to a sigmoid function (Panel A). This is actually quite intuitive: for very weak stimuli, no response is exhibited (i.e., the organism does not sense the stimulus); beyond a certain point, the magnitude of the response grows as the magnitude of the stimulus grows; and, beyond a certain point, further increases in stimulus magnitude do not evoke stronger responses (i.e., the system is saturated). Now, let us compare two processes, such as P1 and P2 in Panel B of Figure 7.54. The response of process P1 is clearly different from that of process P2: in process P2, response magnitude grows faster than in process P1. This difference in the slope of the function is what some sensory researchers call "sensitivity." Of course, processes P1 and P3 also differ from each other, even though their slopes are identical. Process P1 shows greater sensitivity than process P3 because it requires weaker stimuli to yield the same response. There can also be a third situation, where two processes differ in their offsets rather than in their slopes or relative positions along the X-axis (Panel C). Here, the same stimulus intensity evokes a greater response in process P2 than in process P1, even though the slopes of the two functions are identical and they have the same position along the X-axis. Although this indicates that process P2 is more sensitive than process P1,

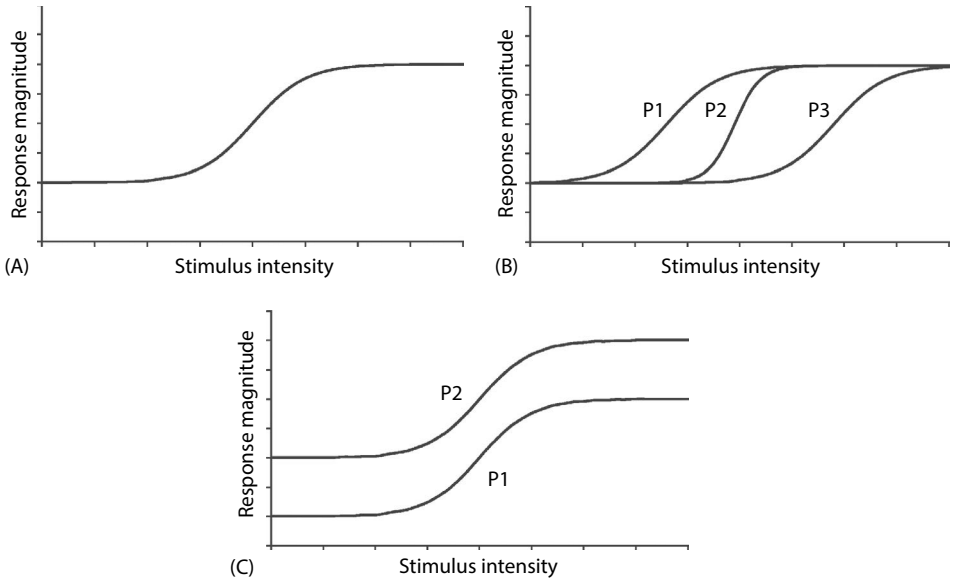


FIGURE 7.54 What is sensitivity? Not all changes in responsiveness are considered to reflect changes in sensitivity. The magnitude of a sensory response is usually a sigmoid function of the intensity of the stimulus (A). Some differences in the function are considered to reflect changes in sensitivity (B) but others are not (C). See text for details.

the term *sensitivity* is usually applied only to the difference in slopes or to the position along the X-axis. The difference in offsets is usually referred to simply as a difference in *responsiveness*. Thus, the increased responsiveness of the circadian system that results from dark adaptation may or may not reflect a change in the *sensitivity* of the system.

In the short time range of 5–60 minutes, a team of researchers found no evidence of reduced photic sensitivity associated with the reduction in responsiveness of the circadian system of the golden hamster caused by previous photic stimulation.[110] Rather, they found evidence for a saturation of the phase-shifting mechanism and consequent compression of the stimulus–response function. In contrast, long-term dark adaptation in the circadian system of *tau*-mutant hamsters was found to be associated with changes in both sensitivity and maximal responsiveness.[280] This issue evidently needs further study.

The phenomenon of circadian dark adaptation observed in mice and hamsters bears resemblance to the phenomenon of *photostasis* in rats. John Penn and Theodore Williams originally described an elevation in dark-adapted rhodopsin levels in rats raised under dim light–dark cycles as compared to rats raised under bright light–dark cycles, an elevation that resulted in the conservation of the number of photons that the retina catches each day (photostasis).[281] Later research established that the elevation of rhodopsin levels in animals maintained in dim light–dark cycles or darkness was not just a one-time developmental phenomenon but could also be produced in adult rats after approximately 3 weeks of treatment.[282,283] Thus, circadian dark adaptation might be an expression of photostasis. In any event, the effect of dark adaptation on the photic responsiveness of the circadian system has important implications for the nonparametric theory of entrainment. Because full dark adaptation of the circadian system requires approximately 3 weeks in darkness, animals maintained under full light–dark cycles in the laboratory or in nature are not dark adapted. They are photically stimulated each day and, consequently, have a reduced responsiveness to light. Based on the regression lines in Figure 7.51, one would expect maximal phase delays and phase advances of 0.2 hour in response to a 1-hour light pulse presented on the first circadian cycle following the termination of an LD 12:12 cycle. This would limit the range of entrainment of mice ($\tau = 23.6$ hours) to the interval between 23.4 and 23.8 hours. Of course, a normal LD cycle contains more than just 1 hour of light per day, but the actual range of entrainment of mice extends at least from 21 to 28 hours.[233] How the additional light contained in an LD cycle produces the full shift required for entrainment remains to be elucidated.

Finally, it is possible to speculate about the evolutionary advantage of dark adaptation in the circadian system. Animals that are in darkness but have recently been exposed to a natural light–dark cycle are likely to have their circadian pacemakers closely in phase with the zeitgeber and, therefore, not to require large shifts in order to reestablish entrainment. On the other hand, animals that have been in darkness for several weeks (in an underground tunnel, for example) are likely to have drifted from the appropriate phase angle of entrainment and, consequently, will require greater phase shifts in order to reestablish entrainment. Thus, greater responsiveness to light in dark-adapted animals may be an adaptive feature of the circadian system. Furthermore, as we will see in Section 7.3, the occurrence of dark adaptation is a *sine qua non* condition for the operation of nonparametric entrainment.

7.2.4 Some Bizarre Parametric Effects

To complete this section on photic parameters, it is necessary to mention some bizarre experimental observations for which there is currently no explanation. Bizarre observations are usually discarded as oddities, but these observations on Siberian hamsters (*P. sungorus*) are too robust to be discarded. Some researchers have observed that Siberian hamsters housed under short photoperiods typical of the winter lose their daily rhythms of sleep and become arrhythmic.[211] Other researchers observed a temporary compression of α in hamsters maintained under long photoperiods and subjected to a single pulse of red light during the night[284] or for no clear reason.[285] More impressively, hamsters maintained under a long photoperiod and subjected to two brief light pulses on two consecutive nights became arrhythmic.[95,284] Arrhythmicity (and sometimes freeruns) was also observed in hamsters subjected simply to a 5-hour delay of the light–dark cycle.[286–288] Evidently, the Siberian hamster is not an ordinary rodent, and further research on this atypical species may help us understand properties of the circadian pacemaker that are not as evident in other species.

7.3 SYNTHESIS AND MODELS

In the two previous sections, we discussed the control of circadian rhythms by the photic environment. We saw that entrainment is established by a nonparametric mechanism and modulated by various photic parameters. A traditional way to coalesce multiple experimental findings into a synthetic view of a complex system is to develop a *model* of the system. Models are valuable research tools that can formally define the important variables affecting a system, summarize existing experimental data, and make accurate predictions of how the system would function under conditions that have not or cannot be tested.[289] In circadian physiology, two general classes of models have been developed: *mathematical models* based on analogies with clocks or oscillators and *functional models* developed as computer programs (Figure 7.55).

7.3.1 Mathematical Models

Mathematical models of the circadian system are often based on an analogy with a swinging pendulum. A frictionless swinging pendulum is a simple, elegant, well-researched timing device. As diagrammed in Figure 7.56, the movement



FIGURE 7.55 Inspirations for models of the circadian system. Two general classes of models have been used to describe and simulate the effects of environmental disturbances on the circadian system. Some models are derived from mathematical constructs of the workings of a clock, whereas other models draw upon the pragmatic functional power of computers. (Copyright ArtToday, Tucson, AZ.)

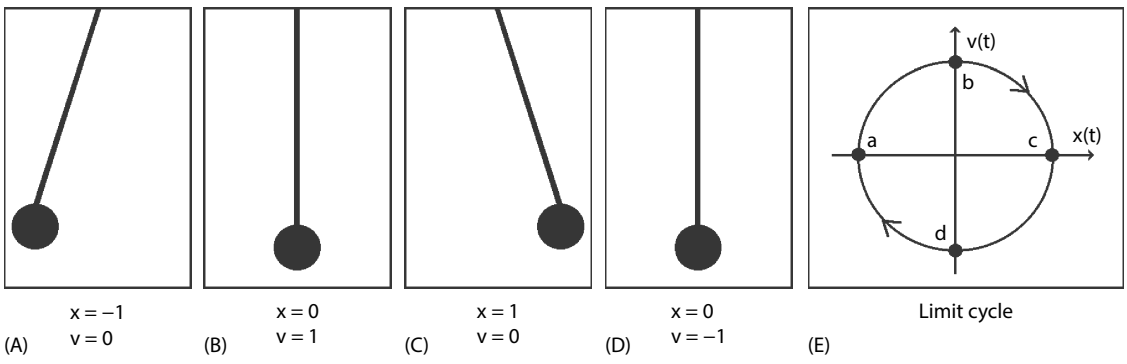


FIGURE 7.56 Defining a limit cycle. If one thinks of the position (x) and velocity (v) of a moving pendulum (A–D), one can easily visualize a limit cycle on a Cartesian plot (E). See text for details.

of the pendulum can be elegantly described by a *limit cycle* defined by two *state variables*: velocity (v) and position in space (x). In Panel A, the pendulum is at the leftmost position (x = –1), where it stops before swinging back to the other side (thus, v = 0). In Panel B, the pendulum is at a central position (x = 0) and is moving with maximal velocity (v = 1). Similarly, Panels C and D show other combinations of position and velocity. Notice that, if we plot the position and velocity values of the four panels on a coordinate system (Panel E), we obtain a circular pattern that describes the pendulum's movement through eternity (unless the cycle is disturbed).

Limit cycles have been used to describe the oscillation of the circadian pacemaker.[290–297] Thinking of the circadian pacemaker as a limit cycle oscillator allows, for instance, the reconciliation of Type 0 and Type 1 PRCs as evoked by strong and weak stimuli. In case you forgot about our discussion in Section 7.1, Type 1 PRCs in invertebrates and nonanimals can become Type 0 PRCs if the intensity or duration of the stimulus is increased. Let us follow the diagrams in Figure 7.57. A Type 0 PRC is explained in Row A. We start with an undisturbed limit cycle (1). Next, we introduce a disturbance in one of the state variables, such as a light pulse (2). This will disturb the limit cycle (3). If the disturbance is strong enough (that is, if the light pulse is very intense), the phase of the cycle may be pushed to point b, thus advancing the cycle by 12 hours (4). Consequently, the PRC will be of Type 0. In contrast, in Row B, we cause a small disturbance of the limit cycle, so that the cycle can resume at a new phase that is different from the old phase but does not constitute as large a shift as that in Row A. Consequently, the PRC will be of Type 1. The elegance of this explanation is unquestionable. A major problem, however, is that the state variables in this limit cycle are not known. In the case of the pendulum, we used position and velocity. In the case of the circadian pacemaker, we have no idea what the two state variables are, so that the variables must be arbitrarily defined. In Chapter 13, we will look at some complex mathematical models that incorporate what is known about the molecular structure of the circadian clock, but a simple limit cycle based on two variables cannot accommodate all the genes that are involved in the system, even if the model's output is mathematically satisfactory.[298] Thus, the elegance of a limit cycle model comes at the cost of low verisimilitude. Another potential problem is that limit cycle models involve the solving of differential equations—and most people (including professional biologists) like advanced calculus as much they like a toothache!

7.3.2 Functional Models

Models are particularly useful when they are parsimonious and can simplify rather than convolute the existing body of knowledge about a physiological system. A major goal of functional models is to avoid the incorporation of assumptions that are not strictly necessary. In particular, assumptions about mathematical properties of the system are restricted to specific functions directly derived from experimental research on actual organisms. Of course, if we are to develop a functional model of appropriate complexity, we need a computer.[299] Most of us take computers for granted these days, but their computational power is truly amazing. Try adding two 3-digit numbers for a minute. Prepare the numbers beforehand, and do the operations as fast as you can. You will be able to complete about 20 additions in one minute. To finish 100,000 additions would take you 83 hours (assuming that you did not fall asleep in the middle of the task). In contrast, a personal computer can do it in 5 hundredths of a second (or less, if it is a fast new machine).

To communicate with a machine, and tell it what we want it to do, we need to use *machine language*. However, if you have any familiarity with computers, you know that contemporary computers come with "translation" programs that convert plain English (or almost plain English) into machine language. Let us take a simple case such as the addition of two numbers (Figure 7.58). We need to send to the computer a precise string of 0s and 1s that depends on the computer chip inside our computer. The sequence shown in Figure 7.58 (machine language) would be appropriate for a Z-80 chip, which was the main processor in the first personal computers that became popular in the 1980s.[300] Instead of typing 0s and 1s (and probably making many mistakes), we could use a shorthand

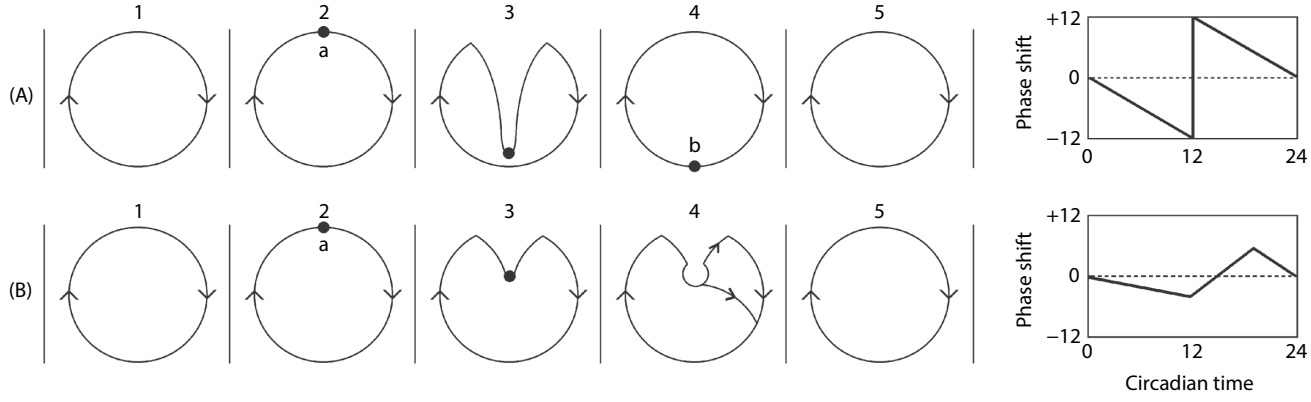


FIGURE 7.57 Type 0 and Type 1 resetting in a limit cycle. If one conceptualizes the circadian pacemaker as a limit cycle oscillator, one can easily associate a Type 0 PRC with strong resetting stimuli (A) and a Type 1 PRC with weak resetting stimuli (B). See text for details.

Task	Add the numbers 320 and 430 and store the result
Machine language	00100001 01000000 00000001 00010001 10101110 00000001 00011001 11101101 01100011 10001000 00010011
Assembly language	LD HL, 320 ′Get 320 LD DE, 430 ′Get 430 ADD HL, DE ′Add LD 5000, HL ′Store
BASIC language	A = 320 + 430

FIGURE 7.58 How to communicate with a computer. If one wants a machine—such as a computer—to perform a task, one must communicate with the machine through machine language. Assembly language was developed early on to help humans "speak" machine language. The BASIC language and other *high-level* languages were developed a little later to provide even easier translation between English and machine language.

system called "assembly language," which at that time was a great improvement.[301] Since then, many *high-level* languages have been developed to facilitate human–computer communication, including BASIC, FORTRAN, COBOL, Pascal, C++, and Java. The *BASIC* language (which is an acronym for Beginners' All-purpose Symbolic Instruction Code)[302,303] was once the standard programming language that, with small differences in dialect, was provided as a component of the operating systems of all personal computers.[304] Having gone through many revisions and upgrades, the language is still alive and strong today as Microsoft's Visual Basic,[305] arguably the world's dominant computer language.[306] In BASIC, the simple and intuitive expression *A = 320 + 430* is all that is needed to cause the computer to add the two numbers and store the result in memory (bottom of Figure 7.58).

For a slightly more sophisticated use of the BASIC language, examine the cartoon in Figure 7.59. The conversation between the two persons may be rather strange, but it is also quite understandable. One can definitely make out that the gentleman is attempting to court the lady and that the lady is not interested in his advances. The only confusing element in the dialog is that, instead of ending each sentence with a period as in standard English, we must end each statement in BASIC with a termination clause, such as "End If" or "Next." The three most useful BASIC statements are "If/Then," "For/Next," and "Select Case" (the latter being just an elaborate form of "If/Then").

Computer models can be extremely simple and yet powerful, allowing instantaneous solutions for complex nonparametric effects of light that could not realistically be conducted by hand.[307] To model a clock, we can start with three lines of code:

```
For Time = 0 to 24
       Print Time
Next
```

These instructions will run the *clock* for 24 hours (in hourly steps) and will print the hours to the computer screen. To model a circadian clock (for which each circadian hour corresponds to 24/τ geophysical hours), which runs for N days with a resolution of 0.1 hour, we need just a few more lines of code:

```
For Day = 1 to N
      For Time = 0.1 to 24 step 0.1
            CT = CT + 24/tau
            If CT > 24 then
                  CT = CT – 24
            End If
      Next Time
Next Day
```

The third line of code advances the circadian clock according to the endogenous period (*tau*), and the fourth line ensures that circadian cycles are limited to 24 circadian hours. Of course, we also need to provide an output for the clock. Because mice run on wheels from approximately CT 12 to CT 20, expression of activity can be coded as the following:

```
If CT >= 12 and CT <= 20 then
       Print actogram_symbol
End If
```

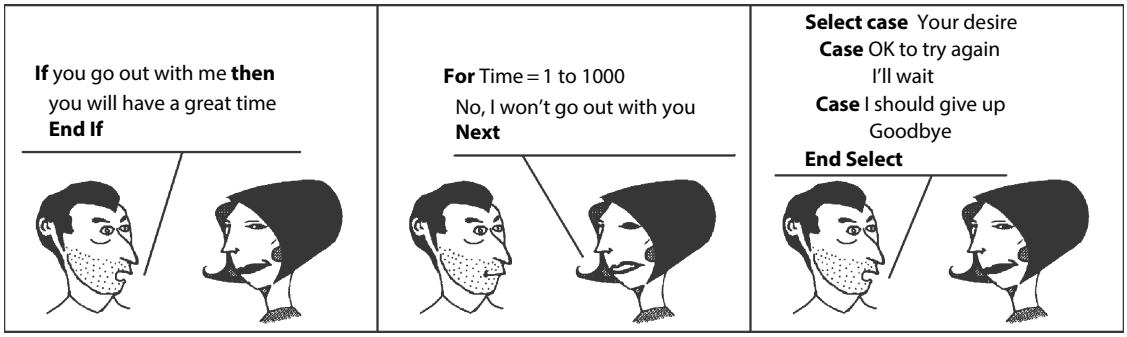


FIGURE 7.59 Speaking BASIC. This conversation structured according to BASIC syntax may sound bizarre, but it can be reasonably well understood by English speakers.

Finally, the PRC can be incorporated as a *Shift* function (taken from the actual mouse PRC) so that, whenever a light pulse is presented, a phase shift *delta* is elicited:

```
If CT >= 11.0 and CT <= 22.9 then
      delta = Shift(CT)   (phase-shift zone)
Else
      delta = 0                    (dead zone)
End if
CT = CT + 24/tau + delta            (new CT)
```

A computer model using these simple elements can incorporate the basic principle of nonparametric entrainment and can simulate freeruns in constant darkness, entrainment to full light–dark cycles, and phase shifts in response to single light pulses, as exemplified in Figure 7.60.

Curiously, the model cannot successfully simulate freeruns in constant light. As shown in Figure 7.61A, the model predicts that a mouse placed under constant light will not exhibit any locomotor activity at all, which is wrong. The lack of activity results from the fact that the pacemaker freezes at CT 10.7, a point in the PRC at which the light-induced phase delay exactly matches the normal advance of the clock. Because CT 10.7 precedes CT 12, the mouse never initiates activity. Pittendrigh noticed this inconsistency of the nonparametric theory of entrainment early on and recognized that provision had to be made for the phenomenon of light adaptation.[121] Strictly speaking, the nonparametric theory of entrainment is incorrect. However, it can be brought back to life if the parametric effect of light adaptation is acknowledged. As we saw in Section 7.2, the circadian system does indeed exhibit dark and

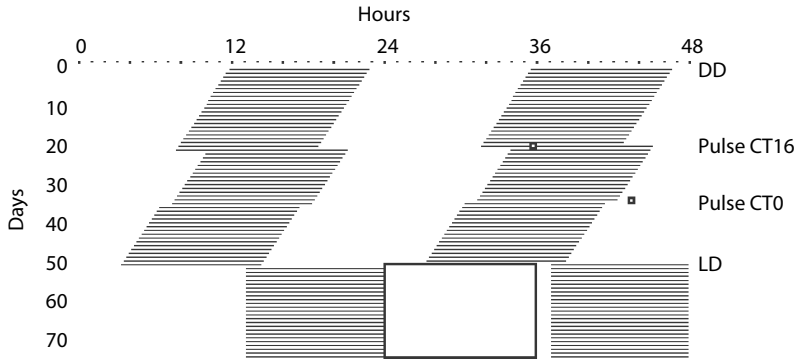


FIGURE 7.60 A computer mouse. This double-plotted actogram shows the simulated activity pattern of a mouse maintained in constant darkness and stimulated with brief light pulses at CT 16 and CT 0 before being subjected to a 24-hour light–dark cycle. The simulated activity pattern resembles the activity pattern of a real mouse relatively well.

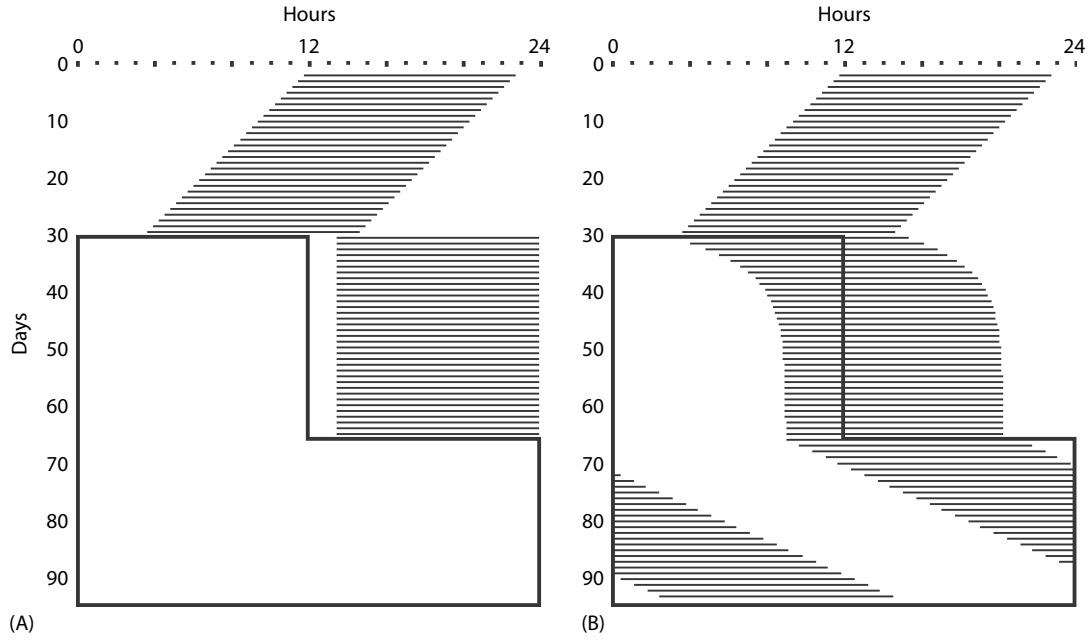


FIGURE 7.61 The need for dark adaptation. A simple computer model of the nonparametric theory of entrainment can simulate the activity pattern of a mouse in constant darkness and under a light–dark cycle but not in constant light (Panel A). If the model incorporates a mechanism of dark adaptation, not only can it simulate activity in constant light but it also provides a better simulation of transients prior to entrainment (Panel B).

light adaptation. If minimal additional code is inserted in the computer program to account for light and dark adaptation, a normal freerun in constant light—with period longer than under constant darkness—is observed (Figure 7.61B). Notice that the change in free-running period conforms to Aschoff's rule. As a matter of fact, the model confirms the prediction that Aschoff's rule derives from the nonparametric effect of light. Notice also that the improved model provides an improved simulation of entrainment to a light–dark cycle by registering the usual transients through which entrainment is normally achieved and by providing a more realistic phase angle of entrainment. A fully operational model that uses the PRC for the golden hamster can be found in the program **Model** (see Exercise 7.4).

The computer model can be used to recapitulate the major concepts covered in this chapter. Entrainment is the result of daily shifts that correct the difference between the period of the pacemaker and the period of the zeitgeber. Consequently, the characteristics of the entrained state depend on three basic elements: the period of the pacemaker, the period of the zeitgeber, and the photic sensitivity of the pacemaker (as described by its PRC). For any given PRC, various characteristics of entrainment can be revealed by manipulation of the period of the pacemaker and the structure of the light–dark cycle. Figure 7.62 shows eight simulations of particular conditions. The top left actogram (*A*) shows a freerun in constant darkness (DD) of a "hamster" with a circadian

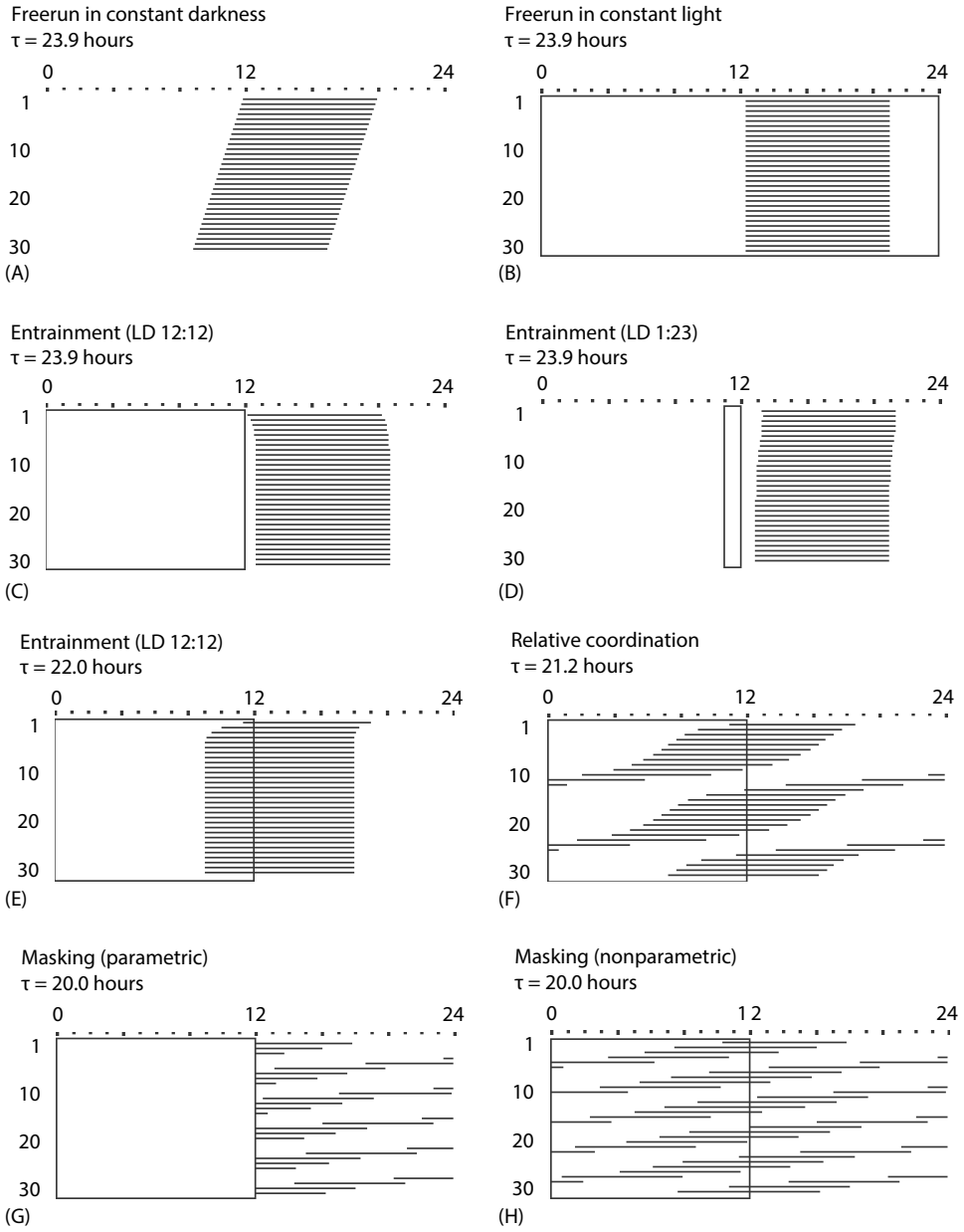


FIGURE 7.62 A good model. The computer model of the nonparametric theory of entrainment that incorporates the mechanism of dark adaptation can produce simulations good enough to summarize most of the photic environmental mechanisms covered in this chapter. See text for details.

period of 23.9 hours. It is evident that this animal's period is shorter than 24 hours, as the rhythm drifts to the left. On the right (*B*), it can be seen that the same animal exhibits a slightly longer period when maintained in constant light (LL), so that the expressed period is 24.0 hours even though the endogenous period is 23.9 hours (Aschoff's rule). We previously discussed the fact that the change in period caused by exposure to constant light is a direct result of the massive stimulation of the PRC. Animals with PRCs that have a greater phase-delay than phase-advance region exhibit greater delays (longer periods) in constant light, whereas animals with PRCs that have a greater phase-advance than phase-delay region exhibit greater advances (shorter periods) in constant light. Of course, you may have noticed that the "hamster" in Figure 7.62 exhibits a longer period in LL than in DD even though its PRC (Figure 7.9) has a greater phase-advance region. The reason for this apparent contradiction is that what really matters is the final integration of discrete phase shifts, not the algebraic summation of the shifts as read off the static PRC. When the animal is exposed to light at all points of the circadian cycle, the actual response to light is given by a greatly distorted PRC (as each pulse causes a shift, thus altering the circadian time of the following pulse).[307,308] If it were not for computer simulations, one would never be able to appreciate this difference.

The second row of actograms in Figure 7.62 shows that a light–dark cycle with a period of 24 hours can easily entrain a pacemaker with a period of 23.9 hours. Although a full LD cycle (*C*) produces entrainment more rapidly than daily 1-hour pulses (*D*), both light schedules produce stable entrainment. In the third row, it can be seen that an LD 12:12 cycle entrains a pacemaker with a period of 22 hours (*E*) but that the phase angle of entrainment (that is, the time between lights off and the onset of locomotor activity) is quite different from that of a pacemaker with a period of 23.9 hours (*C*). The difference is easily explained by the phase-shifting requirements: because a pacemaker with a shorter period needs greater phase delays to match the period of the zeitgeber, more of the phase-delay region of the PRC must be exposed to light (and this is accomplished by moving the circadian system forward into the light phase of the LD cycle). If, however, the period of the pacemaker is too short (*F*), the circadian system cannot shift enough. Relative coordination—a clear *attempt* at entrainment without a stable outcome—is observed instead.

The left actogram in the last row of Figure 7.62 (*G*) shows negative masking that could easily be interpreted as entrainment by a naive observer. The actogram certainly indicates that the animal is consistently inactive during the day and active during the night, as if it were entrained. Inspection of the actogram on the right (*H*), which is identical to the one on the left except for the photic inhibition, reveals that the pacemaker is actually free-running through the light–dark cycle. Thus, in the actogram on the left (*G*), light simply blocked the expression of the activity rhythm without actually affecting the pacemaker. The computer model cannot actually simulate masking (because masking is a parametric process that presumably takes place downstream from the pacemaker), but I manually blocked off that section of the actogram in order to indicate masking. Finally, it should be noticed that the apparent freerun in the last actogram (*H*) is not truly a freerun. Close inspection reveals that the period of the activity rhythm becomes slightly longer, and then slightly shorter, as the animal enters and traverses the light phase of the LD cycle. This is a natural result of the movement of light stimulation along the different segments of the PRC. I call this effect *nonparametric masking*, although this is not an expression commonly used among circadian physiologists.

In addition to parametric masking, the model is unable to simulate the transients sometimes associated with discrete phase shifts (although it does simulate the transients of entrainment quite well) and aftereffects of entrainment. This could be interpreted as a deficiency of the model. However, because the model specifies only pacemaker processes, the failure to simulate the two phenomena can also be interpreted as evidence that single-pulse transients and aftereffects are generated at the level of effector organs and are *not* related to the operation of the pacemaker. Indeed, as previously discussed, there is considerable evidence that light-induced phase shifts of the pacemaker are instantaneous, which means that transients must be generated at the level of effector organs. The issue of aftereffects remains to be resolved.

SUMMARY

1. According to the nonparametric theory of entrainment, entrainment of circadian rhythms is attained by discrete daily phase shifts of the circadian pacemaker that amount to the difference between the period of the pacemaker and the period of the zeitgeber ($\Delta\phi = \tau - T$). Every species has a limited range of entrainment, but this range may be expanded if the zeitgeber frequency is a multiple or submultiple of the circadian frequency.
2. Although entrainment is established by a nonparametric mechanism, it is modulated by various parametric mechanisms. Some important parametric effects of light on circadian rhythms are those associated with masking, aftereffects, and dark adaptation. One of the parametric effects of light—namely, masking—may rival entrainment in the control of the temporal organization of behavioral and autonomic functions.
3. Modeling of the circadian system can help the synthesis of disperse experimental data and the elaboration of hypotheses to be tested by future research. Two general classes of models have been developed: *mathematical models* based on analogies with clocks or oscillators and *functional models* developed as computer programs.

EXERCISES

7.1 Measuring phase shifts and other effects of photic stimulation. In this exercise, we will use the program **Plot** to inspect sample data files containing running-wheel activity records of rodents subjected to various forms of photic stimulation. If you have not done the computer exercises in previous chapters, you should be reminded that you first need to double-click on the **Circadian** icon to open the program banner and then click on **Plot** (the first icon on the left) to start it.

1. Start the program **Plot**.
2. In the *Source* panel, double-click on the Data subfolder to access the folder, and then click on the file **A20**. This file contains running-wheel activity records (with 6-minute resolution) of a domestic mouse maintained in constant darkness for 34 days. The mouse was exposed to a 2-hour light pulse at 11 a.m. on day 22 (circadian time 16). Click on the actogram button (the green button) to see the records.
3. You will see clear circadian rhythmicity with a period shorter than 24.0 hours. A phase delay of 4–5 hours on day 23 should also be clear. Although the position of day 23 in the actogram should be obvious because of the phase shift, notice that the day of each actogram line is indicated when you click on that line.
4. To calculate the exact value of the phase shift, click on the $\Delta\phi$ button (under the display panel) and then click on the first onset after the shift (i.e., day 23). Two red lines will appear on the actogram indicating the prepulse and postpulse linear regressions of onsets. The value of the calculated shift (–4.8 hours) will appear above the display panel. The shift is calculated on the day of the pulse as the time difference between the onsets predicted by the prepulse and postpulse linear regressions.
5. The exact value of the shift may vary slightly depending on the criteria used to determine a shift. Click on the *Settings* item in the menu bar, then change the "No. of days to skip after intervention" to **1**, and then click on *OK*. Next, click on the actogram button again to refresh the screen. Finally, click on the $\Delta\phi$ button again, and then on day 23 once more. Notice that now the calculated shift is –5.0 hours. The value changed because you told the program to ignore the first day after the pulse (when the shift had not been fully completed).
6. Now select file **A21**. This file contains running-wheel activity records (with 6-minute resolution) of a domestic mouse maintained in constant darkness for 29 days. The mouse was exposed to a 2-hour light pulse a few minutes after midnight on the morning of day 14 (circadian time 0). Click on the actogram button to see the records.
7. Like the previous mouse, this mouse has a free-running period shorter than 24.0 hours. The phase shift, however, was much smaller and in the opposite direction (i.e., a phase advance). To calculate the exact value of the shift, click on the $\Delta\phi$ button and then on the first onset after the shift (i.e., day 14 itself). You will receive an error message (above the display window) because the program is unable to calculate the shift.
8. The program was unable to calculate the shift because of the "noisy" pattern of activity on the top left part of the actogram. To clean up the actogram, change the *Clip* filter at the bottom of the *Data* panel to **80**. Then repeat the steps: actogram button, $\Delta\phi$ button, and day 14.
9. A phase shift of +2.0 hours will be reported above the display panel, although you may not be too pleased with the postshift linear regression. The program took into consideration the variations in the amount of running, whereas you may be more concerned about the absolute onsets. A simple way to reach a consensus is to use more days for the linear regression. Go back to *Settings* and change the "No. of days to use after skipped days" to **12**. Then, repeat all steps: *OK*, actogram, $\Delta\phi$, day 14. This computation of a +1.5 hour shift should please everyone.
10. Now select file **A22**. This file contains running-wheel activity records (with 6-minute resolution) of a Nile grass rat (a diurnal rodent) maintained in constant darkness for 25 days and under a light–dark cycle for the following 18 days. Set the *Clip* filter back to **0** and then click on the actogram button to see the records. (If, for any reason, you are currently in black-and-white mode, make sure to return to color mode.)
11. You probably do not need to be told that the light–dark cycle (starting on day 26) had lights on from 4 a.m. to 4 p.m. The blue lines (which indicate moderate activity) allow you to infer this. As a matter of fact, this is an excellent example of *positive masking* by light. Notice that the blue section of the activity records matches the light phase of the light–dark cycle from the very first day when the cycle was imposed (day 26). On the other hand, the yellow and red sections (which indicate higher activity levels) take some 8–9 days to accomplish the 4-hour phase advance required by the light–dark cycle (on day 26, the lights came on at approximately circadian time 20). Thus, entrainment was attained 8–9 days after the imposition of the light–dark cycle, but positive masking was evident right away (and seemed to persist through the 18 days of recording).
12. Finally, select file **A23**. This file contains running-wheel activity records (with 6-minute resolution) of a golden hamster (a nocturnal rodent) maintained under an unusual light–dark cycle with 12-hour

period (two blocks of 7 hours of light and 5 hours of darkness each day). To facilitate inspection of the records, marks of lights off and lights on have been inserted in the file (and can be seen as red dots). Click on the actogram button.

13. Notice that the hamster runs in the wheel during the second dark phase each day (and a little bit during the light phase preceding it) but not during the first dark phase. Because a 12-hour cycle is outside the hamster's range of entrainment, the animal essentially ignores the first of the two daily 12-hour cycles and is entrained by the second cycle.

7.2 Viewing PRCs. In this exercise, we will use the program **PRC** (the sixth icon from the right in the Circadian banner). This program is an atlas of PRCs (i.e., of phase shifts of circadian rhythms evoked by single pulses of environmental stimuli). The data were obtained from published studies and adjusted, if necessary, to comply with the convention that the onset of activity be designated as circadian time twelve (CT 12) for nocturnal organisms and as circadian time zero (CT 0) for diurnal organisms.

1. Start the program **PRC**.
2. Notice that you can choose either *Photic* or *Nonphotic* stimuli. We will stay with the default choice (*Photic*) in this exercise and will deal with *Nonphotic* stimuli in Chapter 8. Notice also the three utility buttons: one allows you to clear the picture when it gets too crowded, the other allows you to resize the Y-axis when needed, and the third allows you to copy the picture to the Windows clipboard (so that you can paste it on a graphics program).
3. Click on the second species name in the list of species (*Arvicanthis ansorgei*). Notice that the common name of the species and the properties of the photic stimulus are displayed. Now *double*-click on the species name. A PRC will be plotted in red. The thick line corresponds to mean values, and the thin lines give an estimate of variability. Basic information (including the source of the data) appears in the lower panel.
4. Because the amplitude of the PRC is rather small, the Y-axis must be resized. Click on the magnifier button twice to set the axis range from –3 to +3 hours. Then double-click on *A. ansorgei* again. Now you can see well that phase delays are evoked between CT 10 and CT 18, and phase advances are evoked during the rest of the circadian cycle (i.e., there does not seem to be a "dead zone").
5. To read a number off the plot, just click on the curve. For instance, if you click on the thick red line at CT 12, a box will appear indicating a phase shift of –1.0 hour.
6. Now double-click on the next species (*A. niloticus*). The PRC is plotted in green. It is similar but not identical to the curve for *A. ansorgei*.
7. Go ahead and inspect as many PRCs as you wish now. In general, photic PRCs have a phase-delay region during early subjective night and a phase-advance region during late subjective night and/or early subjective day. You will need to clear the picture every so often and to resize the Y-axis for some species.

7.3 Understanding entrainment. This is a simple exercise. You will run the program **Entrain** (the seventh icon from the right in the Circadian banner). **Entrain** is a short tutorial on the mechanism of entrainment of circadian rhythms. The tutorial takes only about 6 minutes to complete and is self-explanatory. Go ahead!

7.4 Modeling photic entrainment. In this exercise, we will use the program **Model** (the fifth icon from the right in the Circadian banner) to perform computer modeling of photic entrainment. Although the basic elements of the model apply to any organism and any environmental stimulus, the PRC used in this program is that of the golden hamster for photic stimulation. Details about the program were presented in Section 7.3.

1. Start the program **Model**.
2. At the top of the window, notice that you can stipulate the "endogenous" period of the "organism" and can choose to have one to four blocks of simulations. Set the *Period* to **23.8** hours and the *Blocks* to **2**.
3. In *Block 1*, leave the number of days at **14** but set the *Mode* to **DD** (constant darkness). Set *Block 2* to **42** days in **LD** *Mode* (light–dark cycle). You may leave the default values in the lower panel. Click on *Run*.
4. Notice that the *animal* exhibits a free-running period of 23.8 hours in constant darkness but an entrained period of 24.0 hours under the light–dark cycle. Notice also that entrainment is achieved through transients over a few days. Once entrainment is established, the onset of activity occurs about an hour before lights off. (You can determine the clock time of any point in the actogram by simply clicking on it.)
5. Click on *Close* to return to the main window. Then change the *Period* to **24.1** hours and click on *Run*. Notice the difference in the phase angle of entrainment. The onset of activity after entrainment is established is now more than an hour *after* lights off. Although the PRC has obviously not changed, the change in period caused a change in the phase angle of entrainment.
6. Again, click on *Close* to return to the main window. Then shorten the duration of the light phase of the light–dark cycle by setting the time of *Lights off* in *Block 2* to **2** and click on *Run*. Notice that the pattern of activity did not change much. The actogram under LD 2:22 is essentially the same as under LD 12:12. This is due to the fact that the important part of the light–dark cycle is subjective dawn (because

this is when phase advances are evoked, and an animal with an endogenous period longer than 24 hours needs phase advances in order to entrain to a 24-hour light–dark cycle).

7. Of course, things would be different if the endogenous period were shorter than 24 hours. Click on *Close*, change the *Period* to **23.9** hours, and *Run* another simulation. Now, entrainment was not even achieved. Go back, set the duration in *Block 2* to **110** days, and try again. Now you can see entrainment. Because the period is shorter than 24 hours, light must be present during subjective dusk.
8. Click on *Close*, set the *Period* to **21.7** hours, set *Lights off* to **12**, and *Run* another simulation. Now you see *relative coordination* instead of entrainment. Because the period is very short, the "animal" cannot phase shift enough to achieve entrainment. However, the effects of the light–dark cycle are evident. For at least seven times, there were *attempts* at entrainment (a slowing down of the clock), which, however, were short lived.
9. You are welcome to run additional simulations now. Exercise 8.3 will explore further the issue of phase shifts induced by single light pulses.

SUGGESTIONS FOR FURTHER READING

Meijer, J. H. (2001). Photic entrainment of mammals. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 183–222.

A good review article on the phenomenology and neural bases of photic entrainment in mammals.

Daan, S. and Aschoff, J. (2001). The entrainment of circadian systems. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 7–43.

A review article dealing with general principles of entrainment with emphasis on nonparametric and parametric effects of light.

Aschoff, J. (1981). Freerunning and entrained circadian rhythms. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 81–93.

A typical Aschoff article, filled with interesting facts and insightful generalizations. The treatment of the topic of entrainment is lighter than in the other articles in this list, but entrainment is skillfully placed in the context of modulation of free-running rhythms.

Pittendrigh, C. S. (1981). Circadian systems: Entrainment. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 95–124.

A classic review article by Pittendrigh, the originator of the nonparametric theory of entrainment. This is not an easy-reading article but is worth the effort.

Golombek, D. A. and Rosenstein, R. E. (2010). Physiology of circadian entrainment. *Physiological Reviews* 90: 1063–1102.

A good literature review of photic entrainment mechanisms.

Rea, M. S., Figueiro, M. G., Bierman, A., and Bullough, J. D. (2010) Circadian light. *Journal of Circadian Rhythms* 8: art. 2.

A journal article discussing the meaning of "light" for the circadian system.

WEBSITES TO EXPLORE

International Society for Eye Research: http://www.iser.org.
Lighting supplies: http://www.buylighting.com.
Optical instruments: http://www.edmundoptics.com.
Optical Society of America: http://www.osa.org.
Vision Science website: http://www.visionscience.com.
Sylvania Lighting International: http://www.havells-sylvania.com/.

REFERENCES

1. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences United States of America* 40: 1018–1029.
2. Bünning, E. (1936). Die endonome Tagesrhythmik als Grundlage der photoperiodischen Reaktion. *Berichte der Deutchen Botanischen Gesellschaft* 54: 590–607.
3. Morse, D., Hastings, J. W., and Roenneberg, T. (1994). Different phase responses of the two circadian oscillators in *Gonyaulax*. *Journal of Biological Rhythms* 9: 263–274.
4. Page, T. L., Mans, C., and Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.
5. Saunders, D. S. (1978). An experimental and theoretical analysis of photoperiodic induction in the flesh-fly, *Sarcophaga argyrostoma*. *Journal of Comparative Physiology* 124: 75–95.
6. Barlow, R. B. (1983). Circadian rhythms in the *Limulus* visual system. *Journal of Neuroscience* 3: 856–870.
7. Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A., and Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740.
8. Rothenfluh, A., Abodeely, M., Price, J. L., and Young, M. W. (2000). Isolation and analysis of six *timeless* alleles that cause short- or long-period circadian rhythms in Drosophila. *Genetics* 156: 665–675.
9. Fuchikawa, T. and Shimizu, I. (2008). Parametric and nonparametric entrainment of circadian locomotor rhythm in the Japanese honeybee *Apis cerana japonica*. *Biological Rhythm Research* 39: 57–67.
10. Underwood, H. (1983). Circadian pacemakers in lizards: Phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
11. King, V. M., Bentley, G. E., and Follett, B. K. (1997). A direct comparison of photoperiodic time measurement and the circadian system in European starlings and Japanese quail. *Journal of Biological Rhythms* 12: 431–442.
12. Eskin, A. (1971). Some properties of the system controlling the circadian activity rhythm of sparrows. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 55–80.

13. Zivkovic, B. D., Underwood, H., Steele, C. T., and Edmonds, K. (1999). Formal properties of the circadian and photoperiodic systems of Japanese quail: Phase response curve and effects of T-cycles. *Journal of Biological Rhythms* 14: 378–390.
14. Stetson, M. H. and Gibson, J. T. (1977). The estrous cycle in golden hamsters: A circadian pacemaker times preovulatory gonadotropin release. *Journal of Experimental Zoology* 201: 289–294.
15. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology* 106: 253–266.
16. Takahashi, J. S., DeCoursey, P. J., Mauman, L., and Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.
17. Nelson, D. E. and Takahashi, J. S. (1991). Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *Journal of Physiology* 439: 115–145.
18. Rosenberg, R. S., Zee, P. C., and Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.
19. Shimomura, K. and Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
20. Meijer, J. H. and De Vries, M. J. (1995). Light-induced phase shifts in onset and offset of running-wheel activity in the Syrian hamster. *Journal of Biological Rhythms* 10: 4–16.
21. Grosse, J., Loudon, A. S. I., and Hastings, M. H. (1995). Behavioural and cellular responses to light of the circadian system of *tau* mutant and wild-type Syrian hamsters. *Neuroscience* 65: 587–597.
22. Best, J. D., Maywood, E. S., Smith, K. L., and Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *Journal of Neuroscience* 19: 828–835.
23. Daymude, J. A. and Refinetti, R. (1999). Phase-shifting effects of single and multiple light pulses in the golden hamster. *Biological Rhythm Research* 30: 202–215.
24. Elliott, J. A. and Tarmarkin, L. (1994). Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. *Journal of Comparative Physiology A* 174: 469–484.
25. Christian, C. A. and Harrington, M. E. (2002). Three days of novel wheel access diminishes light-induced phase delays in vivo with no effect on *per1* induction by light. *Chronobiology International* 19: 671–682.
26. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J., and Mrosovsky, M. (2003). Behavioral arousal blocks light-induced phase advances in locomotor rhythmicity but not light-induced Per1 and Fos expression in the hamster suprachiasmatic nucleus. *Neuroscience* 118: 253–261.
27. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2007). Circadian effects of light no brighter than moonlight. *Journal of Biological Rhythms* 22: 356–367.
28. Foster, R. G., Provencio, I., Hudson, D., Fiske, S., De Grip, W., and Menaker, M. (1991). Circadian photoreception in the retinally degenerate mouse (*rd/rd*). *Journal of Comparative Physiology A* 169: 39–50.
29. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
30. Khammanivong, A. and Nelson, D. E. (2000). Light pulses suppress responsiveness within the mouse photic entrainment pathway. *Journal of Biological Rhythms* 15: 393–405.
31. Yoshimura, T., Nishio, M., Goto, M., and Ebihara, S. (1994). Differences in circadian photosensitivity between retinally degenerate CBA/J mice (*rd/rd*) and normal CBA/M mice (+/+). *Journal of Biological Rhythms* 9: 51–60.
32. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
33. Kilduff, T. S., Vugrinic, C., Lee, S. L., Milbrandt, J. D., Mikkelsen, J. D., O'Hara, B. F., and Heller, H. C. (1998). Characterization of the circadian system of *NGFI-A* and *NGFI-A/NGFI-B* deficient mice. *Journal of Biological Rhythms* 13: 347–357.
34. Benloucif, S. and Dubocovich, M. L. (1996). Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN mouse. *Journal of Biological Rhythms* 11: 113–125.
35. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
36. Bullough, J. D., Figueiro, M. G., Possidente, B. P., Parsons, R. H., and Rea, M. S. (2005). Additivity in murine circadian phototransduction. *Zoological Science* 22: 223–227.
37. Seggio, J. A., Fixaris, M. C., Redd, J. D., Logan, R. W., and Rosenwasser, A. M. (2009). Chronic ethanol intake alters circadian phase shifting and free-running period in mice. *Journal of Biological Rhythms* 24: 304–312.
38. Etchegaray, J. P., Machida, K. K., Noton, E., Constance, C. M., Dallmann, R., Di Napoli, M. N., DeBruyne, J. P. et al. (2009). Casein kinase 1 δ regulates the pace of the mammalian circadian clock. *Molecular and Cellular Biology* 29: 3853–3866.
39. Van der Leest, H. T., Rohling, J. H. T., Michel, S., and Meijer, J. H. (2009). Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLOS ONE* 4: e4976.
40. Dallmann, R., DeBruyne, J. P., and Weaver, D. R. (2011). Photic resetting and entrainment in CLOCK-deficient mice. *Journal of Biological Rhythms* 26: 390–401.
41. Van Oosterhout, F., Fisher, S. P., van Diepen, H. C., Watson, T. S., Houben, T., van der Leest, H. T., Thompson, S., Peirson, S. N., Foster, R. G., and Meijer, J. H. (2012). Ultraviolet light provides a major input to non-image-forming light detection in mice. *Current Biology* 22: 1397–1402.
42. Sharma, V. K. (1996). Light-induced phase response curves of the circadian activity rhythm in individual field mice, *Mus booduga*. *Chronobiology International* 13: 401–409.
43. Sharma, V. K., Chandrashekaran, M. K., and Nongkynrih, P. (1997). Daylight and artificial light phase response curves for the circadian rhythm in locomotor activity of the field mouse *Mus booduga*. *Biological Rhythm Research* 28: 39–49.
44. Sharma, V. K. and Chandrashekaran, M. K. (1997). Rapid phase resetting of a mammalian circadian rhythm by brief light pulses. *Chronobiology International* 14: 537–548.
45. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
46. Sharma, V. K. (2003). Effect of light intensity on the phase and period response curves in the nocturnal field mouse *Mus booduga*. *Chronobiology International* 20: 223–231.
47. Shrama, V. K., Chidambaram, R., and Yadunandam, A. K. (2003). Melatonin enhances the sensitivity of circadian pacemakers to light in the nocturnal field mouse *Mus booduga*. *Journal of Experimental Zoology* 297A: 160–168.

48. Summer, T. L., Ferraro, J. S., and McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
49. Bauer, M. S. (1992). Irradiance responsivity and unequivocal type-1 phase responsivity of rat circadian activity rhythms. *American Journal of Physiology* 263: R1110–R1114.
50. Stokes, M. A., Kent, S., and Armstrong, S. M. (1999). The effect of multiple pulses of light on the circadian phase response of the rat. *Journal of Biological Rhythms* 14: 172–184.
51. Stokes, M. A., Kent, S., and Armstrong, S. M. (2002). The relationships between phase and period responses to light pulses. *Biological Rhythm Research* 33: 303–317.
52. Stepien, J. M. and Kennaway, D. J. (2001). Phase response relationships between light pulses and melatonin rhythms in rats. *Journal of Biological Rhythms* 16: 234–242.
53. Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sánchez, R., Ríos, C. D., Freitag, W. O., Richardson, G. S., and Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep–wake cycle. *Science* 233: 667–671.
54. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N., and Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.
55. Honma, K., Honma, S., and Wada, T. (1987). Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. *Experientia* 43: 1205–1207.
56. Honma, K. and Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167–168.
57. Dijk, D. J., Beersma, G. M., Daan, S., and Lewy, A. J. (1989). Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *American Journal of Physiology* 256: R106–R111.
58. Frennan, M., Kripke, D. F., and Gillin, J. C. (1989). Bright light can delay human temperature rhythm independent of sleep. *American Journal of Physiology* 257: R136–R141.
59. Shanahan, T. L. and Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *Journal of Clinical Endocrinology and Metabolism* 73: 227–235.
60. Buresová, M., Dvoráková, M., Zvolsky, P., and Illnerová, H. (1991). Early morning bright light phase advances the human circadian pacemaker within one day. *Neuroscience Letters* 121: 47–50.
61. Dawson, D., Lack, L., and Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102.
62. Boivin, D. B., Duffy, J. F., Kronauer, R. E., and Czeisler, C. A. (1996). Dose–response relationships for resetting of human circadian clock by light. *Nature* 379: 540–542.
63. Honma, K., Honma, S., Nakamura, K., Sasaki, M., Endo, T., and Takahashi, T. (1995). Differential effects of bright light and social cues on reentrainment of human circadian rhythms. *American Journal of Physiology* 268: R528–R535.
64. Tzischinsky, O. and Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.
65. Shanahan, T. L., Kronauer, R. E., Duffy, J. F., Williams, G. H., and Czeisler, C. A. (1999). Melatonin rhythm observed throughout a three-cycle bright-light stimulus designed to reset the human circadian pacemaker. *Journal of Biological Rhythms* 14: 237–253.
66. Zeitzer, J. M., Dijk, D. J., Kronauer, R. E., Brown, E. N., and Czeisler, C. A. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: Melatonin phase resetting and suppression. *Journal of Physiology* 526: 695–702.
67. Khalsa, S. B. S., Jewett, M. E., Cajochen, C., and Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.
68. Lockley, S. W., Brainard, G. C., and Czeisler, C. A. (2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *Journal of Clinical Endocrinology and Metabolism* 88: 4502–4505.
69. Warman, V. L., Dijk, D. J., Warman, G. R., Arendt, J., and Skene, D. J. (2003). Phase advancing human circadian rhythms with short wavelength light. *Neuroscience Letters* 342: 37–40.
70. Burgess, H. J., Fogg, L. F., Young, M. A., and Eastman, C. I. (2004). Bright light therapy for winter depression: Is phase advancing beneficial? *Chronobiology International* 21: 759–775.
71. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C., and Weber, J. M. (2004). Evening melatonin and bright light administration induce additive phase shifts in dim light melatonin onset. *Journal of Pineal Research* 36: 192–194.
72. St. Hilaire, M. A., Gooley, J. J., Khalsa, S. B. S., Kronauer, R. E., Czeisler, C. A., and Lockley, S. W. (2012). Human phase response curve to a 1 h pulse of bright white light. *Journal of Physiology* 590: 3035–3045.
73. Rüger, M., St Hilaire, M. A., Brainard, G. C., Khalsa, S. S., Kronauer, R. E., Czeisler, C. A., and Lockley, S. W. (2013). Human phase response curve to a single 6.5 h pulse of short-wavelength light. *Journal of Physiology* 591: 353–363.
74. Mien, I. H., Chua, E. C., Lau, P., Tan, L. C., Lee, I. T., Yeo, S. C., Tan, S. S., and Gooley, J. J. (2014). Effects of exposure to intermittent versus continuous red light on human circadian rhythms, melatonin suppression, and pupillary constriction. *PLOS ONE* 9: e96532.
75. Pohl, H. (1983). Light pulses entrain the circadian activity rhythm of a diurnal rodent (*Ammospermophilus leucurus*). *Comparative Biochemistry and Physiology B* 76: 723–729.
76. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P., and Challet, E. (2003). Circadian profile and photic regulation of clock genes in the suprachiasmatic nucleus of a diurnal mammal, *Arvicanthis ansorgei*. *Neuroscience* 116: 583–591.
77. Mahoney, M., Bult, A., and Smale, L. (2001). Phase response curve and light-induced Fos expression in suprachiasmatic nucleus and adjacent hypothalamus of *Arvicanthis niloticus*. *Journal of Biological Rhythms* 16: 149–162.
78. Refinetti, R. (2004). Parameters of photic resetting of the circadian system of a diurnal rodent, the Nile grass rat. *Acta Scientiae Veterinariae* 32: 1–6.
79. Tavernier, R. J., Largen, A. L., and Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
80. Honma, S. and Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.

81. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
82. DeCoursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science* 131: 33–35.
83. Pohl, H. (1985). The circadian system of the Turkish hamster, *Mesocricetus brandti*: Responses to light. *Comparative Biochemistry and Physiology A* 81: 613–618.
84. Lee, T. M. and Labyak, S. E. (1997). Free-running rhythms and light- and dark-pulse phase response curves for diurnal *Octodon degus* (Rodentia). *American Journal of Physiology* 273: R278–R286.
85. Kas, M. J. H. and Edgar, D. M. (2000). Photic phase response curve in *Octodon degus*: Assessment as a function of activity phase preference. *American Journal of Physiology* 278: R1385–R1389.
86. Puchalski, W. and Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: Effects of photoperiodic pretreatment and artificial selection. *American Journal of Physiology* 261: R670–R676.
87. Kramm, K. R. and Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.
88. Meijer, J. H., Daan, S., Overkamp, G. J. F., and Hermann, P. M. (1990). The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *Journal of Biological Rhythms* 5: 1–16.
89. Schilling, A., Richard, J. P., and Servière, J. (1999). Duration of activity and period of circadian activity-rest rhythm in a photoperiod-dependent primate, *Microcebus murinus*. *Comptes Rendus de l'Académie des Sciences* 322: 759–770.
90. Glass, J. D., Tardif, S. D., Clements, R., and Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
91. Slotten, H. A., Krekling, S., and Pévet, P. (2005). Photic and nonphotic effects on the circadian activity rhythm in the diurnal rodent *Arvicanthis ansorgei*. *Behavioural Brain Research* 165: 91–97.
92. Lahmam, M., El M'rabet, A., Ouarour, A., Pévet, P., Challet, E., and Vuillez, P. (2008). Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent *Lemniscomys barbarus*. *Chronobiology International* 25: 882–904.
93. Flôres, D. E. F., Tomotani, B. M., Tachinardi, P., Oda, G. A., and Valentinuzzi, V. S. (2013). Modeling natural photic entrainment in a subterranean rodent (*Ctenomys knighti*), the tuco-tuco. *PLOS ONE* 8: e68243.
94. Kumar, D. and Singaravel, M. (2014). Phase and period responses to short light pulses in a wild diurnal rodent, *Funambulus pennanti*. *Chronobiology International* 31: 320–327.
95. Fernandez, F., Lu, D., Ha, P., Costacurta, P., Chavez, R., Heller, H. C., and Ruby, N. F. (2014). Dysrhythmia in the suprachiasmatic nucleus inhibits memory processing. *Science* 346: 854–857.
96. Aschoff, J. (1965). Response curves in circadian periodicity. In: Aschoff, J. (Ed.), *Circadian Clocks*. Amsterdam, the Netherlands: North-Holland, pp. 95–111.
97. Johnson, C. H. (1999). Forty years of PRCs: What have we learned? *Chronobiology International* 16: 711–743.
98. Colwell, C. S., Kaufman, C. M., Menaker, M., and Ralph, M. R. (1993). Light-induced phase shifts and Fos expression in the hamster circadian system: The effects of anesthetics. *Journal of Biological Rhythms* 8: 179–188.
99. Meijer, J. H., Rusak, B., and Gänshirt, G. (1992). The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms. *Brain Research* 598: 257–263.
100. Muscat, L. and Morin, L. P. (2005). Binocular contributions to the responsiveness and integrative capacity of the circadian rhythm system to light. *Journal of Biological Rhythms* 20: 513–525.
101. Agostino, P. V., Harrington, M. E., Ralph, M. R., and Golombek, D. A. (2009). Casein kinase 1 ε and circadian photic responses in hamsters. *Chronobiology International* 26: 126–133.
102. Erkert, H. G. (2004). Extremely low threshold for photic entrainment of circadian activity rhythms in molossid bats (*Molossus molossus*; Chiroptera, Molossidae). *Mammalian Biology* 69: 361–374.
103. Erkert, H. G., Gburek, V., and Scheideler, A. (2006). Photic entrainment and masking of prosimian circadian rhythms (*Otolemur garnettii*, Primates). *Physiology and Behavior* 88: 39–46.
104. Rimmer, D. W., Boivin, D. B., Shanahan, T. L., Kronauer, R. E., Duffy, J. F., and Czeisler, C. A. (2000). Dynamic resetting of the human circadian pacemaker by intermittent bright light. *American Journal of Physiology* 279: R1574–R1579.
105. Middleton, B., Stone, B. M., and Arendt, J. (2002). Human circadian phase in 12:12 h, 200:8 lux and 1000:8 lux light–dark cycles, without scheduled sleep or activity. *Neuroscience Letters* 329: 41–44.
106. Barlow, H. B. and Mollon, J. D. (1982). Psychophysical measurements of visual performance. In: Barlow, H. B. and Mollon, J. D. (Eds.), *The Senses*. New York: Cambridge University Press, pp. 114–132.
107. Goldstein, E. B. (2002). *Sensation and Perception*, 6th edn. Pacific Grove, CA: Wadsworth.
108. Comas, M., Beersma, D. G. M., Spoelstra, K., and Daan, S. (2006). Phase and period responses of the circadian system of mice (*Mus musculus*) to light stimuli of different duration. *Journal of Biological Rhythms* 21: 362–372.
109. Van den Pol, A. N., Cao, V., and Heller, H. C. (1998). Circadian system of mice integrates brief light stimuli. *American Journal of Physiology* 275: R654–R657.
110. Nelson, D. E. and Takahashi, J. S. (1999). Integration and saturation within the circadian photic entrainment pathway of hamsters. *American Journal of Physiology* 277: R1351–R1361.
111. Gronfier, C., Wright, K. P., Kronauer, R. E., Jewett, M. E., and Czeisler, C. A. (2004). Efficacy of a single sequence of intermittent bright light pulses for delaying circadian phase in humans. *American Journal of Physiology* 287: E174–E181.
112. Winfree, A. T. (1971). Corkscrews and singularities in fruitflies: Resetting behavior of the circadian eclosion rhythm. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 81–106.
113. Shaw, J. and Brody, S. (2000). Circadian rhythms in *Neurospora*: A new measurement, the reset zone. *Journal of Biological Rhythms* 15: 225–240.
114. Strogatz, S. H. (1990). Interpreting the human phase response curve to multiple bright-light exposures. *Journal of Biological Rhythms* 5: 169–174.
115. Beersma, D. G. M. and Daan, S. (1993). Strong or weak phase resetting by light pulses in humans? *Journal of Biological Rhythms* 8: 340–347.
116. Jewett, M. E., Kronauer, R. E., and Czeisler, C. A. (1994). Phase-amplitude resetting of the human circadian pacemaker via bright light: A further analysis. *Journal of Biological Rhythms* 9: 295–314.

117. Pittendrigh, C. S. (1981). Circadian systems: Entrainment. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 95–124.
118. Daan, S. (2000). Colin Pittendrigh, Jürgen Aschoff, and the natural entrainment of circadian systems. *Journal of Biological Rhythms* 15: 195–207.
119. Aschoff, J. (1999). Masking and parametric effects of high-frequency light–dark cycles. *Japanese Journal of Physiology* 49: 11–18.
120. Aschoff, J. (1979). Circadian rhythms: Influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
121. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. III. Heavy water and constant light: Homeostasis of frequency? *Journal of Comparative Physiology* 106: 267–290.
122. Aschoff, J. (1990). From temperature regulation to rhythm research. *Chronobiology International* 7: 179–186.
123. Trivedi, A. K., Rani, S., and Kumar, V. (2006). Natural daylight restricted to twilights delays the timing of testicular regression but does not affect the timing of the daily activity rhythm of the house sparrow (*Passer domesticus*). *Journal of Circadian Rhythms* 4: art. 5.
124. Sharma, V. K. and Chidambaram, R. (2002). Intensity-dependent phase-adjustments in the locomotor activity rhythm of the nocturnal field mouse *Mus booduga*. *Journal of Experimental Zoology* 292: 444–459.
125. Borbély, A. A. and Neuhaus, H. U. (1978). Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. *Journal of Comparative Physiology* 128: 37–46.
126. Rosenwasser, A. M., Boulos, Z., and Terman, M. (1983). Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiology and Behavior* 30: 353–359.
127. Terman, M. and Terman, J. (1985). A circadian pacemaker for visual sensitivity? *Annals of the New York Academy of Sciences* 453: 147–161.
128. Beaulé, C., Barry-Shaw, J., and Amir, S. (2004). Fos expression in the suprachiasmatic nucleus during photic entrainment of circadian rhythms in retinally damaged rats. *Journal of Molecular Neuroscience* 22: 223–230.
129. Patton, D. F., Parfyonov, M., Gourmelen, S., Opiol, H., Pavlovski, I., Marchant, E. G., Challet, E., and Mistlberger, R. E. (2013). Photic and pineal modulation of food anticipatory circadian activity rhythms in rodents. *PLOS ONE* 8: e81588.
130. Goldman, B. D., Goldman, S. L., Riccio, A. P., and Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
131. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1982). Circadian entrainment of the squirrel monkey by extreme photoperiods: Interactions between the phasic and tonic effects of light. *Physiology and Behavior* 29: 637–641.
132. Joshi, D. S. and Vanlalnghaka, C. (2005). Non-parametric entrainment by natural twilight in the microchiropteran bat, *Hipposideros speoris*, inside a cave. *Chronobiology International* 22: 631–640.
133. Ebling, F. J. P., Lincoln, G. A., Wollnik, F., and Anderson, N. (1988). Effects of constant darkness and constant light on circadian organization and reproductive responses in the ram. *Journal of Biological Rhythms* 3: 365–384.
134. DeCoursey, P. J., Krulas, J. R., Mele, G., and Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior* 62: 1099–1108.
135. Hamblen-Coyle, M. J., Wheeler, D. A., Rutila, J. E., Rosbash, M., and Hall, J. C. (1992). Behavior of period-altered circadian rhythm mutants of *Drosophila* in light:dark cycles (Diptera: Drosophilidae). *Journal of Insect Behavior* 5: 417–446.
136. Rieger, D., Fraunholz, C., Popp, J., Bichler, D., Dittmann, R., and Helfrich-Förster, C. (2007). The fruit fly *Drosophila melanogaster* favors dim light and times its activity peaks to early dawn and late dusk. *Journal of Biological Rhythms* 22: 387–399.
137. Pohl, H. (1976). Proportional effect of light on entrained circadian rhythms of birds and mammals. *Journal of Comparative Physiology* 112: 103–108.
138. Riccio, A. P. and Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
139. Honma, K., Honma, S., and Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
140. Wever, R. A. (1989). Light effects on human circadian rhythms: A review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
141. Beeler, J. A., Prendergast, B., and Zhuang, X. (2006). Low amplitude entrainment of mice and the impact of circadian phase on behavior tests. *Physiology and Behavior* 87: 870–880.
142. Walmsley, L., Hanna, L., Mouland, J., Martial, F., West, A., Smedley, A. R., Bechtold, D. A., Webb, A. R., Lucas, R. J., and Brown, T. M. (2015). Colour as a signal for entraining the mammalian circadian clock. *PLoS Biology* 13: e1002127.
143. Kasai, M. and Kiyohara, S. (2010). Food- and light-entrainable oscillators control feeding and locomotor activity rhythms, respectively, in the Japanese catfish, *Plotosus japonicus*. *Journal of Comparative Physiology A* 196: 901–912.
144. Fuller, C. A. and Edgar, D. M. (1986). Effects of light intensity on the circadian temperature and feeding rhythms in the squirrel monkey. *Physiology and Behavior* 36: 687–691.
145. Ebihara, S. and Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
146. Lovegrove, B. G., Heldmaier, G., and Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
147. Lovegrove, B. G. and Papenfus, M. E. (1995). Circadian activity rhythms in the solitary Cape molerat (*Georychus capensis*: Bathyergidae) with some evidence of splitting. *Physiology and Behavior* 58: 679–685.
148. Oosthuizen, M. K., Cooper, H. M., and Bennett, N. C. (2003). Circadian rhythms of locomotor activity in solitary and social species of African mole-rats (Family: Bathyergidae). *Journal of Biological Rhythms* 18: 481–490.
149. Rappold, I. and Erkert, H. G. (1994). Re-entrainment, phase-response and range of entrainment of circadian rhythms in owl monkeys (*Aotus lemurinus g.*) of different age. *Biological Rhythm Research* 25: 133–152.
150. Refinetti, R. (2007). Absence of circadian and photoperiodic conservation of energy expenditure in three rodent species. *Journal of Comparative Physiology B* 177: 309–318.

151. Häster, L. and Erkert, H. G. (1993). Alteration of circadian period length does not influence the ovarian cycle length in common marmosets, *Callithrix j. jacchus* (Primates). *Chronobiology International* 10: 165–175.
152. Wheeler, D. A., Hamblen-Coyle, M. J., Dushay, M. S., and Hall, J. C. (1993). Behavior in light–dark cycles of *Drosophila* mutants that are arrhythmic, blind, or both. *Journal of Biological Rhythms* 8: 67–94.
153. Waterhouse, J., Minors, D., Folkard, S., Owens, D., Atkinson, G., Macdonald, I., Reilly, T., Sytnik, N., and Tucker, P. (1998). Light of domestic intensity produces phase shifts of the circadian oscillator in humans. *Neuroscience Letters* 245: 97–100.
154. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A., and Dijk, D. J. (1999). Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *American Journal of Physiology* 277: R1152–R1163.
155. Simpson, H. W., Lobban, M. C., and Halberg, F. (1970). Urinary near-24-hour rhythms in subjects living on a 21-hour routine in the Arctic. *Arctic Anthropology* 7: 144–164.
156. Carmichael, M. S., Nelson, R. J., and Zucker, I. (1981). Hamster activity and estrous cycles: Control by a single versus multiple circadian oscillator(s). *Proceedings of the National Academy of Sciences United States of America* 78: 7830–7834.
157. Refinetti, R., Nelson, D. E., and Menaker, M. (1992). Social stimuli fail to act as entraining agents of circadian rhythms in the golden hamster. *Journal of Comparative Physiology A* 170: 181–187.
158. Reebs, S. G. and Doucet, P. (1997). Relationship between circadian period and size of phase shifts in Syrian hamsters. *Physiology and Behavior* 61: 661–666.
159. Shimomura, K., Nelson, D. E., Ihara, N. L., and Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
160. Osiel, S., Golombek, D. A., and Ralph, M. R. (1998). Conservation of locomotor behavior in the golden hamster: Effects of light cycle and a circadian period mutation. *Physiology and Behavior* 65: 123–131.
161. Chiesa, J. J., Anglèe-Pujolràs, M., Díez-Noguera, A., and Cambras, T. (2005). Activity rhythm of golden hamsters (*Mesocricetus auratus*) can be entrained to a 19-h light–dark cycle. *American Journal of Physiology* 289: R998–R1005.
162. Chiesa, J. J., Díez-Noguera, A., and Cambras, T. (2007). Effects of transient and continuous wheel running activity on the upper and lower limits of entrainment to light–dark cycles in female hamsters. *Chronobiology International* 24: 215–234.
163. Davis, F. C. and Menaker, M. (1981). Development of the mouse circadian pacemaker: Independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
164. Perrigo, G., Bryant, W. C., and vom Saal, F. S. (1990). A unique neural timing system prevents male mice from harming their own offspring. *Animal Behaviour* 39: 535–539.
165. Aton, S. J., Block, G. D., Tei, H., Yamazaki, S., and Herzog, E. D. (2004). Plasticity of circadian behavior and the suprachiasmatic nucleus following exposure to non-24-hour light cycles. *Journal of Biological Rhythms* 19: 198–207.
166. Udo, R., Hamada, T., Horikawa, K., Iwahana, E., Miyakawa, K., Otsuka, K., and Shibata, S. (2004). The role of *Clock* in the plasticity of circadian entrainment. *Biochemical and Biophysical Research Communications* 318: 893–898.
167. Hamasaka, Y., Watari, Y., Arai, T., Numata, H., and Shiga, S. (2001). Retinal and extraretinal pathways for entrainment of the circadian activity rhythm in the blow fly, *Protophormia terraenovae*. *Journal of Insect Physiology* 47: 867–875.
168. Weber, A. L. and Adler, N. T. (1978). Dissociation of persistent estrus and a free-running activity cycle in different light cycles. *Biology of Reproduction* 19: 425–430.
169. Sridaran, R. and McCormack, C. E. (1980). Parallel effects of light signals on the circadian rhythms of running activity and ovulation in rats. *Journal of Endocrinology* 85: 111–120.
170. Vilaplana, J., Cambras, T., Campuzano, A., and Díez-Noguera, A. (1997). Simultaneous manifestations of free-running and entrained rhythms in the rat motor activity explained by a multioscillatory system. *Chronobiology International* 14: 9–18.
171. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M., and Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
172. Usui, S., Takahashi, Y., and Okazaki, T. (2000). Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. *American Journal of Physiology* 278: R1148–R1156.
173. Canal-Corretger, M. M., Cambras, T., and Díez-Noguera, A. (2003). Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiology International* 20: 21–35.
174. Cambras, T., Chiesa, J., Araujo, J., and Díez-Noguera, A. (2004). Effects of photoperiod on rat motor activity rhythm at the lower limit of entrainment. *Journal of Biological Rhythms* 19: 216–225.
175. Hut, R. A., Mrosovsky, N., and Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
176. Boulos, Z., Macchi, M. M., and Terman, M. (2002). Twilights widen the range of photic entrainment in hamsters. *Journal of Biological Rhythms* 17: 353–363.
177. Gorman, M. R., Kendall, M., and Elliott, J. A. (2005). Scotopic illumination enhances entrainment of circadian rhythms to lengthening light:dark cycles. *Journal of Biological Rhythms* 20: 38–48.
178. Anglès-Pujolràs, M., Chiesa, J. J., Díez-Noguera, A., and Cambras, T. (2006). Motor activity rhythms of forced desynchronized rats subjected to restricted feeding. *Physiology and Behavior* 88: 30–38.
179. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
180. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
181. Refinetti, R. (2010). Entrainment of circadian rhythm by ambient temperature cycles in mice. *Journal of Biological Rhythms* 25: 247–256.
182. Liu, C. and Reppert, S. M. (2000). GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron* 25: 123–128.
183. Sharma, V. K., Chidambaram, R., and Chandrashekaran, M. K. (2000). Probing the circadian pacemaker of a mouse using two light pulses. *Journal of Biological Rhythms* 15: 67–73.
184. Watanabe, K., Deboer, T., and Meijer, J. H. (2001). Light-induced resetting of the circadian pacemaker: Quantitative analysis of transient versus steady-state phase shifts. *Journal of Biological Rhythms* 16: 564–573.

185. Asai, M., Yamaguchi, S., Isejima, H., Janouchi, M., Moriya, T., Shibata, S., Kobayashi, M., and Okamura, H. (2001). Visualization of *mPer1* transcription in vitro: NMDA induces a rapid shift of *mPer1* gene in cultured SCN. *Current Biology* 11: 1524–1527.
186. Tapp, W. N. and Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
187. Takamure, M., Murakami, N., Takahashi, K., Huroda, H., and Etoh, T. (1991). Rapid reentrainment of the circadian clock itself, but not the measurable activity rhythms, to a new light–dark cycle in the rat. *Physiology and Behavior* 50: 443–449.
188. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O., and Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
189. Basu, P. and Singaravel, M. (2013). Accurate and precise circadian locomotor activity rhythms in male and female Indian pygmy field mice, *Mus terricolor. Biological Rhythm Research* 44: 531–539.
190. Hut, R. A., Paolucci, S., Dor, R., Kyriacou, C. P., and Daan, S. (2013). Latitudinal clines: An evolutionary view on biological rhythms. *Proceedings of the Royal Society B* 280: 20130433.
191. Bünning, E. (1960). Circadian rhythms and the time measurement in photoperiodism. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 249–256.
192. Rieger, D., Stanewsky, R., and Helfrich-Förster, C. (2003). Cryptochrome, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster. Journal of Biological Rhythms* 18: 377–391.
193. Chakraborty, S. C., Ross, L. G., and Ross, B. (1992). The effect of photoperiod on the resting metabolism of carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology A* 101: 77–82.
194. Herrero, M. J., Madrid, J. A., and Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
195. Foà, A. and Bertolucci, C. (2001). Temperature cycles induce a bimodal activity pattern in ruin lizards: Masking or clock-controlled event? *Journal of Biological Rhythms* 16: 574–584.
196. Basco, P. S., Rashotte, M. E., and Stephan, F. K. (1996). Photoperiod duration and energy balance in the pigeon. *Physiology and Behavior* 60: 151–159.
197. Veghte, J. H. (1965). Thermal and metabolic responses of the gray jay to cold stress. *Physiological Zoology* 37: 316–328.
198. Brandstätter, R., Kumar, V., Abraham, U., and Gwinner, E. (2000). Photoperiodic information acquired and stored *in vivo* retained *in vitro* by a circadian oscillator, the avian pineal gland. *Proceedings of the National Academy of Sciences United States of America* 97: 12324–12328.
199. Haim, A., Yedidia, I., Haim, D., and Zisapel, N. (1994). Photoperiodicity in daily rhythms of body temperature, food and energy intake of the golden spiny mouse (*Acomys russatus*). *Israel Journal of Zoology* 40: 145–150.
200. Haim, A. and Zisapel, N. (1995). Oxygen consumption and body temperature rhythms in the golden spiny mouse: Responses to changes in day length. *Physiology and Behavior* 58: 775–778.
201. Haim, A., McDevitt, R. M., and Speakman, J. R. (1995). Thermoregulatory responses to manipulations of photoperiod in wood mice *Apodemus sylvaticus* from high latitudes. *Journal of Thermal Biology* 20: 437–443.
202. Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. *Physiology and Behavior* 82: 285–294.
203. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J., and Simonneaux, V. (2003). Mechanisms regulating the marked seasonal variation in melatonin synthesis in the European hamster pineal gland. *American Journal of Physiology* 284: R1043–R1052.
204. Vuillez, P., Jacob, N., Teclemariam-Mesbah, R., and Pévet, P. (1996). In Syrian and European hamsters, the duration of sensitive phase to light of the suprachiasmatic nuclei depends on the photoperiod. *Neuroscience Letters* 208: 37–40.
205. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2004). Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. *American Journal of Physiology* 286: R539–R546.
206. Mrugala, M., Zlomanczuk, P., Jagota, A., and Schwartz, W. J. (2000). Rhythmic multiunit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *American Journal of Physiology* 278: R987–R994.
207. Messager, S., Ross, A. W., Barrett, P., and Morgan, P. J. (1999). Decoding photoperiodic time through *Per1* and ICER gene amplitude. *Proceedings of the National Academy of Sciences United States of America* 96: 9938–9943.
208. Pitrosky, B., Kirsch, R., Vivien-Roels, B., Georg-Bentz, I., Canguilhem, B., and Pévet, P. (1995). The photoperiodic response in Syrian hamster depends upon a melatonin-driven circadian rhythm of sensitivity to melatonin. *Journal of Neuroendocrinology* 7: 889–895.
209. Anchordoquy, H. C. and Lynch, G. R. (2000). Timing of testicular recrudescence in Siberian hamsters is unaffected by pinealectomy or long-day photoperiod after 9 weeks in short days. *Journal of Biological Rhythms* 15: 406–416.
210. Gorman, M. R. (2003). Independence of circadian entrainment state and responses to melatonin in male Siberian hamsters. *BMC Physiology* 3: art. 10.
211. Deboer, T., Vyazovskiy, V. V., and Tobler, I. (2000). Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 429–436.
212. Larkin, J. E., Freeman, D. A., and Zucker, I. (2001). Low ambient temperature accelerates short-day responses in Siberian hamsters by altering responsiveness to melatonin. *Journal of Biological Rhythms* 16: 76–86.
213. Sumová, A., Jác, M., Sládek, M., Sauman, I., and Illnerová, H. (2003). Clock gene daily profiles and their phase relationship in the rat suprachiasmatic nucleus are affected by photoperiod. *Journal of Biological Rhythms* 18: 134–144.
214. Humlová, M. and Illnerová, H. (1992). Resetting of the rat circadian clock after a shift in the light/dark cycle depends on the photoperiod. *Neuroscience Research* 13: 147–153.
215. Sumová, A., Trávnícková, Z., and Illnerová, H. (1995). Memory on long but not on short days is stored in the rat suprachiasmatic nucleus. *Neuroscience Letters* 200: 191–194.
216. Sumová, A., Trávnícková, Z., and Illnerová, H. (2000). Spontaneous c-Fos rhythm in the rat suprachiasmatic nucleus: Location and effect of photoperiod. *American Journal of Physiology* 279: R2262–R2269.

217. Jác, M., Sumová, A., and Illnerová, H. (2000). c-Fos rhythm in subdivisions of the rat suprachiasmatic nucleus under artificial and natural photoperiods. *American Journal of Physiology* 279: R2270–R2276.
218. Haim, A., Downs, C. T., and Raman, J. (2001). Effects of adrenergic blockade on the daily rhythms of body temperature and oxygen consumption of the black-tailed tree rat (*Thallomys nigricauda*) maintained under different photoperiods. *Journal of Thermal Biology* 26: 171–177.
219. Schaap, J., Albus, H., vander Leest, H. T., Eilers, P. H. C., Détári, L., and Meijer, J. H. (2003). Heterogeneity of rhythmic suprachiasmatic nucleus neurons: Implications for circadian waveform and photoperiodic encoding. *Proceedings of the National Academy of Sciences United States of America* 100: 15994–15999.
220. Larkin, J. E., Jones, J., and Zucker, I. (2002). Temperature dependence of gonadal regression in Syrian hamsters exposed to short day lengths. *American Journal of Physiology* 282: R744–R752.
221. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2005). Circadian entrainment and phase resetting differ markedly under dimly illuminated versus completely dark nights. *Behavioural Brain Research* 162: 116–126.
222. Cheng, M. Y., Bittman, E. L., Hattar, S., and Zhou, Q. Y. (2005). Regulation of prokineticin 2 expression by light and the circadian clock. *BMC Neuroscience* 6: art. 17.
223. Weinert, D., Weinandy, R., and Gattermann, R. (2007). Photic and non-photic effects on the daily activity pattern of Mongolian gerbils. *Physiology and Behavior* 90: 325–333.
224. Monecke, S. and Wollnik, F. (2005). Seasonal variations in circadian rhythms coincide with a phase of sensitivity to short photoperiods in the European hamster. *Journal of Comparative Physiology B* 175: 167–183.
225. Williams, C. T., Barnes, B. M., and Buck, C. L. (2012). Daily body temperature rhythms persist under the midnight sun but are absent during hibernation in free-living Arctic ground squirrels. *Biology Letters* 8: 31–34.
226. Lincoln, G., Messager, S., Andersson, H., and Hazlerigg, D. (2002). Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: Evidence for an internal coincidence timer. *Proceedings of the National Academy of Sciences United States of America* 99: 13890–13895.
227. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2001). Seasonal variation in endogenous serum melatonin profiles in goats: A difference between spring and fall? *Journal of Biological Rhythms* 16: 254–263.
228. Génin, F. and Perret, M. (2003). Daily hypothermia in captive grey mouse lemurs (*Microcebus murinus*): Effects of photoperiod and food restriction. *Comparative Biochemistry and Physiology B* 136: 71–81.
229. Perret, M. and Aujard, F. (2001). Daily hypothermia and torpor in a tropical primate: Synchronization by 24-h light–dark cycle. *American Journal of Physiology* 281: R1925–R1933.
230. Wehr, T. A., Aeschbach, D., and Duncan, W. C. (2001). Evidence for a biological dawn and dusk in the human circadian timing system. *Journal of Physiology* 535: 937–951.
231. Andersson, H., Johnston, J. D., Messager, S., Hazlerigg, D., and Lincoln, G. (2005). Photoperiod regulates clock gene rhythms in the ovine liver. *General and Comparative Endocrinology* 142: 357–363.
232. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
233. Aschoff, J. (1981). Freerunning and entrained circadian rhythms. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 81–93.
234. Mrosovsky, N. (1999). Masking: History, definitions, and measurement. *Chronobiology International* 16: 415–429.
235. Aschoff, J. and Von Saint Paul, U. (1976). Brain temperature in the unanesthetized chicken: Its circadian rhythm of responsiveness to light. *Brain Research* 101: 1–9.
236. Strubbe, J. H., Spiteri, N. J., and Alingh Prins, A. J. (1986). Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats. *Physiology and Behavior* 36: 647–651.
237. Lincoln, G. A., Andersson, H., and Hazlerigg, D. (2003). Clock genes and the long-term regulation of prolactin secretion: Evidence for a photoperiodic/circannual timer in the pars tuberalis. *Journal of Neuroendocrinology* 15: 390–397.
238. Cajochen, C., Dijk, D. J., and Borbély, A. A. (1992). Dynamics of EEG slow-wave activity and core body temperature in human sleep after exposure to bright light. *Sleep* 15: 337–343.
239. Sone, Y., Hyun, K., Nishimura, S., Lee, Y., and Tokura, H. (2003). Effects of dim or bright-light exposure during the daytime on human gastrointestinal activity. *Chronobiology International* 20: 123–133.
240. De Groot, M. H. M. and Rusak, B. (2002). Entrainment impaired, masking spared: An apparent genetic abnormality that prevents circadian rhythm entrainment to 24-h lighting cycles in California mice. *Neuroscience Letters* 327: 203–207.
241. Gall, A. J., Smale, L., Yan, L., and Nunez, A. A. (2013). Lesions of the intergeniculate leaflet lead to a reorganization in circadian regulation and a reversal in masking responses to photic stimuli in the Nile grass rat. *PLOS ONE* 8: e67387.
242. van der Veen, D. R. and Archer, S. N. (2010). Light-dependent behavioral phenotypes in PER3-deficient mice. *Journal of Biological Rhythms* 25: 3–8.
243. Morin, L. P., Lituma, P. J., and Studholme, K. M. (2010). Two components of nocturnal locomotor suppression by light. *Journal of Biological Rhythms* 25: 197–207.
244. Doi, M., Yujnovsky, I., Hirayama, J., Malerba, M., Tirotta, E., Sassone-Corsi, P., and Borrelli, E. (2006). Impaired light masking in dopamine D2 receptor-null mice. *Nature Neuroscience* 9: 732–734.
245. Doyle, S. E., Castrucci, A. M., McCall, M., Provencio, I., and Menaker, M. (2006). Nonvisual light responses in the *Rpe65* knockout mouse: Rod loss restores sensitivity to the melanopsin system. *Proceedings of the National Academy of Sciences United States of America* 103: 10432–10437.
246. Li, X., Gilbert, J., and Davis, F. C. (2005). Disruption of masking by hypothalamic lesions in Syrian hamsters. *Journal of Comparative Physiology A* 191: 23–30.
247. Jung, C. M., Khalsa, S. B. S., Scheer, F. A. J., Cajochen, C., Lockley, S. W., Czeisler, C. A., and Wright, K. P. (2010). Acute effects of bright light exposure on cortisol levels. *Journal of Biological Rhythms* 25: 208–216.
248. Mrosovsky, N., Foster, R. G., and Salmon, P. A. (1999). Thresholds for masking responses to light in three strains of retinally degenerate mice. *Journal of Comparative Physiology A* 184: 423–428.
249. Redlin, U. and Mrosovsky, N. (1999). Masking of locomotor activity in hamsters. *Journal of Comparative Physiology A* 184: 429–437.
250. Redlin, U. and Mrosovsky, N. (1999). Masking by light in hamsters with SCN lesions. *Journal of Comparative Physiology A* 184: 439–448.

251. Edelstein, K. and Mrosovsky, N. (2001). Behavioral responses to light in mice with dorsal lateral geniculate lesions. *Brain Research* 918: 107–112.
252. Mrosovsky, N. and Hattar, S. (2003). Impaired masking responses to light in melanopsin-knockout mice. *Chronobiology International* 20: 989–999.
253. Mrosovsky, N. (2003). Contribution of classic photoreceptors to entrainment. *Journal of Comparative Physiology A* 189: 69–73.
254. Redlin, U., Cooper, H. M., and Mrosovsky, N. (2003). Increased masking response to light after ablation of the visual cortex in mice. *Brain Research* 965: 1–8.
255. Redlin, U. and Mrosovsky, N. (2004). Nocturnal activity in a diurnal rodent (*Arvicanthis niloticus*): The importance of masking. *Journal of Biological Rhythms* 19: 58–67.
256. Mrosovsky, N. and Hattar, S. (2005). Diurnal mice (*Mus musculus*) and other examples of temporal niche switching. *Journal of Comparative Physiology A* 191: 1011–1024.
257. Refinetti, R. (2000). Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 73: 575–583.
258. Beersma, D. G. M., Daan, S., and Hut, R. A. (1999). Accuracy of circadian entrainment under fluctuating light conditions: Contributions of phase and period responses. *Journal of Biological Rhythms* 14: 320–329.
259. Sharma, V. K. and Daan, S. (2002). Circadian phase and period responses to light stimuli in two nocturnal rodents. *Chronobiology International* 19: 659–670.
260. Stokes, M. A., Kent, S., and Armstrong, S. M. (2001). The effect of repeated pulses of light at the same time on period responses of the rat circadian pacemaker. *Chronobiology International* 18: 187–201.
261. Comas, M., Beersma, D. G. M., Spoelstra, K., and Daan, S. (2007). Circadian response reduction in light and response restoration in darkness: A "skeleton" light pulse PRC study in mice (*Mus musculus*). *Journal of Biological Rhythms* 22: 432–444.
262. Rosenwasser, A. M., Logan, R. W., and Fecteau, M. E. (2005). Chronic ethanol intake alters circadian period-responses to brief light pulses. *Chronobiology International* 22: 227–236.
263. Weisgerber, D., Redlin, U., and Mrosovsky, N. (1997). Lengthening of circadian period in hamsters by novelty-induced wheel running. *Physiology and Behavior* 62: 759–765.
264. Page, T. L., Wassmer, G. T., Fletcher, J., and Block, G. D. (1997). Aftereffects of entrainment on the period of the pacemaker in the eye of the mollusk *Bulla gouldiana*. *Journal of Biological Rhythms* 12: 218–225.
265. Page, T. L. (2000). A novel mechanism for the control of circadian clock period by light. *Journal of Biological Rhythms* 15: 155–162.
266. Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.
267. Bittman, E. L., Costello, M. K., and Brewer, J. M. (2007). Circadian organization of *tau* mutant hamsters: Aftereffects and splitting. *Journal of Biological Rhythms* 22: 425–431.
268. Stewart, K. T., Rosenwasser, A. M., Levine, J. D., McEachron, D. L., Volpicelli, J. R., and Adler, N. T. (1990). Circadian rhythmicity and behavioral depression: II. Effects of lighting schedules. *Physiology and Behavior* 48: 157–164.
269. Endo, T., Honma, S., Hashimoto, S., and Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
270. Pittendrigh, C. S. and Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: I. The stability and lability of spontaneous function. *Journal of Comparative Physiology* 106: 223–252.
271. Winfree, A. T. (1972). Slow dark-adaptation in *Drosophila*'s circadian clock. *Journal of Comparative Physiology* 77: 418–434.
272. Mistlberger, R. E. and Holmes, M. M. (2000). Behavioral feedback regulation of circadian rhythm phase angle in light–dark entrained mice. *American Journal of Physiology* 279: R813–R821.
273. Brooks, E., Patel, D., and Canal, M. M. (2014). Programming of mice circadian photic responses by postnatal light environment. *PLOS ONE* 9: e97160.
274. Refinetti, R. (2007). Enhanced circadian photoresponsiveness after prolonged dark adaptation in seven species of diurnal and nocturnal rodents. *Physiology and Behavior* 90: 431–437.
275. Hébert, M., Martin, S. K., Lee, C., and Eastman, C. I. (2002). The effects of prior light on the suppression of melatonin by light in humans. *Journal of Pineal Research* 33: 198–203.
276. Smith, K. A., Schoen, M. W., and Czeisler, C. A. (2004). Adaptation of human pineal melatonin suppression by recent photic history. *Journal of Clinical Endocrinology and Metabolism* 89: 3610–3614.
277. Jasser, S. A., Hanifin, J. P., Rollag, M. D., and Brainard, G. C. (2006). Dim light adaptation attenuates acute melatonin suppression in humans. *Journal of Biological Rhythms* 21: 394–404.
278. Prichard, J. R., Fahy, J. L., Obermeyer, W. H., Behan, M., and Benca, R. M. (2004). Sleep responses to light and dark are shaped by early experience. *Behavioral Neuroscience* 118: 1262–1273.
279. Hannibal, J., Georg, B., Hindersson, P., and Fahrenkrug, J. (2005). Light and darkness regulate melanopsin in the retinal ganglion cells of the albino Wistar rat. *Journal of Molecular Neuroscience* 27: 147–155.
280. Shimomura, K., Kornhauser, J. M., Wisor, J. P., Umezu, T., Yamazaki, S., Ihara, N. L., Takahashi, J. S., and Menaker, M. (1998). Circadian behavior and plasticity of light-induced *c-fos* expression in SCN of *tau* mutant hamsters. *Journal of Biological Rhythms* 13: 305–314.
281. Penn, J. S. and Williams, T. P. (1986). Photostasis: Regulation of daily photon-catch by rat retinas in response to various cyclic illuminances. *Experimental Eye Research* 43: 915–928.
282. Penn, J. S. (1998). Early studies of the photostasis phenomenon. In: Williams, T. P. and Thistle, A. B. (Eds.), *Photostasis and Related Phenomena*. New York: Plenum, pp. 1–16.
283. Williams, T. P., Squitieri, A., Henderson, R. P., and Webbers, J. P. P. (1999). Reciprocity between light intensity and rhodopsin concentration across the rat retina. *Journal of Physiology* 516: 869–874.
284. Klante, G. and Steinlechner, S. (1995). A short red light pulse during dark phase of LD-cycle perturbs the hamster's circadian clock. *Journal of Comparative Physiology A* 177: 775–780.
285. Weinert, D. and Schottner, K. (2007). An inbred lineage of Djungarian hamsters with a strongly attenuated ability to synchronize. *Chronobiology International* 24: 1065–1079.
286. Ruby, N. F., Saran, A., Kang, T., Franken, P., and Heller, H. C. (1996). Siberian hamsters free run or become arrhythmic after a phase delay of the photocycle. *American Journal of Physiology* 271: R881–R890.
287. Ruby, N. F., Joshi, N., and Heller, H. C. (2002). Constant darkness restores entrainment to phase-delayed Siberian hamsters. *American Journal of Physiology* 283: R1314–R1320.

288. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P., and Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
289. Klerman, E. B. and Jewett, M. E. (1999). Model building, quantitative testing, and model comparison. *Journal of Biological Rhythms* 14: 621–624.
290. Pavlidis, T. (1981). Mathematical models. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 41–54.
291. Lakin-Thomas, P. L., Brody, S., and Coté, G. G. (1991). Amplitude model for the effects of mutations and temperature on period and phase resetting of the *Neurospora* circadian oscillator. *Journal of Biological Rhythms* 6: 281–297.
292. Díez-Noguera, A. (1994). A functional model of the circadian system based on the degree of intercommunication in a complex system. *American Journal of Physiology* 267: R1118–R1135.
293. Pedersen, M. and Johnsson, A. (1994). A study of the singularities in a mathematical model for circadian rhythms. *BioSystems* 33: 193–201.
294. Brown, E. N., Choe, Y., Luithardt, H., and Czeisler, C. A. (2000). A statistical model of the human core-temperature circadian rhythm. *American Journal of Physiology* 279: E669–E683.
295. Kunz, H. and Achermann, P. (2003). Simulation of circadian rhythm generation in the suprachiasmatic nucleus with locally coupled self-sustained oscillators. *Journal of Theoretical Biology* 224: 63–78.
296. Johnson, C. H., Elliott, J. A., and Foster, R. (2003). Entrainment of circadian programs. *Chronobiology International* 20: 741–774.
297. Indic, P., Gurdziel, K., Kronauer, R. E., and Klerman, E. B. (2006). Development of a two-dimension manifold to represent high dimension mathematical models of the intracellular mammalian circadian clock. *Journal of Biological Rhythms* 21: 222–232.
298. Forger, D. B. and Kronauer, R. E. (2002). Reconciling mathematical models of biological clocks by averaging on approximate manifolds. *SIAM Journal on Applied Mathematics* 62: 1281–1296.
299. Garfinkel, D. (1980). Computer modeling, complex biological systems, and their simplifications. *American Journal of Physiology* 239: R1–R6.
300. Conlan, R. (Ed.) (1985). *Understanding Computers: Computer Basics*. Alexandria, VA: Time-Life Books.
301. Miller, A. R. (1981). *The 8080/Z80 Assembly Language: Techniques for Improved Programming*. New York: Wiley.
302. Galbraith, R. (1982). *Professional Programming Techniques Starting with the BASICs*. Blue Ridge Summit, PA: TAB Books.
303. Microsoft Corporation (1985). *Microsoft GW-BASIC Interpreter User's Guide*. Seattle, WA: Microsoft Corporation.
304. Crider, B. (Ed.) (1984). *BASIC Program Conversions: How to Convert Programs from One Computer to Another.* Tucson, AZ: HP Books.
305. Zak, D. (2014). *Programming with Microsoft Visual Basic 2012*, 6th edn. Boston, MA: Cengage Learning.
306. Aho, A. V. (2004). Software and the future of programming languages. *Science* 303: 1331–1333.
307. Refinetti, R. (1993). A functional model of the mammalian circadian pacemaker. *International Journal of Biomedical Computing* 32: 45–60.
308. Roenneberg, T., Hut, R., Daan, S., and Merrow, M. (2010). Entrainment concepts revisited. *Journal of Biological Rhythms* 25: 329–339.
309. Barbacka-Surowiak, G. (2000). Is the PRC for dark pulses in LL a mirror image of the PRC for light pulses in DD in mice? *Biological Rhythm Research* 31: 531–544.
310. Comolli, J., Taylor, W., and Hastings, J. W. (1994). An inhibitor of protein phosphorylation stops the circadian oscillator and blocks light-induced phase shifting in *Gonyaulax polyedra*. *Journal of Biological Rhythms* 9: 13–26.

8 Nonphotic Environmental Mechanisms

8.1 NONPHOTIC ENTRAINMENT

In Chapter 7, we discussed how light synchronizes (or *entrains*) the circadian clock through a mostly nonparametric mechanism and how it otherwise affects the expression of circadian rhythms. Although light is the *zeitgeber* that has been most thoroughly studied, it is certainly not the only one. As diagramed in Figure 8.1, stimuli that have been shown to entrain circadian rhythms include not only light but also several so-called nonphotic stimuli such as ambient temperature, food availability, physical activity (exercise), and social contact. At least in mammals, none of these stimuli seems to have as strong an influence on the circadian system as light has. Nonetheless, all of these stimuli can significantly affect circadian rhythms, particularly when a light–dark (LD) cycle is not simultaneously present.

8.1.1 Ambient Temperature

It is no surprise that variations in ambient temperature can affect behavioral and autonomic processes in living organisms (Figure 8.2), and we will discuss this matter in some detail in Chapter 10. It is noteworthy, however, that daily cycles in ambient temperature can *entrain* circadian rhythms not only in cold-blooded animals but also in warm-blooded animals whose core temperature is minimally affected by variations in the temperature of the environment.[1,2] An example of entrainment by cycles of ambient temperature is shown in Figure 8.3. A golden hamster (*Mesocricetus auratus*) was initially kept under a regimen of dim constant light (1 lux) and constant ambient temperature (28°C), and its running-wheel activity was continuously monitored. Under these conditions, the rhythm freeran with a period slightly longer than 24.0 hours. Later on, ambient temperature was raised to 33°C for several hours each day (as indicated by the rectangles). Notice the two hallmarks of entrainment in both occasions: the period and phase of the activity rhythm were consistently altered by the zeitgeber, and the rhythm freeran from the phase of entrainment after the zeitgeber was removed. In the particular case of this hamster, the possibility that the changes in period and phase were due to *masking*, rather than to entrainment, can be further excluded by the fact that running took place during the presumably aversive (hot) phase of the temperature cycle. This is not always the case, but convincing evidence of entrainment by ambient temperature cycles has been provided in studies on plants, fungi, and invertebrates,[3–8] reptiles and birds,[9–12] and mammals.[13–25] In general, it has been found that ambient temperature is a weak zeitgeber in vertebrates, in the sense that not all animals exposed to the cycle actually entrain and, even in those that entrain, entrainment is often rather unstable. At least in mice, however, this weakness seems to be due to a lack of masking strength, not to a lack of zeitgeber strength.[24] When light or temperature is presented for only 1 hour each day (thus preserving the zeitgeber effect but eliminating masking), the two zeitgebers seem to have comparable entraining strengths, as exemplified in Figure 8.4. Notice the hallmarks of entrainment both under a photic T cycle (Panel A) and under a thermal T cycle (Panel B): the period and phase of the activity rhythm are consistently altered by the zeitgeber, and the rhythm freeruns from the phase of entrainment after the zeitgeber is removed. The actual zeitgeber strength of temperature and light under these conditions was indistinguishable, as measured by percentage of animals that entrained, rhythm robustness in the entrained state, stability of activity onsets, and stability of acrophases.[24]

The range of variation in ambient temperature necessary to produce entrainment has not been thoroughly studied. In a study on little pocket mice (*Perognathus longimembris*), a range of about 2°C was sufficient to produce entrainment, and wider ranges (up to 10°C) did not produce more robust entrainment.[13] In a study on antelope ground squirrels (*Ammospermophilus leucurus*), ranges of 4°C or 6°C were weak zeitgebers, whereas ranges of about 10°C were stronger zeitgebers.[18] In a study on domestic mice (*Mus musculus*), a warm range of 8°C (24°C–32°C) and a cold range of 12°C (12°C–24°C) were used, and 70% of the animals entrained to the cycle regardless of whether it was a neutral-to-warm or cold-to-neutral cycle and regardless of whether the warm/cold phase lasted 8 hours or only 1 hour.[23] When the cold/warm phase was 8-hours long, the mice did most of the running slightly before, during, and slightly after the exposure to the cold, but they ran mostly before the exposure to the heat. This suggests that the circadian resetting properties of cold and warmth may be distinct, although further experimentation is needed to clarify the distinction. Consistently with the concept of distinct resetting properties of cold and warmth, it has long been known that the cold and warmth senses are psychophysically distinct in human subjects[26,27] and are served by distinct sensory receptors and distinct afferent pathways in all species that have been studied.[28,29]

In addition to entrainment by full and skeleton temperature cycles, the effect of ambient temperature on the circadian system has been documented with the use of discrete temperature pulses, which can phase-shift circadian rhythms recorded under otherwise constant conditions.[30–33] This suggests that entrainment by temperature, like entrainment by light, is attained by a nonparametric mechanism. The nonparametric action of temperature is expected in view of the fact, previously discussed in Chapter 6, that the circadian clock is temperature compensated (which implies that the

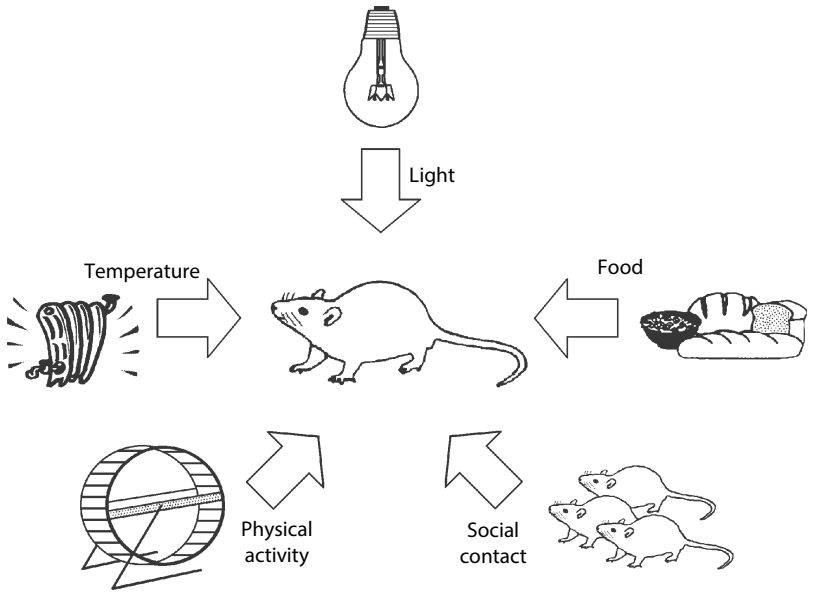


FIGURE 8.1 Many zeitgebers. Several classes of environmental stimuli can synchronize the circadian clock, including light, ambient temperature, food, physical activity, and social contact.



FIGURE 8.2 A cold opossum. Large variations in ambient temperature affect the physiology of living organisms. (Copyright ArtToday, Tucson, AZ.)

period of the clock is not parametrically affected by temperature). Of course, fluctuations in ambient temperature can also *mask* circadian rhythms if they are large enough to inhibit locomotor activity or to overtax the thermoregulatory system.

8.1.2 Food Availability

Food availability is another important environmental variable (Figure 8.5) that is capable of entraining circadian rhythms.[34–36] Restriction of food availability to a few hours a day has been shown to entrain circadian rhythms in insects,[37] fishes,[38–40] birds,[41,42] and mammals.[43–68] An example of entrainment of the running-wheel activity rhythm by restricted feeding is shown in Figure 8.6. A Nile grass rat (*Arvicanthis niloticus*) was initially kept under a regimen of constant light (10 lux) and constant ambient temperature (24°C) with food

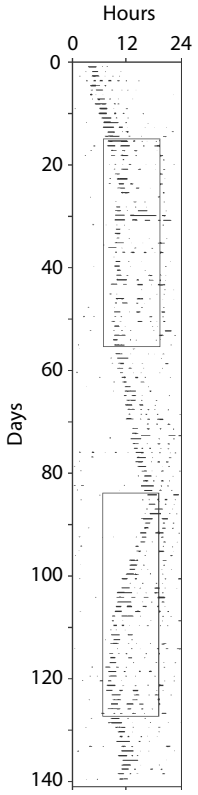


FIGURE 8.3 Cycle of ambient temperature as a zeitgeber. This actogram shows the rhythm of running-wheel activity of a golden hamster (*Mesocricetus auratus*) maintained under constant dim illumination (1 lux) and exposed to cycles of ambient temperature. The rectangles indicate the times of day when ambient temperature was 33°C (91°F, warm for a hamster); during the rest of the time, ambient temperature was approximately 26°C (79°F, neutral for a hamster at rest). Entrainment clearly occurred in the two occasions when the temperature cycle was present. (Adapted from Pohl, H., *Biol. Rhythm Res.*, 29, 311, 1998.)

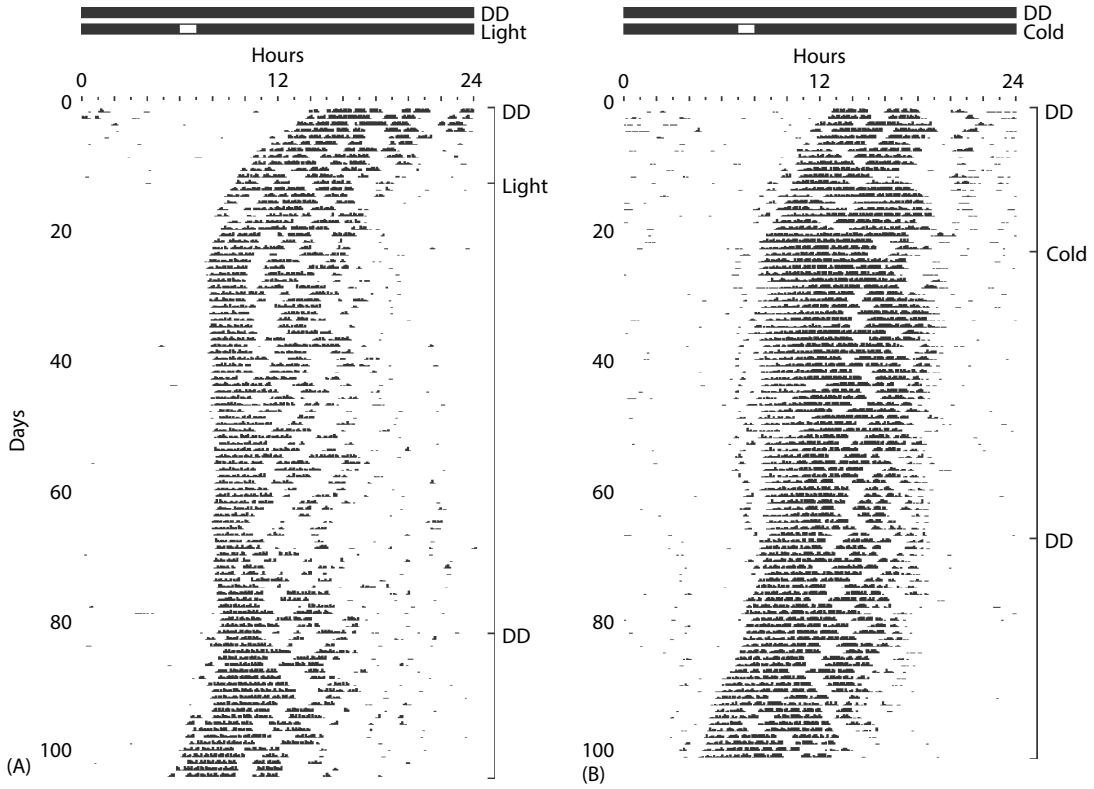


FIGURE 8.4 Light and temperature. These actograms show the running-wheel activity rhythms of a mouse initially maintained in constant darkness and then exposed to light for 1 hour each day (A) and of a mouse also maintained initially under constant darkness but then exposed to a lower ambient temperature for 1 hour each day (B). Both animals entrained to the environmental cycles and freeran when constant darkness was reinstated. The horizontal black and white bars above the actograms indicate the timing of light or temperature stimulation. (Data from Refinetti, R., *J. Physiol. Sci*., 65, 359, 2015.)



FIGURE 8.5 May I have more? In Charles Dickens' classic fictional book, *Oliver Twist*, Oliver surprised everyone by asking for more food after a meal. Feeding schedules have been shown to affect the period and phase of circadian rhythms. (Copyright ArtToday, Tucson, AZ.)

freely available at all times. At the beginning of the records shown in Figure 8.6, a schedule of food restriction (FR) was imposed. Each day, 90% of the free-feeding amount of food was made available at 10:30 a.m. The animal ingested all the food within about 2.5 hours (as indicated by the rectangle). Notice that the activity rhythm was initially free-running with a period longer than 24.0 hours but that it slowly adjusted to the 24-hour schedule of feeding. When food became freely available again, the rhythm resumed its freerun from the phase of entrainment.

As was the case for ambient temperature, food availability seems to be a weaker zeitgeber than light. Although food is more essential for an animal's survival than light is, light exerts a finer control over the activity rhythm than food availability does. This is exemplified in Figure 8.7. Two domestic mice (*M. musculus*) were initially kept under an LD cycle (as indicated by the clear rectangles) and later subjected to either a schedule of FR (Panel A) or a daily light pulse (Panel B). Notice that entrainment occurred in both cases, as indicated by the control of the period and phase of the rhythms by the zeitgebers, as well as by the freeruns from the appropriate phases once the zeitgebers were discontinued. However, notice also that the phase angles of entrainment were very different for the two mice. The phase delay necessary for entrainment was provided by light in the early subjective night but by food in the late subjective night. Perhaps even

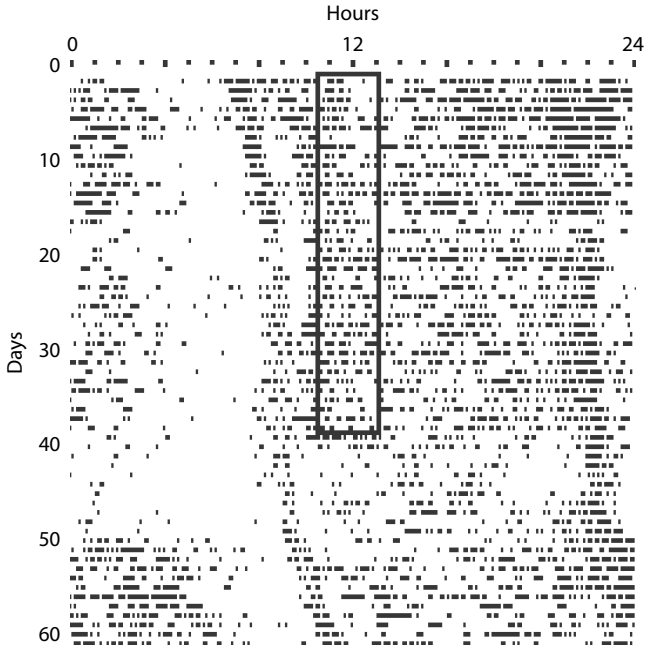


FIGURE 8.6 Feeding schedule as a zeitgeber. This actogram shows the running-wheel activity rhythm of a Nile grass rat (*Arvicanthis niloticus*) maintained under constant illumination (10 lux) and constant ambient temperature (24°C) and exposed to a cycle of food availability. The rectangle indicates the time of day when food was available (90% of the daily free-feeding amount of food was delivered at 10:30 hours each day, and the animal took approximately 2½ hours to consume the food). Entrainment was gradually established over several weeks. (Data from the archives of the Refinetti lab.)

more conspicuous is the difference in the robustness of the rhythms. The daily onsets of activity were rather variable under the FR schedule but were quite regular under the light-pulse schedule, which indicates greater control of the rhythms by the photic zeitgeber than by the nonphotic zeitgeber. As a matter of fact, even this weak entrainment by FR is unusual in mice. As will be discussed in greater detail in Section 8.3, food as a zeitgeber has the peculiar characteristic that, in many situations, it paradoxically yields entrainment at the same time as it does not do so. Particularly in mice, daily FR can control the phase and period of the activity rhythm without actually entraining the master circadian pacemaker. The actogram in Figure 8.8 shows that the activity rhythm is drastically affected by the restricted-feeding schedule. Notice that the mouse is not simply responding to the arrival of food each day but is actually *anticipating* the arrival. Notice also that the period of the rhythm, which was longer than 24 hours during the first 3 weeks with food freely available, eventually becomes exactly 24.0 hours. Yet, as soon as unrestricted access to food is resumed (on the 74th day), the activity rhythm resumes from the point extrapolated from its phase before the restricted-feeding regime was introduced (on the 29th day), and not from the phase of presumed entrainment—thus indicating that true entrainment did not take place!

8.1.3 Physical Exercise

Physical exercise (Figure 8.9) is another stimulus capable of entraining circadian rhythms. An example is shown in Figure 8.10. A mouse was kept under dim red light (which is the same as constant darkness for the circadian system of the mouse), and its drinking activity was continuously monitored. The rhythm clearly freeran with a period shorter than 24 hours. From day 40 to day 90, a running wheel was made available (as indicated by the double-plotted rectangles). Although not shown in the figure, the mouse ran vigorously on the wheel. Entrainment, followed by freerun from the appropriate phase, is clearly seen. Entrainment by voluntary or forced exercise has been documented in various species.[69–78] In addition, it has been shown that discrete *pulses* of exercise can phase-shift circadian rhythms recorded under otherwise constant conditions.[79–87] This suggests that entrainment by exercise, like entrainment by light, is attained by a nonparametric mechanism. Furthermore, because animals given free access to running wheels use the wheels only during part of each day, they end up "pulsing" themselves on a restricted region

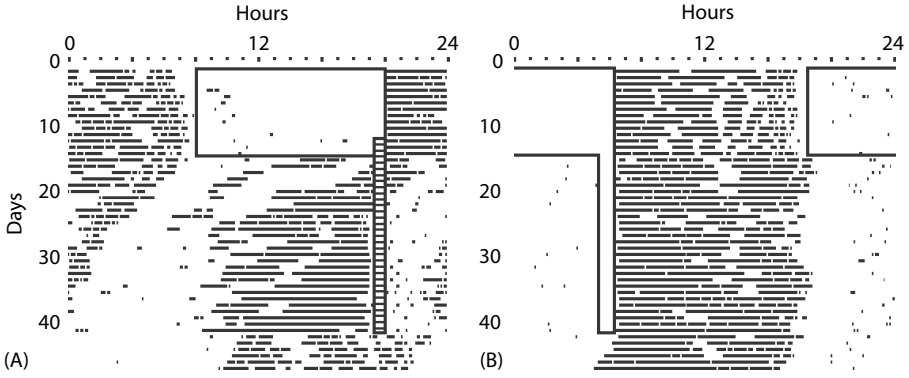


FIGURE 8.7 Comparing food and light as zeitgebers. The actograms show the running-wheel activity rhythms of two domestic mice (*Mus musculus*) maintained first under an LD cycle (LD 12:12) and then in constant darkness under either a daily schedule of food availability (A) or a daily schedule of brief exposure to light (B). The striped vertical rectangle indicates the time of food delivery. The clear rectangles indicate the duration of exposure to light. Entrainment can be seen in both cases, but the activity patterns differ considerably from each other. (Data from Refinetti, R., *Chronobiol. Int.*, 20, 417, 2003.)

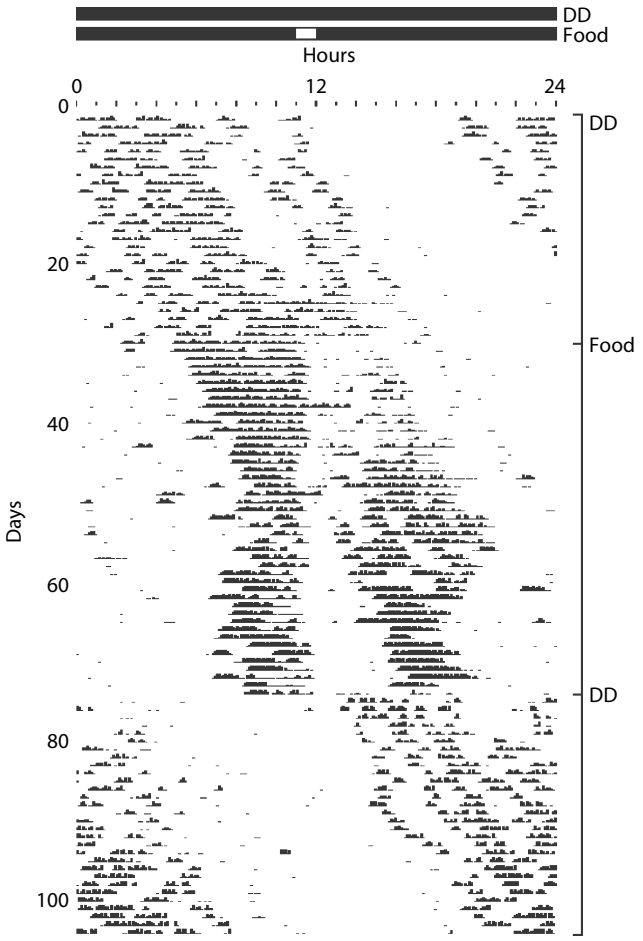


FIGURE 8.8 Entrained or not? This actogram shows the running-wheel activity records of a mouse maintained in constant darkness, initially with food freely available at all times, then with restricted food (85% of free-feeding amount) provided at the time of the white notch on the horizontal bar above the actogram, and then with food freely available again. Restricted feeding caused a change in period and phase of the activity rhythm but did not effect true entrainment of the master circadian clock. (Data from Refinetti, R., *J. Physiol. Sci*., 65, 359, 2015.)



FIGURE 8.9 Jogging in the woods. Schedules of physical activity have been shown to affect the period and phase of circadian rhythms. (Copyright ArtToday, Tucson, AZ.)

of the nonphotic phase–response curve (PRC), which results in a change in their free-running periods. Thus, laboratory rodents (mice, rats, and hamsters) exhibit shorter free-running periods when they have access to running wheels than when they do not have access.[88–97] Curiously, shifts induced by exercise can counteract shifts induced by photic stimulation.[98–100]

An intriguing phenomenon in various rodents is the induction of exercise by cage changes. Rodents seem to be very peculiar about their home cages. When dirty cages are replaced with clean ones, the animals often spend several hours rearranging the bedding and running on the wheel. An example for a Siberian hamster (*Phodopus sungorus*) is shown in Figure 8.11. Although the animal ordinarily showed very little activity during the light phase of the LD cycle, it showed quite a bit of activity immediately after a cage change during the light phase (arrow). Whereas the disrupted activity pattern seems to last only a few hours when the animals are under an LD cycle, cage changes for animals kept in constant darkness can actually phase-shift the circadian clock (presumably because of the exercise *pulse*).[101–103]

Figure 8.12 shows a PRC describing phase shifts evoked by 3-hour pulses of exercise presented at various circadian times. In golden hamsters, exercise pulses can be easily dispensed by transfer of the animal to a different cage fitted with a novel running wheel. Hamsters voluntarily exercise for hours under these conditions. Notice that large phase advances can be evoked during midsubjective day. For comparison, a PRC for light pulses is also shown (dashed line). As seen in the previous chapter, large phase advances are evoked by light during midsubjective night. Because of this 12-hour difference in the two PRCs, it was suggested that there may exist two types of PRCs with particular characteristics: photic and nonphotic curves.[104] This is probably an overgeneralization. Whereas we saw in Chapter 7 that photic PRCs do indeed display considerable similarity across species, there does not seem to be a consistent pattern of PRCs for nonphotic stimuli of various types across species. As shown in Figure 8.13, some nonphotic curves have mostly phase-advance regions, some have mostly phase-delay regions, and the circadian time of the phase-advance region is not consistent among the various conditions. Additional nonphotic PRCs can be inspected in the program **PRC** (see Exercise 8.2). A consistent, reproducible pattern of nonphotic PRCs can be found only for restricted groups of stimuli within restricted groups of organisms (especially the

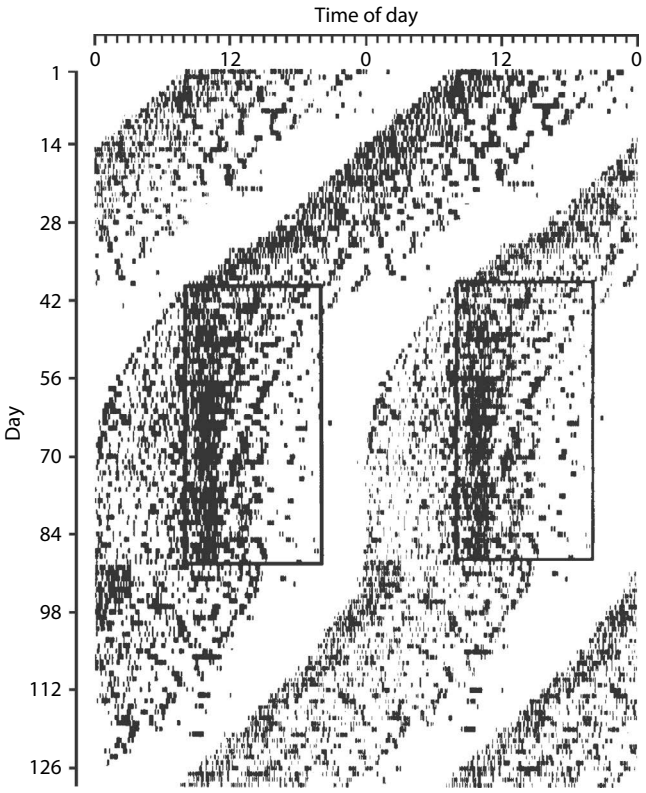


FIGURE 8.10 Exercise schedule as a zeitgeber. This double-plotted actogram shows the rhythm of drinking activity of a domestic mouse (*Mus musculus*) maintained in constant darkness and allowed access to a running wheel for a fraction of each day, as indicated by the rectangles. Entrainment, preceded and followed by freerun, is clearly seen. (Reproduced from Edgar, D.M. and Dement, W.C., *Am. J. Physiol.*, 261, R928, Copyright 1991, American Physiological Society. With permission.)

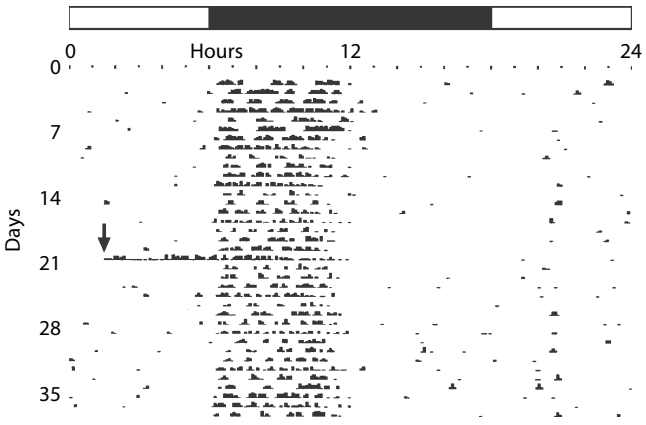


FIGURE 8.11 Please leave my home alone. This actogram shows the running-wheel activity rhythm of a Siberian hamster (*Phodopus sungorus*) maintained under an LD cycle, as indicated by the horizontal bar at the top. On day 21, at the time indicated by the arrow, the animal's cage was replaced with a fresh cage otherwise identical to the old one. The cage change clearly disturbed the animal's activity rhythm. (Data from archives of the Refinetti lab.)

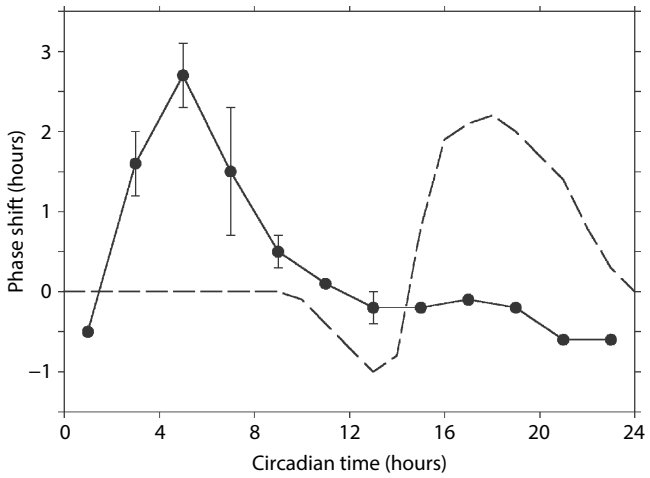


FIGURE 8.12 Comparing the phase-shifting effects of exercise and light. The graph shows phase–response curves (PRCs) for resetting of the circadian clock of the golden hamster (*Mesocricetus auratus*) as determined by phase shifts of the rhythm of running-wheel activity. The circles correspond to the mean shifts (±SE) exhibited by hamsters maintained in constant darkness and induced to run in a novel wheel for 3 hours at various circadian times. The dashed line corresponds to the PRC for brief light pulses, as previously described in Figure 7.8. The two PRCs are clearly different. (Data from Mrosovsky, N. et al., *J. Biol. Rhythms*, 7, 41, 1992; Takahashi, J.S. et al., *Nature*, 308, 186, 1984.)

golden hamster), as exemplified in Figure 8.14. In this case, the various curves are quite similar, with a phase-advance region from CT 4 to CT 12 and almost no response during the rest of the circadian cycle.

8.1.4 Social Interaction

Many animals, particularly humans, engage in extensive social interaction (Figure 8.15), which provides a potential opportunity for social entrainment. In actuality, the experimental evidence in support of the role of social interaction as a zeitgeber is very limited. There is some evidence that social stimuli may be effective in the interaction between a mother and her offspring,[105–107] as exemplified in Figure 8.16. Siberian hamster dams previously maintained under an LD cycle (LD 16:8) were kept in constant darkness from the beginning of pregnancy. The onset times of running-wheel activity of the pups were monitored shortly after weaning, approximately 18 days after birth. Panel A shows the onset time of the dam (triangle), as inferred from the time of lights off of the preceding LD cycle, and the expected onset times of the pups (circles) under the assumption of no social entrainment. Panel B shows the actual data. Notice that the onset times of the pups are clustered around the onset time of the dam. This clustering suggests that the circadian systems of the pups were entrained by the dam.[106]

Entrainment of the offspring's rhythms by the mother is perhaps less surprising during gestation, when the fetuses share the mother's circulatory system.[108–111] However, an ingenious study in sheep showed that environmental light

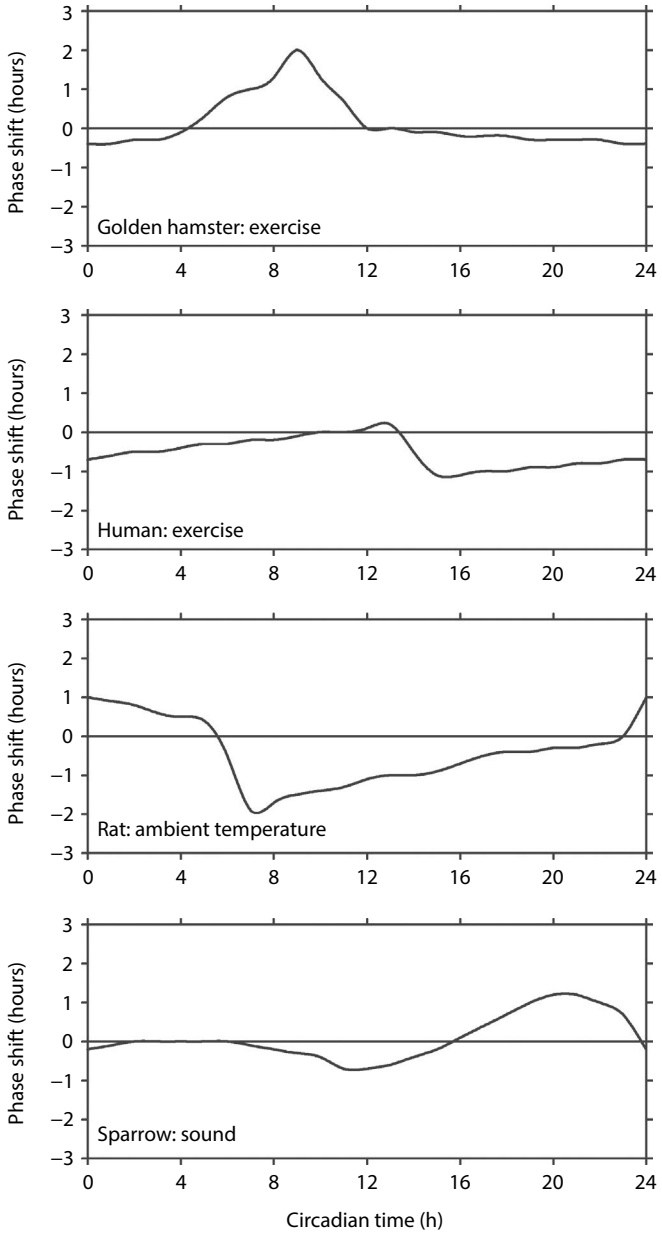


FIGURE 8.13 Nonphotic phase–response curves (PRCs). The graphs show PRCs for the resetting of circadian rhythms by nonphotic stimuli in four vertebrate species. There is little similarity between the various curves. (Data from Wickland, C.R. and Turek, F.W., *Am. J. Physiol.*, 261, R1109, 1991; Buxton, O.M. et al., *Am. J. Physiol.*, 284, R714, 2003; Francis, A.J.P. and Coleman, G.J., *Physiol. Behav.*, 62, 1211, 1997; Reebs, S.G., *Ethology*, 80, 172, 1989.)

penetrates the mother's abdominal wall and produces a robust LD cycle inside the uterus,[112] which means that the circadian clocks of the fetuses could be entrained by the LD cycle rather than by biological rhythms in the mother's body. On the other hand, changes in the feeding time of the rat dam are reported to synchronize the clocks of the embryonic pups,[111,113] and destruction of the master pacemaker of the dam disrupts pup synchronization,[108] which means that fetal synchronization must result from more than just environmental stimulation.

Social entrainment in adults remains very controversial. There is no doubt that social interaction can transiently disturb circadian rhythms,[114–118] but it is not clear whether it can reset the circadian clock. Some researchers claim to have documented social entrainment,[101,119–124] or at least some form of action of social stimuli on the circadian system.[125–129] In many cases, the data presented as evidence of entrainment can much more appropriately be classified as social masking.[130–138] Carefully conducted studies have described various masking effects but have not provided evidence of social entrainment in rodents[139–145] or other mammals.[54,146–150] In birds, socially relevant sounds (conspecific songs) seem to be an effective zeitgeber,[151,152] although their mechanism of action is not known. It is possible that they cause entrainment indirectly by stimulating physical activity.

An example from a study that revealed no evidence of social entrainment in rodents is shown in Figure 8.17. Pairs of golden hamsters were housed together under a 23.3-hour T cycle (1 hour of light per cycle). One of the hamsters was blinded, so that it could not entrain to the T cycle. The actogram clearly shows a 23.3-hour rhythm that corresponds to the sighted hamster and a 24.1-hour rhythm that corresponds to the blind hamster. There is no indication that the sighted hamster affected the free-running rhythm of the blind one. On the contrary, it seems that the two hamsters totally ignored each other. Equivalent results were obtained in 10 other pairs of hamsters.[142] Of course, one could argue that male golden hamsters are solitary animals that exhibit only aggressive behavior or indifference toward other males. However, as shown in Figure 8.18, pairs of male and female hamsters do not exhibit social entrainment either. Male–female pairs of *tau*-mutant hamsters were housed together in constant darkness and were continuously filmed by time-lapse video photography. The activity onsets are indicated by the circles. Even though the animals had sex every five circadian cycles, there is no indication of long-term synchronization of the activity rhythms. Each member of the pair seemed to freerun independently from the other.[153]

A well-conducted study in rats showed that male rats could learn to anticipate having sex with three receptive females each day, but it was not clear whether the circadian system was being controlled by the social stimulus or by the excessive eating in which the male rat indulged after having sex.[154]

The concept of social entrainment of circadian rhythms is related to the concept of social synchronization of estrous cycles, which has a longer history. In 1971, it was reported that social interaction could synchronize the menstrual cycles of young women living in a college dormitory.[155] Several studies claiming to support the idea that social contact can synchronize the reproductive cycle have been conducted in rodents[156,157] and humans.[158–161] All these studies are flawed, however. In none of the studies were reproductive cycles examined carefully over many cycles with adequate individual monitoring. They relied instead on short-term group-synchrony data. Therefore, it is impossible to distinguish short-term coincidence from actual entrainment in any of these studies. Studies on humans have also suffered from other methodological deficiencies.[162,163]

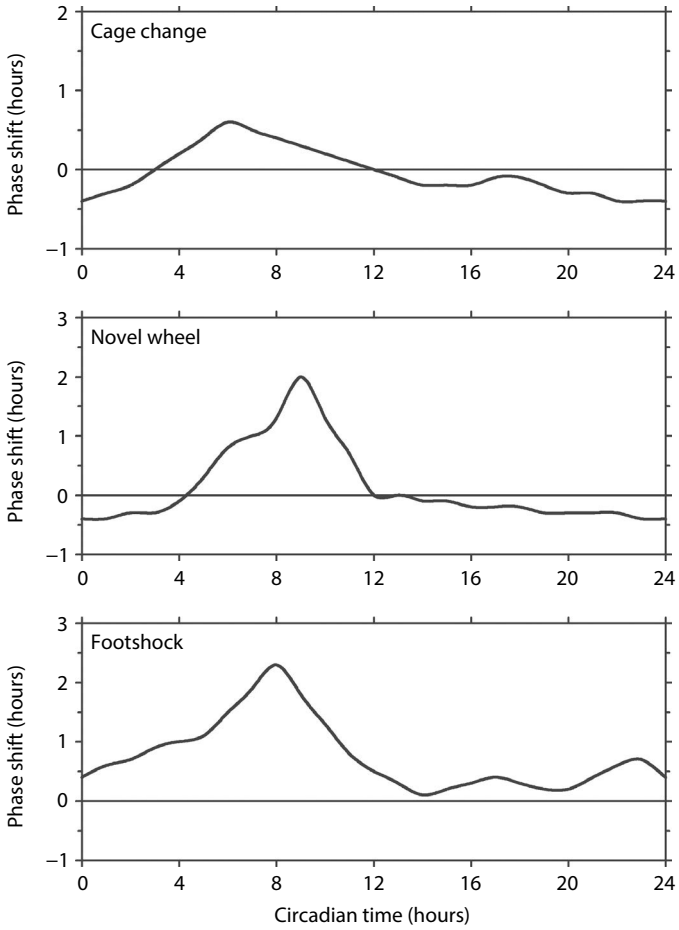


FIGURE 8.14 Consistent nonphotic phase–response curves (PRCs). The graphs show PRCs for the resetting of circadian rhythms by three nonphotic stimuli in the golden hamster (*Mesocricetus auratus*). In a situation like this—where a single species and a limited set of stimuli are considered—there is similarity between the different curves (specifically, all three curves exhibit a phase-advance region between CT 4 and CT 12 and almost no response at other times). (Data from Mrosovsky, N., *J. Comp. Physiol. A*, 162, 35, 1988; Wickland, C.R. and Turek, F.W., *Am. J. Physiol.*, 261, R1109, 1991; Cain, S.W. et al., *Behav. Neurosci.*, 118, 131, 2004.)



FIGURE 8.15 Napping together. Social interaction, such as that between a father and his newborn child, can certainly affect one's daily schedule of activity. It is not clear, however, whether the effect corresponds to circadian entrainment or it is merely masking. (Photo by K. Z. Refinetti.)

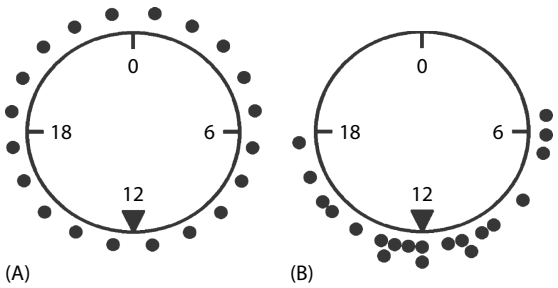


FIGURE 8.16 Maternal entrainment. Female Siberian hamsters (*Phodopus sungorus*) were maintained in constant darkness from the beginning of pregnancy. Shortly after weaning, the pups were given access to running wheels, so that the phase of their circadian clocks could be determined. The small dark circles indicate the times of the onsets of activity of the pups (20 pups from 5 litters). Panel A shows the distribution of onsets as expected by chance. Panel B shows the actual distribution. The estimated time of the activity onset of the dams (ZT 12 of the LD cycle preceding constant darkness) is indicated by the arrowheads. The distribution of onsets of the pups (B) is significantly different from that expected by chance (A), which suggests entrainment of the pups' rhythms by the dams. (Adapted from Duffield, G.E. and Ebling, F.J.P., *J. Biol. Rhythms*, 13, 315, 1998.)

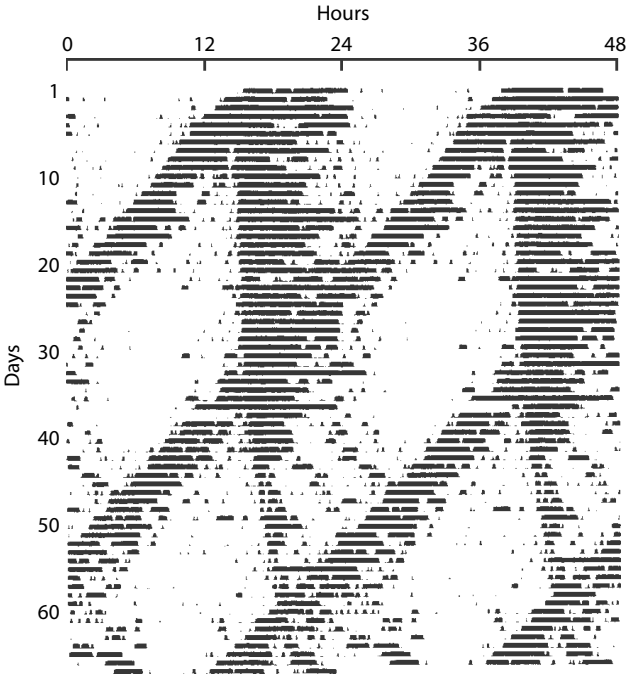


FIGURE 8.17 Lack of social entrainment. This double-plotted actogram shows the combined running-wheel activity rhythms of two male golden hamsters (*Mesocricetus auratus*) housed in a single cage with two running wheels. One of the animals was blinded and the other was exposed to a 23.3-hour LD cycle (LD 1:22.3). The blinded animal seems to freerun in total independence from its cage mate (whose circadian period is clearly controlled by the LD cycle). (Adapted from Refinetti, R. et al., *J. Comp. Physiol. A*, 170, 181, 1992.)

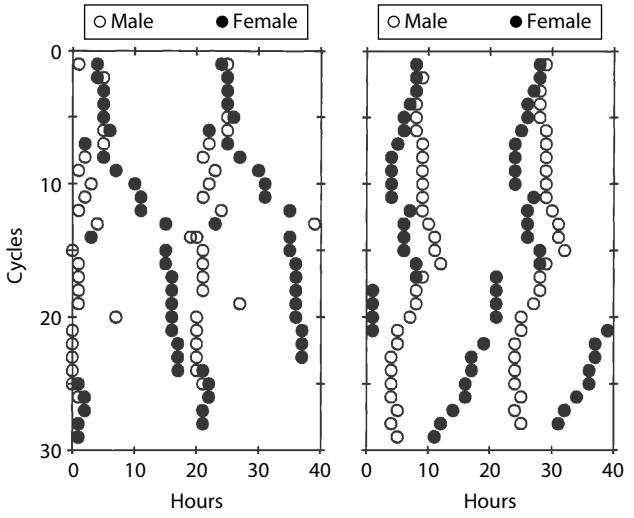


FIGURE 8.18 Lack of social entrainment (again). Male–female pairs of *tau*-mutant hamsters were maintained in constant darkness. The females had been surgically sterilized by tubal ligation to prevent pregnancy. Onsets of activity (as determined by inspection of infrared time-lapse video photography records) are plotted in double-plot actogram format. Social entrainment is not apparent in either pair of hamsters. (Data from Refinetti, R. et al., *FASEB J.*, 5(5), A1126, 1991.)

8.1.5 Dark Pulses

Dark pulses presented against a background of constant light are also considered to be nonphotic stimuli, essentially because they are *nonlight* (that is, they are literally a temporary interruption of photic stimulation). An example of a phase shift induced by a dark pulse is shown in Figure 8.19. A golden hamster was maintained in constant light and received a single 15-minute dark pulse at the time indicated by the dark circle. As indicated by the regression lines fit to the prepulse and postpulse onsets, the dark pulse caused a phase advance of approximately 2 hours. Phase shifts evoked by dark pulses have been documented in various species.[164–174]

A natural question to be asked is whether the PRC for dark pulses is a mirror image of the PRC for light pulses. After all, a brief dark pulse against a background of constant light may be thought of as a long light pulse against a background of darkness. One might try to calculate the expected dark-pulse-evoked shift by integrating the area under the curve of the photic PRC, but assumptions would have to be made about the integration process. Thus, the best approach, at least at a first stage, is to empirically determine the curves for both

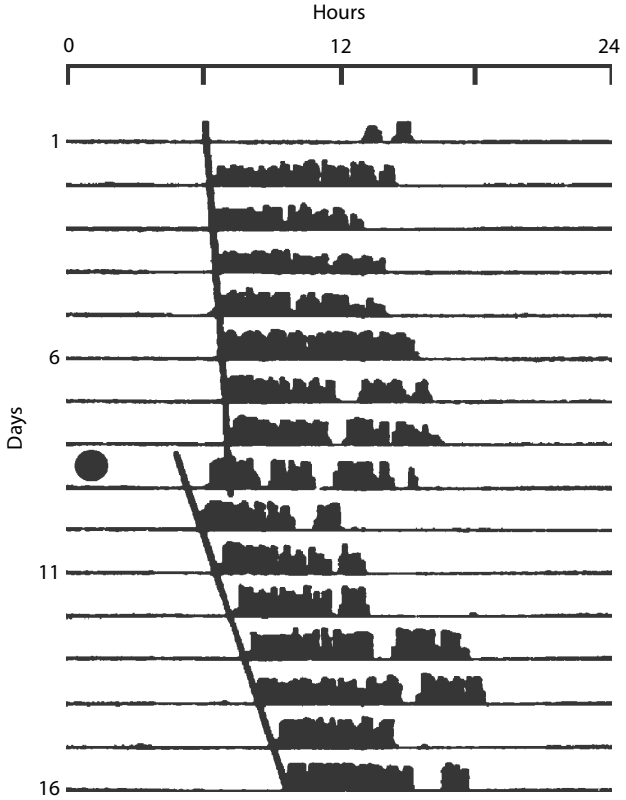


FIGURE 8.19 Dark pulse causes phase shift. The actogram shows the running-wheel activity rhythm of a golden hamster (*Mesocricetus auratus*) maintained in constant light and presented with a single 15-minute pulse of darkness at the time indicated by the dark circle. The oblique lines are linear regressions of the activity onsets that help visualization of the 2-hour phase advance caused by the dark pulse. (Adapted from Rosenwasser, A.M. and Dwyer, S.M., *J. Biol. Rhythms*, 17, 238, 2002.)

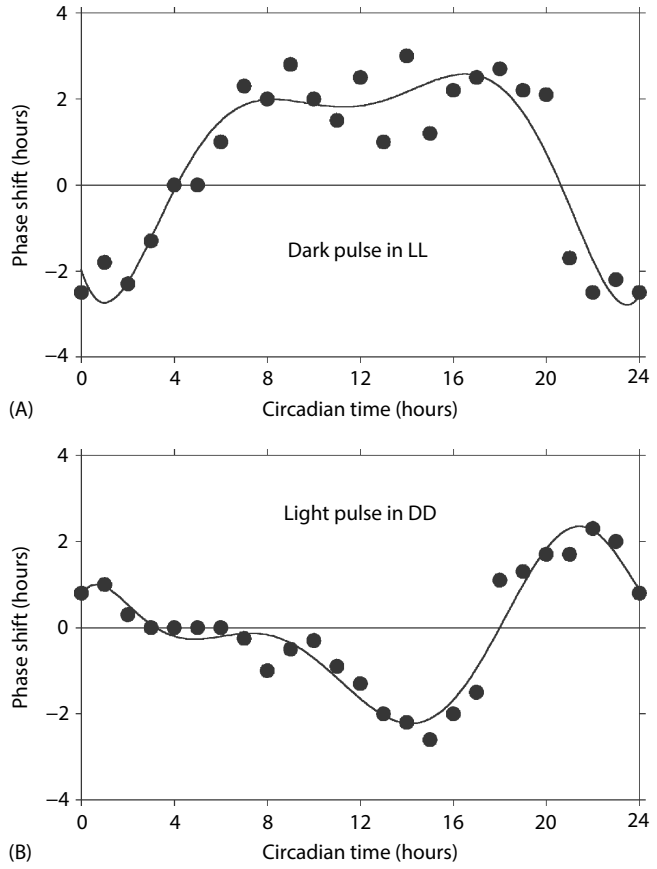


FIGURE 8.20 Dark-pulse PRC. The graphs show the phase–response curves (PRCs) for 1-hour dark pulses presented to mice in constant darkness (A) and for 1-hour light pulses presented to mice in constant darkness (B). The PRCs are very different from each other. (Data from Barbacka-Surowiak, G., *Biol. Rhythm Res.*, 31, 531–544, 2000.)

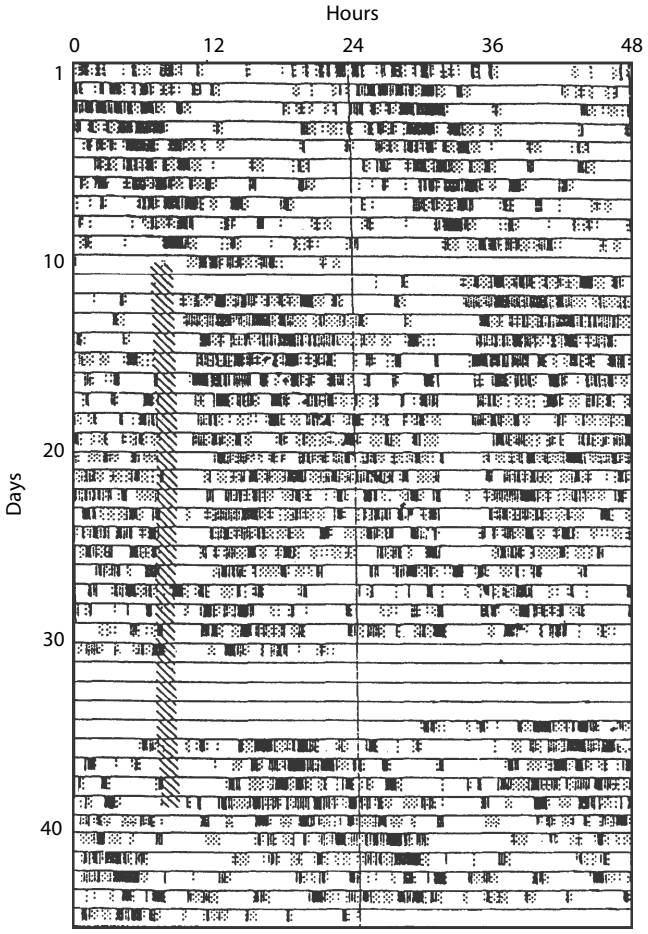


FIGURE 8.21 Entrainment by hypergravity. This double-plotted actogram shows the digitized rhythm of body temperature of a laboratory rat (*Rattus norvegicus*) maintained in constant light (30 lux) for 6 weeks. For 4 weeks, the animal was exposed to 2 *g* gravity (by centrifugation) for 1 hour a day (hatched bar). Notice that the circadian period shortens to match that of the zeitgeber and that the freerun after entrainment starts at the appropriate phase. (Data were lost from day 31 to day 35.) (Adapted from Demaria-Pesce, V.H. et al., *Médecine Aéronautique et Spatiale*, 30, 416, 1991.)

types of stimuli. The results of one such effort are shown in Figure 8.20. Mice were either kept in constant darkness and received 1-hour light pulses (150 lux) or kept in constant light (400 lux) and received 1-hour dark pulses. As expected, the resulting PRCs are quite different from each other. They are not mirror images of each other, but one would not expect them to be. Nonetheless, it has been shown that the PRC for dark pulses can be modeled as the combination of a mirror image of the *photic* curve during early subjective night and a straight image of the *exercise* curve during subjective day and late subjective night.[170] No one has actually tried to model the dark-pulse curve as a mirror image of the integrated photic curve.

8.1.6 Other Stimuli

Another potential zeitgeber is *gravity*. There is no sizable daily cycle of gravity on Earth, but changes in gravity can be simulated by centrifugation. Only one study has documented the entrainment of circadian rhythms by a daily cycle of gravity,[175] as shown in Figure 8.21. Rats were kept in constant light and were centrifuged (to 2 *g*) for 1 hour a day. The actogram shows both an appropriate control of phase of the rhythm by the zeitgeber and a freerun from the phase of entrainment upon removal of the zeitgeber. However, because the animals were maintained in constant light, one cannot exclude the possibility that centrifugation affected light perception (and, therefore, that light rather than gravity was the true zeitgeber). Other studies dealing with the effects of gravity on circadian rhythms have investigated only masking.[176–179] Other nonphotic stimuli that have been claimed to reset the circadian pacemaker, but that have not been investigated in detail, include environmental vibration,[180] seizures,[181] hypoxia,[182] nicotine injections,[183] performance of a demanding cognitive task,[184] and sleep.[185,186] As previously mentioned in Chapter 6, injections of benzodiazepines have consistently been shown to be able to reset the circadian clock.[187–191]

8.2 SEARCHING FOR A COMMON MECHANISM

After discussing these various nonphotic zeitgebers, it is sensible to ask whether there is a mechanism of action common to all of them. Is there a basic nonphotic stimulus that underlies the action of all other stimuli? The differences in the shapes of the PRCs for different nonphotic stimuli across various species (Figure 8.13) do not suggest the existence of a universal common mechanism. However, the similarities observed in restricted domains (e.g., Figure 8.14) encourage the search for a unifying principle. Ralph Mistlberger (Figure 8.22), a psychology professor at Simon Fraser University (in Canada), has conducted a great deal of research aimed at finding such a principle. He was not the first one to realize that all so-called nonphotic stimuli have one thing in common: they cause *arousal*.[101] However, Mistlberger's studies have shown that the notion of arousal itself is quite complex.[192] It certainly makes sense to think that arousal is elicited by abrupt changes in ambient temperature, food availability, physical activity, social interaction, and dark pulses—but what exactly is arousal?

Phase shifts of the circadian clock or acceleration/deceleration of reentrainment after a phase shift of the LD cycle have been obtained by various manipulations intended to increase arousal, such as handling of the animal by the experimenter,[193,194] opportunity for sexual contact,[125] eating after food deprivation (FD),[195] receipt of an electric footshock,[196] restraint stress,[197] or cage agitation combined with water sprinkling.[198] However, phase shifts were not obtained by other arousing manipulations such as engagement in a fight leading to defeat,[143] receipt of a caffeine injection,[199] receipt of a glucocorticoid injection,[200] cage disturbance,[58] or exposure to a brief loud noise.[201] In addition, the fact that physical exercise can inhibit phase shifts induced by light[202–204] led researchers to try to inhibit photic phase shifts by manipulations of arousal such as induction of conditioned fear,[205,206] exposure to a strong olfactory stimulus,[207] or sleep deprivation.[208] Mistlberger's studies suggest that arousal alone is not sufficient to evoke nonphotic phase shifts, although arousal is a necessary component of the process.[171,209–211] The results are summarized in Figure 8.23. The graph shows the mean phase shifts for groups of 12–33 golden hamsters subjected to 3-hour pulses of different stimuli during subjective day (when hamsters are mostly asleep). Arousal was obtained by physical restraint in a small tube (which causes arousal but prevents elevated physical activity and only briefly interferes with sleep). Wakefulness was induced by sleep deprivation on a small pedestal over water (which prevents elevated physical activity and causes only minimal stress if the animals are previously habituated to the apparatus). The combination of arousal and wakefulness was obtained by restraint in a small tube combined with sleep deprivation attained by the presentation of air puffs. A condition of exercise without arousal and wakefulness is not feasible, but a combination of the three stimuli was obtained by the presentation of a novel running wheel (which elicits running and, therefore, exercise, arousal, and wakefulness). Notice that arousal alone did not evoke phase shifts larger



FIGURE 8.22 Ralph Mistlberger. This circadian physiologist from Simon Fraser University (Canada) is the most active researcher of the mechanisms underlying nonphotic phase shifts. (Photo courtesy of Ralph Mistlberger.)

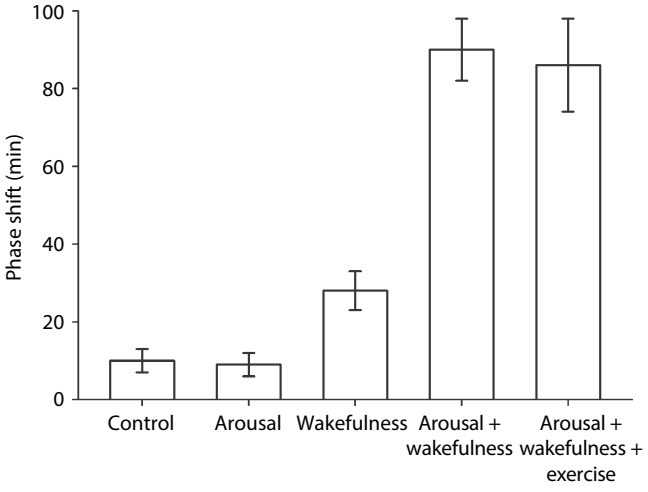


FIGURE 8.23 Dissecting the mechanism of nonphotic phase shifting. The graph shows the mean phase shifts evoked by various stimuli presented for 3 hours at circadian time 6 to male golden hamsters (*Mesocricetus auratus*). Each bar corresponds to the mean (±SE) of 12–33 animals. Arousal alone was obtained by restraint of the animal in a small tube (which is arousing but does not induce activity and does not prevent sleep). Wakefulness alone was obtained by sleep deprivation using the pedestal-over-water method (which does not induce activity and presumably is not arousing). The arousal and wakefulness combination was obtained by restraint in a small tube combined with sleep deprivation by the presentation of frequent air puffs. Presentation of a novel wheel provided the combination of arousal, wakefulness, and exercise. The results indicate that arousal alone or wakefulness alone does not evoke large phase shifts. (Data from Mistlberger, R.E. et al., *J. Biol. Rhythms*, 11, 208, 1996; Mistlberger, R.E. et al., *Am. J. Physiol.*, 285, R917, 2003.)

than those evoked by no stimulus at all (the control condition). Wakefulness alone evoked a somewhat larger shift, but arousal combined with wakefulness evoked the largest shift, which was not further increased by the addition of exercise.

Of course, it is possible that the null effect of exercise noted in Figure 8.23 was an artifact—because the maximal possible shift had already been attained by the combination of arousal and wakefulness. This issue must remain unresolved because exercise cannot be presented by itself (after all, sleeping animals cannot run). Nevertheless, it is clear that neither arousal alone nor wakefulness alone is sufficient to evoke large phase shifts. Therefore, there is not a mechanism of action common to all nonphotic zeitgebers. A combination of arousal and wakefulness, perhaps enhanced by exercise, might underlie the phase-shifting effects of changes in ambient temperature, food availability, physical activity, social interaction, dark pulses, and other nonphotic zeitgebers. Alternatively, each zeitgeber could have its own route to the master clock in the brain, as will be discussed in Chapter 11.

Even if arousal and wakefulness do underlie the phase-shifting effects of nonphotic zeitgebers in nocturnal animals, this connection cannot apply to diurnal animals. In diurnal animals, as in nocturnal ones, nonphotic stimuli are effective only during subjective day.[78,198] Whereas nocturnal animals are asleep during most of subjective day, diurnal animals are awake during this time and, therefore, will not be sleep deprived by the presentation of nonphotic stimuli, so that arousal and wakefulness cannot reliably mediate nonphotic entrainment in diurnal animals.

8.3 SEPARATE FOOD-ENTRAINABLE PACEMAKER

In Chapter 9, we will discuss the possible evolutionary advantages of circadian rhythmicity. We need not discuss, however, the importance of feeding. If you do not eat (or obtain nutrients through some form of artificial infusion), you will soon die. Eating is not an optional activity. Thus, food restriction (FR) as a zeitgeber occupies a special place among nonphotic zeitgebers. In the previous section, it was mentioned that FR seems to be a weaker zeitgeber than light. Yet, animals do not die because of lack of light, whereas they do die because of lack of food. Evidently, functions that are essential for the survival of an organism need not be essential for the synchronization of circadian rhythms. This can lead to conflicts between homeostatic and circadian mechanisms in the body, as will be discussed in Chapter 10.

In most laboratory studies of circadian rhythms, organisms are fed *ad libitum* (AL) (i.e., food is available at all times). In studies on the role of food availability as a zeitgeber, organisms are usually maintained under constant darkness or constant light. Consequently, conditions for the appearance of conflicts between the zeitgeber roles of food and light are usually not present. It would be very wrong, however, to say that the issue of conflicts has escaped the attention of circadian physiologists. A substantial number of studies have examined circadian rhythms of organisms exposed to simultaneous but conflicting cycles of illumination and food availability. Studies have been

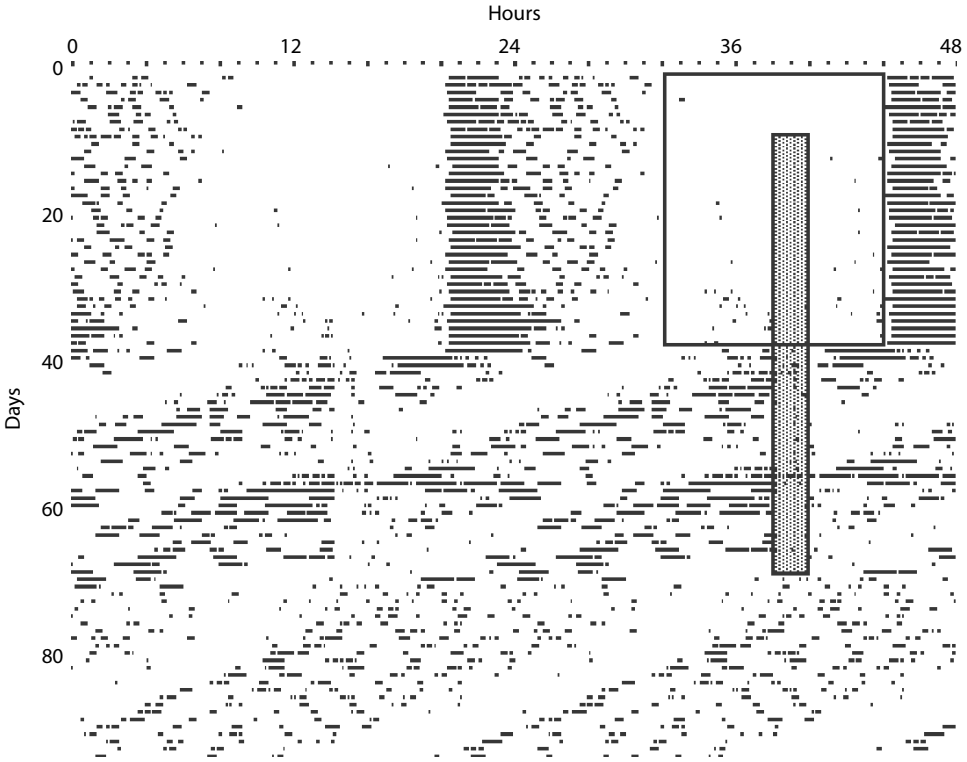


FIGURE 8.24 Conflict of light and food as zeitgebers: light wins. This double-plotted actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained under an LD cycle (as indicated by the single-plotted clear rectangle) and a cycle of food availability (as indicated by the stippled rectangle). The cycle of food availability had very little effect on the activity rhythm when presented simultaneously with the LD cycle or when presented alone. (Data from the archives of the Refinetti lab.)

conducted in fishes,[38,212] birds,[213,214] rodents,[52,63,64,85,203,215–229] and other mammals.[44,230–232] An example is shown in Figure 8.24. This double-plotted actogram shows the rhythm of running-wheel activity of a domestic mouse (*M. musculus*) maintained first under an LD cycle with food available AL, then under simultaneous cycles of food availability and illumination, then under only a cycle of food availability, and finally in constant darkness with food available AL. Notice the robust pattern of entrainment under the LD cycle, which was essentially unaffected by the addition of the cycle of FR. When only FR was present, some masking (and perhaps relative coordination) was noticeable, but entrainment was not established or maintained. It is possible that the implementation of FR (daily delivery of 85% of maintenance diet instead of timed access to a food bin) did not ensure the saliency of the cycle of food availability in this study. However, visual observation of the animal indicated that the food was consumed immediately upon presentation each day (i.e., within 2 hours of delivery). In anthropomorphical terms, the mouse knew that the food would be delivered once a day, he was hungry and motivated to eat, but he did not seem to care about the circadian time of feeding.

A stronger effect of FR can be seen in the records shown in Figure 8.25. In this case, a significant change in the activity

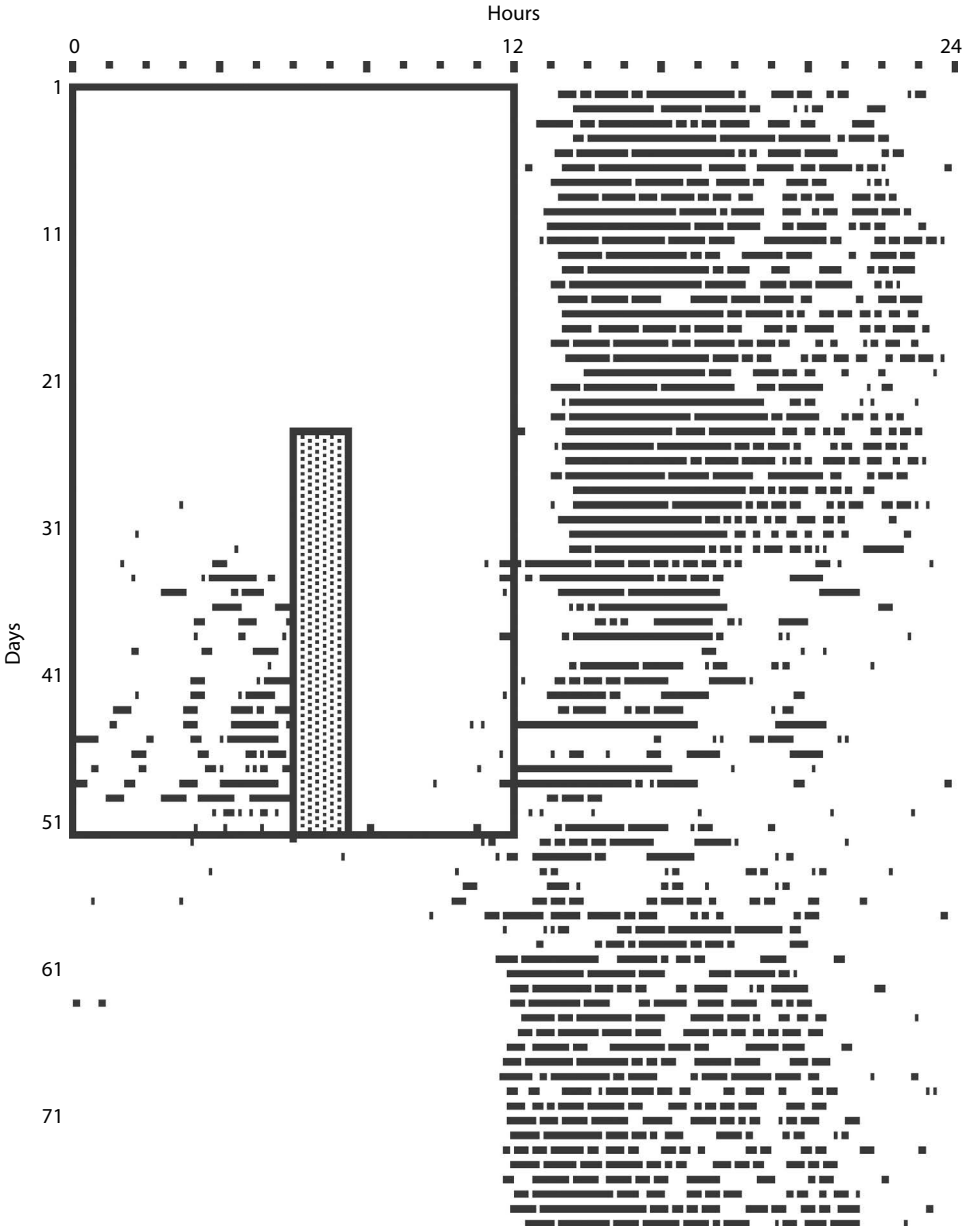


FIGURE 8.25 Conflict of light and food as zeitgebers: food wins. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained under an LD cycle (as indicated by the clear rectangle) and a cycle of food availability (as indicated by the stippled rectangle). Although the cycle of food availability did not cause a shift of the activity rhythm (as evidenced by the free run later on), it clearly induced extra activity during the light phase. The extra activity preceded the time of food access by several hours. (Data from the archives of the Refinetti lab.)

pattern took place a few days after the FR schedule was introduced. Running activity became less regular during the night, and a good deal of activity was exhibited for several hours prior to feeding. Thus, the mouse seemed to anticipate the delivery of food each day. This phenomenon of *food anticipatory activity* (FAA) has been observed in numerous studies in many species, including insects,[37] fishes,[40,233] birds,[214,234] mice,[24,52,59,62,64,217,235–243] rats,[48,51,60,61,227,228,244–255] and other mammals.[44,66,107,256–258]

In principle, FAA could be just a physiological curiosity. By consulting their circadian clocks, animals could literally *anticipate* the delivery of food. In anthropomorphical terms, they could become excited by the expectation of a meal, and the locomotor activity preceding food access would reflect this excitement. As it turns out, however, FAA is a much more interesting phenomenon. Consider Figure 8.26. To avoid photic masking of activity (and to reduce the strength of the photic zeitgeber), the mouse was placed under an LD cycle consisting of only 15 minutes of light each day. Notice the appropriate control of period and phase by the photic zeitgeber during the first month of recording. When the FR schedule was introduced, anticipatory activity developed rapidly. When both zeitgebers were discontinued (that is, when food became available AL in constant darkness), there was a 1-day rebound of activity, which was followed by a very interesting phenomenon. The main component of the activity rhythm (which had been previously entrained by the photic zeitgeber) started to freerun with the short period typical of mice. However, a second component can also be seen. Although short-lived (i.e., lasting about a week), the component associated with the previous FAA freeran with a period much longer than that of the other component. This dissociation of components has been observed by various research teams.[44,237,253,259,260] Because one pacemaker cannot generate two rhythmic components with nonharmonic periods, it is tempting to assume that two pacemakers are involved. Thus, there may be a *food-entrainable oscillator* in addition to the master pacemaker (which is often called the *light-entrainable oscillator* for comparative purposes, even though the master pacemaker is entrainable by various stimuli besides light).

Friedrich Stephan (Figure 8.27), a psychology professor at Florida State University, has spent most of his professional life studying the properties of the food-entrainable oscillator.[35] He would be understandably disappointed if

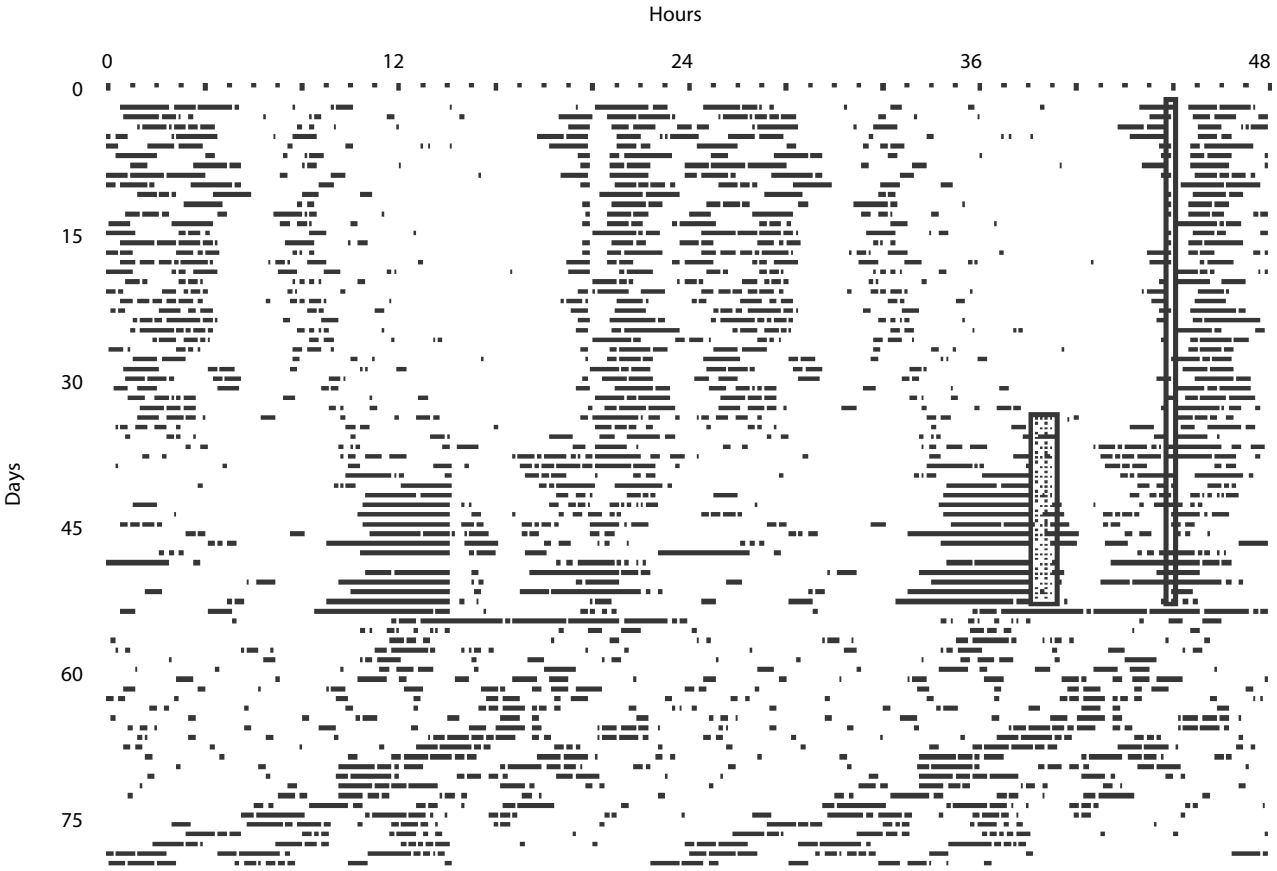


FIGURE 8.26 Conflict of light and food as zeitgebers: two systems revealed. This double-plotted actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained under a skeleton LD cycle (as indicated by the narrow, single-plotted clear rectangle) and a cycle of food availability (as indicated by the stippled rectangle). Clear anticipatory activity was evident in the presence of the cycle of food availability. When both environmental cycles were discontinued on day 54, two components of the activity rhythm freeran for several days (although only the component previously entrained by light persisted indefinitely). (Data from the archives of the Refinetti lab.)

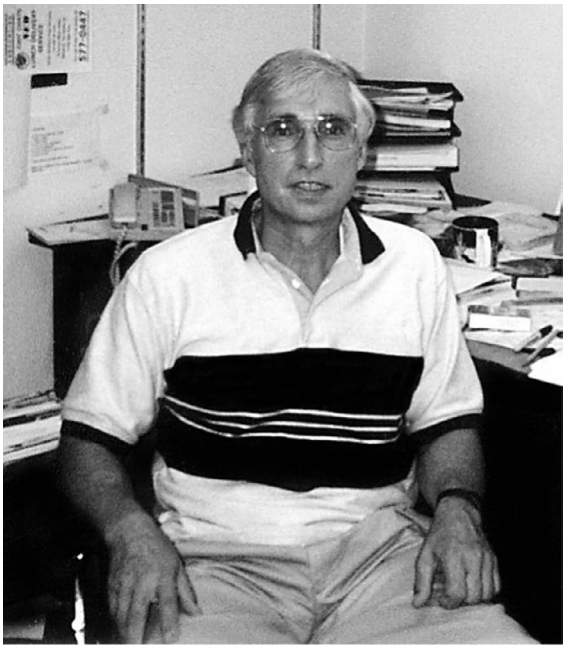


FIGURE 8.27 Friedrich Stephan. This circadian physiologist from Florida State University (Tallahassee) has researched extensively the properties of the food-entrainable pacemaker in rats. (Photo courtesy of Friedrich Stephan.)

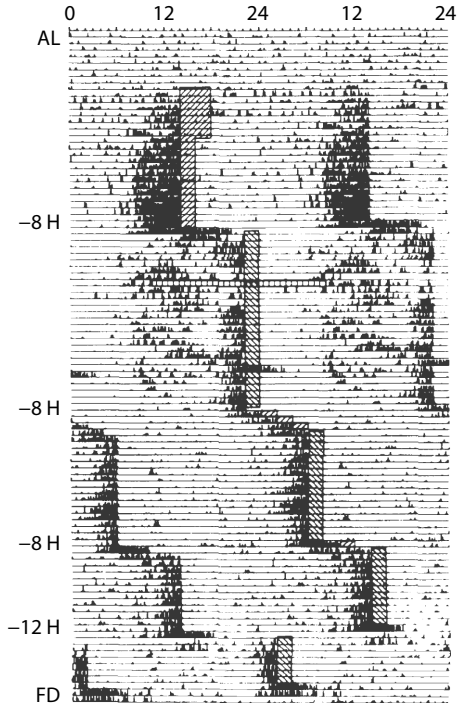


FIGURE 8.28 Confirming the existence of a separate food-entrainable pacemaker. This double-plotted actogram shows the running-wheel activity rhythm of a laboratory rat (*Rattus norvegicus*) rendered arrhythmic by destruction of the master circadian clock in the brain and exposed to cycles of food availability (as indicated by the hatched rectangles). Notice the absence of rhythmicity during the initial days under free-feeding conditions (AL) and the presence of clear rhythmicity under the restricted-feeding schedules. Notice also the persistence of rhythmicity during the two days of total food deprivation (FD). (Reproduced from *Physiol. Behav.*, 52, Stephan, F.K., Resetting of a feeding-entrainable circadian clock in the rat, 985–995. Copyright 1992, with permission from Elsevier.)

we remarked that the concept of a food-entrainable oscillator that is distinct from the master pacemaker resembles the concept of morning and evening oscillators that we discredited in Chapter 6. Stephan has nothing to fear, however. His two-oscillator concept has very strong empirical bases. The strongest evidence that a food-entrainable oscillator really exists is that FAA can be evoked by restricted feeding in animals whose master pacemaker has been destroyed surgically[59,61,109,227,240,253,256,261–269] or inactivated by genetic engineering.[238,239,270–272] As shown in Figure 8.28, animals with these discrete brain lesions are arrhythmic when kept in constant darkness with food available AL. Imposition of an FR schedule evokes anticipatory activity, and this activity pattern is phase-shifted when the time of food access is shifted. Notice that, if the rat is food deprived for a few days (FD), the rhythmic component persists. Rats (like most animals) cannot be food deprived for many days (otherwise they will die), but the persistence of the activity pattern for a few days is evidence that the food-entrainable oscillator is a true oscillator—and not just an hourglass mechanism that is restarted daily by food access. Studies of the coupling of the two mechanisms indicate that the master pacemaker has stronger effects on the food-entrainable oscillator than the latter has on the former,[273–275] but the food-entrainable oscillator is certainly a genuine oscillator, even if its anatomical location is yet to be determined.[254,276–278] The caloric content of food, rather than just bulk, seems to be the relevant signal for resetting of the oscillator,[279] and glucose seems to be more effective than lipids in this regard[280] (see Figure 8.29).

The existence of the food-entrainable oscillator implies that the circadian system must be a hierarchical system with two or more pacemakers. A conceptual diagram of the system is shown in Figure 8.30. The master pacemaker exerts control over slave pacemakers (such as the food-entrainable oscillator) or directly over passive slaves (effector organs) to generate a multitude of overt rhythms. External stimuli can affect the system at any of the three levels as masking agents, or at the pacemaker levels as zeitgebers. Very likely, slave actions can feedback on the master pacemaker.

A final reminder is necessary here. When evidence about the food-entrainable oscillator started to appear, there was no *a priori* reason to doubt that both the food-entrainable oscillator and the master pacemaker could be responsive to FR. However, an early review article referred to the master pacemaker as the *light-entrainable oscillator* to contrast it with the *food-entrainable oscillator*, thus making the assumption of distinct input modalities to the two clocks[34]—and the dichotomy caught on. This misleading dichotomy was later bolstered by the observation that rhythms of gene expression in a peripheral organ such as the liver were affected more rapidly by a change in the cycle of food availability than by a change in the LD cycle.[281,282] Yet, the assertion that FR entrains

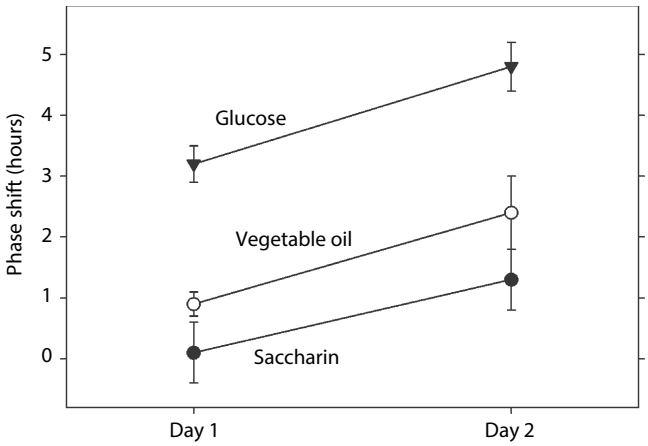


FIGURE 8.29 Dissecting the food-entrainable pacemaker. The graph shows the effects of different nutrients on the pace of reentrainment of the feeding rhythms of laboratory rats (*Rattus norvegicus*) after an 8-hour delay of the schedule of FR. The data points indicate the mean phase shifts (±SE) of 7–8 rats on the first and second days after the 8-hour shift of the feeding schedule. All rats had been previously rendered arrhythmic by destruction of the master circadian clock in the brain and had been entrained by restricted feeding of normal rat food for 2 weeks. Notice that glucose feeding led to greater phase shifts than vegetable oil or saccharin did. (Data from Stephan, F.K. and Davidson, A.J., *Physiol. Behav.*, 65, 277, 1998.)

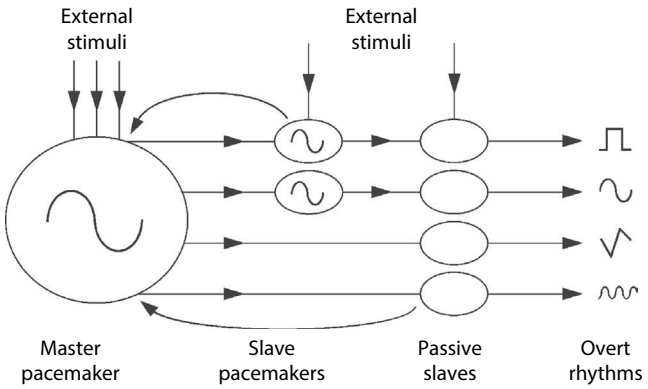


FIGURE 8.30 A hierarchy of pacemakers. The existence of a separate food-entrainable pacemaker requires a reconceptualization of the circadian system as a hierarchy of master and slave pacemakers (and perhaps passive slave pacemakers as well), each of them modulated (or not) by environmental stimuli. (Adapted from Turek, F.W., *Recent Progr. Hormone Res.*, 49, 43, 1994.)

the food-entrainable pacemaker does not imply the assertion that FR does *not* entrain the master pacemaker. As shown in Figure 8.31, FR entrains both pacemakers.[236] The mouse whose activity records are shown in the figure was initially maintained under simultaneous cycles of illumination and feeding. In this condition (LD), both FAA (open arrows) and a light-entrained component (closed arrows) can be clearly seen. When the schedule of FR was maintained in constant darkness (DD), the anticipatory activity component was slightly unmasked but persisted otherwise

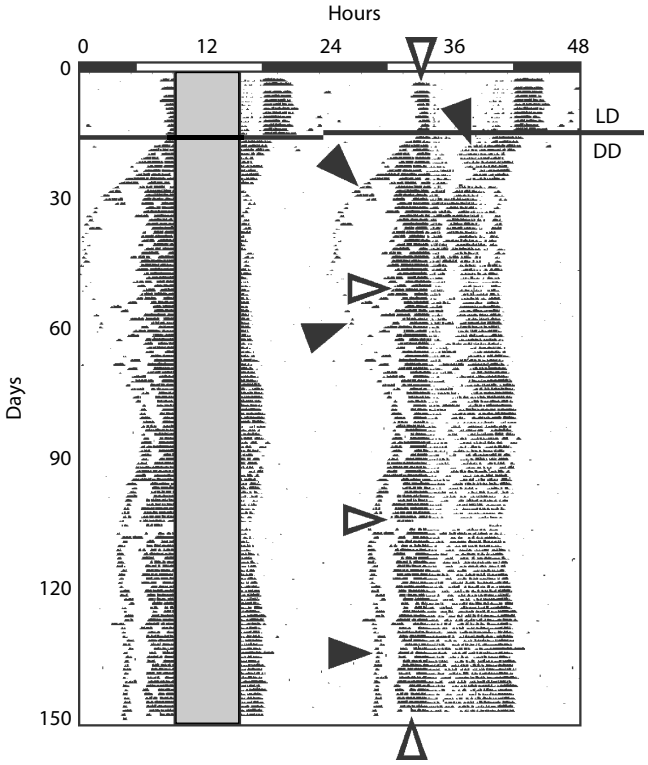


FIGURE 8.31 But let's not get carried away. The *light-entrainable* component of the activity rhythm can definitely be entrained by FR, as shown in this double-plot actogram of the running-wheel activity rhythm of a domestic mouse (*Mus musculus*). A 24-hour LD cycle (denoted by the white and dark horizontal bars at the top) was present for 2 weeks. Food availability was restricted to 6 hours per day (as indicated by the single-plotted gray box) throughout. The *food-entrainable* component (white arrowheads) exhibits entrainment from the beginning of the record. The *light-entrainable* component (closed arrowheads) slowly switches from entrainment by light to entrainment by FR after the LD cycle is eliminated. (Courtesy of Abel Bult-Ito.)

unaltered. The main activity component initially freeran with the short period typical of mice but soon started to slow down, eventually entraining to the feeding schedule. Measurements conducted directly in the pacemaker *post mortem* (clock gene expression in the brain) confirmed entrainment.[236] Thus, the master pacemaker *can* be entrained by a schedule of FR.

Another clear demonstration of entrainment of the master pacemaker by hypocaloric FR was provided by Jorge Mendoza and colleagues at the University of Strasbourg (in France). Using Sudanian grass rats (*Arvicanthis ansorgei*), they obtained rapid entrainment of the main component of running-wheel activity by restricted feeding, with an anticipatory component embedded in the main component, and freerun from the appropriate phase after return to AL feeding.[66]

Having discussed endogenous mechanisms of circadian rhythmicity in Chapter 6, photic environmental mechanisms in Chapter 7, and nonphotic environmental mechanisms in this chapter, we shall now move on to a discussion of the integration of mechanisms in Chapter 9.

SUMMARY

1. Although light is the zeitgeber that has been most thoroughly studied, several nonphotic environmental stimuli have been shown to also entrain circadian rhythms. They include ambient temperature, food availability, physical activity (exercise), and social contact. Nonphotic stimuli seem not to have as strong an influence on the circadian system as light has.
2. There is some evidence that different nonphotic zeitgebers act on the circadian system through a common mechanism, but the nature of this common mechanism is still not clear.
3. Food availability can act directly on a slave pacemaker (the *food-entrainable oscillator*), as well as on the master pacemaker, to entrain circadian rhythms.

EXERCISES

8.1 Studying entrainment by FR in mice. If you assembled the data acquisition system in Exercise 2.3 or if you have access to a data acquisition system for rodent activity in a researcher's laboratory, you can conduct this exercise. In many countries, you will also need an institutionally approved protocol to perform this instructional activity involving a vertebrate species. If you are a university student using this book as part of a formal course, your professor should be able to take care of the necessary paperwork. The goal of the exercise is to provide you with firsthand experience on the process of nonphotic entrainment.

1. At first, you will place mice in individual cages with running wheels under an LD cycle with 12 hours of light and 12 hours of darkness per day. After you determine that the rhythm of running-wheel activity is properly entrained, you must find out how much food your mice eat each day.
2. To find out how much each mouse eats, empty each food hopper and then fill it with a previously weighed amount of food (at least 50 g, or 1.8 oz). After 3 days, empty the food hopper again and weigh the food (but do not forget to refill the hopper when you are finished!). The difference between the amount that you put in and the amount that you took out is approximately the amount that the mouse ate in 3 days (there may have been some spillage). Divide the amount by 3 to find out how much the mouse ate per day. It should be around 5 g (or 0.18 oz).
3. If you feed the mouse the amount of food that it normally eats, food will not be a conspicuous stimulus. A safe but effective level of FR is 85% of the free-feeding level. Multiply each mouse's daily consumption by 0.85 to determine the daily ration for FR.
4. When you are ready to start the actual study, place the animals in constant darkness for a few days (still with food freely available). Make sure to weigh the animals at least once so that you can monitor weigh losses later. Then empty the food hoppers and start dropping the daily ration in the hoppers at the same time each day. The ideal time (to speed up the study) is about 4 hours before the onset of activity on the first day.
5. Inspect the records of running-wheel activity at least every 2 days. About a week into the study, you should be able to notice an increase in activity several hours before the feeding time. Although FR to 85% of the free-feeding level is reasonably safe, you should weigh the animals regularly to ensure that they are not losing too much weight. If an animal's weight drops below 80% of its initial weight, you must increase the daily ration!
6. Two or three weeks after the beginning of the FR schedule, you should be able to notice clear entrainment. The daily onsets of activity will occur more or less regularly several hours before the time of feeding. At this point, you should fill the food hoppers and record activity for a few more days so that you can determine if the rhythms freerun from the phase of entrainment. If they do not, then you are probably dealing with *masking*, not with true entrainment. Actually, if you do use mice as I suggested, you will likely observe strong anticipatory activity but not true entrainment. True entrainment is more likely in other species.
7. When the exercise is finished, reinstate the LD cycle and make sure that someone is in charge of caring for the animals after you are gone.

8.2 Viewing PRCs. In this exercise, we will use the program **PRC** (the sixth icon from the right in the Circadian banner). As already mentioned in Exercise 7.2, this program is an atlas of PRCs (i.e., of phase shifts of circadian rhythms evoked by single pulses of environmental stimuli). The data were obtained from published studies and adjusted, if necessary, to comply with the convention that the onset of activity be designated as CT 12 for nocturnal organisms and as CT 0 for diurnal organisms.

1. Start the program **PRC**.
2. Notice that you can choose either *Photic* or *Nonphotic* stimuli. We looked at photic PRCs in Exercise 7.2, so you should click on *Nonphotic* now. Notice also the three utility buttons: one allows you to clear the picture when it gets too crowded, the other allows you to resize the Y-axis when needed, and the third allows you to copy the picture to the Windows clipboard (so that you can paste it on a graphics program).
3. The operation of the program is rather simple and has been explained in Exercise 7.2. Now explore the nonphotic PRCs of the various species at your leisure. Most curves have small amplitude and will fit in the smallest Y-axis scale (from –3 to +3 hours). Notice that some curves refer to

nonphotic agents, such as particular drugs, that mimic the effect of light. Consequently, they look like photic PRCs (with a phase-delay region during early subjective night and a phase-advance region during late subjective night and/or early subjective day).

8.3 Building PRCs. In this exercise, we will use the program **Model** (the fifth icon from the right in the Circadian banner) to simulate phase shifts of the circadian system and build a PRC. Although the PRC used by the program is that for light pulses, the general process is the same as for nonphotic stimuli.

1. Start the program **Model**.
2. At the top panel, change *Period* to **23.5** hours and set the number of *Blocks* to **2**.
3. At the *Block 1* panel, leave all values at their defaults (*Days* at 14, *Mode* at LD, *Lights on* at 0, and *Lights off* at 12, which means that the "animal" will be under a 24-hour LD cycle, with lights on at midnight and off at noon, for 14 days).
4. At the *Block 2* panel, change *Days* to **28**, change the *Mode* to **pulse**, change *Days in DD* to **14**, and leave *Circadian time* at 20 and *Duration* at 42. This means that the animal will be in constant darkness for 28 days and will receive a pulse at circadian time 20 on the 15th day. Circadian time 20 is 8 circadian hours after the onset of activity (CT12). Because the free-running period is different from 24.0 hours, 1 circadian hour is $\tau/24$ of a real hour. The computer will make the necessary adjustment (for $\tau = 23.5$, 1 circadian hour equals 0.98 hour).
5. Now click on *Run* to start the simulation. You will observe a phase advance of 1.6 hours following the pulse. To confirm this value, you may just click on the onset before the pulse to see the clock time, then click on the onset after the pulse, and then subtract the two values. However, because of the slope in the actogram, you will overestimate the shift to be 1.9 hours. Try it now.
6. To correct for the slope, the prepulse phase and the postpulse phase must be compared on the same day. In order to accomplish this, you may extend an imaginary line going from the onsets before the pulse down to the first postpulse onset, then extend another imaginary line going from the onsets after the pulse up to the first postpulse onset, and then subtract the onset times on that day. If you do this, you should obtain a shift of 1.6 hours. (If this seems a bit confusing, you may want to redo Exercise 7.1, where the computer does all these corrections for you.)
7. There is one more complication, although it is often negligible. Because each physical hour is $24/\tau$ of a circadian hour, you must multiply the observed shift by 1.02 (i.e., 24/23.5) to obtain the actual shift of +1.63 circadian hours (instead of +1.60 hours). As you can see, the correction is rather small. However, the correction would be larger if the period were farther from 24.0 hours. For a period of 20.0 hours, a shift of 1.6 clock hours would be a shift of 1.92 circadian hours (a difference of 0.32 hour, or almost 20 minutes).
8. Because the adjustment for the slope and the conversion between physical hour and circadian hour can be confusing, let us construct the PRC using an animal with a period of 24.0 hours, so that 1 circadian hour is the same as one physical hour. Click on *Close* to return to the main window. At the top panel, change the *Period* back to **24**. In the *Block 2* panel, change the *Circadian time* to **0**. Then click on *Run*.
9. You should see no phase shift (a phase shift of 0.0 circadian hours). Write down the circadian time of the pulse and the resulting phase shift. Then, repeat the previous step 11 times, replacing only the *Circadian time* (2, 4, 6, …, 22). You will observe phase delays at CT 12 and CT 14 and phase advances from CT 16 to CT 22.
10. When you have collected all 12 data points, plot them on a graph with circadian time on the abscissa and phase shifts on the ordinate. Notice that the resulting PRC has the characteristics of a photic PRC (that is, phase delays elicited during early subjective night and phase advances elicited during late subjective night). Nonphotic PRCs usually have a phase-advance region during subjective day and not always have a phase-delay region.

SUGGESTIONS FOR FURTHER READING

Stephan, F. K. (2001). Food-entrainable oscillators in mammals. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 223–246.

A thorough review of research on the entrainment of circadian rhythms by FR.

Mrosovsky, N. (1996). Locomotor activity and non-photic influences on circadian clocks. *Biological Reviews of the Cambridge Philosophical Society* 71: 343–372.

A good review of studies on the entrainment of circadian rhythms by nonphotic agents, particularly physical exercise.

Schibler, U., Ripperger, J., and Brown, S. A. (2003). Peripheral circadian oscillators in mammals: Time and food. *Journal of Biological Rhythms* 18: 250–260.

An excellent review article that discusses the relationship between central and peripheral pacemakers as affected by photic and nonphotic stimuli.

Webb, I. C., Antle, M. C., and Mistlberger, R. E. (2014). Regulation of circadian rhythms in mammals by behavioral arousal. *Behavioral Neuroscience* 128: 304–325.

A thorough review of nonphotic entrainment with emphasis on its implications for neuroscience research.

WEBSITES TO EXPLORE

Automated Feeding Systems (Sweeney): http://www.sweeneyfeeders.com.

Environmental Chambers (Thermotron): http://www.thermotron.com/.

Refrigerated Incubators (Thermo Scientific): http://www.thermoscientific.com/en/products/refrigerated-incubators.html.

Temperature Control Equipment (Digital Control Systems): http://www.dcs-inc.net/temperature.htm.

Treadmills for Rodents (TSE Systems): http://www.tse-systems.com/products/behavior/motor-function/treadmill.htm.

REFERENCES

1. Sweeney, B. M. and Hastings, J. W. (1960). Effects of temperature upon diurnal rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 87–103.
2. Rensing, L. and Ruoff, P. (2002). Temperature effect on entrainment, phase shifting, and amplitude of circadian clocks and its molecular bases. *Chronobiology International* 19: 807–864.
3. Michael, T. P., Salomé, P. A., and McClung, C. R. (2003). Two *Arabidopsis* circadian oscillators can be distinguished by differential temperature sensitivity. *Proceedings of the National Academy of Sciences United States America* 100: 6878–6883.
4. Roenneberg, T., Dragovic, Z., and Merrow, M. (2005). Demasking biological oscillators: Properties and principles of entrainment exemplified by the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences United States America* 102: 7742–7747.
5. Page, T. L. (1985). Circadian organization in cockroaches: Effects of temperature cycles on locomotor activity. *Journal of Insect Physiology* 31: 235–242.
6. North, R. D. (1993). Entrainment of the circadian rhythm of locomotor activity in wood ants by temperature. *Animal Behaviour* 45: 393–397.
7. Currie, J., Goda, T., and Wijnen, H. (2009). Selective entrainment of the *Drosophila* circadian clock to daily gradients in environmental temperature. *BMC Biology* 7: art. 49.
8. Kannan, N. N., Reveendran, R., Dass, S. H., Manjunatha, T., and Sharma, V. K. (2012). Temperature can entrain egg laying rhythm of *Drosophila* but may not be a stronger zeitgeber than light. *Journal of Insect Physiology* 58: 245–255.
9. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
10. Foà, A. and Bertolucci, C. (2001). Temperature cycles induce a bimodal activity pattern in ruin lizards: Masking or clock-controlled event? *Journal of Biological Rhythms* 16: 574–584.
11. Ellis, D. J., Firth, B. T., and Belan, I. (2009). Thermocyclic and photocyclic entrainment of circadian locomotor activity rhythms in sleepy lizards, *Tiliqua rugosa*. *Chronobiology International* 26: 1369–1388.
12. Eskin, A. (1971). Some properties of the system controlling the circadian activity rhythm of sparrows. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 55–80.
13. Lindberg, R. G. and Hayden, P. (1974). Thermoperiodic entrainment of arousal from torpor in the little pocket mouse, *Perognathus longimembris*. *Chronobiologia* 1: 356–361.
14. Aschoff, J. and Tokura, H. (1986). Circadian activity rhythms in squirrel monkeys: Entrainment by temperature cycles. *Journal of Biological Rhythms* 1: 91–99.
15. Francis, A. J. P. and Coleman, G. J. (1990). Ambient temperature cycles entrain the free-running circadian rhythms of the stripe-faced dunnart, *Sminthopsis macroura*. *Journal of Comparative Physiology A* 167: 357–362.
16. Francis, A. J. P. and Coleman, G. J. (1988). The effect of ambient temperature cycles upon circadian running and drinking activity in male and female laboratory rats. *Physiology and Behavior* 43: 471–477.
17. Rajaratnam, S. M. W. and Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
18. Pohl, H. (1998). Temperature cycles as zeitgeber for the circadian clock of two burrowing rodents, the normothermic antelope ground squirrel and heterothermic Syrian hamster. *Biological Rhythm Research* 29: 311–325.
19. Goldman, B. D., Goldman, S. L., Riccio, A. P., and Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
20. Herzog, E. D. and Huckfeldt, R. M. (2003). Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 763–770.
21. Tokura, H. and Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
22. Pálková, M., Sigmund, L., and Erkert, H. G. (1999). Effect of ambient temperature on the circadian activity rhythm in common marmosets, *Callithrix j. jacchus* (Primates). *Chronobiology International* 16: 149–161.
23. Refinetti, R. (2010). Entrainment of circadian rhythm by ambient temperature cycles in mice. *Journal of Biological Rhythms* 25: 247–256.
24. Refinetti, R. (2015). Comparison of light, food, and temperature as environmental synchronizers of the circadian rhythm of activity in mice. *Journal of Physiological Sciences* 65: 359–366.
25. El Allali, K., Achaâban, M. R., Bothorel, B., Piro, M., Bouâouda, H., El Allouchi, M., Ouassat, M., Malan, A., and Pévet, P. (2013). Entrainment of the circadian clock by daily ambient temperature cycles in the camel (*Camelus dromedarius*). *American Journal of Physiology* 304: R1044–R1052.
26. Stevens, J. C. and Stevens, S. S. (1960). Warmth and cold: Dynamics of sensory intensity. *Journal of Experimental Psychology* 60: 183–192.
27. Refinetti, R. (1990). Is there modal opponency in the thermal senses? *Journal of Thermal Biology* 15: 255–258.
28. Hensel, H. (1974). Thermoreceptors. *Annual Review of Physiology* 36: 233–249.
29. Schepers, R. J. and Ringkamp, M. (2009). Thermoreceptors and thermosensitive afferents. *Neuroscience and Biobehavioral Reviews* 33: 205–212.
30. Webb, H. M., Bennet, M. F., Graves, R. C., and Stephens, G. C. (1953). Relationship between time of day and inhibiting influence of low temperature on the diurnal chromatophore rhythm of *Uca*. *Biological Bulletin* 105: 386–387.
31. Edery, I., Rutila, J. E., and Rosbash, M. (1994). Phase shifting of the circadian clock by induction of the *Drosophila period* protein. *Science* 263: 237–240.
32. Francis, A. J. P. and Coleman, G. L. (1997). Phase response curves to ambient temperature pulses in rats. *Physiology and Behavior* 62: 1211–1217.
33. Ruby, N. F., Burns, D. E., and Heller, H. C. (1999). Circadian rhythms in the suprachiasmatic nucleus are temperature-compensated and phase-shifted by heat pulses *in vitro*. *Journal of Neuroscience* 19: 8630–8636.

34. Boulos, Z. and Terman, M. (1980). Food availability and daily biological rhythms. *Neuroscience and Biobehavioral Reviews* 4: 119–131.
35. Stephan, F. K. (2001). Food-entrainable oscillators in mammals. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 223–246.
36. Mendoza, J. (2007). Circadian clocks: Setting time by food. *Journal of Neuroendocrinology* 19: 127–137.
37. Frisch, B. and Aschoff, J. (1987). Circadian rhythms in honeybees: Entrainment by feeding cycles. *Physiological Entomology* 12: 41–49.
38. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S., and Tabata, M. (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *Journal of Comparative Physiology A* 181: 121–132.
39. Sánchez-Vásquez, F., Aranda, A., and Madrid, J. A. (2001). Differential effects of meal size and food energy density on feeding entrainment in goldfish. *Journal of Biological Rhythms* 16: 58–65.
40. Kasai, M. and Kiyohara, S. (2010). Food- and light-entrainable oscillators control feeding and locomotor activity rhythms, respectively, in the Japanese catfish, *Plotosus japonicus*. *Journal of Comparative Physiology A* 196: 901–912.
41. Hau, M. and Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
42. Hau, M. and Gwinner, E. (1996). Food as a circadian zeitgeber for house sparrows: The effect of different food access durations. *Journal of Biological Rhythms* 11: 196–207.
43. Jilge, B. (1991). Restricted feeding: A nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
44. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
45. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1996). Daily restricted feeding effects on the circadian activity rhythms of the stripe-faced dunnart, *Sminthopsis macroura*. *Journal of Biological Rhythms* 11: 188–195.
46. Challet, E., Losee-Olson, S., and Turek, F. W. (1999). Reduced glucose availability attenuates circadian responses to light in mice. *American Journal of Physiology* 276: R1063–R1070.
47. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
48. Edmonds, S. C. and Adler, N. T. (1977). Food and light as entrainers of circadian running activity in the rat. *Physiology and Behavior* 18: 915–919.
49. Coleman, G. J. and Francis, A. J. P. (1991). Food deprivation and reinstatement phase shifts rat activity rhythms in constant light but not constant dark. *Physiology and Behavior* 50: 167–171.
50. Rusak, B., Mistlberger, R. E., Losier, B., and Jones, C. H. (1988). Daily hoarding opportunity entrains the pacemaker for hamster activity rhythms. *Journal of Comparative Physiology A* 164: 165–171.
51. Challet, E., Malan, A., and Pévet, P. (1996). Daily hypocaloric feeding entrains circadian rhythms of wheel-running and body temperature in rats kept in constant darkness. *Neuroscience Letters* 211: 1–4.
52. Holmes, M. M. and Mistlberger, R. E. (2000). Food anticipatory activity and photic entrainment in food-restricted BALB/c mice. *Physiology and Behavior* 68: 655–666.
53. Sharma, V. K., Chidambaram, R., Subbaraj, R., and Chandrashekaran, M. K. (2000). Effects of restricted feeding cycle on the locomotor activity rhythm in the mouse *Mus booduga*. *Physiology and Behavior* 70: 81–87.
54. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
55. Boulos, Z., Frim, D. M., Dewey, L. K., and Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
56. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Feeding time synchronizes primate circadian rhythms. *Physiology and Behavior* 18: 775–779.
57. White, W. and Timberlake, W. (1995). Two meals promote entrainment of rat food-anticipatory and rest-activity rhythms. *Physiology and Behavior* 57: 1067–1074.
58. Mendoza, J., Angeles-Castellanos, M., and Escobar, C. (2005). A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *European Journal of Neuroscience* 22: 2855–2862.
59. Marchant, E. G. and Mistlberger, R. E. (1997). Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6J mice. *Brain Research* 765: 273–282.
60. Gooley, J. J., Schomer, A., and Saper, C. B. (2006). The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Nature Neuroscience* 9: 398–407.
61. Jansen, H. T., Sergeeva, A., Stark, G., and Sorg, B. A. (2012). Circadian discrimination of reward: Evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiology International* 29: 454–468.
62. Mendoza, J., Graff, C., Dardente, H., Pévet, P., and Challet, E. (2005). Feeding cues alter clock gene oscillations and photic responses in the suprachiasmatic nuclei of mice exposed to a light–dark cycle. *Journal of Neuroscience* 25: 1514–1522.
63. Abe, H., Honma, S., and Honma, K. (2007). Daily restricted feeding resets the circadian clock in the suprachiasmatic nucleus of CS mice. *American Journal of Physiology* 292: R607–R615.
64. Feillet, C. A., Ripperger, J. A., Magnone, M. C., Dulloo, A., Albrecht, U., and Challet, E. (2006). Lack of food anticipation in *Per2* mutant mice. *Current Biology* 16: 2016–2022.
65. Mendoza, J., Clesse, D., Pévet, P., and Challet, E. (2010). Food-reward signalling in the suprachiasmatic clock. *Journal of Neurochemistry* 112: 1489–1499.
66. Mendoza, J., Gourmelen, S., Dumont, S., Sage-Ciocca, D., Pévet, P., and Challet, E. (2012). Setting the main circadian clock of a diurnal mammal by hypocaloric feeding. *Journal of Physiology* 590: 3155–3168.
67. Piccione, G., Bertolucci, C., Caola, G., and Foà, A. (2007). Effects of restricted feeding on circadian activity rhythms of sheep: A brief report. *Applied Animal Behaviour Science* 107: 233–238.
68. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
69. Reebs, S. G. and Mrosovsky, N. (1989). Effects of induced wheel running on the circadian activity rhythms of Syrian hamsters: Entrainment and phase response curve. *Journal of Biological Rhythms* 4: 39–48.
70. Mistlberger, R. E. (1991). Effects of daily schedules of forced activity on free-running rhythms in the rat. *Journal of Biological Rhythms* 6: 71–80.

71. Edgar, D. M. and Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
72. Marchant, E. G. and Mistlberger, R. E. (1996). Entrainment and phase shifting of circadian rhythms in mice by forced treadmill running. *Physiology and Behavior* 60: 657–663.
73. Laemle, L. K. and Ottenweller, J. E. (1999). Nonphotic entrainment of activity and temperature rhythms in anophthalmic mice. *Physiology and Behavior* 66: 461–471.
74. Dallmann, R., Lemm, G., and Mrosovsky, N. (2007). Toward easier methods of studying nonphotic behavioral entrainment in mice. *Journal of Biological Rhythms* 22: 458–461.
75. Yamanaka, Y., Honma, S., and Honma, K. (2008). Scheduled exposures to a novel environment with a running-wheel differentially accelerate re-entrainment of mice peripheral clocks to new light–dark cycles. *Genes to Cells* 13: 497–507.
76. Power, A., Hughes, A. T. L., Samuels, R. E., and Piggins, H. D. (2010). Rhythm-promoting actions of exercise in mice with deficient neuropeptide signaling. *Journal of Biological Rhythms* 25: 235–246.
77. Yamanaka, Y., Honma, S., and Honma, K. (2013). Daily exposure to a running wheel entrains circadian rhythms in mice in parallel with development of an increase in spontaneous movement prior to running-wheel access. *American Journal of Physiology* 305: R1367–R1375.
78. Hut, R. A., Mrosovsky, N., and Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
79. Wickland, C. R. and Turek, F. W. (1991). Phase-shifting effects of acute increases in activity on circadian locomotor rhythms in hamsters. *American Journal of Physiology* 261: R1109–R1117.
80. Mrosovsky, N., Salmon, P. A., Menaker, M., and Ralph, M. R. (1992). Nonphotic phase shifting in hamster clock mutants. *Journal of Biological Rhythms* 7: 41–49.
81. Janik, D. and Mrosovsky, N. (1993). Nonphotically induced phase shifts of circadian rhythms in the golden hamster: Activity–response curves at different ambient temperatures. *Physiology and Behavior* 53: 431–436.
82. Gannon, R. L. and Rea, M. A. (1995). Twelve-hour phase shifts of hamster circadian rhythms elicited by voluntary wheel running. *Journal of Biological Rhythms* 10: 196–210.
83. Eastman, C. I., Hoese, E. K., Youngstedt, S. D., and Liu, L. (1995). Phase-shifting human circadian rhythms with exercise during the night shift. *Physiology and Behavior* 58: 1287–1291.
84. Weisgerber, D., Redlin, U., and Mrosovsky, N. (1997). Lengthening of circadian period in hamsters by novelty-induced wheel running. *Physiology and Behavior* 62: 759–765.
85. Challet, E., Takahashi, J. S., and Turek, F. W. (2000). Nonphotic phase-shifting in *Clock* mutant mice. *Brain Research* 859: 398–403.
86. Buxton, O. M., Lee, C. W., L'Hermite-Balériaux, M., Turek, F. W., and Van Cauter, E. (2003). Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase. *American Journal of Physiology* 284: R714–R724.
87. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2004). Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. *American Journal of Physiology* 286: R539–R546.
88. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S., and Takahashi, K. (1988). Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiology and Behavior* 42: 87–91.
89. Edgar, D. M., Martin, C. E., and Dement, W. C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *Journal of Biological Rhythms* 6: 185–199.
90. Edgar, D. M., Kilduff, T. S., Martin, C. E., and Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior* 50: 373–378.
91. Kuroda, H., Fukushima, M., Nakai, M., Katayama, T., and Murakami, N. (1997). Daily wheel running activity modifies the period of free-running rhythms in rats via intergeniculate leaflet. *Physiology and Behavior* 61: 633–637.
92. Mistlberger, R. E., Bossert, J. M., Holmes, M. M., and Marchant, E. G. (1998). Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. *Behavioural Brain Research* 96: 93–99.
93. Mrosovsky, N. (1999). Further experiments on the relationship between the period of circadian rhythms and locomotor activity levels in hamsters. *Physiology and Behavior* 66: 797–801.
94. Mistlberger, R. E. and Holmes, M. M. (2000). Behavioral feedback regulation of circadian rhythm phase angle in light–dark entrained mice. *American Journal of Physiology* 279: R813–R821.
95. Deboer, T. and Tobler, I. (2000). Running wheel size influences circadian rhythm period and its phase shift in mice. *Journal of Comparative Physiology A* 186: 969–973.
96. Koteja, P., Swallow, J. G., Carter, P. A., and Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.
97. Weinert, D. and Schottner, K. (2007). An inbred lineage of Djungarian hamsters with a strongly attenuated ability to synchronize. *Chronobiology International* 24: 1065–1079.
98. Mistlberger, R. E. and Antle, M. C. (1998). Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. *Brain Research* 786: 31–38.
99. Christian, C. A. and Harrington, M. E. (2002). Three days of novel wheel access diminishes light-induced phase delays in vivo with no effect on *per1* induction by light. *Chronobiology International* 19: 671–682.
100. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J., and Mrosovsky, M. (2003). Behavioral arousal blocks light-induced phase advances in locomotor rhythmicity but not light-induced Per1 and Fos expression in the hamster suprachiasmatic nucleus. *Neuroscience* 118: 253–261.
101. Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology A* 162: 35–46.
102. Pellowski, M. W. and Rosenwasser, A. M. (1996). Circadian phase shifting associated with routine cage maintenance: A retrospective analysis. *Biological Rhythm Research* 27: 130–137.
103. Evans, J. A., Elliott, J. A., and Gorman, M. R. (2007). Circadian effects of light no brighter than moonlight. *Journal of Biological Rhythms* 22: 356–367.
104. Smith, R. D., Turek, F. W., and Takahashi, J. S. (1992). Two families of phase–response curves characterize the resetting of the hamster circadian clock. *American Journal of Physiology* 262: R1149–R1153.
105. Davis, F. C. and Gorski, R. A. (1985). Development of hamster circadian rhythms. I. Within-litter synchrony of mother and pup activity rhythms at weaning. *Biology of Reproduction* 33: 353–362.

106. Duffield, G. E. and Ebling, F. J. P. (1998). Maternal entrainment of the developing circadian system in the Siberian hamster (*Phodopus sungorus*). *Journal of Biological Rhythms* 13: 315–329.
107. Jilge, B., Kuhnt, B., Landerer, W., and Rest, S. (2001). Circadian temperature rhythms in rabbit pups and in their does. *Laboratory Animals* 35: 364–373.
108. Davis, F. C. and Gorski, R. A. (1988). Development of hamster circadian rhythms: Role of the maternal suprachiasmatic nucleus. *Journal of Comparative Physiology A* 162: 601–610.
109. Weaver, D. R. and Reppert, S. M. (1989). Periodic feeding of SCN-lesioned pregnant rats entrains the fetal biological clock. *Developmental Brain Research* 46: 291–296.
110. Parraguez, V. H., Valenzuela, G. J., Vergara, M., Ducsay, C. A., Yellon, S. M., and Serón-Ferré, M. (1996). Effect of constant light on fetal and maternal prolactin rhythms in sheep. *Endocrinology* 137: 2355–2361.
111. Ohta, H., Xu, S., Moriya, T., Iigo, M., Watanabe, T., Nakahata, N., Chisaka, H. et al. (2008). Maternal feeding controls fetal biological clock. *PLOS ONE* 3: e2601.
112. Parraguez, V. H., Sales, F., Valenzuela, G. J., Vergara, M., Catalán, L., and Serón-Ferré, M. (1998). Diurnal changes in light intensity inside the pregnant uterus in sheep. *Animal Reproduction Science* 52: 123–130.
113. Nováková, M., Sládek, M., and Sumová, A. (2010). Exposure of pregnant rats to restricted feeding schedule synchronizes the SCN clocks of their fetuses under constant light but not under a light–dark regime. *Journal of Biological Rhythms* 25: 350–360.
114. Greco, A. M., Gambardella, P., Sticchi, R., D'Aponte, D., di Renzo, G., and de Franciscis, P. (1989). Effects of individual housing on circadian rhythms of adult rats. *Physiology and Behavior* 45: 363–366.
115. Tornatzky, W. and Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior* 53: 983–993.
116. Meerlo, P., De Boer, S. F., Koolhaas, J. M., Daan, S., and Van den Hoofdakker, R. H. (1996). Changes in daily rhythms of body temperature and activity after a single social defeat in rats. *Physiology and Behavior* 59: 735–739.
117. Keeney, A. J., Hogg, S., and Marsden, C. A. (2001). Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. *Physiology and Behavior* 74: 177–184.
118. Cambras, T., Castejón, L., and Díez-Noguera, A. (2012). Social interaction with a rhythmic rat enhances the circadian pattern of the motor activity and temperature of LL-induced arrhythmic rats. *Physiology and Behavior* 105: 835–840.
119. Wever, R. (1970). Zur Zeitgeber-Stärke eines Licht-Dunkel-Wechsels für die circadiane Periodik des Menschen. *Pflügers Archiv* 321: 133–142.
120. Aschoff, J., Fatranská, M., Giedke, H., Doerr, P., Stamm, D., and Wisser, H. (1971). Human circadian rhythms in continuous darkness: Entrainment by social cues. *Science* 171: 213–215.
121. Marimuthu, G., Rajan, S., and Chandrashekaran, M. K. (1981). Social entrainment of the circadian rhythm in the flight activity of the microchiropteran bat *Hipposideros speoris*. *Behavioral Ecology and Sociobiology* 8: 147–150.
122. Moritz, R. F. A. and Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.
123. Governale, M. M. and Lee, T. M. (2001). Olfactory cues accelerate reentrainment following phase shifts and entrain free-running rhythms in female *Octodon degus* (Rodentia). *Journal of Biological Rhythms* 16: 489–501.
124. Levine, J. D., Funes, P., Dowse, H. B., and Hall, J. C. (2002). Resetting the circadian clock by social experience in *Drosophila melanogaster*. *Science* 298: 2010–2012.
125. Honrado, G. I. and Mrosovsky, N. (1989). Arousal by sexual stimuli accelerates the re-entrainment of hamsters to phase advanced light–dark cycles. *Behavioral Ecology and Sociobiology* 25: 57–63.
126. Goel, N. and Lee, T. M. (1995). Social cues accelerate reentrainment of circadian rhythms in diurnal female *Octodon degus* (Rodentia: Octodontidae). *Chronobiology International* 12: 311–323.
127. Goel, N. and Lee, T. M. (1997). Social cues modulate free-running circadian activity rhythms in the diurnal rodent, *Octodon degus*. *American Journal of Physiology* 273: R797–R804.
128. Jechura, T. J., Walsh, J. M., and Lee, T. M. (2003). Testosterone suppresses circadian responsiveness to social cues in the diurnal rodent *Octodon degus*. *Journal of Biological Rhythms* 18: 43–50.
129. Paul, M. J. and Schwartz, W. J. (2007). On the chronobiology of cohabitation. *Cold Spring Harbor Symposia on Quantitative Biology* 72: 615–621.
130. Kavanau, J. L. (1963). The study of social interaction between small animals. *Animal Behaviour* 11: 263–273.
131. Møller, U. and Bojsen, J. (1974). The circadian temperature rhythm in Syrian hamsters as a function of the number of animals per cage. *Journal of Interdisciplinary Cycle Research* 5: 61–69.
132. Regal, P. J. and Connolly, M. S. (1980). Social influences on biological rhythms. *Behaviour* 72: 171–199.
133. Crowley, M. and Bovet, J. (1980). Social synchronization of circadian rhythms in deer mice (*Peromyscus maniculatus*). *Behavioral Ecology and Sociobiology* 7: 99–105.
134. Büttner, D. (1992). Social influences on the circadian rhythm of locomotor activity and food intake of guinea pigs. *Journal of Interdisciplinary Cycle Research* 23: 100–112.
135. Frisch, B. and Koeniger, N. (1994). Social synchronization of the activity rhythms of honeybees within a colony. *Behavioral Ecology and Sociobiology* 35: 91–98.
136. Tornatzky, W., Cole, J. C., and Miczek, K. A. (1998). Recurrent aggressive episodes entrain ultradian heart rate and core temperature rhythms. *Physiology and Behavior* 63: 845–853.
137. Rajaratnam, S. M. W. and Redman, J. R. (1999). Social contact synchronizes free-running activity rhythms of diurnal palm squirrels. *Physiology and Behavior* 66: 21–26.
138. Melo, P., Gonçalves, B., Menezes, A., and Azevedo, C. (2013). Socially adjusted synchrony in the activity profiles of common marmosets in light–dark conditions. *Chronobiology International* 30: 818–827.
139. Richter, C. P. (1970). Dependence of successful mating in rats on functioning of the 24-hour clocks of the male and female. *Communications in Behavioral Biology* 5: 1–5.
140. Davis, F. C., Stice, S., and Menaker, M. (1987). Activity and reproductive state in the hamster: Independent control by social stimuli and a circadian pacemaker. *Physiology and Behavior* 40: 583–590.
141. Aschoff, J. and von Goetz, C. (1988). Masking of circadian activity rhythms in male golden hamsters by the presence of females. *Behavioral Ecology and Sociobiology* 22: 409–412.

142. Refinetti, R., Nelson, D. E., and Menaker, M. (1992). Social stimuli fail to act as entraining agents of circadian rhythms in the golden hamster. *Journal of Comparative Physiology A* 170: 181–187.
143. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M., and Daan, S. (1997). Stress-induced changes in circadian rhythms of body temperature and activity in rats are not caused by pacemaker changes. *Journal of Biological Rhythms* 12: 80–92.
144. Gattermann, R. and Weinandy, R. (1997). Lack of social entrainment of circadian activity rhythms in the solitary golden hamster and in the highly social Mongolian gerbil. *Biological Rhythm Research* 28(S): 85–93.
145. Meerlo, P. and Daan, S. (1998). Aggressive and sexual social stimuli do not phase shift the circadian temperature rhythm in rats. *Chronobiology International* 15: 231–240.
146. Miles, L. E. M., Raynal, D. M., and Wilson, M. A. (1977). Blind man living in normal society has circadian rhythms of 24.9 hours. *Science* 198: 421–423.
147. Kleinknecht, S. (1985). Lack of social entrainment of free-running circadian activity rhythms in the Australian sugar glider (*Petaurus breviceps*: Marsupialia). *Behavioral Ecology and Sociobiology* 16: 189–193.
148. Erkert, H. G., Nagel, B., and Stephani, I. (1986). Light and social effects on the free-running circadian activity rhythm in common marmosets (*Callithrix jacchus*, Primates): Social masking, pseudo-splitting, and relative coordination. *Behavioral Ecology and Sociobiology* 18: 443–452.
149. Kennaway, D. J. and Van Dorp, C. F. (1991). Free-running rhythms of melatonin, cortisol, electrolytes, and sleep in humans in Antarctica. *American Journal of Physiology* 260: R1137–R1144.
150. Steel, G. D., Callaway, M., Suedfeld, P., and Palinkas, L. (1995). Human sleep–wake cycles in the high Arctic: Effects of unusual photoperiodicity in a natural setting. *Biological Rhythm Research* 26: 582–592.
151. Gwinner, E. (1966). Entrainment of a circadian rhythm in birds by species-specific song cycles. *Experientia* 22: 765–766.
152. Reebs, S. G. (1989). Acoustical entrainment of circadian activity rhythms in house sparrows: Constant light is not necessary. *Ethology* 80: 172–181.
153. Refinetti, R., Rissman, E., and Menaker, M. (1991). Circadian organization of sex behavior in pairs of tau-mutant hamsters. *FASEB Journal* 5(5): A1126.
154. Landry, G. J., Opiol, H., Marchant, E. G., Pavlovski, I., Mear, R. J., Hamson, D. K., and Mistlberger, R. E. (2012). Scheduled daily mating induces circadian anticipatory activity rhythms in the male rat. *PLOS ONE* 7: e40895.
155. McClintock, M. K. (1971). Menstrual synchrony and suppression. *Nature* 229: 244–245.
156. Handelmann, G., Ravizza, R., and Ray, W. J. (1980). Social dominance determines estrous entrainment among female hamsters. *Hormones and Behavior* 14: 107–115.
157. McClintock, M. K. (1984). Estrous synchrony: Modulation of ovarian cycle length by female pheromones. *Physiology and Behavior* 32: 701–705.
158. Preti, G., Cutler, W. B., Garcia, C. R., Huggins, G. R., and Lawley, H. J. (1986). Human axillary secretions influence women's menstrual cycles: The role of donor extract of females. *Hormones and Behavior* 20: 474–482.
159. Weller, A. and Weller, L. (1993). Menstrual synchrony between mothers and daughters and between roommates. *Physiology and Behavior* 53: 943–949.
160. Weller, A. and Weller, L. (1995). The impact of social interaction factors on menstrual synchrony in the workplace. *Psychoneuroendocrinology* 20: 21–31.
161. Stern, K. and McClintock, M. K. (1998). Regulation of ovulation by human pheromones. *Nature* 392: 177–179.
162. Schank, J. C. (2001). Menstrual-cycle synchrony: Problems and new directions for research. *Journal of Comparative Psychology* 115: 3–15.
163. Harris, A. L. and Vitzthum, V. J. (2013). Darwin's legacy: An evolutionary view of women's reproductive and sexual functioning. *Journal of Sex Research* 50: 207–246.
164. Boulos, Z. and Rusak, B. (1982). Circadian phase response curves for dark pulses in the hamster. *Journal of Comparative Physiology A* 146: 411–417.
165. Meijer, J. H., Daan, S., Overkamp, G. J. F., and Hermann, P. M. (1990). The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *Journal of Biological Rhythms* 5: 1–16.
166. Van Reeth, O., Zhang, Y., Zee, P. C., and Turek, F. W. (1992). Aging alters feedback effects of the activity-rest cycle on the circadian clock. *American Journal of Physiology* 263: R981–R986.
167. Lee, T. M. and Labyak, S. E. (1997). Free-running rhythms and light- and dark-pulse phase response curves for diurnal *Octodon degus* (Rodentia). *American Journal of Physiology* 273: R278–R286.
168. Barbacka-Surowiak, G. (2000). Is the PRC for dark pulses in LL a mirror image of the PRC for light pulses in DD in mice? *Biological Rhythm Research* 31: 531–544.
169. Dwyer, S. M. and Rosenwasser, A. M. (2000). Effects of light intensity and restraint on dark-pulse-induced circadian phase shifting during subjective night in Syrian hamsters. *Journal of Biological Rhythms* 15: 491–500.
170. Rosenwasser, A. M. and Dwyer, S. M. (2001). Circadian phase shifting: Relationships between photic and nonphotic phase-response curves. *Physiology and Behavior* 73: 175–183.
171. Mistlberger, R. E., Belcourt, J., and Antle, M. C. (2002). Circadian clock resetting by sleep deprivation without exercise in Syrian hamsters: Dark pulses revisited. *Journal of Biological Rhythms* 17: 227–237.
172. Rosenwasser, A. M. and Dwyer, S. M. (2002). Phase shifting the hamster circadian clock by 15-minute dark pulses. *Journal of Biological Rhythms* 17: 238–247.
173. Mendoza, J. Y., Dardente, H., Escobar, C., Pevét, P., and Challet, E. (2004). Dark pulse resetting of the suprachiasmatic clock in Syrian hamsters: Behavioral phase-shifts and clock gene expression. *Neuroscience* 127: 529–537.
174. Canal, M. M. and Piggins, H. D. (2006). Resetting of the hamster circadian system by dark pulses. *American Journal of Physiology* 290: R785–R792.
175. Demaria-Pesce, V. H., Murakami, D., and Fuller, C. A. (1991). Modulation des rythmes circadiens de la température corporelle chez des rats soumis a 2 G pendant une heure par jour. *Médecine Aéronautique et Spatiale* 30: 416–421.
176. Fuller, C. A., Hoban-Higgins, T. M., Griffin, D. W., and Murakami, D. M. (1994). Influence of gravity on the circadian timing system. *Advances in Space Research* 14(8): 399–408.
177. Murakami, D. M., Tang, I., and Fuller, C. A. (1998). Chronic 2G exposure affects c-Fos reactivity to a light pulse within the rat suprachiasmatic nucleus. *Journal of Gravitational Physiology* 5: 71–78.
178. Holley, D. C., DeRoshia, C. W., Moran, M. M., and Wade, C. E. (2003). Chronic centrifugation (hypergravity) disrupts the circadian system of the rat. *Journal of Applied Physiology* 95: 1266–1278.

179. Hoban-Higgins, T. M., Alpatov, A. M., Wassmer, G. T., Rietveld, W. J., and Fuller, C. A. (2003). Gravity and light effects on the circadian clock of a desert beetle, *Trigonoscelis gigas*. *Journal of Insect Physiology* 49: 671–675.
180. Simoni, A., Wolfgang, W., Topping, M. P., Kavlie, R. G., Stanewsky, R., and Albert, J. T. (2014). A mechanosensory pathway to the *Drosophila* circadian clock. *Science* 343: 525–528.
181. Quigg, M., Straume, M., Smith, T., Menaker, M., and Bertram, E. H. (2001). Seizures induce phase shifts of rat circadian rhythms. *Brain Research* 913: 165–169.
182. Jarsky, T. M. and Stephenson, R. (2000). Effects of hypoxia and hypercapnia on circadian rhythms in the golden hamster. *Journal of Applied Physiology* 89: 2130–2138.
183. Gillman, A. G., Leffel, J. K., Kosobud, A. E. K., and Timberlake, W. (2013). Behavioral characteristics and pharmacological manipulations of a nicotine-entrainable circadian oscillator. *Chronobiology International* 30: 855–869.
184. Gritton, H. J., Sutton, B. C., Martinez, V., Sarter, M., and Lee, T. M. (2009). Interactions between cognition and circadian rhythms: Attentional demands modify circadian entrainment. *Behavioral Neuroscience* 123: 937–948.
185. Goichot, B., Weibel, L., Chapotot, F., Gronfier, C., Piquard, F., and Brandenberger, G. (1998). Effect of the shift of the sleep–wake cycle on three robust endocrine markers of the circadian clock. *American Journal of Physiology* 275: E243–E248.
186. Danilenko, K. V., Cajochen, C., and Wirz-Justice, A. (2003). Is sleep per se a zeitgeber in humans? *Journal of Biological Rhythms* 18: 170–178.
187. Van Reeth, O. and Turek, F. W. (1987). Adaptation of circadian rhythmicity to shift in light–dark cycle accelerated by a benzodiazepine. *American Journal of Physiology* 253: R204–R207.
188. Wickland, C. and Turek, F. W. (1991). Phase-shifting effect of triazolam on the hamster's circadian rhythm of activity is not mediated by a change in body temperature. *Brain Research* 560: 12–16.
189. Van Reeth, O. and Turek, F. W. (1992). Changes in the phase response curve of the circadian clock to a phase-shifting stimulus. *Journal of Biological Rhythms* 7: 137–147.
190. Biello, S. M. and Mrosovsky, N. (1993). Circadian phase-shifts induced by chlordiazepoxide without increased locomotor activity. *Brain Research* 622: 58–62.
191. Vansteensel, M. J., Deboer, T., Dahan, A., and Meijer, J. H. (2003). Differential responses of circadian activity onset and offset following GABA-ergic and opioid receptor activation. *Journal of Biological Rhythms* 18: 297–306.
192. Webb, I. C., Antle, M. C., and Mistlberger, R. E. (2014). Regulation of circadian rhythms in mammals by behavioral arousal. *Behavioral Neuroscience* 128: 304–325.
193. Hastings, M. H., Mead, S. M., Vindlacheruvu, R. R., Ebling, F. J. P., Maywood, E. S., and Grosse, J. (1992). Non-photic phase shifting of the circadian activity rhythm of Syrian hamsters: The relative potency of arousal and melatonin. *Brain Research* 591: 20–26.
194. Antle, M. C. and Mistlberger, R. E. (2000). Circadian clock resetting by sleep deprivation without exercise in the Syrian hamster. *Journal of Neuroscience* 20: 9326–9332.
195. Challet, E., Pévet, P., and Malan, A. (1997). Effect of prolonged fasting and subsequent refeeding on free-running rhythms of temperature and locomotor activity in rats. *Behavioural Brain Research* 84: 275–284.
196. Cain, S. W., Verwey, M., Hood, S., Leknickas, P., Karatsoreos, I., Yeomans, J. S., and Ralph, M. R. (2004). Reward and aversive stimuli produce similar nonphotic phase shifts. *Behavioral Neuroscience* 118: 131–137.
197. Mohawk, J. A. and Lee, T. M. (2005). Restraint stress delays reentrainment in male and female diurnal and nocturnal rodents. *Journal of Biological Rhythms* 20: 245–256.
198. Glass, J. D., Tardif, S. D., Clements, R., and Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
199. Antle, M. C., Steen, N. M., and Mistlberger, R. E. (2001). Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. *NeuroReport* 12: 2901–2905.
200. Janik, D., Nest, K. J., and Janiga, M. A. (2001). Exogenous corticosteroid and shifts of circadian rhythms in hamsters. *Chronobiology International* 18: 203–213.
201. Vilaplana, J., Cambras, T., and Díez-Noguera, A. (1995). Sound does not entrain motor activity circadian rhythms of rats. *Physiology and Behavior* 58: 975–978.
202. Ralph, M. R. and Mrosovsky, N. (1992). Behavioral inhibition of circadian responses to light. *Journal of Biological Rhythms* 7: 353–359.
203. Mistlberger, R. E. (1991). Scheduled daily exercise or feeding alters the phase of photic entrainment in Syrian hamsters. *Physiology and Behavior* 50: 1257–1260.
204. Sinclair, S. V. and Mistlberger, R. E. (1997). Scheduled activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods. *Behavioural Brain Research* 87: 127–137.
205. Amir, S. and Stewart, J. (1998). Conditioned fear suppresses light-induced resetting of the circadian clock. *Neuroscience* 86: 345–351.
206. Amir, S. and Stewart, J. (1999). The effectiveness of light on the circadian clock is linked to its emotional value. *Neuroscience* 88: 339–345.
207. Amir, S., Cain, S., Sullivan, J., Robinson, B., and Stewart, J. (1999). Olfactory stimulation enhances light-induced phase shifts in free-running activity rhythms and Fos expression in the suprachiasmatic nucleus. *Neuroscience* 92: 1165–1170.
208. Challet, E., Turek, F. W., Laute, M. A., and Van Reeth, O. (2001). Sleep deprivation decreases phase-shift responses of circadian rhythms to light in the mouse: Role of serotonergic and metabolic signals. *Brain Research* 909: 81–91.
209. Mistlberger, R. E., Marchant, E. G., and Sinclair, S. V. (1996). Nonphotic phase-shifting and the motivation to run: Cold exposure reexamined. *Journal of Biological Rhythms* 11: 208–215.
210. Mistlberger, R. E., Sinclair, S. V., Marchant, E. G., and Neil, L. (1997). Phase shifts to refeeding in the Syrian hamster mediated by running activity. *Physiology and Behavior* 61: 273–278.
211. Mistlberger, R. E., Antle, M. C., Webb, I. C., Jones, M., Weinberg, J., and Pollock, M. S. (2003). Circadian clock resetting by arousal in Syrian hamsters: The role of stress and activity. *American Journal of Physiology* 285: R917–R925.
212. Chen, W. M. and Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
213. Hau, M. and Gwinner, E. (1997). Adjustment of house sparrow circadian rhythms to a simultaneously applied light and food zeitgeber. *Physiology and Behavior* 62: 973–981.
214. Reierth, E. and Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
215. Nelson, W., Scheving, L., and Halberg, F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *Journal of Nutrition* 105: 171–184.

216. Hotz, M. M., Connolly, M. S., and Lynch, C. B. (1987). Adaptation to daily meal-timing and its effect on circadian temperature rhythms in two inbred strains of mice. *Behavior Genetics* 17: 37–51.
217. Dudley, C. A., Erbel-Sieler, C., Estill, S. J., Reick, M., Franken, P., Pitts, S., and McKnight, S. L. (2003). Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science* 301: 379–383.
218. Shido, O., Sakurada, S., and Nagasaka, T. (1991). Effect of heat acclimation on diurnal changes in body temperature and locomotor activity in rats. *Journal of Physiology* 433: 59–71.
219. Krieger, D. T. (1974). Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* 95: 1195–1201.
220. Panksepp, J. and Krost, K. (1975). Modification of diurnal feeding patterns by palatability. *Physiology and Behavior* 15: 673–677.
221. Brinkhof, M. W. G., Daan, S., and Strubbe, J. H. (1998). Forced dissociation of food- and light-entrainable circadian rhythms of rats in a skeleton photoperiod. *Physiology and Behavior* 65: 225–231.
222. Lax, P., Zamora, S., and Madrid, J. A. (1999). Food-entrained feeding and locomotor circadian rhythms in rats under different lighting conditions. *Chronobiology International* 16: 281–291.
223. White, W. and Timberlake, W. (1999). Meal-engendered circadian-ensuing activity in rats. *Physiology and Behavior* 65: 625–642.
224. Challet, E., Pévet, P., Vivien-Roels, B., and Malan, A. (1997). Phase-advanced daily rhythms of melatonin, body temperature, and locomotor activity in food-restricted rats fed during daytime. *Journal of Biological Rhythms* 12: 65–79.
225. Challet, E., Pévet, P., and Malan, A. (1997). Lesion of the serotonergic terminals in the suprachiasmatic nuclei limits the phase advance of body temperature rhythm in food-restricted rats fed during daytime. *Journal of Biological Rhythms* 12: 235–244.
226. Boulamery-Velly, A., Simon, N., Vidal, J., Mouchet, J., and Bruguerolle, B. (2005). Effects of three-hour restricted food access during the light period on circadian rhythms of temperature, locomotor activity, and heart rate in rats. *Chronobiology International* 22: 489–498.
227. Angeles-Castellanos, M., Salgado-Delgado, R., Rodriguez, K., Buijs, R. M., and Escobar, C. (2010). The suprachiasmatic nucleus participates in food entrainment: A lesion study. *Neuroscience* 165: 1115–1126.
228. Patton, D. F., Parfyonov, M., Gourmelen, S., Opiol, H., Pavlovski, I., Marchant, E. G., Challet, E., and Mistlberger, R. E. (2013). Photic and pineal modulation of food anticipatory circadian activity rhythms in rodents. *PLOS ONE* 8: e81588.
229. Froy, O., Chapnik, N., and Miskin, R. (2008). The suprachiasmatic nuclei are involved in determining circadian rhythms during restricted feeding. *Neuroscience* 155: 1152–1159.
230. Jilge, B. and Stähle, H. (1993). Restricted food access and light–dark: Impact of conflicting zeitgebers on circadian rhythms of the rabbit. *American Journal of Physiology* 264: R708–R715.
231. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1995). Entrainment of circadian wheel-running rhythms of the northern brown bandicoot, *Isoodon macrourus*, by daily restricted feeding schedules. *Chronobiology International* 12: 176–187.
232. Ware, J. V., Nelson, O. L., Robbins, C. T., and Jansen, H. T. (2012). Temporal organization of activity in the brown bear (*Ursus arctos*): Roles of circadian rhythms, light, and food entrainment. *American Journal of Physiology* 303: R890–R902.
233. Reebs, S. G. and Lague, M. (2000). Daily food-anticipatory activity in golden shiners: A test of endogenous timing mechanisms. *Physiology and Behavior* 70: 35–43.
234. Eckerman, D. A. (1999). Scheduling reinforcement about once a day. *Behavioural Processes* 45: 101–114.
235. Keith, D. R., Hart, C. L., Robotham, M., Tariq, M., Le Sauter, J., and Silver, R. (2013). Time of day influences the voluntary intake and behavioral response to methamphetamine and food reward. *Pharmacology, Biochemistry and Behavior* 110: 117–126.
236. Castillo, M. R., Hochstetler, K. J., Tavernier, R. J., Greene, D. M., and Bult-Ito, A. (2004). Entrainment of the master circadian clock by scheduled feeding. *American Journal of Physiology* 287: R551–R555.
237. Challet, E., Solberg, L. C., and Turek, F. W. (1998). Entrainment in calorie-restricted mice: Conflicting zeitgebers and free-running conditions. *American Journal of Physiology* 274: R1751–R1761.
238. Sheward, W. J., Maywood, E. S., French, K. L., Horn, J. M., Hastings, M. H., Seckl, J. R., Holmes, M. C., and Harmar, A. J. (2007). Entrainment to feeding but not to light: Circadian phenotype of $VPAC_2$ receptor-null mice. *Journal of Neuroscience* 27: 4351–4358.
239. Takasu, N. N., Kurosawa, G., Tokuda, I. T., Mochizuki, A., Todo, T., and Nakamura, W. (2012). Circadian regulation of food-anticipatory activity in molecular clock-deficient mice. *PLOS ONE* 7: e48892.
240. Malloy, J. N., Paulose, J. K., Li, Y., and Cassone, V. M. (2012). Circadian rhythms of gastrointestinal function are regulated by both central and peripheral oscillators. *American Journal of Physiology* 303: G461–G473.
241. Luby, M. D., Hsu, C. T., Shuster, S. A., Gallardo, C. M., Mistlberger, R. E., King, O. D., and Steele, A. D. (2012). Food anticipatory activity behavior of mice across a wide range of circadian and non-circadian intervals. *PLOS ONE* 7: e37992.
242. Wolff, G. and Esser, K. A. (2012). Scheduled exercise phase shifts the circadian clock in skeletal muscle. *Medicine and Science in Sports and Exercise* 44: 1663–1670.
243. Gallardo, C. M., Hsu, C. T., Gunapala, K. M., Parfyonov, M., Chang, C. H., Mistlberger, R. E., and Steele, A. D. (2014). Behavioral and neural correlates of acute and scheduled hunger in C57BL/6 mice. *PLOS ONE* 9: e95990.
244. Crystal, J. D. (2001). Circadian time perception. *Journal of Experimental Psychology: Animal Behavior Processes* 27: 68–78.
245. White, W., Schwartz, G. J., and Moran, T. H. (1999). Meal-synchronized CEA in rats: Effects of meal size, intragastric feeding, and subdiaphragmatic vagotomy. *American Journal of Physiology* 276: R1276–R1288.
246. Díaz-Muñoz, M., Vázquez-Martínez, O., Aguilar-Roblero, R., and Escobar, C. (2000). Anticipatory changes in liver metabolism and entrainment of insulin, glucagon, and corticosterone in food-restricted rats. *American Journal of Physiology* 279: R2048–R2056.
247. Pecoraro, N., Gomez, F., Laugero, K., and Dallman, M. F. (2002). Brief access to sucrose engages food-entrainable rhythms in food-deprived rats. *Behavioral Neuroscience* 116: 757–776.
248. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M., and Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.
249. Mistlberger, R. E. (1992). Anticipatory activity rhythms under daily schedules of water access in the rat. *Journal of Biological Rhythms* 7: 149–160.

250. Meynard, M. M., Valdés, J. L., Recabarren, M., Serón-Ferré, M., and Torrealba, F. (2005). Specific activation of histaminergic neurons during daily feeding anticipatory behavior in rats. *Behavioural Brain Research* 158: 311–319.
251. Mendoza, J., Angeles-Castellanos, M., and Escobar, C. (2005). Differential role of the accumbens shell and core subterritories in food-entrained rhythms of rats. *Behavioural Brain Research* 158: 133–142.
252. Sabath, E., Salgado-Delgado, R., Guerrero-Vargas, N. N., Guzman-Ruiz, M. A., Basualdo, M. C., Escobar, C., and Buijs, R. M. (2014). Food entrains clock genes but not metabolic genes in the liver of suprachiasmatic nucleus lesioned rats. *FEBS Letters* 588: 3104–3110.
253. Clarke, J. D. and Coleman, G. J. (1986). Persistent meal-associated rhythms in SCN-lesioned rats. *Physiology and Behavior* 36: 105–113.
254. Acosta-Galvan, G., Yi, C. X., van der Vliet, J., Jhamandas, J. H., Panula, P., Angeles-Castellanos, M., Basualdo, M. C., Escobar, C., and Buijs, R. M. (2011). Interaction between hypothalamic dorsomedial nucleus and the suprachiasmatic nucleus determines intensity of food anticipatory behavior. *Proceedings of the National Academy of Sciences United States America* 108: 5813–5818.
255. Slotten, H. A., Pitrosky, B., and Pévet, P. (1999). Influence of the mode of daily melatonin administration on entrainment of rat circadian rhythms. *Journal of Biological Rhythms* 14: 347–353.
256. Paul, M. J., Kauffman, A. S., and Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
257. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
258. Morgado, E., Juárez, C., Melo, A. I., Domíinguez, B., Lehman, M. N., Escobar, C., Meza, E., and Caba, M. (2011). Artificial feeding synchronizes behavioral, hormonal, metabolic and neural parameters in mother-deprived neonatal rabbit pups. *European Journal of Neuroscience* 34: 1807–1816.
259. Honma, K., von Goetz, C., and Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.
260. Phillips, D. L., Rautenberg, W., Rashotte, M. E., and Stephan, F. K. (1993). Evidence for a separate food-entrainable circadian oscillator in the pigeon. *Physiology and Behavior* 53: 1105–1113.
261. Krieger, D. T., Hauser, H., and Krey, L. C. (1977). Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. *Science* 197: 398–399.
262. Stephan, F. K., Swann, J. M., and Sisk, C. L. (1979). Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. *Behavioral and Neural Biology* 25: 545–554.
263. Kurumiya, S. and Kawamura, H. (1991). Damped oscillation of the lateral hypothalamic multineuronal activity synchronized to daily feeding schedules in rats with suprachiasmatic nucleus lesions. *Journal of Biological Rhythms* 6: 115–127.
264. Stephan, F. K. (1992). Resetting of a feeding-entrainable circadian clock in the rat. *Physiology and Behavior* 52: 985–995.
265. Abe, H. and Rusak, B. (1992). Anticipatory activity and entrainment of circadian rhythms in Syrian hamsters exposed to restricted palatable diets. *American Journal of Physiology* 263: R116–R124.
266. Mistlberger, R. E. (1992). Nonphotic entrainment of circadian activity rhythms in suprachiasmatic nuclei ablated hamsters. *Behavioral Neuroscience* 106: 192–202.
267. Mistlberger, R. E. (1993). Circadian properties of anticipatory activity to restricted water access in suprachiasmatic-ablated hamsters. *American Journal of Physiology* 264: R22–R29.
268. Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M., and Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research* 739: 12–18.
269. Pezuk, P., Mohawk, J. A., Yoshikawa, T., Sellix, M. T., and Menaker, M. (2010). Circadian organization is governed by extra-SCN pacemakers. *Journal of Biological Rhythms* 25: 432–441.
270. Pitts, S., Perone, E., and Silver, R. (2003). Food-entrained circadian rhythms are sustained in arrhythmic *Clk/Clk* mutant mice. *American Journal of Physiology* 285: R57–R67.
271. Fuller, P. M., Lu, J., and Saper, C. B. (2008). Differential rescue of light- and food-entrainable circadian rhythms. *Science* 320: 1074–1077.
272. Pendergast, J. S., Oda, G. A., Niswender, K. D., and Yamazaki, S. (2012). *Period* determination in the food-entrainable and methamphetamine-sensitive circadian oscillator(s). *Proceedings of the National Academy of Sciences United States America* 109: 14218–14223.
273. Stephan, F. K. (1986). Coupling between feeding- and light-entrainable circadian pacemakers in the rat. *Physiology and Behavior* 38: 537–544.
274. Ottenweller, J. E., Tapp, W. N., and Natelson, B. H. (1990). Phase-shifting the light–dark cycle resets the food-entrainable circadian pacemaker. *American Journal of Physiology* 258: R994–R1000.
275. Rashotte, M. E. and Stephan, F. K. (1996). Coupling between light- and food-entrainable circadian oscillators in pigeons. *Physiology and Behavior* 59: 1005–1010.
276. Mistlberger, R. E. and Rusak, B. (1988). Food-anticipatory circadian rhythms in rats with paraventricular and lateral hypothalamic ablations. *Journal of Biological Rhythms* 3: 277–291.
277. Davidson, A. J. and Stephan, F. K. (1998). Circadian food anticipation persists in capsaicin deafferented rats. *Journal of Biological Rhythms* 13: 422–429.
278. Davidson, A. J., Poole, A. S., Yamazaki, S., and Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain and Behavior* 2: 32–39.
279. Stephan, F. K. (1997). Calories affect zeitgeber properties of the feeding entrained circadian oscillator. *Physiology and Behavior* 62: 995–1002.
280. Stephan, F. K. and Davidson, A. J. (1998). Glucose, but not fat, phase shifts the feeding-entrained circadian clock. *Physiology and Behavior* 65: 277–288.
281. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes and Development* 14: 2950–2961.
282. Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y., and Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science* 291: 490–493.

9 Integration of Mechanisms

9.1 INTERNAL TEMPORAL ORDER

Up to this point in the book, we have talked about circadian rhythms as if organisms exhibited only one circadian rhythm at a time. We have certainly talked about rhythmicity in numerous variables, such as locomotor activity, body temperature, melatonin secretion, and blood pressure—but we have treated each variable as if it were the only variable. It is now time to recognize that circadian rhythmicity is exhibited by many variables simultaneously. This will raise a multitude of new problems. The most obvious problem is perhaps how the multiple rhythms relate to each other—the problem of *internal temporal order*. Do all rhythms peak at the same time, or do some peak earlier or later than others? Do all rhythms have the same waveform, so that we can legitimately compare their temporal structures? The next obvious problem is that of causation. If one rhythm lags behind another, is it because it is caused by the earlier rhythm? Does the circadian pacemaker generate each and every rhythm individually, or are most rhythms simply derived from a few clock-controlled rhythms? We do not have definite answers to these questions, but research has advanced enough to allow us to at least start to answer them.

Let us begin by looking at three variables measured simultaneously. Figure 9.1 shows 5-day sections of the records of rectal temperature, plasma concentration of urea, and plasma concentration of cholesterol of a goat (*Capra hircus*). Notice that the waveforms of the three rhythms are relatively similar (at least at this low temporal resolution of one measurement every 3 hours), but their timing is not. The rhythms of temperature and urea concentration have peaks and troughs at about the same time, but the rhythm of cholesterol concentration is 180° out of phase with the other two rhythms. Figure 9.2 shows 5-day sections of the records of heart rate, intra-abdominal temperature, and locomotor activity of a golden hamster (*Mesocricetus auratus*). The waveforms are more dissimilar in this case (perhaps because of the higher resolution of one measurement every hour), but the three rhythms peak at about the same time each day. Figure 9.3 shows 20-day averages of the daily rhythms of locomotor activity, defecation, feeding, drinking, and urination of a rabbit (*Oryctolagus cuniculus*). In this case, all five rhythms peak at night, but the waveforms are rather dissimilar.

Figure 9.4 is a classic figure originally published by Halberg (one of the forefathers of circadian physiology, as explained in Chapter 1) in 1979.[1] It shows the acrophases (times of peak) of 62 variables measured in the domestic mouse. The figure is mostly of historical and heuristic value because the different variables were measured on different animals, often in different laboratories. Nevertheless, its message is very clear: if we bother to search, we can find at least one rhythm peaking at any given time of the day. Understanding how the various rhythms relate to each other is an enormous task that circadian physiologists have yet to undertake in earnest. Careful monitoring of multiple variables simultaneously in individual animals over many circadian cycles is the first step in this laborious enterprise. This has been done occasionally in plants[2] and animals,[3–14] as well as in humans.[15–22] Two interesting recent developments were characterizations of metabolomes by mass spectroscopy using blood samples from mice[23] and humans.[24] Of the hundreds of metabolites indentified in the samples, 15%–20% exhibited daily oscillation.

When we analyze the phase relationships among various variables that are simultaneously measured, we must keep in mind the distinction between the two meanings of *synchronization* discussed in Chapter 6. By one meaning, the 62 rhythms in Figure 9.4 are not synchronized—because they peak at different times of the day. By the other—and more relevant—meaning, the rhythms must be synchronized, otherwise they would not keep a constant phase relationship day after day. That is, if two variables differ in their acrophases, but the difference is constant day after day, then they *are* synchronized. Also, transient episodes of desynchronization must be distinguished from true desynchronized states and from plain biological noise. Consider Figure 9.5. Groups of rats were maintained under a light–dark cycle (LD 12:12) that was reversed on day 0 (that is, the light–dark cycle was phase-shifted by 12 hours). The graphs show the mean amounts of food and water ingested during the light and dark phases of the light–dark cycle after the reversal. The authors of the study claim that the feeding and drinking rhythms were temporarily desynchronized, because the drinking rhythm reentrained to the new light–dark cycle in 4–6 days, whereas the feeding rhythm did so in 7–9 days.[25] I do not see any evidence of desynchronization in these data. The reversal of the feeding rhythm seems *noisier* than the reversal of the drinking rhythm, but a new stable day–night differentiation is attained in 8–9 days in both rhythms. *Noise*, but no real desynchronization, is also seen in the relationship between the rhythms of body temperature and locomotor activity of a golden hamster in Figure 9.6. In this case, the light–dark cycle was phase-advanced by 6 hours (on the day indicated by the arrowhead), and it took several days for the rhythms to readjust to the new phase. We will return to this issue when we discuss the topic of *jet lag* in Chapter 15.

Before we examine the subject of temporal order in greater detail, I should probably point out why the analysis of phase relationships is important. In Chapter 3, we saw that circadian rhythms can be characterized by six parameters: mean

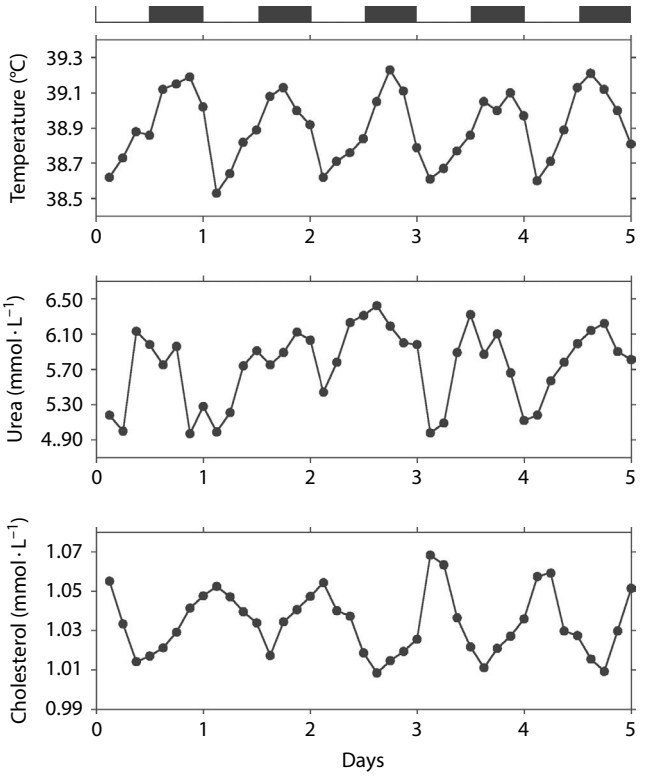


FIGURE 9.1 Three rhythms in a goat. The graphs show 5-day segments of simultaneous records of rectal temperature, plasma urea concentration, and plasma cholesterol concentration of a female goat (*Capra hircus*). The horizontal bars at the top indicate the timing of the light–dark cycle. Notice that the rhythms of rectal temperature and urea concentration have similar phases (peaking in the middle of the night), but the rhythm of cholesterol concentration has the opposite phase (peaking in the middle of the day). (Data from Piccione, G. et al., *Comp. Biochem. Physiol. A*, 134, 563, 2003.)

level, amplitude, phase, period, waveform, and robustness. The *mean levels* and *amplitudes* of different rhythms cannot be compared because they refer to distinct physical quantities. For instance, in the golden hamster, body temperature oscillates from 36.2°C to 37.0°C with a mean of 36.6°C,[26] heart rate oscillates from 370 to 460 bpm with a mean of 400 bpm,[27] and melatonin content in the pineal gland oscillates from 0 to 700 pg with a mean of 90 pg.[28] As long as oranges and apples cannot be compared, the mean levels and amplitudes of different rhythms cannot be compared. The *periods* of different rhythms could certainly be compared, but, under natural conditions, all rhythms are normally entrained by the 24-hour alternation of day and night, so that they all have the same period. Differences in *waveform* and *robustness* could also be compared, but they are already taken into account (if feasible) in the analysis of *phase* relationships. Therefore, the principal parameter in the comparison of multiple rhythms is their phase relationship. Of course, temporal order is fundamental for the proper operation of the body. As a simple confirmatory example, think of how inappropriate it would be to perform the full process of digestion before any food is ingested!

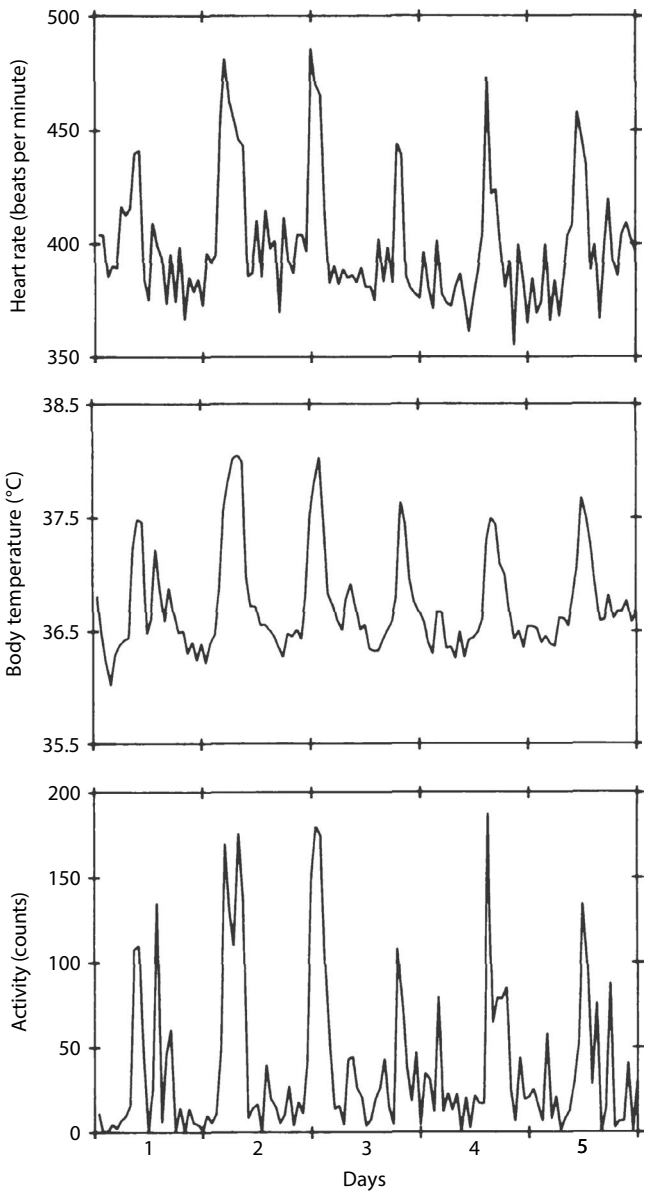


FIGURE 9.2 Three rhythms in a hamster. The graphs show 5-day segments of simultaneous records of heart rate, body temperature, and locomotor activity of a *tau*-mutant hamster (*Mesocricetus auratus*) maintained in constant light (100 lux). The data were collected by telemetry at 6-minute intervals and averaged hourly. Notice the close synchronization of the three rhythms. (Reproduced from Refinetti, R. and Menaker, M., *Am. J. Physiol.*, 264, R235, Copyright 1992, American Physiological Society. With permission.)

9.1.1 Relationship between the Body Temperature Rhythm and the Activity Rhythm

We saw in Chapter 5 that the most extensively studied rhythms are those of locomotor activity and body temperature. Therefore, it makes sense to start the investigation of the relationship between different rhythms by examining the relationship between the rhythms of temperature and activity. These two rhythms have been simultaneously monitored in many studies on various species.[3,26,29–48] Examples for two species are shown in Figure 9.7. The temporal courses of the

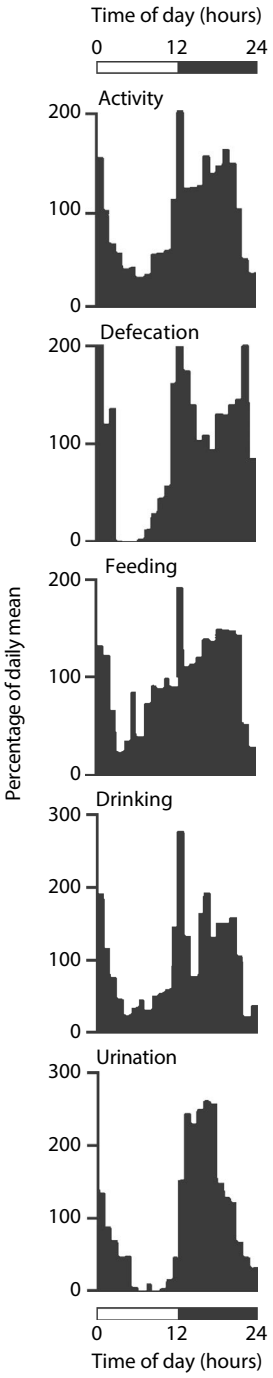


FIGURE 9.3 Five rhythms in a rabbit. The graphs show the daily distribution of values of five behavioral variables recorded from a rabbit (*Oryctolagus cuniculus*). All values are plotted as percentages of the daily mean. Each graph is the average of 20 consecutive days. The horizontal bars at the top and bottom indicate the timing of the light–dark cycle. Notice that all five rhythms peak at night, even though their waveforms vary considerably. (Data from Jilge, B. and Stähle, H., *Am. J. Physiol.*, 264, R708, 1993.)

two rhythms are very similar in the two species. As expected in a diurnal animal, the activity and body temperature rhythms of the tree shrew (*Tupaia belangeri*) exhibit high values during the day and low values during the night. As expected in a nocturnal animal, the activity and body temperature rhythms of the flying squirrel (*Glaucomys volans*) exhibit high values during the night and low values during the day. Figure 9.8 provides a closer look (at higher temporal resolution) of the rhythms of the flying squirrel. Notice that body temperature rises when the animal becomes more active and falls when activity is reduced, even though individual bouts of activity are not necessarily correlated with individual rises in body temperature.

In contrast to the data shown in Figure 9.8, the human body temperature rhythm has been consistently reported to start to ascend several hours before awakening.[22,30,49] The relationship between the two rhythms has rarely been analyzed systematically in other animals, but two studies of mine, one in rats[50] and another in eight different mammalian species,[51] provided results equivalent to those shown in Figure 9.8 (that is, an almost perfect synchrony between the two rhythms). As shown in Figure 9.9, body temperature and activity of the laboratory rat ascend past the daily mean value at exactly the same time—which happens to be the time of lights off. This suggests that humans and rodents differ in the phase relationship between the rhythms of activity and body temperature. However, the difference lies more on the way the data are analyzed than on the data themselves. In both humans and rodents, temperature starts to ascend slowly several hours before awakening and then rises abruptly (more so in rodents than in humans) at wake time.[51] Accordingly, you may notice in Figure 9.9 that the temperature rhythm of the rat reaches its nadir early in the light phase and then rises very slowly until the abrupt elevation at the time of lights off. Arguments about phase relationships of various rhythms are very often clouded by the simple but fundamental fact that different rhythms have different waveforms. Use of the acrophase of a cosine function fit to the data (see Chapter 3) can circumvent the problem numerically but cannot eliminate it.

The alleged phase lead of the temperature rhythm in humans is said to become stronger when the rhythms are allowed to freerun under constant environmental conditions.[22,30,49] Inspection of published records of temperature and activity rhythms in rodents does not suggest an advance of several hours in the temperature rhythm of animals maintained in constant darkness or constant light.[32,52–54] In my study on rats, I compared the phase relationships of the two rhythms in animals maintained under a full light–dark cycle (LD 12:12), a skeleton photoperiod (LD 1:23), and constant darkness.[50] The results are shown in Figure 9.10. The time when the rhythms ascended past the daily mean value was slightly delayed in constant darkness (DD) because the animals were freerunning with periods longer than 24.0 hours, but the delay was the same for the temperature rhythm and the activity rhythm. In other words, the phase relationship of the two rhythms was not changed in the freerun state.

With or without a delay between them, the rhythms of body temperature and activity proceed so closely together—both under a light–dark cycle and in constant conditions—that it is quite reasonable to wonder whether the temperature

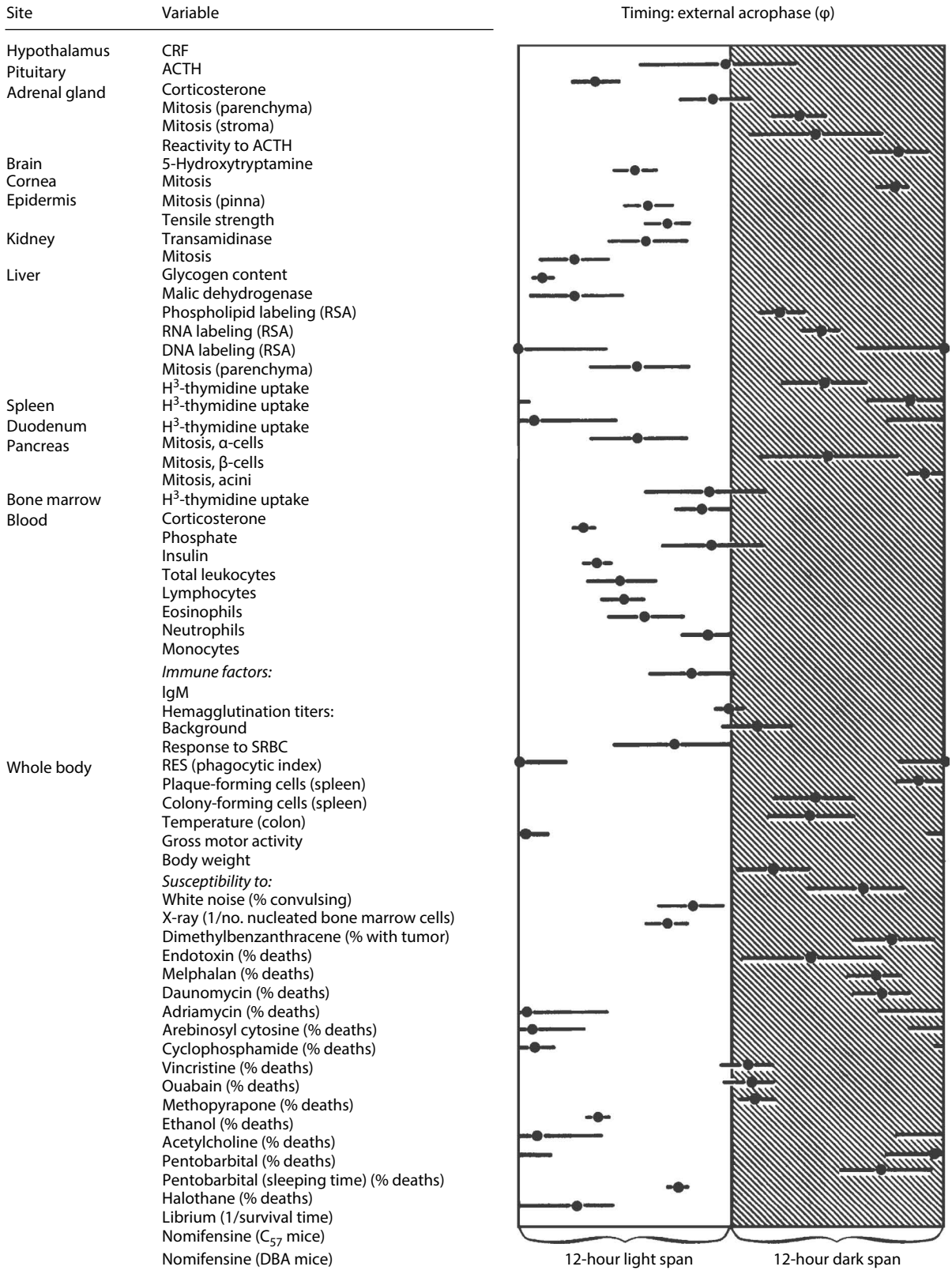


FIGURE 9.4 Sixty rhythms in mice. Shown are the mean acrophases (± SE) of over 60 rhythms measured in groups of mice (*Mus musculus*) maintained under a light–dark cycle (LD 12:12). Notice that just as many rhythms peak during the day as during the night in this nocturnal species. (Reproduced from Halberg F. et al., *Chronobiologia*, 6, 405, Copyright 1979, Franz Halberg. With permission.)

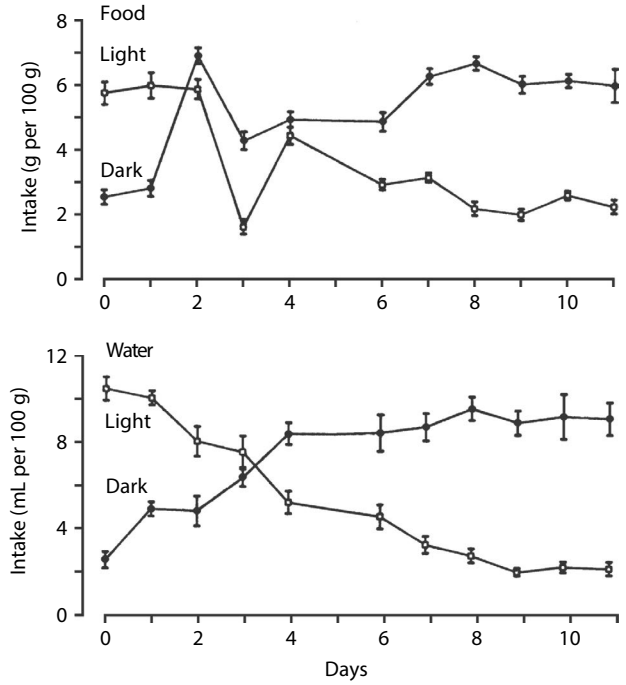


FIGURE 9.5 Jet-lagged rats. The graphs show the daily ingestion of food and water of rats (*Rattus norvegicus*) subjected to a full inversion (12-hour shift) of the light–dark cycle on day 0. Each data point corresponds to the mean (± SE) of 8 rats. Although adaptation to the inverted light–dark cycle seems to proceed more slowly for food intake than for water intake during the first few days, full inversion of the day–night differentiation of intake is attained in 8 or 9 days for both food and water. (Adapted from Zerath, E. et al., *Med. Sci. Res.*, 22, 53, 1994.)

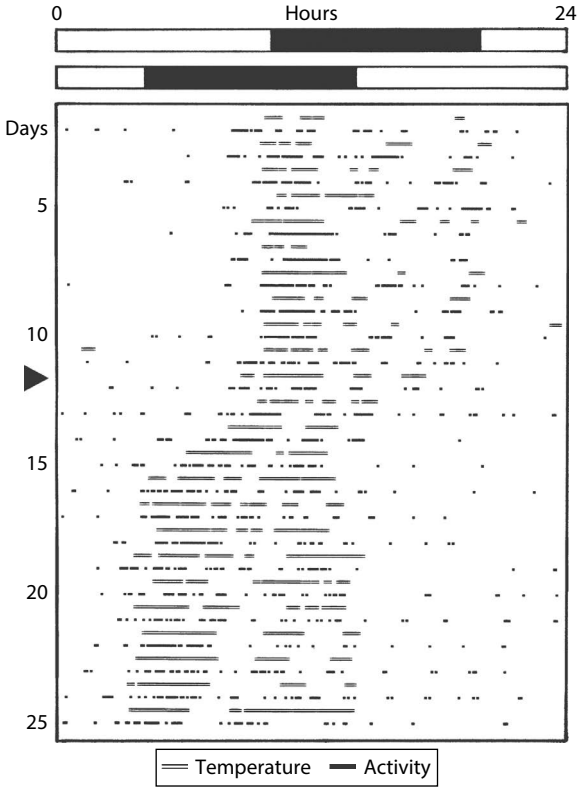


FIGURE 9.6 Jet-lagged hamster. This composite actogram shows the body temperature and locomotor activity rhythms of a golden hamster (*Mesocricetus auratus*) subjected to a 6-hour phase advance of the light–dark cycle. The timing of the light–dark cycles is indicated by the horizontal bars above the actogram, and the day of the shift is indicated by the arrowhead on the left margin. After the advance of the light–dark cycle, both rhythms advanced gradually over several days. (Adapted from Refinetti, R. and Menaker, M., *Pharmacol. Biochem. Behav.*, 45, 27, 1993.)

rhythm is not simply a consequence of the activity rhythm. Indeed, the fact that acute episodes of physical activity and exercise can elevate body temperature has been extensively documented in humans[55–61] and other vertebrates.[62–68] An example is shown in Figure 9.11. Horses were either exercised or allowed to rest for 2 hours in the afternoon. On the rest day, body temperature rose slowly throughout the afternoon as a natural expression of the daily rhythm. On the exercise day, a sharp elevation of body temperature was observed during the exercise session. This shows that physical activity can significantly raise body temperature. Consequently, the daily elevation in body temperature associated with circadian rhythmicity might be a direct result of increased activity.

In order to investigate the potential causal link between the activity rhythm and the temperature rhythm, several researchers recorded the body temperature rhythm of human subjects maintained in continuous bed rest[69–71] or undergoing a *constant-routine* protocol, which involves bed rest as well as sleep deprivation and the ingestion of frequent, equal-size meals.[17,72–74] Although the amplitude of the rhythm is reduced under this condition, robust rhythmicity persists, as exemplified in Figure 9.12. Thus, while the activity rhythm may alter the amplitude and shape of the body temperature rhythm, it does not cause it. This fact is further clarified by the data shown in Figure 9.13. In this study, young men and women were kept for 60 consecutive hours under an unusual sleep–wake cycle lasting 90 minutes (with 30 minutes in bed and 60 minutes awake). The influence of these cycles can be easily seen as small 90-minute oscillations superimposed on the circadian oscillation of body temperature.[75] Clearly, the circadian oscillation is independent of the short sleep–wake cycle, even if its waveform is affected by the sleep–wake cycle.

Bed rest cannot be used with animals—because they do not comply with requests for voluntary rest—so that we cannot place animals in bed rest to verify whether the body temperature rhythm persists in the absence of the activity rhythm. There are, however, at least two strong pieces of evidence in support of the argument that the activity rhythm does not cause the temperature rhythm in animals. One of them has to do with the direction of potential causality. If the activity rhythm causes the temperature rhythm, one would expect it to be at least as strong as (if not stronger than) the temperature rhythm—especially in small animals with low thermal inertia. Yet, as shown in Figure 9.14, the robustness of the temperature rhythm is greater than the robustness of the

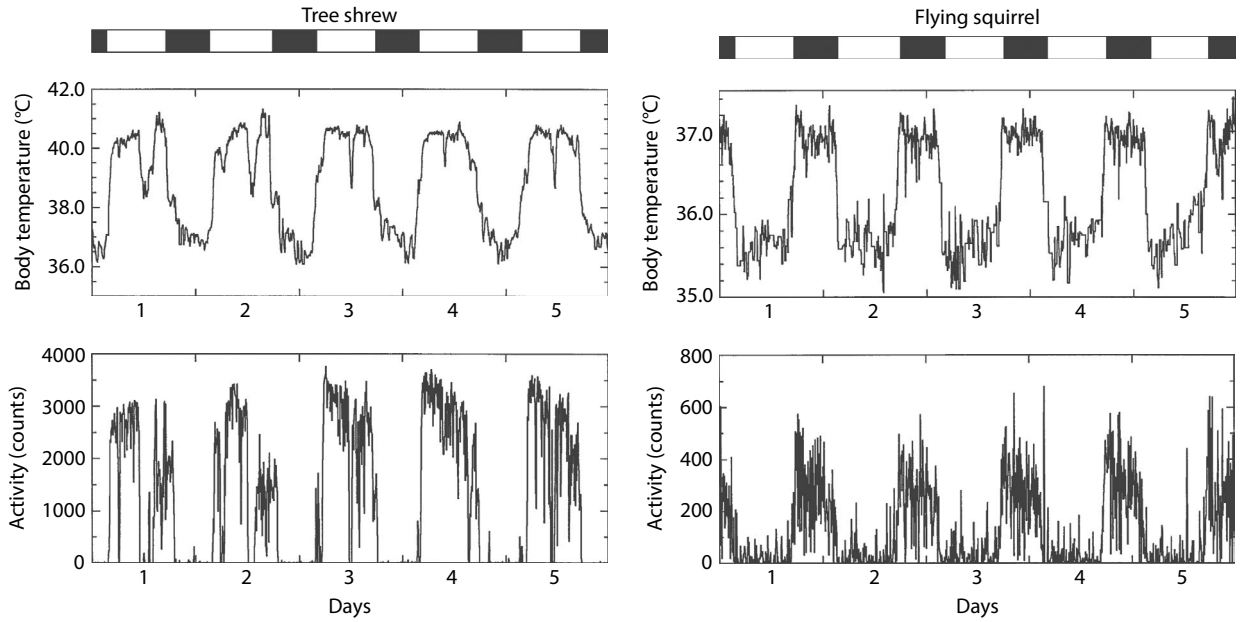


FIGURE 9.7 Synchronization of the rhythms of body temperature and locomotor activity. The graphs show 5-day segments of the records of body temperature and locomotor activity (recorded by telemetry with 6-minute resolution) of a tree shrew (*Tupaia belangeri*) and a flying squirrel (*Glaucomys volans*). The horizontal black and white bars indicate the duration of the dark and light phases of the prevailing light–dark cycle. Notice that both body temperature and activity level are high during the day in the diurnal tree shrew but are high during the night in the nocturnal flying squirrel. (Data from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999.)

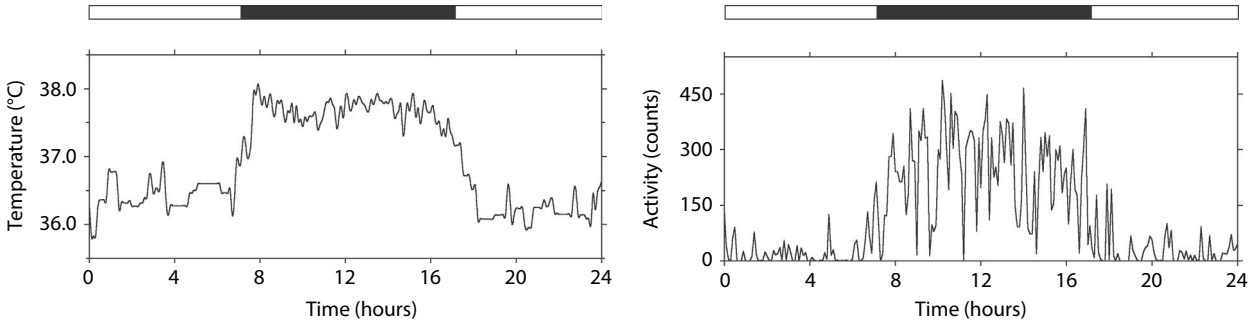


FIGURE 9.8 A closer look at the synchrony of body temperature and activity in the flying squirrel. The graphs show 24-hour segments of the records of body temperature and locomotor activity (recorded by telemetry with 6-minute resolution) of a flying squirrel (*Glaucomys volans*). The horizontal white and dark bars indicate the duration of the light and dark phases of the prevailing light–dark cycle. Notice the close temporal relationship of the two variables. (Data from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999.)

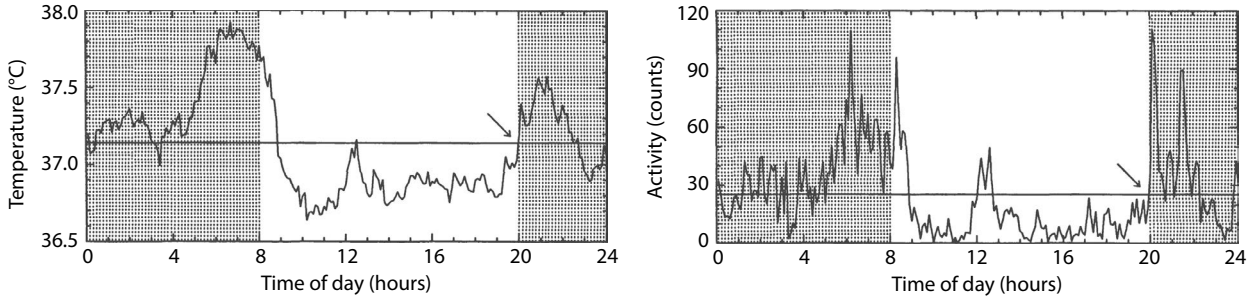


FIGURE 9.9 A closer look at the synchrony of body temperature and activity in the rat. The graphs show the rhythms of body temperature and locomotor activity of a laboratory rat (*Rattus norvegicus*). The data were collected by telemetry every 6 minutes and averaged over 7 consecutive days. The shading indicates the dark phase of the light–dark cycle. The horizontal line corresponds to the mean value for the 7 days. The arrows point to the time of day when the variable (temperature or activity) ascends past the mean. (Data from Refinetti, R., *Biol. Rhythm Res.*, 28, 19, 1997.)

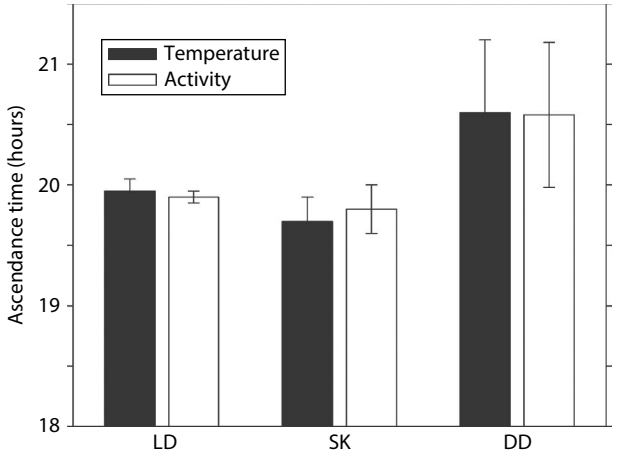


FIGURE 9.10 Persistent synchrony of the rhythms of body temperature and activity. The graph shows the mean time of ascendance past the mean of the rhythms of body temperature and locomotor activity of laboratory rats (*Rattus norvegicus*) maintained under a 24-hour light–dark cycle (LD) and a 24-hour skeleton photoperiod with 1 hour of light per day (SK) and in constant darkness (DD). Each bar corresponds to the mean (± SE) of six rats. In all three conditions, the ascendance time for the temperature rhythm is statistically indistinguishable from the ascendance time for the activity rhythm. (Data from Refinetti, R., *Biol. Rhythm Res.*, 28, 19, 1997.)

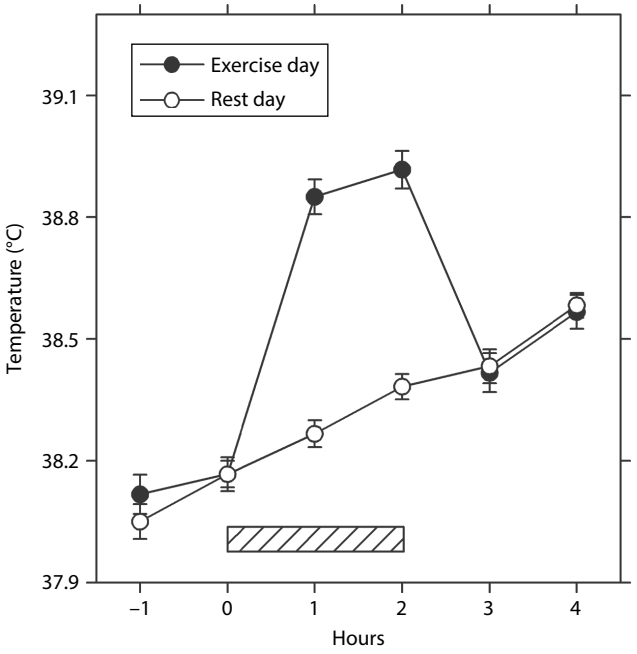


FIGURE 9.11 Exercise causes elevation of body temperature. The graph shows the variation in rectal temperature of horses on a day in which they underwent a 2-hour exercise session and on day in which they rested in the stables. Each data point corresponds to the mean (± SE) of measurements on six horses. The hatched bar indicates the timing of the exercise session. Notice the circadian elevation of body temperature on the rest day and the acute effect of exercise on the exercise day. (Adapted from Piccione, G. et al., *Chronobiol. Int.*, 21, 571, 2004.)

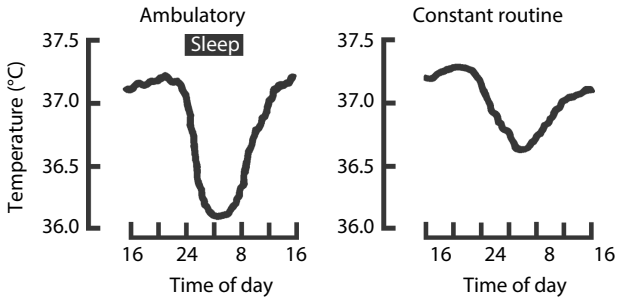


FIGURE 9.12 Activity rhythm does not cause temperature rhythm in humans. The graphs show the average profiles of the body temperature rhythms of healthy young men under ambulatory (with variations in activity) and constant-routine (bed rest, no sleep) conditions. Notice that the constant-routine condition reduces the amplitude of, but does not eliminate, the body temperature rhythm. (Adapted from Czeisler, C.A. and Dijk, D.J., Human circadian physiology and sleep–wake regulation, in: J.S. Takahashi et al. (Eds.), *Circadian Clocks*, Kluwer/Plenum, New York, 2001, pp. 531–569.)

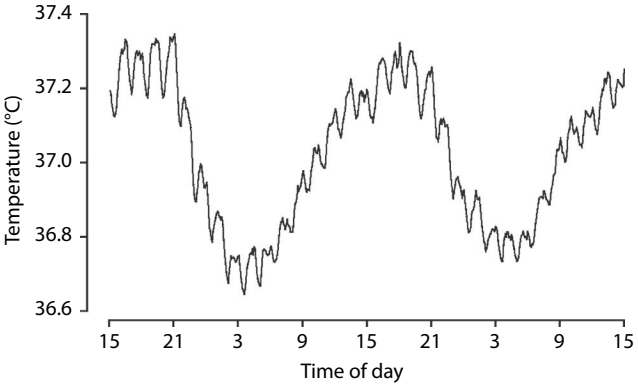


FIGURE 9.13 Very short days in the lab. Nineteen young men and women (one at a time) were kept in the laboratory for 60 consecutive hours under very short "days" of 90 minutes (30 minutes in bed, 60 minutes awake). The graph shows their average body temperature during the middle 48 hours. The short sleep–wake cycles had a noticeable but rather small effect on the circadian rhythm of body temperature. (Adapted from Buysse, D.J. et al., *Sleep*, 28, 1365, 2005.)

activity rhythm in six species of small rodents. Therefore, if there were a causal connection between the two rhythms, the temperature rhythm should cause the activity rhythm—and not the other way around.

The second piece of evidence has to do with the day–night difference in the correlation between the rhythms of activity and temperature. Although nocturnal animals are generally more active at night than during the day, their body temperature is higher at night regardless of the actual activity level.[32,36,76–78] Conversely, the body temperature of diurnal animals is higher during the *day* regardless of the actual activity level.[51] These relationships are illustrated in Figure 9.15. Notice that, for the nocturnal animals (left column), body temperature is higher at night for all levels of activity, even though there is a small effect of activity level on body temperature. For the diurnal animals (right column), body temperature is higher during the day for all

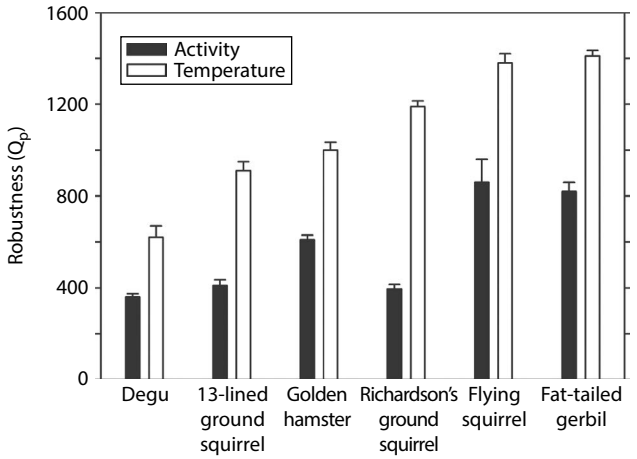


FIGURE 9.14 Activity rhythm is unlikely to cause temperature rhythm in rodents. The graph shows the mean robustness (± SE) of the daily rhythms of locomotor activity and body temperature of 3–8 individuals of each of six species of small rodents. Notice that the temperature rhythm is consistently more robust than the activity rhythm, which suggests that the activity rhythm cannot be responsible for the temperature rhythm. (Adapted from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999.)

levels of activity. Thus, we may confidently say that the body temperature rhythm in animals, as in humans, is not caused by the activity rhythm.

9.1.2 Other Dependencies

We saw earlier that the human body temperature rhythm persists during inactivity and sleep deprivation in the constant-routine protocol.[17,72–74] We also saw that variations in activity can alter ("mask") the temperature rhythm. Can sleep per se (i.e., separately from the obvious association between sleep and inactivity) affect body temperature? Yes, it has been shown that the occurrence of sleep leads to a lowering of core temperature, even if the magnitude of the temperature change is much smaller than that associated with the normal circadian oscillation.[75,79,80] As discussed in Chapter 10, the lowering of core temperature is due, to a great extent, to increased heat loss associated with increased skin temperature in the hands and feet—at least in humans.[81–83] As sleep progresses, the regulation of body temperature is virtually blocked during the REM stage, so that the body passively cools off if ambient temperature is lower than body temperature.[84–97]

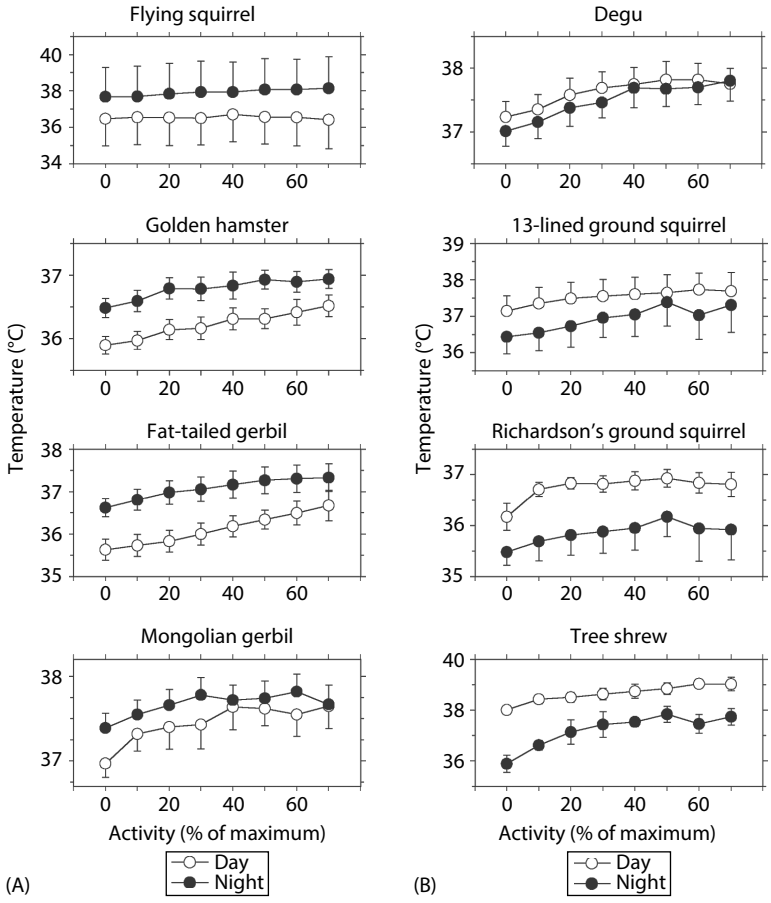


FIGURE 9.15 Activity rhythm does not cause temperature rhythm in small mammals. The graphs show the mean body temperatures associated with different levels of activity, separately for day and night, in 8 species of small mammals. The data points correspond to the means (± SE) of 3–8 animals per species. Nocturnal species are represented in Panel A, and diurnal species in Panel B. Notice that, in all species, body temperature is higher during the active phase of the daily cycle than during the inactive phase at all activity levels. (Adapted from Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999.)

Curiously, photic stimulation prior to sleep prevents the normal sleep-induced fall in body temperature.[98–102]

Because animals do not eat while sleeping, the daily rhythm of activity imposes restrictions on feeding. I am unaware of studies that investigated the feeding rhythm in the absence of the activity rhythm, so I cannot comment on the potential causal link between the two rhythms. However, it is known that food ingestion is associated with an acute rise in body temperature in various species.[85,103–106] Therefore, the daily rhythm of feeding could be responsible for the daily rhythm of body temperature. It is not. In animals fed *ad libitum*, the concentration of feeding during the light phase or the dark phase of the light–dark cycle could possibly result in a chronic elevation of body temperature. However, as shown in Figure 9.16, dogs fed a single meal a day still exhibit a clear daily rhythm of body temperature.[107] Humans and animals fed small meals at regular intervals throughout the day also exhibit rhythmicity in body temperature.[17,72–74,108] As a matter of fact, animals and humans fed no meal at all (that is, subjected to food deprivation) still show daily rhythmicity in body temperature.[11,69,109–116] An example is provided in Figure 9.17. The rectal temperature of a goat was recorded at 3-hour intervals for several days. During the first 3 days, the animal received a single meal each day (indicated by the arrows). For the next 3 days, no food was provided. Food deprivation caused a small decline in body temperature, but rhythmicity was clearly preserved.

Although the feeding rhythm is not the cause of the temperature rhythm, it could very well be the cause of some other rhythm. Figure 9.18 shows that feeding is not the cause of the rhythm of secretion of the hormone leptin in the horse.[117] Neither is it the cause of the rhythm of leptin secretion in the rat.[118] Figure 9.19 shows that feeding is also not responsible for the rhythm of cholesterol production in goats but *is* responsible for the maintenance of the rhythm of urea production.[119] Feeding is not responsible for the daily rhythm of glycemia (blood glucose concentration) in sheep and cattle but *is* responsible for the rhythm of glycemia in dogs and horses.[120] The rhythm of cortisol production in horses is not caused by feeding.[121]

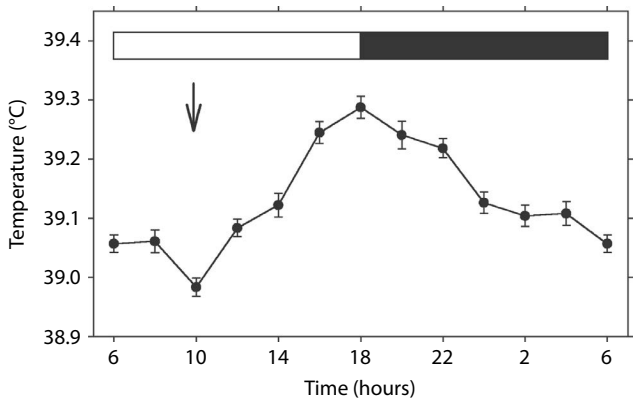


FIGURE 9.16 Body temperature rhythm persists with only one meal per day. The graph shows the mean daily profile of the body temperature rhythm of dogs (*Canis familiaris*) fed one meal a day. The data points correspond to the means (± SE) of seven Beagle dogs over 7 consecutive days. The horizontal bar indicates the timing of the light–dark cycle. The arrow indicates the time of feeding. Notice that body temperature continues to rise for many hours after the daily meal. (*Data* from Refinetti, R. and Piccione, G., *J. Vet. Med. Sci.*, 65, 935, 2003.)

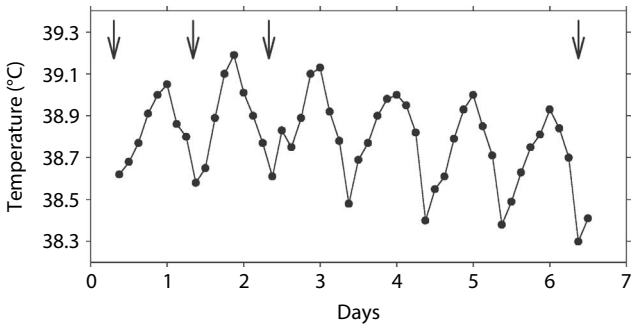


FIGURE 9.17 Body temperature rhythm persists during total food deprivation. The graph shows a segment of the records of rectal temperature of a goat (*Capra hircus*) maintained under a 24-hour light–dark cycle with and without daily meals. The arrows indicate meal times. Notice the persistence of the temperature rhythm (with a small fall in the mean level) through 96 hours of food deprivation. (Data from Piccione, G. et al., *Comp. Biochem. Physiol. A*, 134, 563, 2003.)

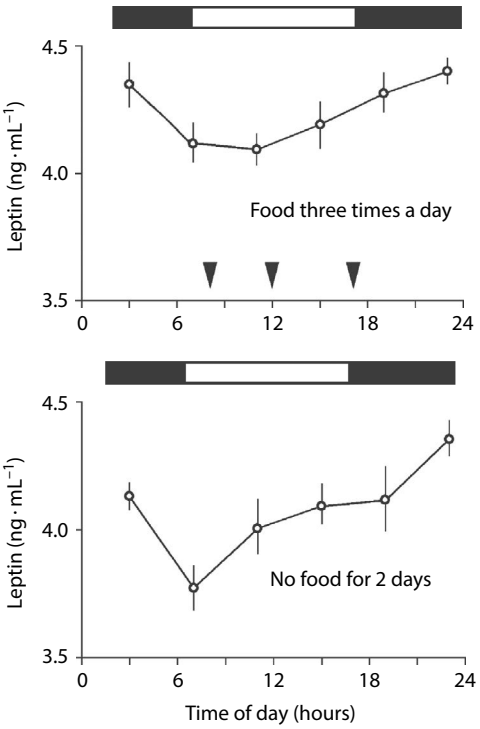


FIGURE 9.18 Rhythm of serum leptin concentration persists during total food deprivation. The graphs show the daily profiles of serum leptin concentration in horses (*Equus caballus*) fed three times a day or maintained without food for 2 days. The data points correspond to the means (± SE) of five horses. The horizontal bars indicate the timing of the light–dark cycle. The inverted triangles indicate feeding times. Notice that the daily rhythm persists (and is even enhanced) during food deprivation. (Data from Piccione, G. et al., *Chronobiol. Int.*, 21, 405, 2004.)

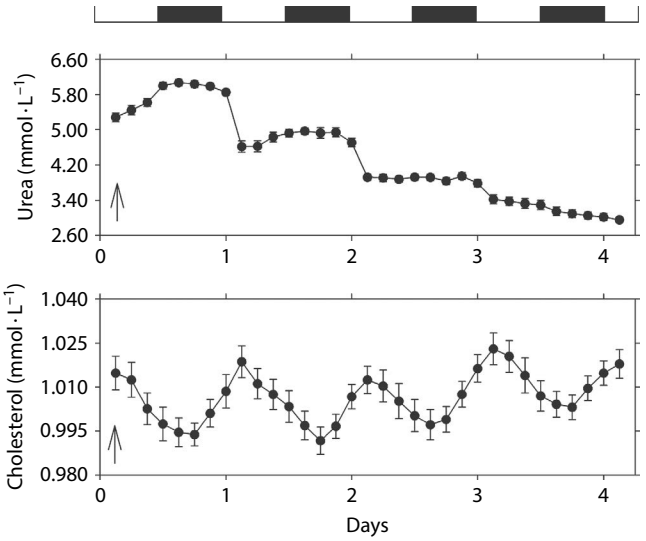


FIGURE 9.19 Rhythm of serum cholesterol concentration persists during total food deprivation, but rhythm of urea concentration does not. The graphs show 4-day segments of the records of serum urea concentration and serum cholesterol concentration of goats (*Capra hircus*) maintained under a light–dark cycle without food. The data points correspond to the means (± SE) of five goats. The horizontal white and dark bars indicate the timing of the light–dark cycle. The arrows indicate the time of the last meal. Notice that the rhythm of cholesterol concentration persists in the absence of daily meals, but the rhythm of urea concentration gradually vanishes. (Data from Piccione, G. et al., *Comp. Biochem. Physiol. A*, 134, 563, 2003.)

Another dependence of one rhythm on another that has received the attention of circadian physiologists is the possible reliance of the rhythm of heart rate on the rhythm of activity. Variations in heart rate are, of course, closely associated with variations in activity. Accordingly, ambulatory recording of heart rate shows that heart rate is lower during sleep, even if the subject changes his or her sleep time, as illustrated in Figure 9.20. However, studies on human subjects maintained in bed rest have consistently indicated that the rhythm of heart rate is *not* caused by the activity rhythm, even though its amplitude is greatly reduced in the absence of the activity rhythm.[17,70,122–125] On the other hand, the rhythm of blood pressure may be generated by the activity rhythm.[122]

9.2 ECOLOGY AND EVOLUTION

If the problem of internal temporal order is a complex one, things can only get more complicated when we try to understand how organisms adapt to their environment—now and throughout evolution. In previous chapters, we discussed several environmental factors that affect circadian rhythms, but we dealt with each one individually. Now, we must add the complexity of interactions among environmental factors to the complexity of the organism's internal temporal order. To be sure, we cannot satisfactorily perform this task at this time. We can, however, attempt to elucidate a few ecological and evolutionary issues related to circadian rhythms.

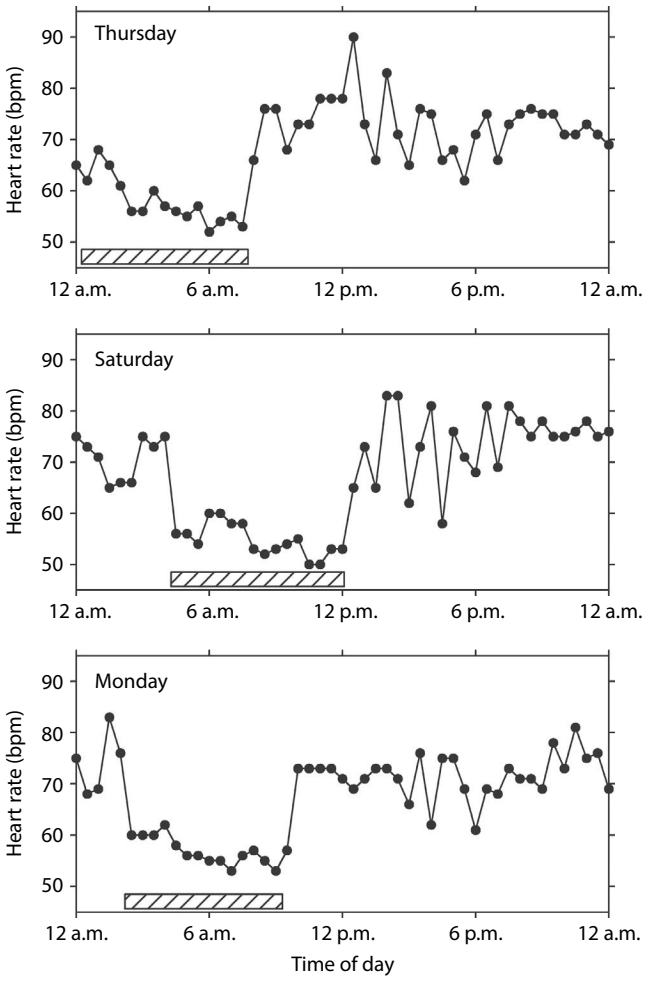


FIGURE 9.20 Rhythm of heart rate is affected by deviation in bedtime. The heart rate of a university professor was monitored for many consecutive days. The three graphs show the readings for three different days. The small hatched horizontal bars in each graph indicate sleep time. Heart rate is lower during sleep whether sleep takes place during the usual time (Thursday) or later than usual (Saturday and Monday). (Data from the archives of the Refinetti lab.)

9.2.1 EVOLUTION

We saw in previous chapters that circadian rhythmicity is a pervasive characteristic of life on Earth. That this characteristic is quite old is evidenced by the fact that circadian rhythmicity is exhibited even by animal species that inhabit subterranean environments and have long lost a functional visual system.[67,126–131] Because these animals are not exposed to daily environmental fluctuations, they had no selective force to drive the evolution of endogenous circadian clocks. The fact that they do have the clocks indicates that they must have inherited them from nonsubterranean ancestors. But how long ago did this happen? Discussions of evolutionary issues in the popular media often invoke the image of dinosaurs, the giant reptiles that populated the Earth about 200 million years ago (Figure 9.21). We must, however, go much further back in time to seek the origins of circadian rhythmicity. We must go back to the beginning



FIGURE 9.21 Raging dinosaur. Dinosaurs, along with many other animals, populated the Earth during the Mesozoic Era. (Copyright ArtToday, Tucson, AZ.)

of life on Earth, some 4 billion years ago.[132] The Earth itself was formed 4½ billion years ago.[133]

As shown in Figure 9.22, all life forms on Earth can be classified into one of three domains: Bacteria, Archaea, and Eukarya.[134,135] The domain of bacteria is the oldest branch of the tree of life, having preceded the archaea and all eukaryotes (i.e., organisms whose cells contain a nuclear membrane). Because extant bacteria exhibit circadian rhythmicity,[136–140] it is usually assumed that rhythmicity was present already in the earliest life forms and was retained in all divergent branches along the evolutionary tree. However, in the absence of fossil evidence, it is equally possible that circadian rhythmicity evolved *de novo* multiple times in various taxonomic groups.[141] As will be discussed in Chapter 12, there are fundamental differences in the molecular machinery of circadian pacemakers among extant organisms of the three domains of life, which supports the hypothesis of multiple evolutionary occurrences. On the other hand, at least one component of the molecular machinery seems to be conserved among animal, plant, and fungal circadian systems,[142] which suggests a common origin of circadian rhythmicity for all eukaryotes. Within the animal kingdom, different sets of clock genes are used by different taxa, but some genes are found in all animal phyla studied so far.[143–145] From a larger evolutionary

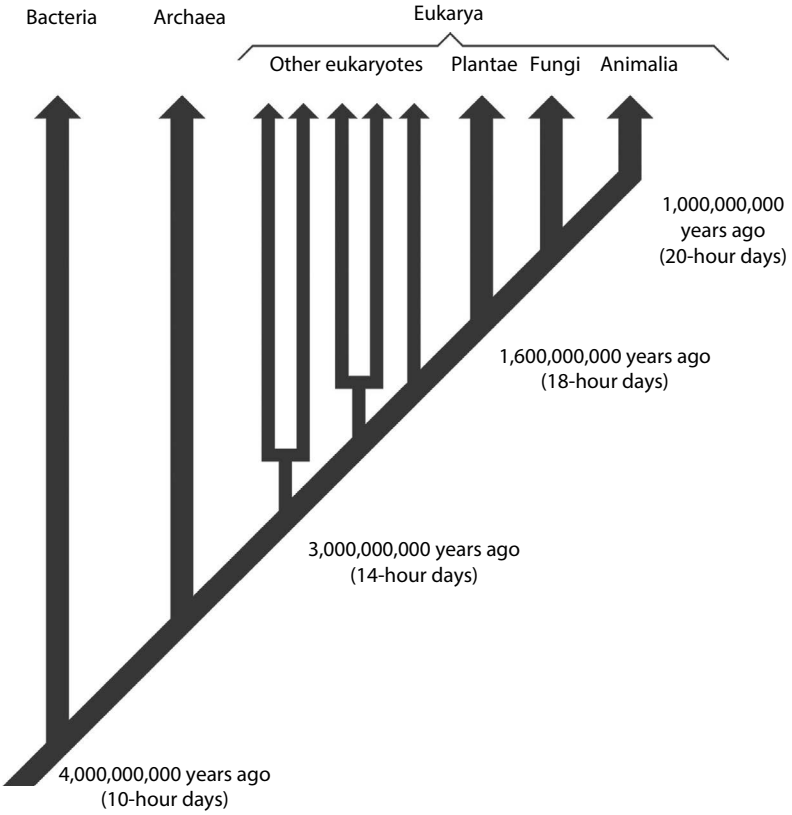


FIGURE 9.22 The tree of life. Life on Earth started not long after the formation of the planet itself, some 4.5 billion years ago. The diagram indicates the approximate time of the divergence of the three domains of life (Bacteria, Archaea, and Eukarya) and of the kingdoms of Eukarya. The approximate duration of a day (which was shorter than 24 hours for most of Earth's life) is also indicated at various timepoints. (Data from Pennisi, E., *Science*, 300, 1694, 2003; Benton, M.J. and Ayala, F.J., *Science*, 300, 1698, 2003; Meyerowitz, E.M., *Science*, 295, 1482, 2002; Doolittle, W.F., *Science*, 284, 2124, 1999; Barnett, J.E., *Time's Pendulum: From Sundials to Atomic Clocks*, Plenum, New York, 1998.)

perspective, a comparison of proteins found in bacteria, archaea, and eukarya revealed that peroxiredoxins exhibit daily rhythmicity in all three domains of life, suggesting that metabolic rhythms are ancient and were likely responsible for the evolution of circadian clocks.[146]

In the strict sense of the term, *circadian* rhythmicity could not possibly have evolved several billion years ago. This is because the duration of a day was outside the circadian range for most of Earth's life. Earth's rotation has been slowing down for billions of years, so that, as indicated in Figure 9.22, the duration of a day was only 10 hours 4 billion years ago and still only 18 hours 1½ billion years ago.[147] Of course, these changes were slow enough to allow natural selection to favor adaptive mutations. Since the origins of the animal kingdom 1 billion years ago, the period of Earth's rotation has lengthened only four hours. *Tau*-mutant hamsters (the critters with endogenous 20-hour periodicity mentioned in Chapter 6) would be right at home on Earth at that time—except that the first placental mammals did not evolve until 100 million years ago[148–150] (Figure 9.23). Our own species (*Homo sapiens*), having appeared only 100–200 thousand years ago,[151–154] has never had to face a day with a period significantly different from 24 hours.

Much more than just the duration of a day has changed during Earth's history. For instance, mean global temperature has oscillated from 0°C (32°F) to almost 30°C (86°F) since the first vertebrates appeared on Earth (Figure 9.24). It could be freezing at summer time in the Bahamas—except that the Bahamas did not exist at that time, as the geography of the Earth has changed as much as its climate[155] (Figure 9.25). With all of these changes in the environment, it is very difficult to determine how circadian rhythmicity evolved over the eons. At the most basic level, it is generally agreed that endogenous rhythmicity evolved as a mechanism that allowed organisms to prepare for predictable daily changes in the environment.[156–161] For instance, photosynthetic plants could wait for sunlight each day, but those with an innate mechanism capable of anticipating sunrise would get an early start by initiating preparatory adjustments during the last part of the night. Similarly, nocturnal rodents could wait for the darkness of the night before getting ready to leave their burrows, but those with an innate mechanism capable of anticipating sunset would prepare in advance for the rigors of foraging. On a limited scale, experimental research has demonstrated enhanced reproductive fitness or survival in normal organisms as compared to organisms with deficient circadian systems.[139,162–168]

The three main eukaryotic kingdoms (fungi, plants, and animals) diverged between 1 and 1.5 billion years ago[169,170] (Figure 9.22) and started to colonize terrestrial environments soon afterward.[171] Most taxa likely originated in the tropics and expanded toward the poles without losing their tropical presence.[172] Mammals did not appear until 300 million years ago and did not evolve a placenta until 100 million years ago[173] (Figure 9.23). It seems that mammals originated in Asia and did not disperse to Europe and North America until 50 million years ago.[174] Of particular interest in mammalian evolution is the development of endothermy (the ability to produce enough metabolic heat to elevate body temperature above ambient temperature), which allowed the evolution of an endogenously generated rhythm of body temperature. As will be discussed in greater detail in Chapter 10, birds and mammals are the only extant groups of organisms in which endothermy is widespread. Consequently, it is assumed that endothermy never evolved in other groups. It has been speculated that dinosaurs and other Mesozoic reptiles may have been endothermic,[175–178] but it is more likely that they achieved stable body temperature because of the thermal latency associated with their gigantic body sizes.[179–181] Cross-taxa comparisons of basal metabolic

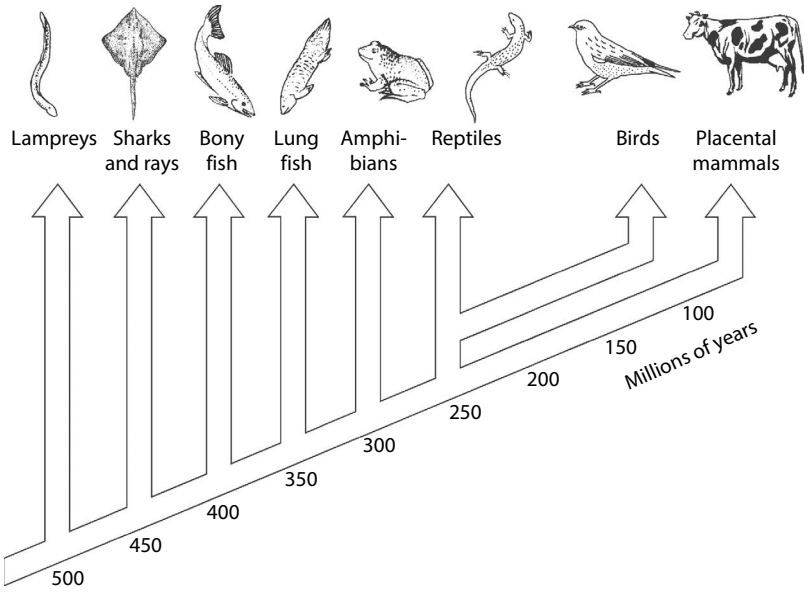


FIGURE 9.23 The vertebrate tree of life. Vertebrates have been on Earth for 500 million years. The diagram indicates the approximate time of the divergence of several branches of this subphylum of the phylum Chordata. (Data from Pennisi, E., *Science*, 300, 1694, 2003; Benton, M.J. and Ayala, F.J., *Science*, 300, 1698, 2003; Stokstad, E., *Science*, 291, 232, 2001.)

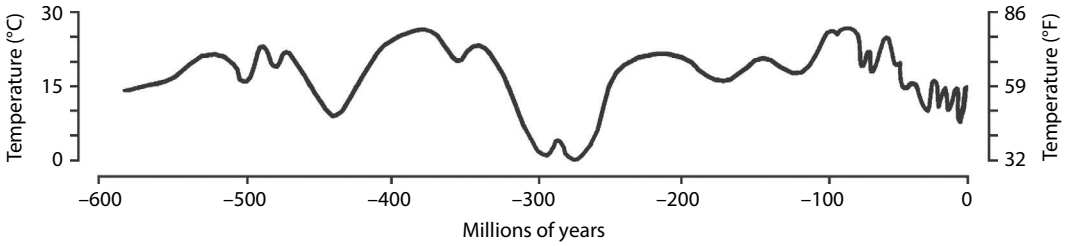


FIGURE 9.24 Ups and downs of Earth's temperature. The graph shows the variation in mean global surface temperature over the last 600 million years. Notice the relatively small range of variation during the last 50 million years (when most extant mammalian species evolved). (Data from Bradley, R.S., *Paleoclimatology*, 2nd edn., Academic Press, San Diego, CA, 1999; Menne, M., Global long-term mean land and sea surface temperatures, Global surface temperature anomalies report, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, 2000.)

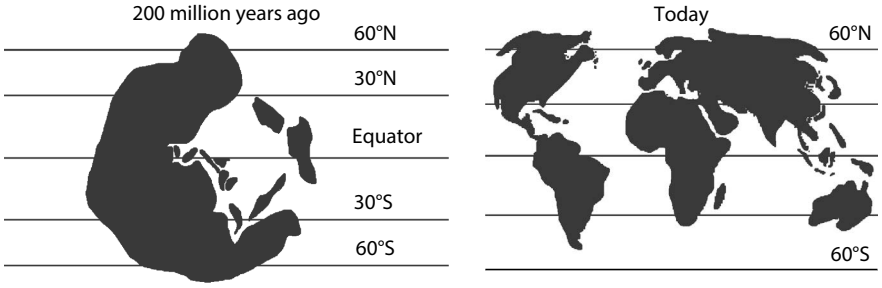


FIGURE 9.25 Geography of ancient Earth. Since the early vertebrates evolved (500 million years ago), not only the climate but also the geography of Earth has changed considerably. The diagrams show the organization of continents 200 million years ago and today. (Adapted from Torsvik, T.H., *Science*, 300, 1379, 2003.)

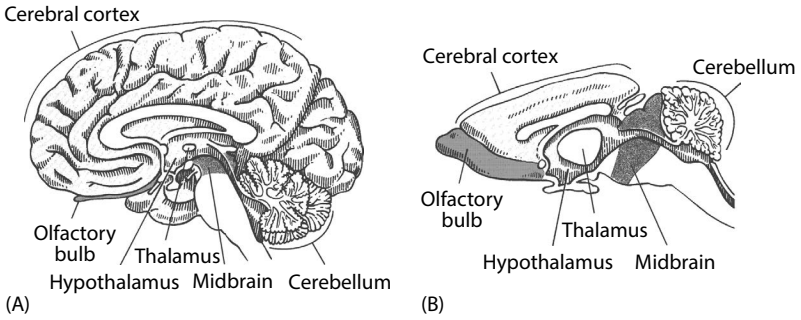


FIGURE 9.26 Of man and rat. These diagrams of midsagittal views of the human brain (A) and the rat brain (B) emphasize similarities and differences in the nervous systems of the two species. Differences are particularly evident in the relative sizes of the cerebral cortex, olfactory bulb, and thalamus. (Adapted from Rosenzweig, M.R. and Leiman, A.L., *Physiological Psychology*, D. C. Heath, Lexington, MA, 1982.)

rate of extant mammalian species suggest that endothermy did not appear until 70 million years ago,[182–184] perhaps not even until 10 million years ago.[185] A sharp fall of about 20°C in global temperature between 50 and 20 million years ago[186] (Figure 9.24) may have provided a thermal environment that favored mammalian species with high metabolic rates.[185,187]

There are 5–50 million species on Earth, although only 1.5 million of them have been identified and classified.[188,189] All of these species share the property of being alive, but close neighbors in a taxonomic branch share much more. Particularly relevant for our discussion of the physical substrates of the circadian system in later chapters is the fact that the nervous systems of vertebrates—and particularly of mammals—share many anatomical and physiological characteristics.[190] As diagrammed in Figure 9.26, the human brain and the rat brain have many common features. Major differences include a much larger cerebral cortex in humans than in rats and much larger olfactory bulb and thalamus in rats than in humans. These anatomical differences are reflected in the superior cognitive abilities of humans and in the keen sense of smell in rats.

9.2.2 Diurnal and Nocturnal Niches

Perhaps the most fundamental ecological issue in circadian physiology is an organism's adoption of a *nocturnal* niche or a *diurnal* niche (Figure 9.27). Yet, it is also a most obscure



FIGURE 9.27 Birds of a feather. Some birds, like the hawk (A), are active during the day; others, like the owl (B), are active at night. (Courtesy of National Image Library, United States Fish and Wildlife Service.)

issue. In terms of evolution, it is not even certain whether the choice of a temporal niche was relevant to early life forms. If the first organisms were *photoautotrophic* and relied on energy from the Sun, then the choice of a diurnal niche would certainly have been important. However, if the first organisms were *chemoautotrophic* and relied on geothermal energy from deep-ocean vents,[191–193] then the alternation of day and night on Earth's surface would have been of very little importance. Eventually, of course, all organisms exposed to sunlight had to deal with the conflict between obtaining the needed energy from solar radiation and being damaged by its strong ultraviolet emissions. Resolution of this conflict—in the form of daily vertical migration in the ocean—may have been the driving force for the evolution of circadian rhythmicity.[194,195] Millions of years later, when living beings—particularly *heterotrophic* ones, such as animals—abandoned the ocean and colonized terrestrial environments, the choice of a nocturnal niche was probably necessary as a means of preventing desiccation.[196] Thus, invasion of the diurnal niche became possible only after the evolution of integuments capable of preventing water loss. Most ancient mammals probably evolved in a nocturnal niche,[197] at a time when dinosaurs filled both the diurnal and the nocturnal niches.[198] One can only speculate whether endothermy evolved in a nocturnal niche[185] or in a diurnal one.[187]

Although many animals today can be classified as either diurnal (day active) or nocturnal (night active), many others defy classification.[199–201] A few examples are shown in Figure 9.28. Dogs and eagles, for example, are diurnal. Cats and owls are nocturnal. Giraffes are *crepuscular*, which means that they are active at dawn and dusk but not during most of the day or most of the night. Generally, but with a good number of exceptions, birds and large mammals tend to be diurnal. Small mammals abound in both nocturnal and diurnal niches, as do insects. Plants cannot photosynthesize without light and, therefore, might be labeled diurnal.

From the perspective of circadian physiologists, accurate assessment of the diurnal or nocturnal nature of a species requires controlled investigations in which all environmental variables except the one under investigation (usually the light–dark cycle) are maintained constant. The activity pattern of a

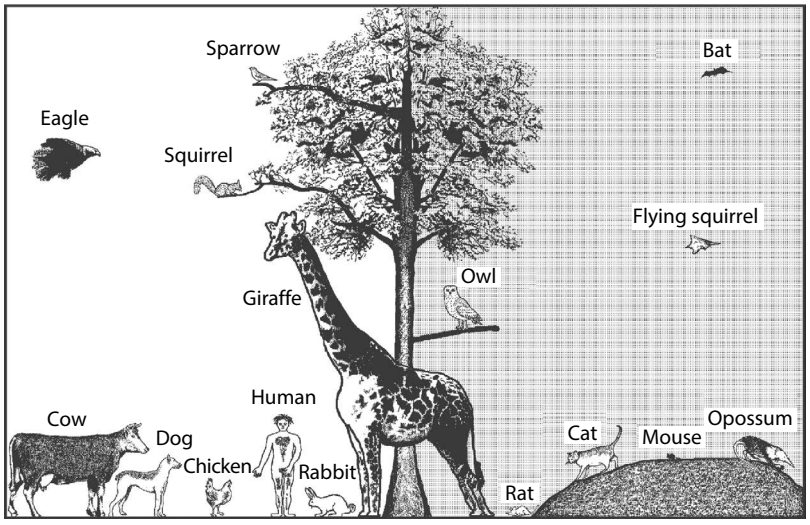


FIGURE 9.28 Temporal niches. The diagram depicts some mammals and birds with diurnal habits (left side), with nocturnal habits (right side), or with crepuscular habits (giraffe and rabbit).

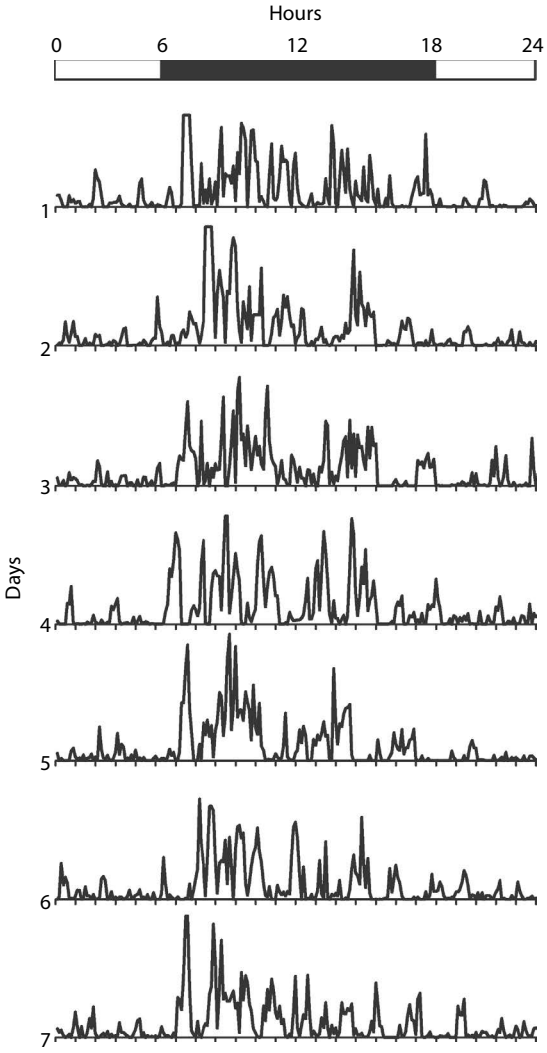


FIGURE 9.29 Golden hamsters are nocturnal animals. This actogram-style plot of a 7-day section of the records of locomotor activity of a golden hamster (*Mesocricetus auratus*) shows that golden hamsters are clearly nocturnal and exhibit very little activity during the light phase of the light–dark cycle. Activity was monitored by telemetry and recorded at 6-minute intervals. The animal was kept in an individual cage (without a running wheel) with food and water freely available at a constant ambient temperature of 24°C under a 24-hour light–dark cycle (indicated by the horizontal bar at the top). (Data from the archives of the Refinetti lab.)

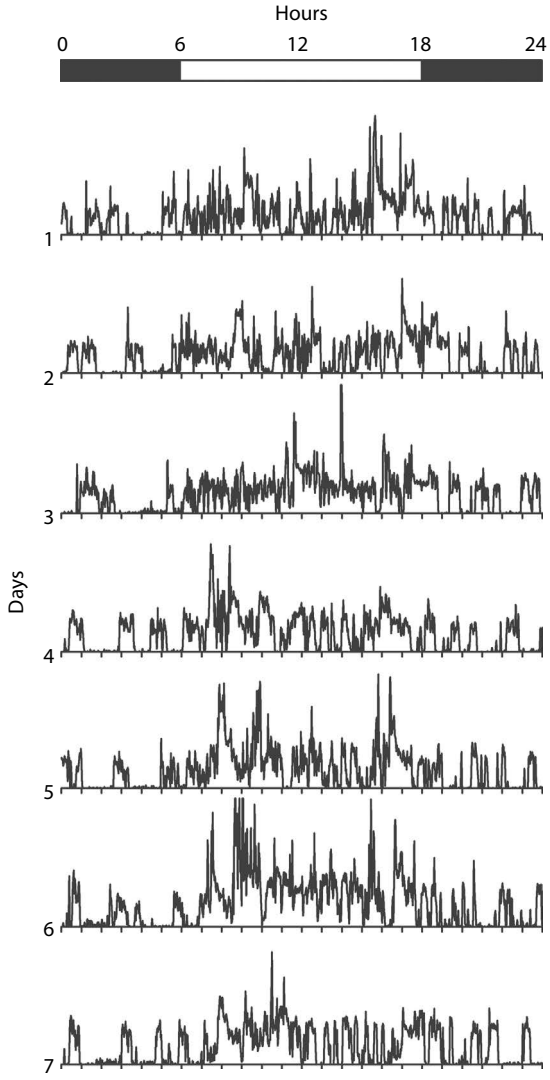


FIGURE 9.30 Horses are diurnal animals—or are they not? This actogram-style plot of a 7-day section of the records of locomotor activity of a horse (*Equus caballus*) shows that horses are slightly more active during the light phase of the light–dark cycle, but some activity is evident throughout the day. Activity was recorded at 1-minute intervals with a datalogger. The animal was kept in an individual stall under a natural light–dark cycle (indicated by the horizontal bar at the top). (Data from the archives of the Refinetti lab.)

golden hamster (*M. auratus*), recorded by telemetry under controlled conditions in the laboratory, is illustrated in Figure 9.29. Notice that, although some activity is exhibited at any time of the day, most activity is concentrated in the dark phase of the light–dark cycle. Thus, the golden hamster is a nocturnal animal.

Figure 9.30 illustrates the activity pattern of a horse (*Equus caballus*) recorded by actigraphy in an indoor stall. Although activity is even more spread out over the whole day than in the hamster, most activity is concentrated in the light phase of the light–dark cycle. Thus, the horse is a diurnal animal. In contrast, Figure 9.31 illustrates the activity pattern of a Mongolian gerbil (*Meriones unguiculatus*) recorded in a running wheel in the laboratory. The pattern is much more irregular, although a short interval of high activity is consistently observed at dawn. Thus, the Mongolian gerbil is a crepuscular animal (or a *matutinal* crepuscular animal, as it does not exhibit a *vespertinal* peak in activity). Some species, particularly among primates, have activity rhythms so irregular that a new word was created to describe their pattern of activity: *cathemerality*.[202] The term *arrhythmicity* would probably have been sufficient, but animals designated as cathemeral are usually animals that alternate between diurnality, nocturnality, and arrhythmicity.[203]

Regarding the crepuscular pattern of activity, you will most likely remember from previous chapters that the most common waveform of the activity rhythm of animals is a bimodal one,[204] which means that enhanced activity at dawn

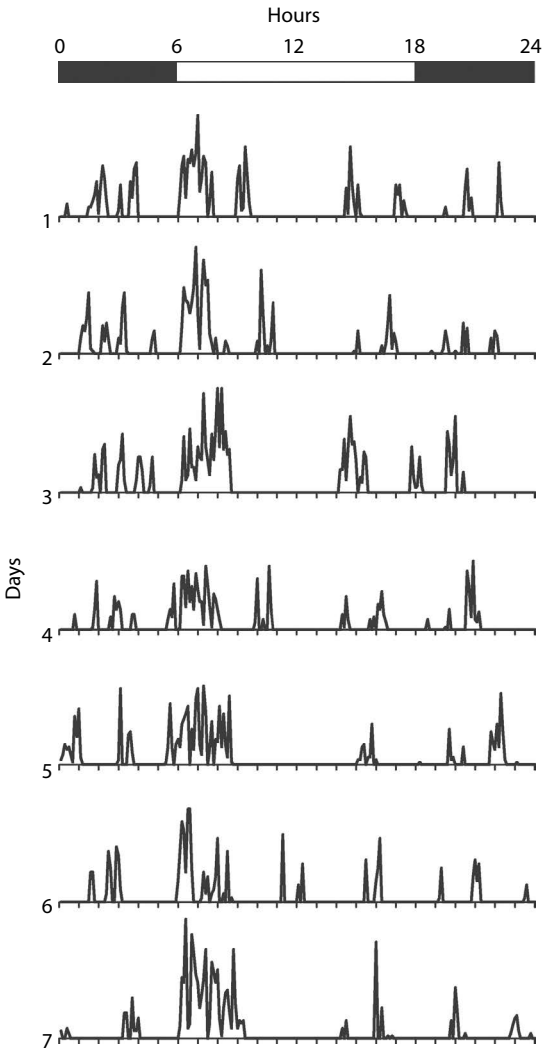


FIGURE 9.31 Mongolian gerbils are not clearly diurnal or nocturnal. This actogram-style plot of a 7-day section of the records of locomotor activity of a Mongolian gerbil (*Meriones unguiculatus*) shows that gerbils are not clearly nocturnal or diurnal. Activity was monitored in a running wheel and recorded at 6-minute intervals. The animal was kept in an individual cage under a 24-hour light–dark cycle (indicated by the horizontal bar at the top). A peak of activity is evident each day immediately after lights on, and this peak is preceded by some activity for a few hours before lights on. Smaller activity bouts are seen also before and after lights off. (Data from the archives of the Refinetti lab.)

and dusk is quite common. In a sense, it could be said that diurnal animals fill the space between the peaks at dawn and dusk, whereas nocturnal animals fill the space between the peaks at dusk and dawn. Crepuscular animals would be those that do not fill either space preferentially. Consequently, their rhythms are less robust.[51,205,206] Although more research is needed on this issue, it seems that weak rhythmicity results from a weak connection between the circadian pacemaker and the effector organs, not from a weak pacemaker. Thus, guinea pigs (*Cavia porcellus*) have very weak (*noisy*) activity rhythms even though simultaneous electrophysiological recording of brain cells indicates robust rhythmicity in the circadian pacemaker.[207]

I must point out that, even in species that exhibit clearly diurnal or clearly nocturnal activity patterns, the distinction between diurnality and nocturnality is not always a straightforward one. For instance, in goldfish (*Carassius auratus*), about 80% of individuals tested in the laboratory are diurnal, whereas 10% are nocturnal, and 10% are just "messy."[208] In sea bass (*Dicentrarchus labrax*), about 40% of individuals tested in the laboratory are diurnal, whereas 60% are nocturnal.[209] In carpenter ants (*Camponotus compressus*), approximately 70% of individually tested animals are nocturnal, whereas 30% are diurnal.[210] In Mongolian gerbils (*M. unguiculatus*), about 75% of individuals tested in the laboratory are diurnal, whereas 25% are nocturnal.[211] Likewise, in subterranean mole rats of various species, some members of the species are diurnal and some are nocturnal.[130,212]

Although interindividual differences in circadian period could account for small interindividual differences in the phase angle of entrainment (see Chapter 7), differences large enough to justify the labeling of some animals as diurnal and some as nocturnal require a much more powerful mechanism. Not only powerful, the mechanism must be quite flexible—because, in some species, the same individual may be diurnal under some circumstances and nocturnal under other circumstances. For instance, wolves (*Canis lupus*) are normally nocturnal; however, when traveling over long distances, they travel during the day.[213] Conversely, migratory birds are normally diurnal, but they do most of their migratory flight at night.[214–216] Also, humans are normally diurnal; however, when searching for a new mate, we do most of our work at night—in nightclubs, private parties, or even the Internet!

The flexibility of the mechanism responsible for the selection of temporal niche is perhaps the most evident in a small rodent from Chile, the degu (*Octodon degus*). These animals, which are diurnal in the wild,[217] can instantly become nocturnal in the laboratory simply by gaining access to a running wheel.[218,219] An example is shown in Figure 9.32. This double-plotted actogram depicts the body temperature rhythm of a degu with and without access to a wheel (as indicated on the right margin). With access to the wheel, the animal is clearly nocturnal, exhibiting high temperature values during the dark phase of the light–dark cycle. When the light–dark cycle is discontinued, the rhythm freeruns appropriately from the preceding phase of entrainment. Amazingly, subjective day and subjective night are reversed instantly upon the removal of the running wheel. The reversal is confirmed by the entrainment pattern in the final section of the records. According to the authors of the study, the reversal of subjective day and subjective night observed in this individual is typical of all degus.[218] A similar phenomenon—but limited to only a subgroup of animals—has been reported in the Nile grass rat (*Arvicanthis niloticus*).[220,221] As previously discussed in Chapter 5, the grass rats that were observed to be nocturnal when given access to running wheels seemed to have been members of an idiosyncratic subgroup. In my laboratory, all grass rats housed with running wheels have been diurnal.[222,223] A third research team observed increased crepuscular activity in grass rats housed with wheels, but the activity pattern was still predominantly diurnal.[224]

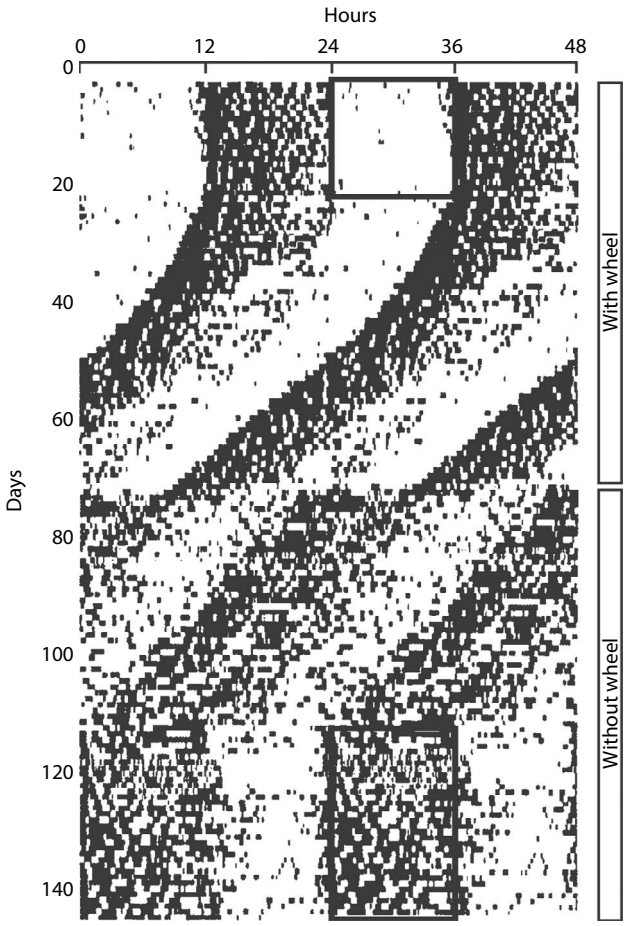


FIGURE 9.32 Degus can be either diurnal or nocturnal. This double-plotted actogram shows the rhythm of body temperature of a degu (*Octodon degus*) maintained in constant darkness or under an LD 12:12 cycle (as indicated by the single-plotted rectangles). Plotted time bins correspond to values of body temperature above a 72-hour running mean; values below the mean are not plotted. Notice that, during the first 70 days, the animal had access to a running wheel, and its body temperature was higher at night (as expected of a nocturnal animal). During the remaining days, the animal did not have access to a running wheel, and its body temperature rhythm switched to a diurnal pattern. (Adapted from Kas, M.J.H. and Edgar, D.M., *J. Neurosci.*, 19, 328, 1999.)

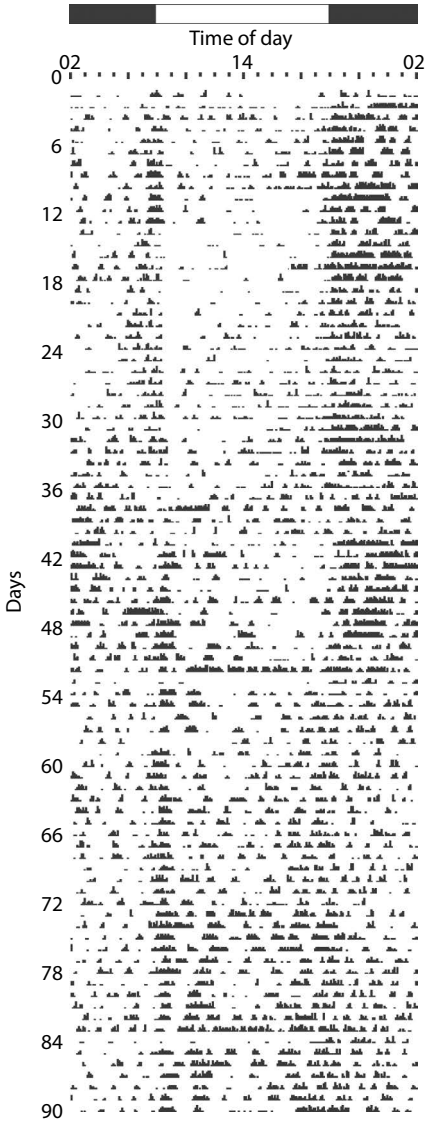


FIGURE 9.33 Drifting degu. This single-plotted actogram shows the rhythm of running-wheel activity of a degu (*Octodon degus*) maintained under an LD 12:12 cycle (as indicated by the horizontal bars at the top). This animal was more active at night in the beginning of the records but more active during the day by the end of the 3-month interval. (Adapted from Refinetti, R., *J. Comp. Physiol. A*, 192, 701, 2006.)

The surprising change of temporal niche exhibited by degus upon gaining access to running wheels is not as surprising as the change I have observed in my own laboratory. If given enough time, many degus will switch from a diurnal niche to a nocturnal niche (or vice versa) spontaneously, even if having access to a running wheel from the beginning.[211] An example is shown in Figure 9.33. The degu whose records are shown in this figure was initially nocturnal but started drifting around day 50 and soon became preponderantly active during the light phase. Spontaneous shifting between diurnal and nocturnal niches has also been reported in cotton rats (*Sigmodon hispidus*).[225]

Fruit flies (*Drosophila melanogaster*) are diurnal, but they don't like bright light. Thus, if the light–dark cycle is bright, they restrict their activity to the very beginning and very end of the day. If the light–dark cycle is not as bright, they expand their activity time to the middle of the day.[226] In a more extreme fashion, Norway lobsters (*Nephrops norvegicus*) tested in the laboratory are nocturnal if the brightness of the light–dark cycle is 10 lux (the brightness experienced at a depth of about 20 m in the ocean) but diurnal if the brightness is 0.1 lux (the brightness experienced at a depth of about 400 m).[227] Similarly, white-fronted lemurs (*Eulemur fulvus*) are clearly nocturnal when the brightness of the light–dark cycle is 0.1 lux but diurnal if the brightness is 0.0001 lux or less.[43]

Even in animals that are consistent about their choice of temporal niche, a radical distinction between diurnal and nocturnal species is rarely justified. A detailed comparison of temporal niche selection in a number of rodent species

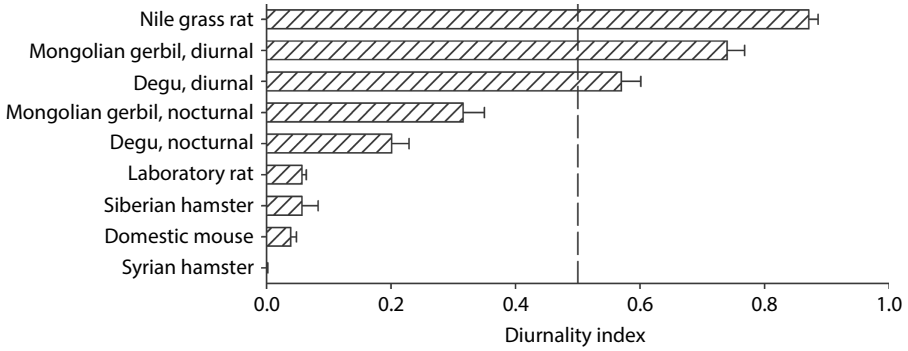


FIGURE 9.34 A natural continuum. The graph shows that, among a group of rodent species, there is a gradient from diurnality (index = 1.0) to nocturnality (index = 0.0) rather than a dichotomous separation. (Adapted from Refinetti, R., *J. Comp. Physiol. A*, 192, 701, 2006.)

revealed a gradient of diurnality rather than a dichotomous division.[211] As shown in Figure 9.34, Nile grass rats are on the diurnal end of the continuum, whereas Syrian (golden) hamsters are on the nocturnal end, and other species (or subgroups within a species) lie between the two extremes. Diurnal monkeys monitored in the wild are found to occasionally make excursions into the night.[228]

A curious form of phase reversal is found in mice of the genus *Acomys*. In natural settings in rocky deserts of the Middle East, common spiny mice (*A. cahirinus*) share a foraging microhabitat with golden spiny mice (*A. russatus*). Normally, common spiny mice are nocturnal, whereas golden spiny mice are diurnal. However, if the common spiny mice are removed from the area, the golden spiny mice become nocturnal.[229] This suggests that the golden spiny mice are normally pushed into the diurnal niche by the competition for resources. Indeed, when golden spiny mice are trapped in the field and immediately tested individually in the laboratory, they exhibit a nocturnal pattern of activity,[230] even if their response to photic masking is not as clear-cut as that of common spiny mice.[231] Thus, although the phase reversal in spiny mice may be less interesting than that in degus from the point of view of circadian mechanisms, it is quite interesting from an ecological point of view. It shows how masking mechanisms may supplant entrainment mechanisms in the determination of the temporal niche of species in the wild. In a related example, wild rats are usually nocturnal, but they can switch their foraging to the daytime when a nocturnal predator (fox) is present.[232]

Not as dramatic as the phase reversal of spiny mice, but still worth noting, is the change in the temporal distribution of activity of Syrian hamsters in the wild. As indicated earlier (and shown in Figure 9.34), golden hamsters are almost exclusively nocturnal when housed under laboratory conditions. A study of a wild population in Syria yielded unexpected results, which are shown in Figure 9.35. Panel A in this figure shows the average activity pattern of hamsters housed in the laboratory—which is predominantly nocturnal, as expected. Panel B shows the average activity pattern of hamsters in the wild. In the wild, golden hamsters are clearly crepuscular instead of nocturnal.[233] The difference between the pattern observed in the laboratory and the pattern observed in the wild probably results from two main factors. Ecologically, it

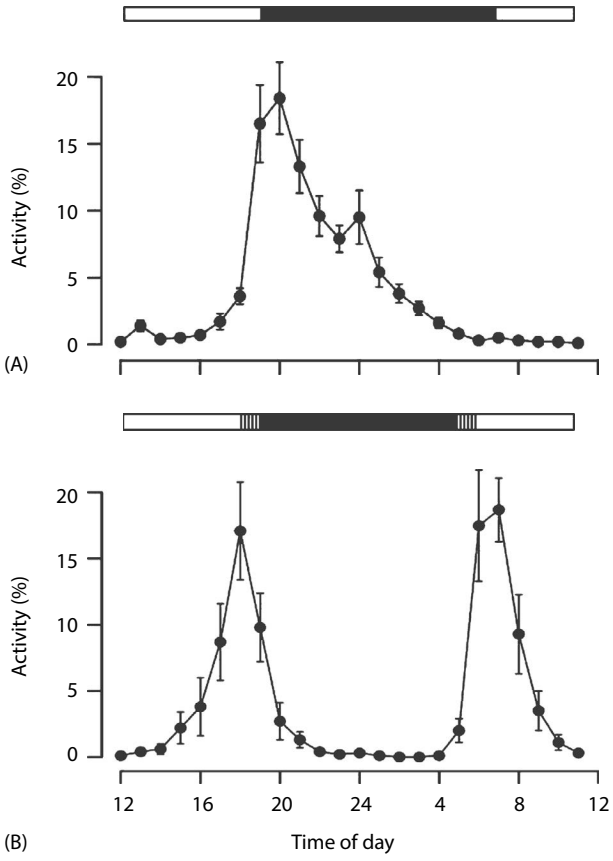


FIGURE 9.35 Domestic and wild hamsters. Golden hamsters (*Mesocricetus auratus*) are common pets that have been bred in captivity for almost a century. In the laboratory, they are clearly nocturnal (A), but in the wild, they are active almost exclusively at dawn and dusk (B). The duration of the light and dark phases of the light–dark cycle is indicated by the white and dark segments of the horizontal bars above the graphs (with the striped area denoting twilight). (Adapted from Gattermann, R. et al., *Biol. Lett.*, 4, 253, 2008.)

is reasonable to assume that nighttime predators in the wild would restrict the hamsters' aboveground activity at night, whereas the absence of predators in the laboratory would allow exploratory behavior throughout the night. It is also the case, however, that the researchers recorded only aboveground activity, so that it is perfectly possible that the hamsters in

the wild were active inside their burrows throughout the night but the researchers failed to record this activity. Of course, the large peak of activity at dawn in the wild is not present in the laboratory records and cannot be explained easily by methodological deficiency. More comparative studies of this type would contribute to the understanding of mechanisms of temporal niche determination.

It is often assumed that smaller, subdominant predator species coexist with their larger, dominant counterparts through temporal partitioning of resources. An actual study of smaller (wild dogs and cheetahs) and larger (spotted hyenas and lions) predators in the wild in Africa contradicted this assumption by showing extensive overlap of the main activity periods of the two groups of animals during the night and twilights.[234]

Given the blurred line separating diurnal from nocturnal organisms, it is not surprising that there is limited knowledge of anatomical features that can differentiate diurnal organisms from nocturnal organisms. Having eyes specialized for day vision (that is, possessing retinal cones in addition to retinal rods) evidently facilitates adaptation to a diurnal niche, but, as we will see in Chapter 11, image-forming photoreception is essentially independent from circadian photoreception. An animal with a diurnal circadian system will entrain to the light–dark cycle with a diurnal phase angle regardless of the sophistication of its visual system. Diurnality (or nocturnality) is a property of the circadian system. Direct your attention to Figure 9.36, which shows the acrophases (times of the daily peak) of the body temperature rhythms of 10 mammalian species determined under very similar environmental conditions. The two most evident features of the figure are the high interspecies variability of acrophases and the high intraspecies variability in some species (as indicated by the error bars). Not as evident but equally worthy of notice is the fact that the acrophases of diurnal animals are concentrated in the second half of the light phase, whereas the acrophases of nocturnal animals are concentrated in the first half of the dark phase. The finding of acrophases during the day in diurnal animals and acrophases during the night in nocturnal animals is expected. However, it is not at all obvious why body temperature reaches its daily peak late in the active phase of diurnal animals but early in the active phase of nocturnal animals.

Even if not necessary for circadian entrainment, there are unquestionable differences in the eyes of diurnal and nocturnal animals. Eye size in primates is *not* related to diurnality or nocturnality, but nocturnal primates tend to have larger orbital aperture diameter.[235] Examination of the eyes of two diurnal rodents (*Arvicanthis ansorgei* and *Lemniscomys barbarus*) revealed that 33% of the photoreceptors in the retina were cones ("daytime" photoreceptors), whereas cones account for less than 3% of the photoreceptors in the retina of nocturnal rats and mice.[236] Examination of the eyes of 12 mammalian species detected acetylcholine in the corneal epithelium of all of the diurnal species but not in the corneal epithelium of any of the nocturnal species.[237] Similarly, more ascorbate was found in the corneal epithelium of diurnal species than in the corneal epithelium of nocturnal species, which is presumably related to protection of the eye from ultraviolet radiation.[238]

Aschoff suggested that nocturnal animals tend to have free-running periods shorter than 24 hours, whereas diurnal animals tend to have free-running periods longer than 24 hours.[239] Comparison of free-running periods (τ) of 50 species in Figure 9.37, however, reveals no significant difference between diurnal and nocturnal animals. Also, no significant difference is found for the duration of the active phase of the circadian cycle (α). Researchers who have tried to identify the mechanisms responsible for diurnality or nocturnality have generally found that there is no clear difference between diurnal and nocturnal organisms except for the obvious difference in the phase angles of entrainment.[240–245] Several recent studies compared the neural circuits involved in the control of circadian rhythms in the diurnal Nile grass rat and the nocturnal laboratory rat (Figure 9.38). The operation of the circadian pacemaker, as assessed by measures of gene expression in the brain, was found to be essentially identical in the two species, and any observed differences were downstream from the pacemaker.[246–248] Various instances of temporal niche inversion in mice were also attributed to downstream mechanisms.[249,250]

Comparison of the phase–response curves (PRCs) for photic resetting of the circadian pacemaker in various species of diurnal and nocturnal animals reveals no substantial differences. For example, Figure 9.39 shows the photic PRCs for the domestic mouse (which is nocturnal) and the Nile grass rat. In Panel A, phase shifts are plotted as a function of hours

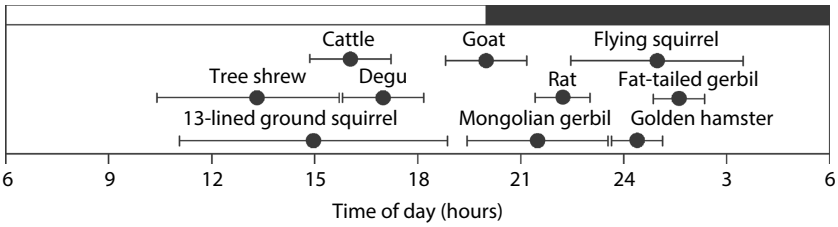


FIGURE 9.36 Tell me where your acrophase is. The graph shows the mean acrophase (and 99% confidence interval) of the body temperature rhythm of 10 mammalian species maintained under very similar environmental conditions (ambient temperature around 24°C, summerlike photoperiod). The thick horizontal bar at the top indicates the timing of the prevailing light–dark cycle. Generally, the acrophase of the body temperature rhythm occurs during the day in diurnal animals and during the night in nocturnal animals. (Data Refinetti, R., *J. Exp. Zool.*, 275, 67, 1996; Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999; Piccione, G. et al., *Comp. Biochem. Physiol. A*, 134, 563, 2003; Piccione, G. et al., *BMC Physiol.*, 3, art. 7, 2003.)

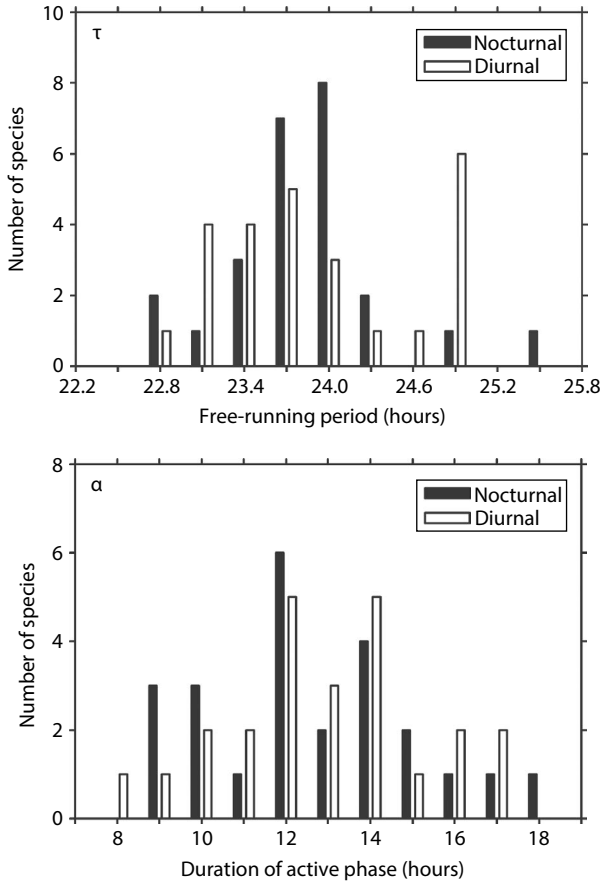


FIGURE 9.37 Free-running periods and duration of active phase of diurnal and nocturnal organisms. The graphs show the frequency distributions of free-running periods (τ) and durations of the active phase (α) of many nocturnal and diurnal species as reported in the literature. Organisms included are vertebrate and invertebrate animals, as well as nonanimals. Kolmogorov–Smirnov tests reveal no significant difference between the distributions of diurnal and nocturnal organisms either for τ ($D = 0.167$, $p > 0.05$) or for α ($D = 0.113$, $p > 0.05$). (Data from References 6, 18, 30, 49, 54, 127, 129, 130, 208, 218, 252, 254, 264, 272, 278, 310, 311, 313, 322, 384, 389, 405, 423, 425, 426, 504, 510, 554–637.)

since the onset of activity. The two curves are very different, confirming that the mouse is nocturnal and the grass rat is diurnal. In Panel B, however, phase shifts are plotted as a function of circadian time—that is, taking into consideration that nocturnal animals are active at night (onset time = CT 12). Now the two curves are very similar. The mouse attains larger phase delays than does the grass rat, but the general characteristics of the curves are essentially identical. Figure 9.40 shows the average photic PRC for 22 determinations in 15 diurnal species and the average curve for 29 determinations in 13 nocturnal species. Again, the two curves are similar. The major differences are the presence of a dead zone during subjective day in nocturnal species (but not in diurnal species) and the attainment of greater phase delays in diurnal species. These relatively small differences may result from the limited number of species used. As seen in Figure 9.39, the PRC for the diurnal Nile grass rat does have a dead zone during subjective night and has a shallower phase-delay region than does the curve for the nocturnal mouse. Interestingly, the PRCs for normal (diurnal) degus and Nile grass rats are not different from the PRCs for animals that become nocturnal when given access to running wheels.[251,252] Studies of PRCs for *nonphotic* resetting have also failed to identify any fundamental difference between nocturnal and diurnal animals.[253,254]

So, is the difference between diurnal and nocturnal animals attributable exclusively to a difference in how the pacemaker is connected to the various effector organs? Apparently yes, although recent studies in diurnal rodents have started to unveil possible differences in the central nervous system. Studies in Nile grass rats suggest that there may be differences in the operation of the pacemaker during subjective day.[255,256] Because the differences are small and have been identified only in the comparison of two species (the Nile grass rat and the golden hamster), further studies are necessary before any generalizations can be made. Studies in the striped grass mouse (*L. barbarus*) suggest that one of the clock genes in the brain may behave differently in diurnal and nocturnal organisms, thus providing a potential explanation for the difference in temporal niche selection.[257] Further studies on this matter are eagerly awaited.

9.2.3 Natural Light Exposure

The distinction between diurnal and nocturnal organisms is further complicated by the fact that the proportion of light and darkness in a normal day varies with the seasons (see Chapter 4). Consider Figure 9.41, which shows actograms of the running-wheel activity of a Nile grass rat maintained under a summer photoperiod (LD 16:8) and a winter photoperiod (LD 8:16). The animal is clearly diurnal in the summer (Panel A), as its activity is almost

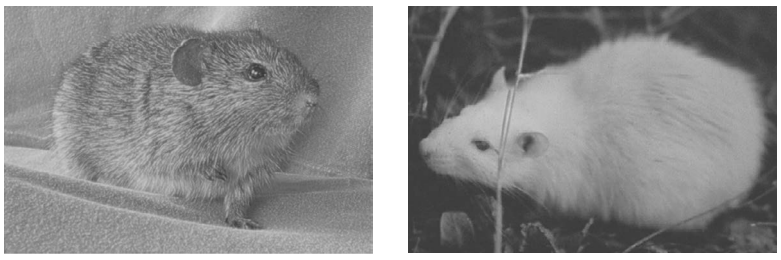


FIGURE 9.38 Furry friends. Some rodents, like the Nile grass rat (A), are active during the day; others, like the laboratory rat (B), are active at night. (Photo by R. Refinetti.)

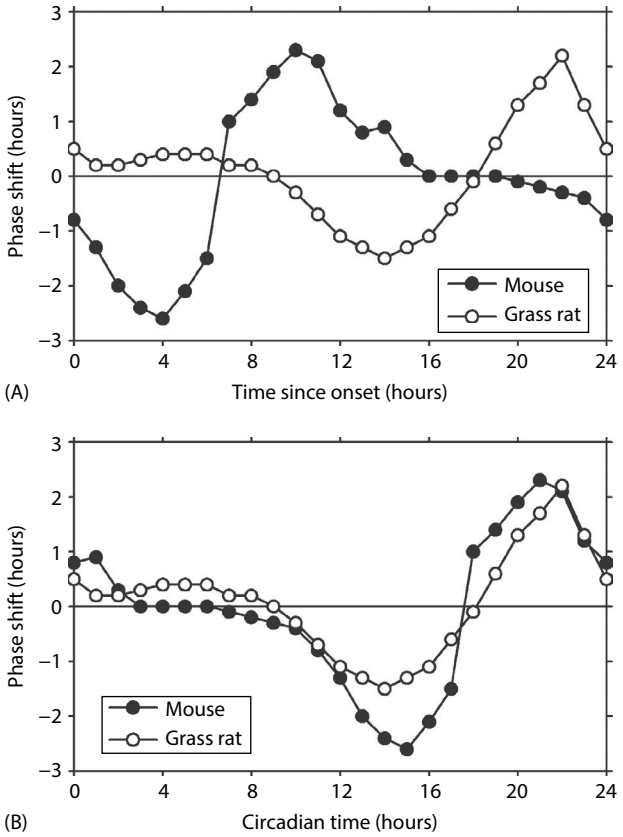


FIGURE 9.39 Timing of light-induced phase shifts in a diurnal and nocturnal rodent. If the phase–response curves (PRCs) for photic resetting of the diurnal Nile grass rat (*Arvicanthis niloticus*) and of the nocturnal domestic mouse (*Mus musculus*) are plotted according to hours since activity onset, the curves reflect the difference in activity timing of the two species (A). However, if the PRCs are plotted according to circadian time (with activity onset being CT 0 for diurnal animals and CT 12 for nocturnal animals), the two curves are almost identical (B). (Data from Refinetti, R., *Acta Scientiae Veterinariae*, 32, 1, 2004; Barbacka-Surowiak, G., *Biol. Rhythm Res.*, 31, 531, 2000.)

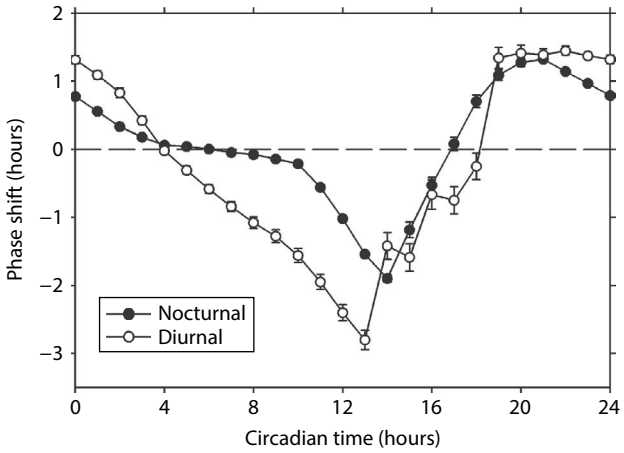


FIGURE 9.40 Timing of light-induced phase shifts in diurnal and nocturnal organisms. The graph shows the average photic phase–response curve (PRC) for 51 species, separately for diurnal and nocturnal species. Organisms included are vertebrate and invertebrate animals, as well as nonanimals. The data points correspond to the means (± SE) of 22 PRCs of 15 diurnal species and 29 PRCs of 13 nocturnal species, as reported in the literature. Notice that the main difference between the two average PRCs is the absence of a *dead zone* during subjective day (circadian time 4 through 10) in the PRC for diurnal organisms. (Data from References 222, 251, 252, 254, 266, 311, 388, 406, 423, 504, 556, 560, 568, 592, 599, 611, 613, 618, 625, 627, 633, 634, 636, and 638–660.)

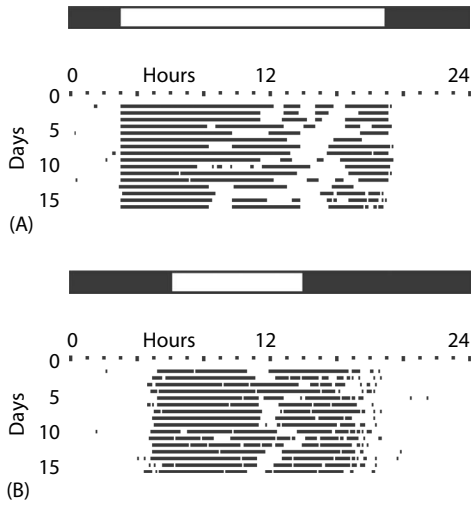


FIGURE 9.41 Like summer, like winter. The actograms show the running-wheel activity rhythms of a Nile grass rat (*Arvicanthis niloticus*) maintained under a summerlike long photoperiod (LD 16:8, Panel A) and under a winterlike short photoperiod (LD 8:16, Panel B). The horizontal bars above the actograms indicate the durations of the light and dark phases of the prevailing light–dark cycles. Notice the occurrence of activity for several hours into the night under the winterlike photoperiod. (Data from the archives of the Refinetti lab.)

entirely concentrated in the light phase of the light–dark cycle. However, in the winter (Panel B), the light phase is so short that, even though the daily duration of activity is compressed, there is considerable activity during the dark phase.

It is rather obvious that the duration of photic stimulation received by an organism each day depends on how many hours of light are available. However, in many cases, organisms are exposed to only a small fraction of the available light. For instance, most humans in urban areas stay indoors most of their time and are exposed to bright light for only a few hours each day. In one study, 10 people in San Diego (California) were asked to wear photocells on the forehead continuously, so that the levels of illuminance could be measured. Out of 10 hours of sunlight per day in the winter, the subjects were exposed to light an average of only 90 minutes.[258] In another study, individuals in San Diego were found to be exposed to sunlight for a little over an hour a day in the winter and a little over 2 hours in the summer, while individuals in Rochester (New York) and Montreal (Canada) were exposed to about 2½ hours of sunlight in the summer but less than half an hour in the winter.[259,260] Written questionnaires given to 500 volunteers in Germany and Switzerland indicated that 90 minutes were spent outdoors on work days and about 4 hours on weekends.[261] Online questionnaires filled out by more than 65,000 people in central Europe

indicated that only 2½ hours per week were spent outdoors in 2002, and the figure went down to 2 hours and 6 minutes in 2010,[262] which suggests that people will not go outdoors at all by 2040!

Nocturnal animals, by necessity, are exposed to very little light each day. As discussed in Chapter 7, this does not present a problem for the circadian system because single brief pulses of light are sufficient to produce entrainment. However, very few studies have actually examined the pattern of light exposure of animals in the wild. We know that many small rodents spend the daylight hours in burrows or nests that shield them from light. Thus, we can expect the typical pattern of entrainment of nocturnal rodents found in the laboratory (Panel A in Figure 9.42) *not* to be typical of the pattern found in the wild. Because nocturnal rodents in the wild are not exposed to the full light–dark cycle, it is possible that they essentially freerun in darkness and only occasionally (every few days) receive light pulses when they come out of the burrow too early or return to the burrow too late. This would result in a zigzagging pattern of activity consistent with entrainment by frequency demultiplication (Panel B in Figure 9.42). Another possibility is that the animals actually wake up well before dusk and perform multiple samplings of the outside illumination, not leaving the burrow until after sunset. This would result in a pattern of activity very similar to the one observed under inescapable light–dark cycles in the laboratory, except perhaps for some irregularities in the onsets of activity (Panel C).

One way to address the issue is to provide animals in the laboratory with light-tight nest boxes, so that they can choose when to expose themselves to the otherwise inescapable light–dark cycle. Representative results from a study conducted with domestic mice are shown in Figure 9.43. The pattern of running-wheel activity exhibited when the mouse did not have access to a light-tight nest box can be compared with the pattern exhibited when the mouse had access to the nest box. Running activity was less intense, and the onsets were less regular, when the nest box was available, but the general activity pattern was not substantially disturbed.[223] Only minor disturbances of the activity pattern were also observed in studies on golden hamsters.[263–265] This seems to suggest that the hypothetical pattern depicted in Panel C of Figure 9.42 is closer to reality than the one depicted in Panel B. Results from a study on flying squirrels (*G. volans*) have often been presented as evidence of entrainment by frequency demultiplication (the pattern in Panel B),[266] but the data do not justify the claims. As shown in Figure 9.44, the activity pattern of a representative flying squirrel with access to a nest box in that study does not show the zigzagging of onsets characteristic of entrainment by frequency demultiplication.

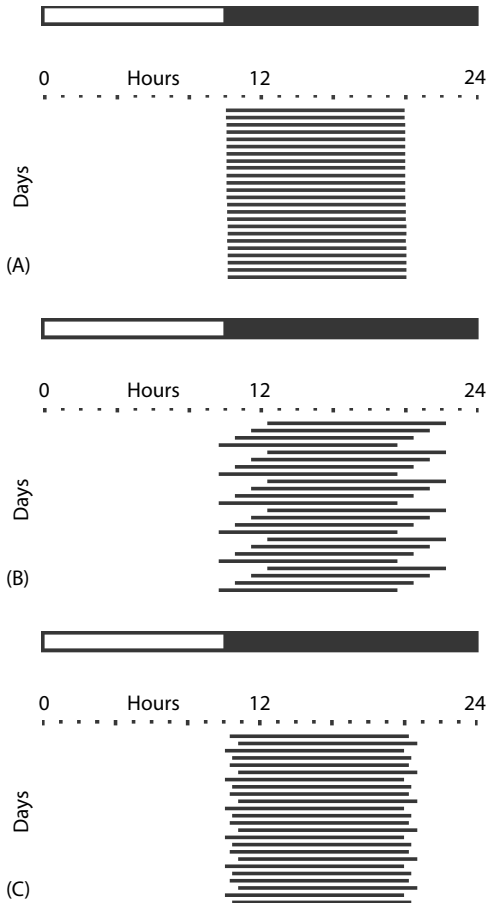


FIGURE 9.42 Hiding from light. These actogram-like diagrams depict three potential patterns of entrainment for a nocturnal animal. Standard entrainment in the presence of an inescapable light–dark cycle is shown in (A). When the light–dark cycle can be mostly avoided by utilization of a shelter, entrainment may be attained by frequency demultiplication (B) or by a slightly disrupted standard process (C), depending on how often the animal exposes itself to light.

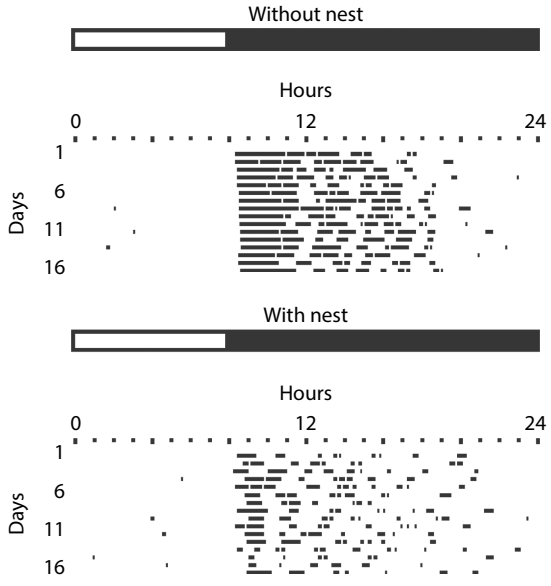


FIGURE 9.43 No zigzagging: mouse. The actograms show the running-wheel activity rhythms of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle (LD 8:16) with and without access to a light-tight nest box. When the nest box is available, the daily onsets of activity are slightly less regular and the running pattern is sparser. However, the zigzagging of onsets characteristic of entrainment by frequency demultiplication is absent. (Adapted from Refinetti, R., *Physiol. Behav.*, 82, 285, 2004.)

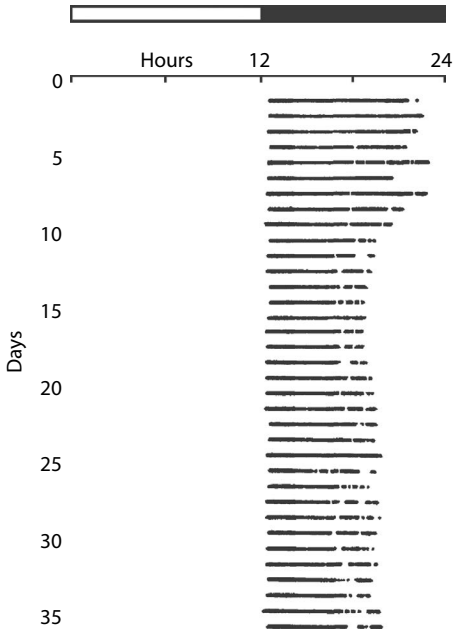


FIGURE 9.44 No zigzagging: flying squirrel. The actogram shows the running-wheel activity rhythm of a flying squirrel (*Glaucomys volans*) maintained under a light–dark cycle (LD 12:12) with access to a light-tight nest box. The daily onsets of activity are very regular and do not exhibit the zigzagging pattern characteristic of entrainment by frequency demultiplication. (Adapted from DeCoursey, P.J., *J. Comp. Physiol. A*, 159, 161, 1986.)

Naturally, the small effect of nest-box availability may be combined with the effect of changes in photoperiod. Figure 9.45 shows the mean variabilities of activity onsets of mice and Nile grass rats housed with or without nest boxes under short and long photoperiods. For the mice, the variability is increased in the nest-box condition only under a short photoperiod (LD 8:16). For the grass rat, variability is greater under a short photoperiod only if the nest box is not available. In any event, it seems clear that frequent light sampling provides enough photic stimulation to produce full entrainment even in animals that spend most of the light phase in a burrow or nest. As shown in Figure 9.46, mice enter and exit the nest box repeatedly throughout the night and also during the day. Although they spend more time inside the nest box during the day than during the night, they are constantly sampling the light outside (Figure 9.47, Panel A). The reverse is true for the diurnal Nile grass rat (Panel B). Extensive light sampling has also been observed in bats[267] and dunnarts.[268]

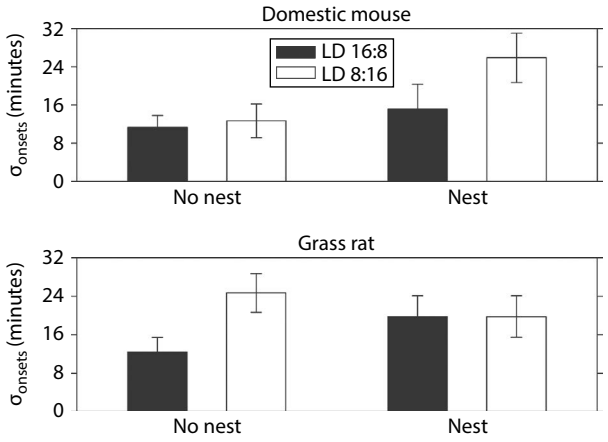


FIGURE 9.45 Variability of onsets. The graphs show the mean variability of onsets (standard deviation of 10 consecutive daily onsets) for groups of domestic mice (*Mus musculus*) and Nile grass rats (*Arvicanthis niloticus*) with and without access to light-tight nest boxes. The bars are the means (± SE) of seven animals per species. Mice exhibit greater variability of onsets when the nest boxes are available, particularly under the winter-like photoperiod (LD 8:16). In grass rats, the effects of photoperiod and nest-box availability are less clear. (Data from Refinetti, R., *Physiol. Behav.*, 82, 285, 2004.)

As you remember from Chapter 7, the important segment of the light–dark cycle is either dusk (for animals with free-running periods shorter than 24 hours) or dawn (for animals with free-running periods longer than 24 hours). The rest of the daily illumination either has a small effect on entrainment (if perceived during subjective night) or is ignored (if perceived during the dead zone of subjective day). Thus, it is essential that light be perceived either at dusk or at dawn. Surprisingly, it has been reported that the European ground squirrel (*Spermophilus citellus*) restricts its aboveground activity to a few hours in the middle of the day, thus avoiding photic stimulation at dawn *and* dusk.[269,270] A diagram depicting the activity pattern under long and short photoperiods is shown in Figure 9.48 (Panel A). For comparison, the figure also shows diagrams for the Nile grass rat—which, like other diurnal rodents,[166,217,271] is above-ground throughout the light phase (Panel B). At least under a long photoperiod, the European ground squirrel seems not to experience either dawn or dusk, which should prevent it from attaining entrainment. The explanation for this apparent anomaly lies on the distinction between α and subjective day. The European ground squirrel clearly has a short α (about 9 hours). However, by definition, subjective day lasts 12 circadian hours. This means that α is not a good estimator of subjective day in this species. If we assume that the three missing hours are at the end of α, then the end of subjective day (and, therefore, the beginning of subjective night) will be very close to dusk. Light sampling by brief excursions to the surface at this time will be enough to maintain appropriate entrainment.

One aspect of the natural light–dark cycle that is rarely reproduced in laboratory studies is the gradual transition between darkness and light that occurs at dawn (and the gradual transition between light and darkness that occurs at dusk). In the laboratory, the lights are turned on abruptly in the morning and turned off abruptly at night. Is this difference significant for the animals? Only a few studies have addressed this issue. Because the transition phases either add to or subtract from the time of lights on, small differences in entrainment patterns under square-wave and twilight-inclusive light–dark cycles cannot be unequivocally interpreted. For the reason that only small differences have been found,[39,264] I consider the issue to be unresolved. I should point out, however, that the range of entrainment (for zeitgeber periods different from 24 hours) seems to be about an hour or two wider when

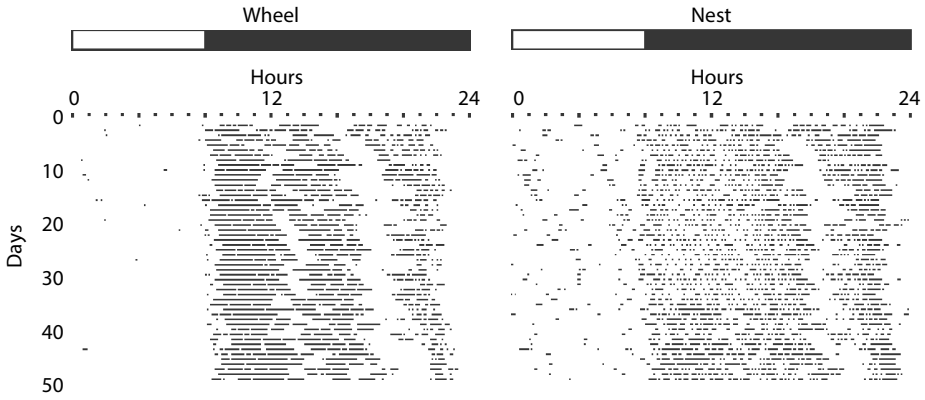


FIGURE 9.46 Lots of light probing. The actograms show the rhythms of running-wheel activity and nest-box visitation of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle (LD 8:16) with access to a light-tight nest box. The animal visited the nest box quite often during the dark phase of the light–dark cycle, when it was most active in the running wheel. Movement in and out of the nest box during the light phase was less frequent but frequent enough to provide many occasions for light probing each day. (Adapted from Refinetti, R., *Physiol. Behav.*, 82, 285, 2004.)

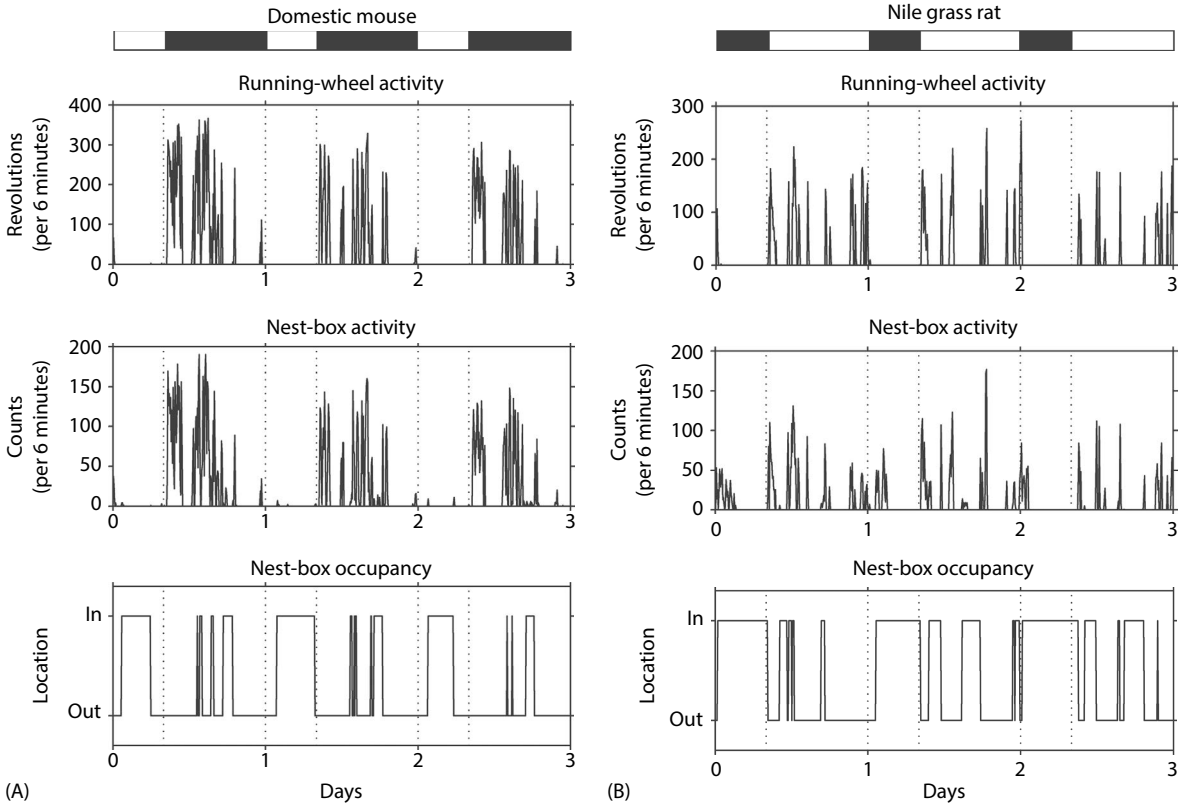


FIGURE 9.47 Staying home. The graphs show 3-day segments of the records of running-wheel activity, movement in and out of the nest box, and nest-box occupancy of a domestic mouse (*Mus musculus*, A) and a Nile grass rat (*Arvicanthis niloticus*, B). Both animals were maintained under a light–dark cycle (LD 8:16 for the mouse, LD 16:8 for the grass rat) with access to a light-tight nest box. Although the mouse spent more time inside the nest box during the light phase of the light–dark cycle than during the dark phase, it moved in and out of the box more frequently during the dark phase. Similarly, although the grass rat spent more time inside the nest box during the dark phase of the light–dark cycle than during the light phase, it moved in and out of the box more frequently during the light phase. (Data from the archives of the Refinetti lab.)

twilight-inclusive light–dark cycles are used, as compared to standard square-wave cycles.[265,272,273]

Finally, one aspect of photic stimulation that is seldom studied in the laboratory or in the wild is the organism's opportunity to control its own stimulation. Although the possibility of self-entrainment is conceivable, the few studies that have been conducted have found that the animals continue to freerun when given full control over the lights.[274–278] Nocturnal rodents turn the lights on only occasionally and remain essentially in darkness.[274–276] An example is shown in

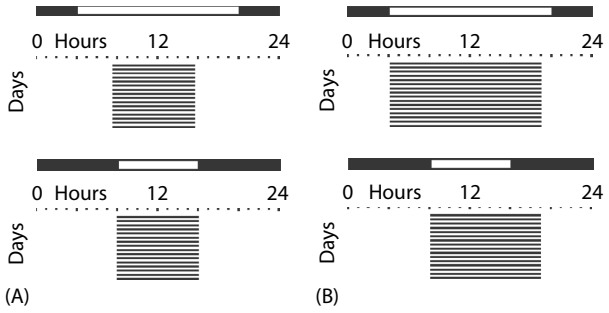


FIGURE 9.48 The difference between European ground squirrels and Nile grass rats. These actogram-type diagrams illustrate the different patterns of activity described for the European ground squirrel (*Spermophilus citellus*) in a natural environment (Panel A) and for the Nile grass rat (*Arvicanthis niloticus*) in a seminatural laboratory environment (Panel B). Whereas the activity pattern of the ground squirrel is not affected by season, the activity pattern of the grass rat is greatly affected by changes in photoperiod.

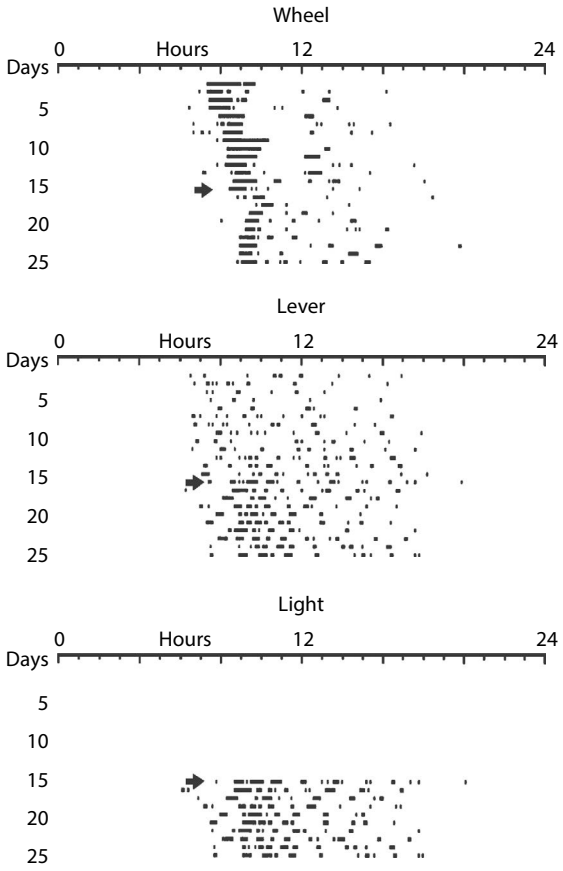


FIGURE 9.49 Self-selected illumination. The actograms show the rhythms of running-wheel activity, lever pressing, and light exposure of a golden hamster (*Mesocricetus auratus*) maintained in constant darkness and allowed to turn on lights (50 lux) by pressing the lever. For the first 2 weeks, lever pressing did not activate the lights. Starting on day 15 (arrows), the lights were on whenever the hamster pressed the lever and maintained it depressed. Notice that a clear phase delay of the wheel activity rhythm occurred only on the first day after the lever became functional, even though the temporal pattern of light exposure was similar in the following days. (Adapted from Refinetti, R., *Behav. Process.*, 40, 107, 1997.)

Figure 9.49. A golden hamster was kept in constant darkness with access to a running wheel and to a small lever attached to the wall of the cage. Starting on the 15th day (arrows), depression of the lever caused the lights to be turned on (and to remain on until the lever was released). The rhythm of running-wheel activity seemed to undergo a phase delay on the day when the lights became available, but no effect of photic self-stimulation was evident afterward. The rhythm of lever pressing became more robust when the lights became available, which indicates that the behavior became goal oriented. The pattern of illumination (bottom actogram) reflected the pattern of lever pressing, although the actogram format gives the incorrect impression that the lights were on for many hours each day (in actuality, all lever presses were very brief, so that the total time of lights on was only a few minutes each day). In short, hamsters do choose to expose themselves to light occasionally, but no consistent effect on the circadian system results from it.

9.2.4 Environmental Conflicts

A new frontier in the experimental study of circadian rhythms—a frontier that is yet to be explored—is the investigation of the combined effects of multiple environmental stimuli on the operation of the circadian system. We saw in previous chapters that many environmental factors—such as light, temperature, food availability, physical exercise, and social interaction—can entrain circadian rhythms. However, except for a brief discussion of the potential conflict between the light–dark cycle and an artificial cycle of food restriction, we have not considered more than one environmental variable at a time. To understand the behavior of animals (including humans) in the real world, we must understand how the multiple variables interact.

According to the principle of *optimal foraging*, behavior has costs and benefits, and natural selection has favored animals that can maximize net benefit.[279] Many laboratory studies have shown, for instance, that thermal comfort[280–282] or even food[283–285] may be put aside if the cost of obtaining them is elevated. Many other laboratory studies have examined conflicts between two motivational drives, such as thermal comfort and light aversion[286,287] or thermal comfort and food availability.[288–292] Unfortunately, none of these studies has addressed the issue of entrainment. One study in golden hamsters showed that the animals will run less on the wheels if they are given the opportunity to engage in alternate activities (*enriched environment*), but no changes in the characteristics of entrainment were observed.[293] A study in sugar gliders, which are small marsupials active exclusively at night in captivity, showed that the animals will become inactive in cold-wet nights and will sometimes be active in the afternoon in the wild.[294]

In two interesting recent studies, mice were required to *work for food*, by running on a wheel multiple times to earn small pieces of food. As the work load increased, the animals (which are nocturnal) started working earlier daily, thus

invading the diurnal niche (with an advanced phase angle of entrainment).[295,296] It is not clear why the heavier labor was not spread out over the night instead of being partially moved into the day, and more studies involving the combined effects of multiple environmental stimuli on the operation of the circadian system would be very welcome.

9.3 LIFETIME CHANGES

Essentially all of the characteristics of circadian rhythms discussed in this and previous chapters pertained to generic adult organisms. There is much more in life, however, than plain adulthood. At the minimum, we need to consider development and aging. Also, circadian rhythms in adulthood are affected by reproductive and seasonal cycles. In addition, circadian processes in adult organisms can potentially be modified by experience (*learning*). All of these issues will be discussed here in Section 9.3. Moreover, because we have dealt essentially with genderless organisms until now, the last subsection of the chapter will address the issue of sexual dimorphism in circadian rhythms.

9.3.1 Intra- and Interindividual Variability

Basic research on circadian rhythms, like basic research on other physiological processes, is normally concerned with mean effects, as opposed to individual differences. Attention to the variability between repeated measures on the same individual or between measures on different individuals is ordinarily limited to the statistical requirement of establishing the significance of differences between means. This practice rests on the assumption that intraindividual and interindividual variabilities are numerically equivalent. However, studies that investigated intra- and interindividual variabilities in various physiological processes have revealed that the two variabilities are often different.[297–304] This difference in variabilities has important implications for the management of individual needs, such as in clinical practice in veterinary or human medicine. If interindividual variability is greater than intraindividual variability, then treatments designed for an *average* patient may turn out to be too weak—or too strong—for a patient who is not average. On the other hand, if intraindividual variability is greater than interindividual variability, then the notion of an average patient may be useful, but administration of the treatment to patients may have to be adjusted on a daily or weekly basis.

Very few researchers have compared intra- and interindividual variabilities of circadian rhythms. I am aware of only four studies that conducted such comparison for the rhythms of body temperature,[305,306] melatonin secretion,[307] and cortisol secretion.[308] A fifth study investigated the rhythm of cortisol secretion in a large number of human volunteers and found that about 15% of the subjects had flat rhythms.[309] This indicates extreme interindividual variability, but the subjects were not studied under standardized conditions, so that exogenous causes for the lack of rhythmicity in some of the subjects cannot be excluded.

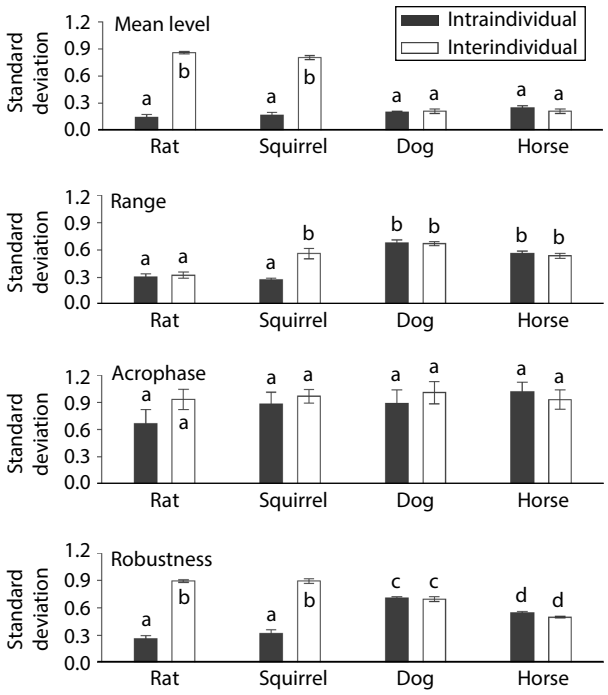


FIGURE 9.50 Intra- and interindividual variability: parameters of body temperature rhythm. The graphs compare the intraindividual and interindividual variabilities of four parameters of the body temperature rhythm of laboratory rats, 13-lined ground squirrels, Beagle dogs, and British Thoroughbred horses. All computations of variability (standard deviation of the mean) are based on measurements conducted on seven individuals for 7 consecutive days with 2-hour resolution. The bars show the mean variability (±SE) for each condition. All animals were maintained under 24-hour light–dark cycles. For each parameter, means sharing the same letter (a or b) are not significantly different from each other. (Data from Refinetti, R. and Piccione, G., *J. Therm. Biol.*, 30, 139, 2005.)

The results from a well-controlled study of the body temperature rhythm in animals are summarized in Figure 9.50. To avoid the potential bias of selection of an idiosyncratic species, four different species were used: the laboratory rat, the 13-lined ground squirrel, the domestic dog, and the horse. All animals exhibited daily rhythmicity. Intra- and interindividual variabilities were computed for the mean level, range of oscillation, acrophase, and robustness of the rhythm. Notice that the variabilities differ in different parameters of the rhythm and in different species but that—whenever there is a difference between interindividual variability and intraindividual variability—the latter is always smaller than the former. That is, the day-to-day variability of an individual's rhythm never exceeds the variability between the rhythms of different individuals. Interindividual variability is consistently larger than intraindividual variability. Thus, the concept of an *average* patient would seem to be a dangerous one. However, for the acrophase—which is perhaps the parameter most often used in clinical practice—interindividual variability is statistically indistinguishable from intraindividual variability in all four species. This justifies the practice of ignoring individual differences.

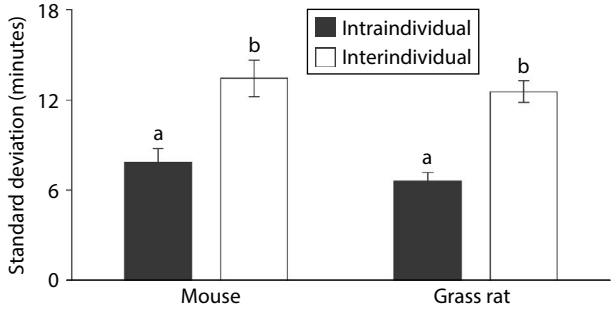


FIGURE 9.51 Intra- and interindividual variability: free-running period. The graph compares the intraindividual and interindividual variabilities of the free-running period of laboratory mice and Nile grass rats, as determined by analysis of the running-wheel activity rhythm in constant darkness. All computations of variability (standard deviation of the mean) are based on measurements conducted on seven individuals for 7 consecutive blocks of 10 days. The bars show the mean variability (± SE) for each condition. Means sharing the same letter (a, b, etc.) are not significantly different from each other. Notice that intraindividual variability is smaller than interindividual variability both in mice and in grass rats. (Data from the archives of the Refinetti lab.)

A few investigators have also examined intra- and interindividual variabilities of the free-running period of the activity rhythm. Here, intraindividual variability has been consistently found to be smaller than interindividual variability,[310–313] as illustrated for the domestic mouse and the Nile grass rat in Figure 9.51. In absolute values, a standard deviation of 6–8 minutes (intraindividual variability) means that the free-running period deviates on average 0.1 hour from the long-term mean. The variability between individuals is twice as large.

9.3.2 Development

Many organisms, including all animals, are *not* born with the full anatomical and functional characteristics of adults (Figure 9.52). Circadian rhythms, as many other processes, must undergo *development*. Developmental processes are controlled by both genetic inheritance (*nature*) and environmental factors (*nurture*), although you probably remember our discussion about free-running periods in Chapter 6. At least in rodents, the free-running period exhibited by adults does not depend on the period of the light–dark cycle experienced during development[314,315] and, in this sense, it is fully controlled by *nature*. As a matter of fact, circadian rhythmicity is truly endogenously generated, so that adult rodents display rhythmicity even if they are raised in constant darkness or constant light.[316–320] This is also true for fruit flies, although in this case the lighting conditions during development may affect the free-running periods of the adults in a more complex way.[321,322]

Although developmental aspects of circadian rhythmicity have not received as much attention as other aspects, some knowledge of the development of circadian rhythms is available.[323,324] We know, for instance, that newborn human babies do not have a daily rhythm of melatonin secretion. Rhythmicity is not apparent until 3 months of age.[325,326] Likewise, human babies do not have a rhythm of body temperature. As shown in Figure 9.53, the body temperature of a newborn oscillates randomly; a daily pattern is noticeable at 3 months of age; and a mature daily rhythm is not reached until a year or more after birth,[327,328] although earlier achievement of the mature pattern at 3 months of age has been reported.[329] One group of researchers claimed to have observed daily rhythmicity of skin temperature in 3-week-old newborns, but the data they presented were rather unconvincing.[330] Actigraphic studies of infant activity seem to agree on the emergence of daily rhythmicity around 6 weeks after birth,[331–333] which is also the time

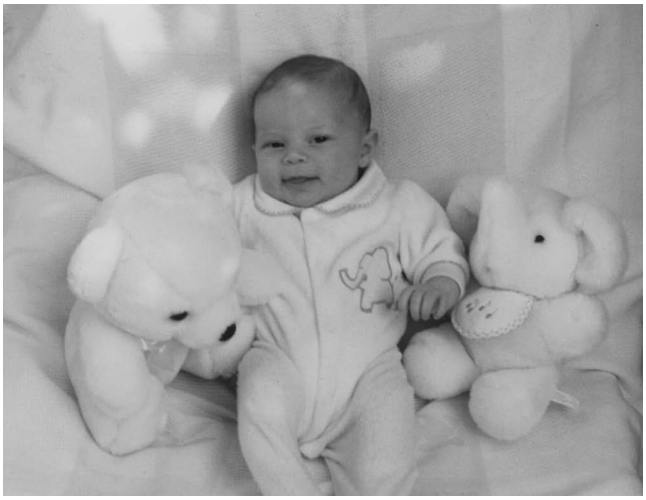


FIGURE 9.52 It's a girl! Babies are born with immature circadian systems. Development of mature circadian rhythms requires many months. (Photo by R. Refinetti.)

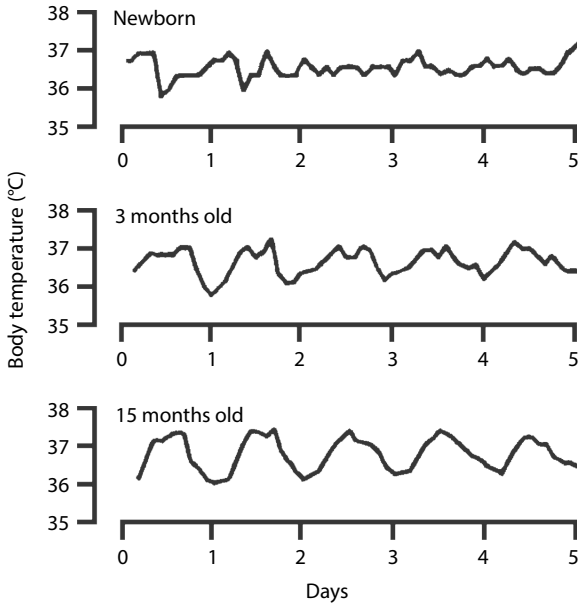


FIGURE 9.53 Maturation of body temperature rhythm in human infants. The graphs show 5-day segments of the records of body temperature of human infants at three stages of development of daily rhythmicity. The adult amplitude of the rhythm (1°C) is attained at approximately 15 months of age. (Adapted from Kleitman, N. et al., *Am. J. Physiol.*, 119, 48, 1937.)

when infants start demanding feeding with daily rhythmicity (that is, during the day but not at night).[334] Studies of the development of the daily rhythm of heart rate and blood pressure are less conclusive and provide estimates of adult patterns as early as the second day after birth or as late as a month or more later.[335–338] On the other hand, human babies do seem to exhibit daily rhythmicity in adrenal cortisol secretion at the time of birth,[339] with an adult pattern being observable by 4 months of age.[340]

In rats, a rhythm of body temperature with a range of oscillation of 2°C–4°C is observed on the day after birth, but it seems to vanish by 15–20 days of age.[341–346] Incipient rhythmicity appears again at 25 days of age and attains the adult range (1.6°C) at 45 days of age,[347] which is approximately the age when the rhythm of corticosterone secretion reaches the adult pattern.[348] Because the early temperature rhythm vanishes in a few days and is observed only when the pups are kept at an ambient temperature below thermoneutrality, this rhythm is probably not a true precursor of the adult rhythm of body temperature but a form of cold-induced torpor.[343,349] In rabbits, temperature rhythmicity can be observed as early as 4 or 5 days after birth in pups allowed to remain with the doe[350,351] but not in pups kept in isolation with continuous intraesophageal feeding.[344] Daily rhythmicity of activity in rabbits can be detected 6 days after birth, although the adult pattern is not attained until 2 months later.[352]

Honeybees start their lives as nurses in the bee colony. As nurses, they are equally active during the day and during the night. After a few weeks, they mature into forager bees. Shortly before they become foragers, they develop daily rhythmicity, which is an important feature for the appropriate collection of pollen from flowers outside the colony.[353] Interestingly, the maturation process is not permanent. If a colony loses its nurses, some of the foragers will revert to being nurses, and, when they do so, they become arrhythmic again.[354]

Newborn calves (Figure 9.54) lack daily rhythmicity of body temperature. Daily rhythms comparable to those of adults are not observed until 2 months after birth.[355] One research group reported the presence of rhythmicity 2 weeks after birth,[356] but their calves were exposed to large daily fluctuations in ambient temperature (about 20°C), which probably caused the fluctuation in body temperature. In calves maintained under constant ambient temperature (Figure 9.55), no difference between measurements taken at dawn and measurements taken at dusk was found for the first 10 days of life. Later on, measurements taken at dawn decreased gradually until a stable dusk–dawn difference of about 1°C was achieved between 50 and 60 days after birth.

Lambs (young sheep) and foals (young horses) also develop daily rhythms of body temperature during early life, although adult rhythms seem to be attained earlier than in calves.[357] As shown in Figure 9.56, a stable dusk–dawn difference is achieved about 1 month after birth. In puppies (young dogs), the body temperature rhythm is fully matured by 40 days after birth.[358] Development of the daily rhythm of body temperature in kids (young goats) seems to be much slower, with



FIGURE 9.54 My mom is a cow! Minutes after giving birth, a young cow attends to her newborn calf. (Photo by Scott Bauer, Agricultural Research Service, United States Department of Agriculture.)

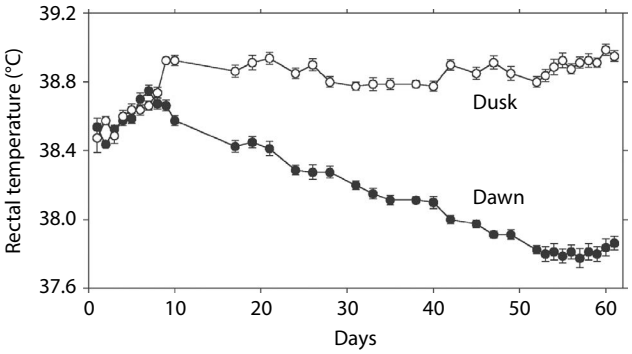


FIGURE 9.55 Maturation of body temperature rhythm in bovine infants. The graph shows the mean rectal temperature (±SE) of 8 calves (*Bos taurus*), as measured at dawn and dusk for 2 months following birth. Day 0 is the day of birth. The adult amplitude of the rhythm (1°C) is attained at approximately 50 days of age. (Data from Piccione, G. et al., *BMC Physiol.*, 3, art. 7, 2003.)

stable day–night differences not being attained until the second year of life.[359] Evidently, different species develop the body temperature rhythm at different rates.

Development at different rates in different species can also be seen in other rhythms. For instance, the rhythm of melatonin secretion is present immediately after birth in seals,[360] 2 weeks after birth in hamsters and rats,[361] 9 weeks after birth in horses,[362] and 3 months after birth in humans.[325,326] It should be pointed out that the delay in the expression of rhythms is most likely due to the development of mechanisms downstream from the circadian pacemaker. At least in rats and sheep, it has been shown that the pacemaker itself is already

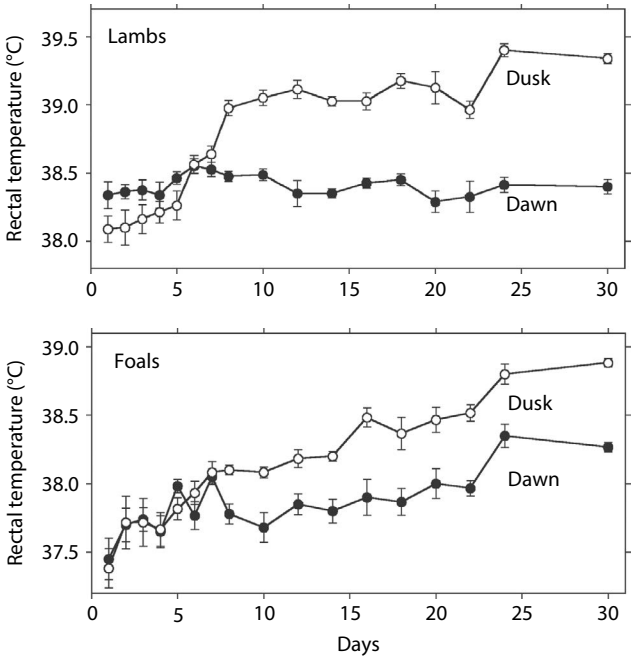


FIGURE 9.56 Maturation of body temperature rhythm in ovine and equine infants. The graphs show the mean rectal temperature (± SE) of 8 lambs (*Ovis aries*) and 6 foals (*Equus caballus*), as measured at dawn and dusk for a month following birth. Day 0 is the day of birth. The adult amplitudes of the rhythms are attained at the end of the first month of life. (Data from Piccione, G. et al., *J. Therm. Biol.*, 27, 333, 2002.)

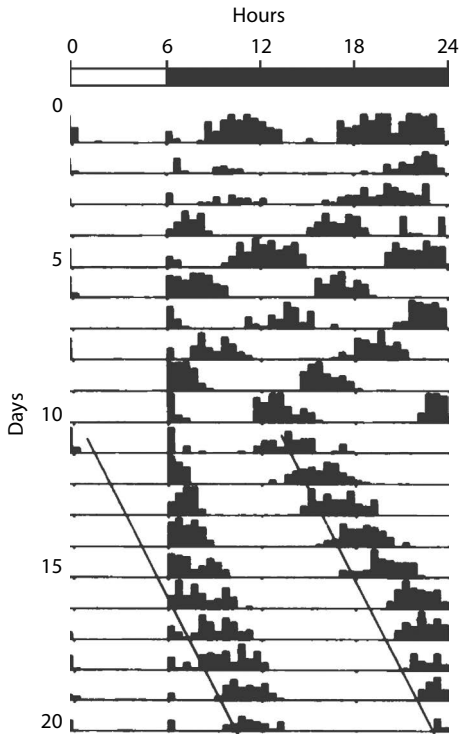


FIGURE 9.57 Circadian or circatidal rhythm? This actogram shows the rhythm of swimming activity of a zooplankton (*Dimorphostylis asiatica*) maintained under a 24-hour light–dark cycle (as indicated by the horizontal white and dark bar at the top). Starting on day 11, a 12.5-hour cycle of hydrostatic pressure was superposed on the light–dark cycle. The diagonal lines indicate the times of maximum hydrostatic pressure. Notice that the light–dark cycle produced masking only, whereas the cycle of hydrostatic pressure produced entrainment. (Adapted from Akiyama, T., *Zool. Sci.*, 21, 29, 2004.)

oscillating at birth![363–368] The circadian clock is already functional during embryonic development in zebra fish as well.[369]

Development progresses all the way through adolescence, and a few studies have examined changes in the circadian system in this time scale. In humans, children tend to prefer activities early in the day (at least earlier than most adults), and this preference does not vanish until the 9th or 10th grade (14 years of age).[370–372] In rats, locomotor activity is much higher at night than during the day in infancy; the level of nocturnal activity is gradually reduced until the adult night–day ratio is reached at 6 months of age[373] (which corresponds to approximately 18 years of age in humans).

9.3.3 Rhythmic Interactions

Various ultradian and infradian rhythms were described in Chapter 4. We have ignored them while discussing the phenomenology and mechanics of circadian rhythms in previous chapters, but we must now face the issue of potential interactions between ultradian/infradian rhythms and circadian rhythms.

We will start with *tidal* rhythms, about which we know relatively little. We know, of course, that animals inhabiting intertidal zones are exposed to two strong environmental cycles: the ebb and flow of tidal waters (which has a period of 12.4 hours) and the alternation of night and day (which has a period of 24 hours). Both laboratory and field observations have shown that the activity of intertidal organisms is affected by the two environmental cycles.[374–381] One example is shown in Figure 9.57. The swimming activity of the zooplankton *Dimorphostylis asiatica* was recorded first under only a 24-hour light–dark cycle and later under both the light–dark cycle and a 12.5-hour simulated tidal cycle. In the absence of the tidal cycle, the circatidal swimming rhythm seemed to freerun with a period shorter than 12 hours but was expressed only during the dark phase of the light–dark cycle. This photic masking of activity also occurred when the tidal cycle was introduced and entrained the rhythm. In this particular example, it appears that the organism does not have a circadian clock and must rely on entrainment of the circatidal clock in combination with daily photic masking. However, studies in this and other species have revealed a multitude of arrangements: presence of a circadian component and absence of a circatidal component,[375] simultaneous presence of both components,[378,382,383] alternation of circadian and circatidal components in the same individual,[377,384] and even absence of both circadian and circatidal endogenous rhythmicity.[374] Some of the differences in results may be due to genuine differences between species, but many more studies are needed to elucidate the issue.

Analysis of the interaction between circadian and circatidal rhythmicities can become quite complex because different organs may exhibit different rhythmic patterns. In the horseshoe crab (*Limulus polyphemus*), it has been shown that visual sensitivity exhibits circadian rhythmicity that can be entrained by a light–dark cycle, whereas locomotion exhibits circatidal rhythmicity that can be entrained by imposed tides.[385] Under a light–dark cycle without tides, the crab can exhibit the two rhythmicities at the same time: entrained daily rhythmicity in visual sensitivity and free-running circatidal rhythmicity in locomotion, as depicted in Figure 9.58. Similarly, the speckled sea louse (*Eurydice pulchra*) exhibits circadian rhythmicity of pigmentation at the same time as circatidal rhythmicity of swimming.[381]

Much more research has been conducted on interactions between circadian and *estrous* rhythms. Golden hamsters and fowl have been used most often in this research, possibly because of the brevity of their estrous cycles (4 days and 1 day, respectively). It all started in the late 1960s, when John Alleva and colleagues at the Food and Drug Administration (in Washington, DC) had the very reasonable idea of investigating whether estrous cycles, like circadian rhythms, would freerun in constant environmental conditions. We saw in Chapter 4 that estrous cycles have been studied for quite some time, and Alleva knew very well that golden hamsters exhibited estrous cyclicity. He also knew that the estrous cycle was very regular—that is, that a female golden hamster becomes sexually receptive a few hours before dark every 4 days. Considering that the period of the estrous cycle varies greatly from species to species (see Chapter 4), he should have expected the cycle to be endogenously generated—and, therefore, to freerun in constant conditions. Nonetheless, he and his colleagues conducted the study.[386] They did find that the estrous cycle freeran when the animals were housed in constant light, but there was a twist. To their surprise, the estrous cycle freeran hand in hand with the circadian rhythm of locomotor activity. That is, the estrous cycle freeran not with a period of 96 hours (4 days) but with a period equal to four circadian cycles (which equals 96 hours only when the circadian period is exactly 24 hours). This finding was replicated in rats and hamsters in other laboratories,[387–391] and an example is shown in Figure 9.59. By adding deuterium oxide to the drinking water (see Chapter 6), the experimenters in this study caused the circadian period of female hamsters to be lengthened to 24.4 hours—and the onsets of sexual receptivity followed along, occurring once every four circadian cycles, not once every 96 hours. These findings were interpreted as evidence that the estrous cycle is generated not by a distinct estrous clock but by the circadian clock, in such a way that the estrous period is a multiple of the circadian period. The interpretation was supported by the observation that estrous cyclicity vanishes when circadian rhythmicity is eliminated by destruction of the master circadian pacemaker through surgical ablation or genetic engineering.[392–395] Of course, the timing of ovulation depends on the timing of maturation of ovarian follicles (which can be thought of as some form of estrous clock or timer), but presumably the timing of follicle maturation is modulated by the circadian clock.

One problem with these studies of the relationship between estrous and circadian rhythmicity is that the changes in period were rather small. In Figure 9.59, the circadian period was 24.4 hours, so that the estrous period was not quite 98 hours (i.e., $4 \times 24.4 = 97.6$). One cannot deny that 98 is larger than 96, but is it larger enough? No one would expect the free-running estrous period to be exactly 96.0 hours, just like no one would expect the circadian period to be exactly 24.0 hours. Thus, the idea that the estrous period is a multiple of the circadian period may be based solely on coincidence. To test this possibility, much greater differences in circadian period are necessary. The homozygous *tau*-mutant hamster discussed in Chapter 6 provided a convenient way to solve the problem. Because the mutants have a circadian period of 20 hours, a 4-cycle multiple should have a period of 80 hours, which is

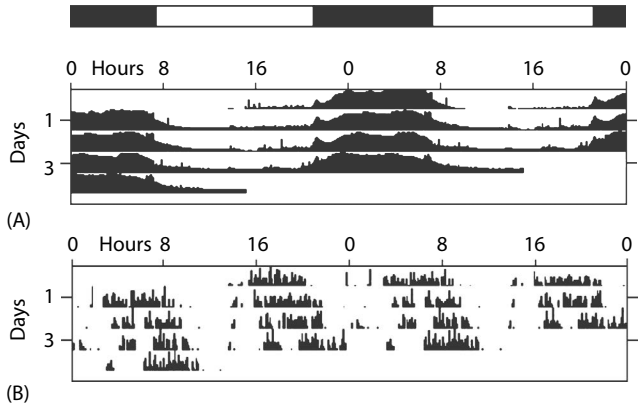


FIGURE 9.58 Circadian and circatidal rhythms. These short double-plotted actograms show the rhythms of visual sensitivity (A) and locomotion (B) of a horseshoe crab (*Limulus polyphemus*) maintained under a 24-hour light–dark cycle (as indicated by the horizontal white and dark bar above the actograms). While visual sensitivity oscillates with an entrained circadian cycle, locomotion freeruns with a circatidal cycle. (Adapted from Watson, W.H. et al., *Biol. Bull.*, 215, 46, 2008.)

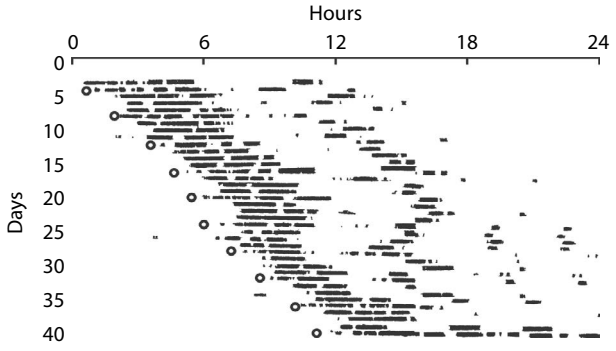


FIGURE 9.59 Dependence of estrous cycle on circadian rhythm in a mammal? This actogram shows the rhythm of running-wheel activity of a female golden hamster (*Mesocricetus auratus*) maintained under constant dim light. The circles denote the onsets of sexual receptivity (which happen every 4 days under a 24-hour light–dark cycle). Notice that the estrous cycle freeruns with a period that is a quadruple multiple of the activity rhythm. (Adapted from Fitzgerald, K.M. and Zucker, I., *Proc. Natl. Acad. Sci. USA*, 73, 2923, 1976.)

considerably shorter than 96 hours. When this study was actually conducted, an estrous period of 100 hours was found.[396] Of course, 100 is a multiple of 20—but it is a 5-cycle multiple, not a 4-cycle multiple. Moreover, 100 is very close to 96. My interpretation is that *there is* an estrous clock with a period of approximately 96 hours in the hamster, but the expression of the clock is gated by the circadian system, in the sense that sexual receptivity is expressed only at the appropriate circadian phase. Thus, in the *tau*-mutant hamster, the estrous cycle is slightly altered from 96 to 100 hours because the interval of five circadian cycles is the closest approximation to the endogenous estrous period of 96 hours. In wild-type hamsters, whose circadian period is almost exactly 24 hours, the estrous period of 96 hours can be matched by four circadian cycles.

If my interpretation is correct, then the period of the estrous cycle is a multiple of the period of the circadian cycle only because the estrous cycle is *gated* by the circadian system, not because it is *generated* by the circadian system. The notion of circadian gating of ovulation may sound unnecessarily complicated, but is not at all a heretic notion. It has been known for quite some time that, if the ovaries are surgically removed to eliminate the hormonal feedback to the brain, then surges of luteinizing hormone (which normally induce ovulation), as well as prolactin surges, are exhibited every day rather than every 4 days.[394,397–402] Thus, the notion that the circadian system gates the operation of the ovaries (through pituitary-hypothalamic hormones) is not controversial. On the other hand, if you have been reading this book from the beginning, you may be surprised that I am advocating a two-clock explanation instead of a more parsimonious, one-clock explanation. The truth is that I have no personal objection to complex systems. I believe that explanations should be as simple as allowed by the facts. In the case of the relationship between estrous and circadian rhythmicity, the facts do not seem to allow the simplest explanation. I must admit, however, that the evidence presented so far is not truly conclusive. Additional evidence comes from studies on primates as well as on birds.

In a study on the common marmoset (*Callithrix jacchus*), whose estrous cycle—like that of humans—lasts 28 days, light–dark cycles with periods of 23 or 26 hours were used. The circadian activity rhythm was entrained by the light–dark cycle in both conditions, but the period of the estrous cycle (monitored by measurement of urinary estrogen concentration) was the same in the two conditions.[403] This is in direct contradiction with the classical studies on wild-type hamsters previously mentioned. Also, in owl monkeys (*Aotus lemurinus*), the ovarian cycle did not maintain a stable phase relationship with the daily rhythm of locomotor activity, although such stability would be expected if the estrous cycle were generated by the circadian clock.[404] But these are small potatoes. Data from studies on fowl are far more dramatic. One example is shown in Figure 9.60. A quail (*Coturnix coturnix*) was maintained first under a 24-hour light–dark cycle (Panel A) and later in constant darkness (Panel B). Oviposition (egg laying) and the feeding rhythm were monitored. Both rhythms were entrained by the light–dark cycle, but they freeran with visibly

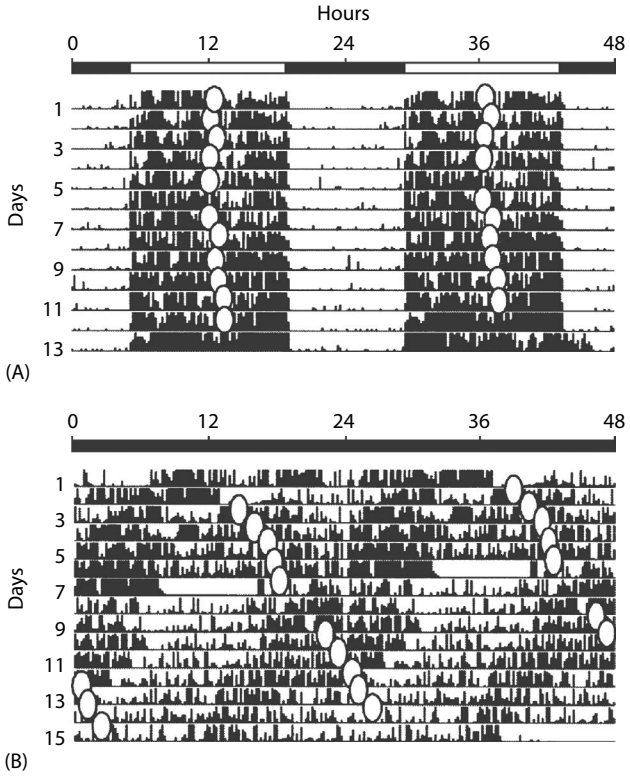


FIGURE 9.60 Independence of circadian rhythm and estrous cycle in a bird. These two double-plotted actograms show the rhythms of feeding activity and egg laying of quails (*Coturnix coturnix*) maintained under a light–dark cycle (A) or in constant darkness (B). The white ovals indicate the time of oviposition. Notice that entrainment of both rhythms is apparent under the light–dark cycle (A) but that the feeding rhythm freeruns with a period shorter than 24 hours, whereas the egg-laying rhythm freeruns with a period longer than 24 hours in constant darkness (B). (Adapted from Houdelier, C. et al., *Chronobiol. Int.*, 19, 1107, 2002.)

different periods in constant darkness. Such a difference in free-running periods can only be explained by the existence of two separate pacemakers. As a matter of fact, in several of the quail maintained under a light–dark cycle, only the feeding rhythm exhibited entrainment; the oviposition rhythm freeran with circadian gating (that is, no egg laying occurred during the dark phase of the light–dark cycle).[405] Similar results were obtained in other studies on quail,[406,407] chicken,[408,409] and turkey,[410] thus substantiating the notion that estrous cycles and circadian rhythms are generated by separate clocks.

Estrous rhythmicity is not the only form of rhythmicity whose interaction with circadian rhythms has been extensively studied. Much research has dealt with the interaction between *annual rhythms* and circadian rhythms. At the most basic level, seasonal changes in day length and ambient temperature affect the daily organization of autonomic and behavioral processes (Figure 9.61). In addition, endogenous circannual rhythms can affect the operation of circadian rhythms, and vice versa.

We saw in Chapter 7 that the seasonal variation in photoperiod causes a temporal compression or expansion of circadian



FIGURE 9.61 Bison in winter. The daily organization of animal behavior must change from summer to winter because of the seasonal changes in day length and ambient temperature and their effects on food availability. (Courtesy of Yellowstone National Park Wildlife Graphics, United States National Park Service.)

rhythms. This has been observed in natural settings[411–420] as well as in the laboratory (where only the photoperiod is changed).[421–426] Given that the hormone melatonin is inhibited by light, it is not surprising that blood concentration of melatonin is compressed in the summer and expanded in the winter,[241,427,428] but locomotor activity is equally affected. Locomotor activity data from a mouse maintained in the laboratory under a simulated natural photoperiod are shown in Figure 9.62. Notice the gradual expansion of the daily active phase (α) as the nights get longer throughout the autumn and first part of winter. Notice also that α is compressed as the nights get shorter in the late winter and early spring. Waveform plots of the activity rhythms of groups of mice under simulated summer, autumn, and winter photoperiods are shown in Figure 9.63. For comparison, waveform plots for the activity rhythms of Nile grass rats under simulated summer and winter photoperiods are shown in Figure 9.64. As nocturnal animals, mice expand α in the winter; as diurnal animals, grass rats compress α in the winter. Notice that compression of α is not complete. Consequently, the mice are active for a few hours before dusk and after dawn in the summer (when the scotophase is short) and grass rats are active for a few hours after dusk in the winter (when the photophase is short).

Of course, compression and expansion of α could very well be artifacts of laboratory conditions. Let us then look at the feeding rhythm of a mouflon sheep (*Ovis musimon*) housed outdoors all year-round (Figure 9.65). As shown in Figure 9.66, α is compressed in the winter and expanded in the summer in this diurnal animal. As a rule, wintertime is associated with rhythm compression in diurnal animals and rhythm expansion in nocturnal animals, whereas summertime is associated with rhythm expansion in diurnal animals and rhythm compression in nocturnal animals. Badgers (*Meles meles*) seem to be an exception to the rule: they are nocturnal but have a longer α in the summer (when the nights are shorter), presumably because their preferred prey (earthworms) is scarce this time of the year, so that the badgers must

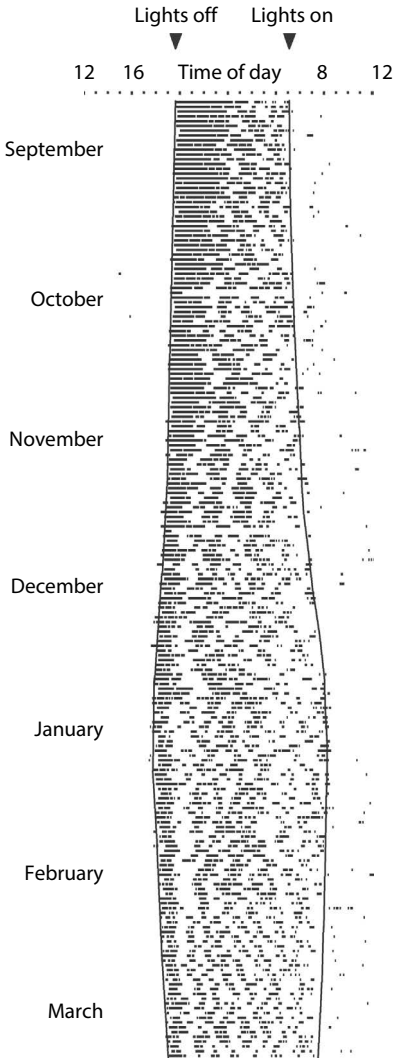


FIGURE 9.62 Following the seasons. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) subjected to a variable 24-hour light–dark cycle that simulated the natural variation in photoperiod at a latitude of 30° North. The vertical lines indicate the times of lights off and lights on. Notice the gradual expansion of the active phase of the daily cycle (α) as the nights become longer in the winter. (Data from the archives of the Refinetti lab.)

spend more time foraging.[429] Humans can also be considered an exception to the rule because our social work conventions make us to be active for the same number of hours each day regardless of season.[430–432]

Seasonal variations have also been documented in other parameters of circadian rhythms, such as phase, amplitude, and period.[111,230,433–442] An interesting seasonal modulation of rhythm *amplitude* is observed in beavers (*Castor canadensis*). During the winter, in Canada and northern United States, beavers remain essentially sequestered in their lodges or underneath the ice cover, so that their daily rhythm of activity is almost flat, whereas robust rhythmicity is present in the summer.[443–445] Another interesting seasonal modulation of activity is the appearance of *migratory restlessness* in migratory birds once a year.[446]

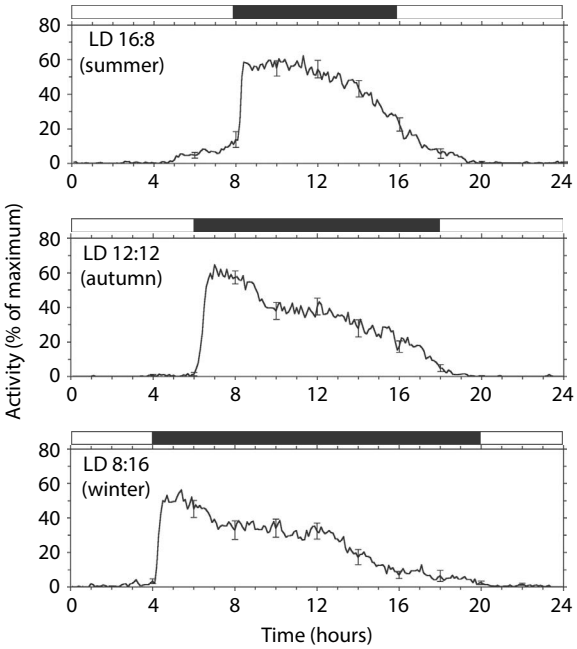


FIGURE 9.63 Modulation of α by photoperiod in mice. The graphs show the mean daily activity patterns of laboratory mice (*Mus musculus*) maintained under three different photoperiods. The curves reflect the means of 30 mice per group (averaged over 6 days for each animal) at 6-minute intervals. Standard errors (SE) are plotted in 2-hour intervals to prevent cluttering of the figures. Notice the compression of the active phase of the daily cycle (α) when the nights are short (LD 16:8) and its expansion when the nights are long (LD 8:16). (Data from Refinetti, R., *Integr. Physiol. Behav. Sci.*, 37, 114, 2002.)

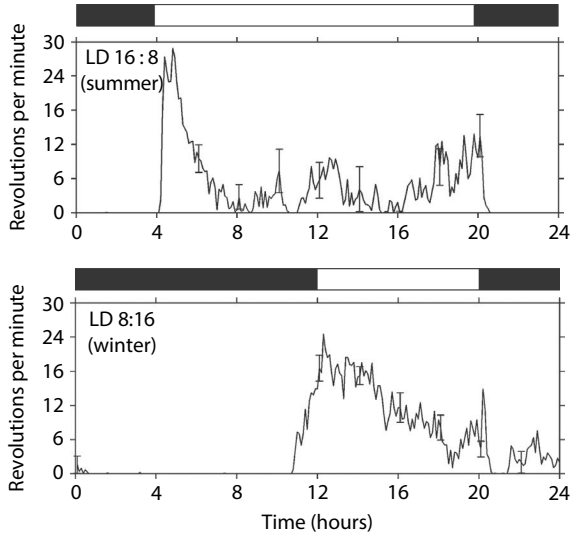


FIGURE 9.64 Modulation of α by photoperiod in grass rats. The graphs show the mean daily activity patterns of Nile grass rats (*Arvicanthis niloticus*) maintained under two different photoperiods, as indicated by the horizontal bars. The curves reflect the means of seven grass rats (averaged over 10 days for each animal) at 6-minute intervals. Standard errors (SE) are plotted in 2-hour intervals to prevent cluttering of the figures. Notice the compression of the active phase of the daily cycle (α) when the photophase is short (lower panel). (Data from Refinetti, R., *Physiol. Behav.*, 82, 285, 2004.)



FIGURE 9.65 Wild sheep. Mouflon sheep (*Ovis musimon*) are ruminants indigenous to southern Europe. (Photo courtesy of Rickey Hunt, Avery, TX.)

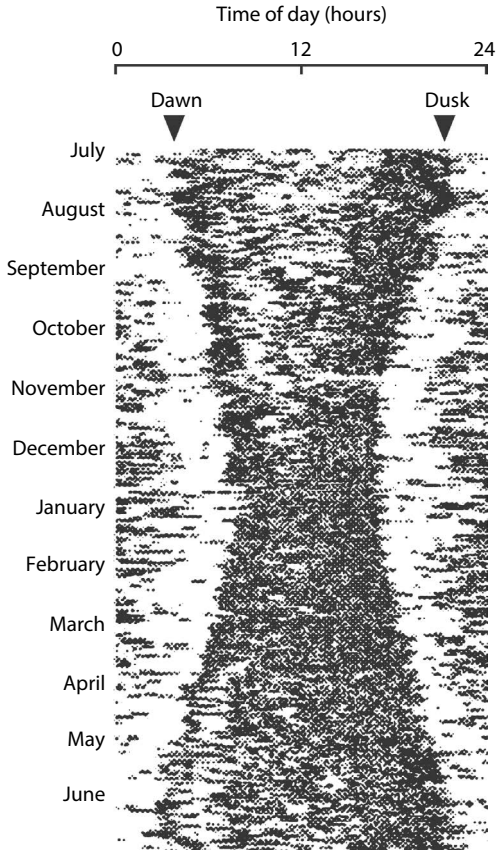


FIGURE 9.66 Modulation of α by photoperiod in mouflon sheep. This actogram shows the feeding rhythm of a mouflon sheep (*Ovis musimon*) maintained outdoors in Germany for a full year. Notice the gradual contraction (and later expansion) of the feeding rhythm as the days become shorter in the winter (and longer again in the summer). (Adapted from Berger, A. et al., *Behav. Res. Methods Instrum. Comput.*, 35, 458, 2003.)

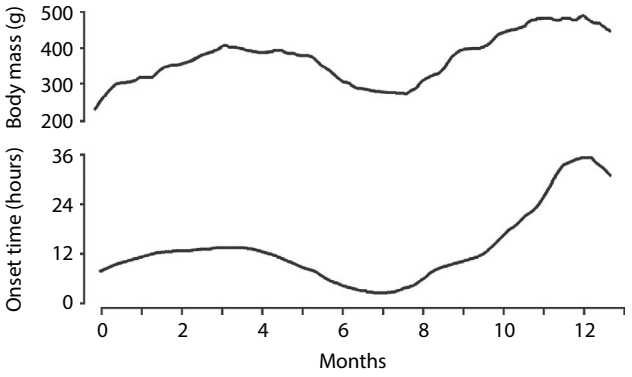


FIGURE 9.67 Circannual modulation of circadian period? The graphs show the annual oscillations of body mass and of circadian activity onset of a golden-mantled ground squirrel (*Callospermophilus lateralis*) maintained in the laboratory under constant ambient temperature (22°C) and constant illumination (LL). Notice that activity onset oscillates in synchrony with body mass, which suggests a possible circannual modulation of circadian period. (Adapted from Mrosovsky, N. et al., *Naturwissenschaften*, 63(6), 298, 1976.)

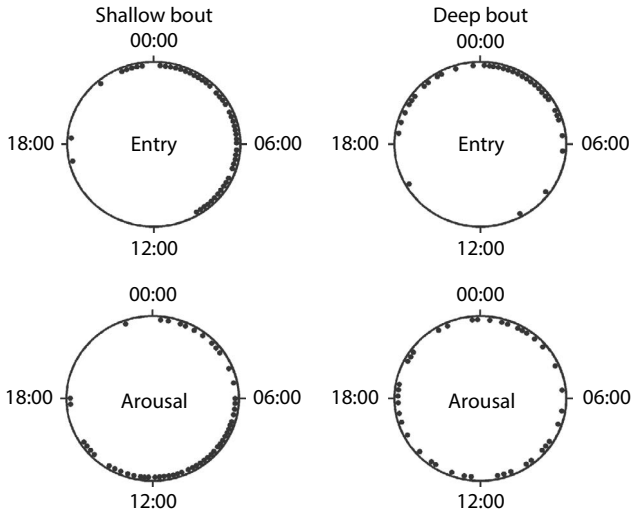


FIGURE 9.68 Circadian modulation of hibernation. The graphs show the distributions of the times of entry into and arousal from hibernation of eight European hamsters (*Cricetus cricetus*) maintained in the laboratory under simulated winter conditions (8°C, LD 8:16). The data are plotted separately for shallow and deep torpor bouts. Notice that, for entry into shallow or deep torpor, the distributions are more or less clustered around the late evening and early morning. (Data from Waßmer, T. and Wollnik, F., *J. Comp. Physiol. B*, 167, 270, 1997.)

Seasonal modulation of the *phase* of circadian rhythms is exemplified by the activity pattern of the golden-mantled ground squirrel (*Callospermophilus lateralis*), as shown in Figure 9.67. The animal was maintained in the laboratory in constant light for over a year. The upper graph shows the circannual variation in body mass. The lower graph shows the variation in onset times of the circadian activity rhythm. The two curves are remarkably similar. The similarity strongly suggests a circannual modulation of the phase of the circadian rhythm. Because the animal was in constant light, the variation in circadian phase was probably caused by a slow variation in circadian *period*. Unfortunately, of 13 animals tested, this was the only one for which reliable activity data could be obtained.[434] Therefore, it is impossible to ascertain whether the conclusion can be generalized to other members of the species, much less to other species.

The interaction between annual and circadian rhythms also occurs in the opposite direction—that is, circadian rhythmicity can affect annual rhythms. Perhaps the best example of this interaction is the circadian modulation of entry into and arousal from hibernation. Consider Figure 9.68. European hamsters (*Cricetus cricetus*) were kept in the laboratory under simulated winter conditions of short photoperiod (LD 8:16) and low ambient temperature (8°C). Each dot in the figure corresponds to an episode of entry into or arousal from hibernation for eight hamsters that exhibited either shallow bouts or deep bouts of hibernation. Although the temporal distribution of episodes is not very tight, it is definitely not random in three of the four variables. For shallow torpor, most entries occurred between midnight and 10 a.m., whereas most arousals occurred between 6 a.m. and 2 p.m. For deep bouts, most entries occurred between 8 p.m. and 4 a.m., whereas the arousals were scattered all over the day.[447] Studies in this and other species of hibernators are in general agreement that entry into torpor is restricted to a narrow segment of the day (and, therefore, is modulated by the circadian system), but there is disagreement about the circadian modulation of arousal. In studies conducted under a light–dark cycle, some investigators have found that arousal is restricted to a narrow segment of the day,[448–450] whereas others have not.[438,451,452] Because the conflicting findings have been obtained in different species, they may be explained by species differences. Of two studies conducted under constant darkness, one found arousal times to be concentrated around the same circadian time,[437] whereas the other did not.[453] The central question is whether the circadian system remains functional during hibernation, as a functional clock is required for the timing of arousal, at least in animals that hibernate deep underground. Circadian rhythmicity of body temperature (with very small amplitude) during hibernation has been reported by some investigators[433,437,450,454] but not by others.[111,438,451,455–458] A study of metabolic activity of various brain areas identified high activity in the site of the master circadian pacemaker during hibernation.[459] Thus, there is some evidence that the circadian system remains functional during hibernation, but further research is needed to clarify the matter.

9.3.4 Aging

Although most of us do not like to think about it, all of us will get old and die. All other living beings will die too—some sooner, some later. The life spans of various species are listed in Table 9.1. Gastrotriches, the members of a phylum of microscopic animals found in fresh water and marine environments, are perhaps the shortest-lived animals. They live for only

TABLE 9.1
Life Span of Animals

Species	Group	Life Span
Gastrotrich	Aquatic invertebrate	3 days
Fruit fly	Flying insect	37 days
Nothobranchius	Freshwater fish	42 days
Ant (worker)	Ground insect	6 months
Bee (worker)	Flying insect	1 year
Golden hamster	Rodent (mammal)	3 years
Quail	Phasianid (bird)	6 years
Rabbit	Lagomorph (mammal)	9 years
Sheep	Small ruminant (mammal)	15 years
Pheasant	Phasianid (bird)	18 years
Squirrel monkey	Primate (mammal)	21 years
Pigeon	Columbid (bird)	26 years
Deer	Ungulate (mammal)	35 years
Bear	Carnivore (mammal)	40 years
Chimpanzee	Primate (mammal)	50 years
Human	Primate (mammal)	80 years
American box turtle	Aquatic reptile	120 years
Galapagos tortoise	Terrestrial reptile	190 years

Sources: All Creatures Veterinary Hospital, Vallejo, CA; Primate Info Net, University of Wisconsin, Madison, WI.



FIGURE 9.69 Short-lived fish. *Nothobranchius furzeri*, a 5 cm long freshwater fish from Africa, is the shortest-lived vertebrate. Its full life cycle is only 42 days long. (Photo by Julian Haffegee, British Killfish Association.)

3 days. The fish *Nothobranchius furzeri* (Figure 9.69) is the shortest-lived vertebrate. It completes its life cycle in 42 days. At the other extreme, the Galapagos tortoise (*Geochelone elephantus*) lives to the ripe age of 190 years. Most humans do not celebrate their 80th birthday. As shown in Figure 9.70, improvements in lifestyle have raised human expected longevity in the last one-and-a-half centuries, but the greatest improvement has been in the prevention of childhood deaths. In the United States, expected longevity at birth rose from under 40 years in 1850 to almost 80 years in 2000. For those who survived until 20 years of age, the gains were much smaller. Women can expect to live a few more years than men, but most of us will

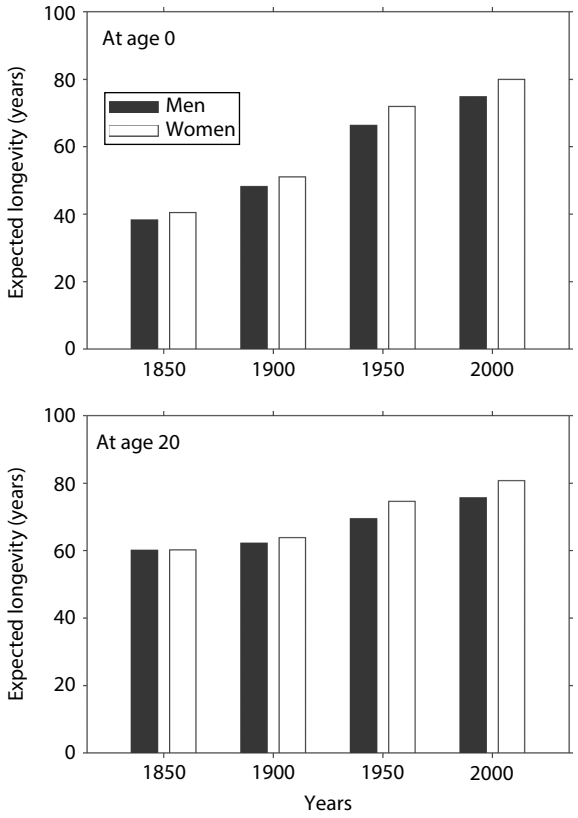


FIGURE 9.70 How long will you live? The graphs show the expected longevity of US residents at birth and at 20 years of age by gender and by half century. The greatest improvement in longevity in the last 150 years was a reduction in childhood mortality. An average 20-year-old American today will be dead before his or her 80th birthday. (Data from National Center for Health Statistics, United States Centers for Disease Control and Prevention.)

be dead before 80 years of age. Although people are not living longer, Europeans in particular are having fewer children, so that the population as a whole is getting older, which has many implications, including the need to consider mechanisms of work redistribution.[460]

Aging brings the decline of many functions well before death. One of many declines is that in the frequency of sexual behavior. As shown in Figure 9.71, young American adults have sex about twice a week on average, whereas people in their fifties have sex about once a week.

Before we move on to other effects of aging more directly related to circadian rhythms, a few comments about the process of aging itself are necessary. Generation after generation, people are born, get old, and die. Therefore, one might be tempted to refer to one's life cycle as a biological rhythm. The standard use of the term *biological rhythm*, however, is to refer to a repetitive process in a biological system. Usually, what we mean by "biological system" is an organism (say, a tree, a mouse, or a human organism). For each organism, life is not a repetitive process. Fantasies about reincarnation aside, we are born and die only once. So, although we may go through a life cycle, this cycle does not repeat itself—and a cycle that occurs only once does not qualify as a rhythmic process. If you go

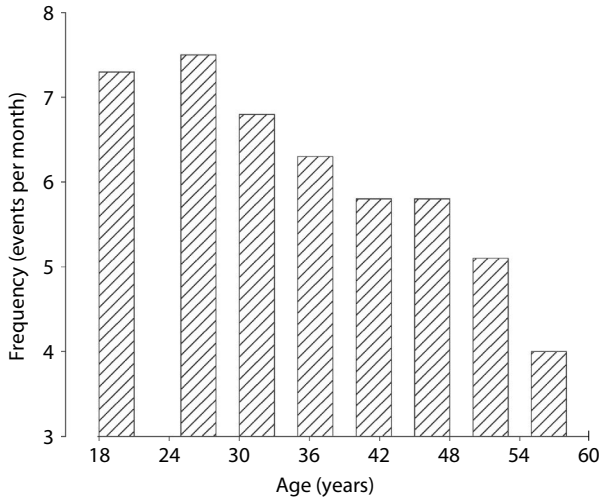


FIGURE 9.71 How often do you have sex? According to a survey of 1200 American adults who had one or more sexual partners during the preceding year, the frequency of sex declines with age. The average American has sex twice a week in the early twenties and once a week in the late fifties. (Data from Laumann, E.O. et al., *The Social Organization of Sexuality*, University of Chicago Press, Chicago, IL, 1994.)

to a given restaurant only once, you cannot say that you go there regularly. Similarly, a wall clock that stops running after 1 day is not really a clock—it is a defective instrument that you will return to the store for a refund. We could consider the biological system to be the species rather than the individual. Species only live once too, but—as long as the species is not extinct—one generation is followed by the next. Thus, there is a cycle of life within the species—and the cycle certainly involves aging. However, once again, the aging of each individual happens only once. No one rejuvenates and starts the aging process again. Therefore, aging is not a repetitive phenomenon and should not be called a biological rhythm. Aging is the result of a disruption in the rhythm of cell reproduction: cells are genetically programmed to undergo a finite number of replications and, when the limit is reached, hundreds of biological changes that characterize aging take place.[461,462]

By far the best-characterized alteration in the circadian system related to aging is a reduction in the *amplitude* of circadian rhythms.[463–465] An example for the rhythm of body temperature is shown in Figure 9.72. Representative records for two rats are shown. Notice that the rhythm of the young rat has an amplitude of almost 2°C, whereas the rhythm of the old rat has an amplitude of only 1°C. Reduction in the amplitude of the body temperature rhythm in old age has been documented in humans[49,466–468] as well as in various rodent species.[469–478] Generally, the level of locomotor activity is greatly reduced in old age, so that the amplitude of the activity rhythm is also reduced.[470,473,476,477,479–489] An example is shown in Figure 9.73. The mean activity level of a group of eight mice at three stages of their lives is shown separately for the dark and light phases of the light–dark cycle. Not surprisingly, mice do not run much on the wheels during the light phase. They run a lot at night when they are young and much less as they get old. Several studies have also documented a reduction in the amplitude of rhythms of other behavioral and autonomic variables.[72,75,486,490–495]

A very interesting—but as yet unreplicated—study indicated that the deteriorating activity rhythm of old hamsters could be rejuvenated by surgical implantation of fetal tissue from the brain region that contains the master circadian pacemaker.[496] The brain grafts not only restored the amplitude of the activity rhythm but also increased the longevity of the animals. I suspect that attempts at replication have been unsuccessful; otherwise, major advances would have been reported since the publication of the article in 1998. The average 4-month increase in hamster longevity[496] corresponds to an increase of about 12 years in human longevity. I am confident that plenty of research funds would be available to scientists who could develop a reliable procedure to extend human life by 12 years.

Aging is also associated with a change in the phase and period of circadian rhythms. A small advance in the phase

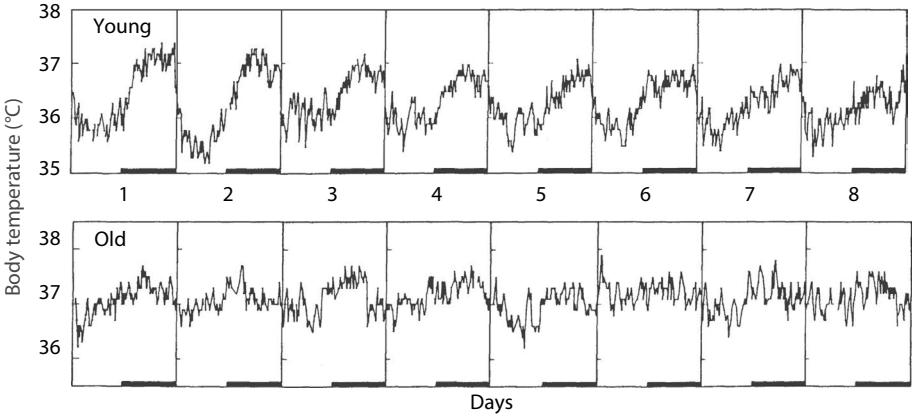


FIGURE 9.72 Reduction in rhythm amplitude with age. The graphs show 8-day segments of the records of body temperature of two representative rats (*Rattus norvegicus*) at a young age (2 months) and at a very old age (29 months). The thin dark horizontal bars indicate the timing of the dark phase of the prevailing light–dark cycle (LD 12:12). The amplitude of the temperature rhythm is clearly reduced in the old animal. (Reproduced from *Exp. Gerontol.*, 25, Refinetti, R., Ma, H., and Satinoff, E., Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders, 533–543, Copyright 1990, with permission from Elsevier.)

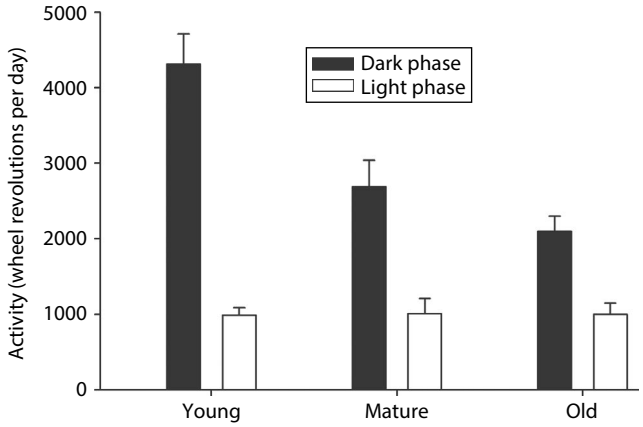


FIGURE 9.73 Reduction in mean level of rhythm with age. The graph shows the variation in the mean number of wheel revolutions (±SE) of 8 mice (*Mus musculus*) during the dark and light phases of the light–dark cycle as they aged. Mice of a fast-aging strain (SAMP8) were used. Notice that running activity during the dark phase, but not during the light phase, is considerably reduced as the animals get older. (Data from Pang, K.C.H. et al., *Neurobiol. Aging*, 25, 111, 2004.)

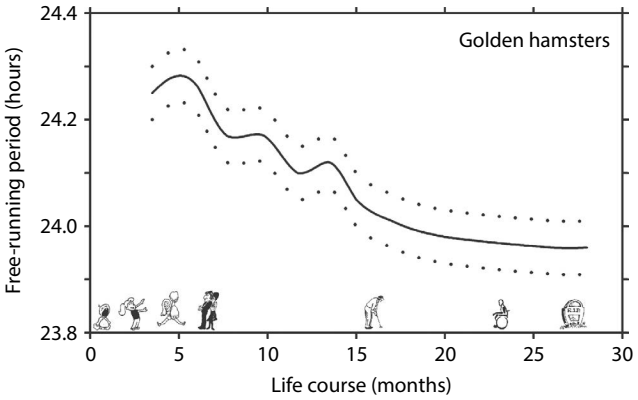


FIGURE 9.74 Shortening of free-running period with age. The graph shows the mean free-running period of golden hamsters (*Mesocricetus auratus*) maintained in constant darkness (by blinding) from 1 month of age to death. The solid line corresponds to the mean of approximately 15 hamsters (dotted lines: ±SE). The approximate correspondence to human age is shown by the drawings at the bottom. The data suggest that the circadian clock speeds up by 1% in the course of a lifetime. (Data from Morin, L.P., *J. Biol. Rhythms*, 3, 237, 1988.)

angle of entrainment in old age has been documented in humans[468,497–501] and rodents.[471,474,483] In humans, an advanced phase angle of entrainment means that older people tend to wake up earlier in the morning. Considering that the human endogenous period is longer than 24 hours and that the phase-advance region of the photic PRC extends into early subjective day, the earlier risetime in old people suggests the occurrence of an age-dependent lengthening of the circadian period. That is, old people would wake up earlier to compensate for the lengthening of their circadian period. However, an earlier risetime in nocturnal rodents with endogenous period shorter than 24 hours would imply a shortening of the circadian period. Is there any direct evidence of a change in period associated with aging? Yes, there is—although the results are conflicting. The results from one study on golden hamsters are shown in Figure 9.74. There is a clear—albeit small—shortening of the free-running period associated with aging. One confounding factor in this study is that the same group of animals was studied from young age to old age. Because the animals were maintained in constant darkness for their whole lives, it is not possible to dissociate the effects of aging from the effects of prolonged exposure to darkness. Nonetheless, studies on golden hamsters have generally agreed that circadian period is shortened in old age.[502–508] Shortening of circadian period was also observed in deer mice,[502] field mice,[480] and laboratory rats.[509] However, lengthening of circadian period was observed in aging domestic mice[314,510,511] and canaries.[512] Several other studies in domestic mice revealed no consistent change in circadian period from middle age to old age.[482,487–489] No change in circadian period with age was reported for owl monkeys.[513] I am aware of only two studies in humans. One study found no difference between the free-running periods of young and old subjects,[278] whereas the other study found shorter periods in older subjects.[49] All in all, it seems that circadian period does change with age in at least some species, but the phenomenon seems to be inconsistent from species to species and not very consistent even within the same species. More studies are needed to clarify the matter.

9.3.5 Learning

In previous chapters, we alluded to the instinctual nature of circadian rhythms. For instance, the free-running period of a species is genetically determined (inherited) and does not rely on learned experiences. Likewise, entrainment of circadian rhythms is established by reflexive phase shifts evoked by environmental stimuli. It is now time to ask whether learning can affect the circadian system. In Chapter 5, we saw that the circadian system can affect the ability to learn. Can the ability to learn affect the circadian system?

We know that the phase of a rhythm can be changed by phase-shifting environmental stimuli, but this is a reflexive response, not a form of learning. Or is it? The study of how animals associate a time with a place (*time–place learning*) has raised doubts about it. The ability of honeybees to return to specific flower beds at particular times of the day (*Zeitgedächtnis*, or *time memory* in German) has been known for over a century.[514] This phenomenon implies that the bees can learn an association between a time and a place.

Figure 9.75 is a diagram of the time–place paradigm used in studies on laboratory rodents. A hungry animal is trained to expect food at one location during the day and at another location during the night. If the animal learns the task, it will soon show a preference for the left feeder during the day and for the right feeder during the night. Of course, the animal could associate the left feeder with light and the right feeder with darkness, regardless of circadian time. To avoid this complication, actual studies use either early morning

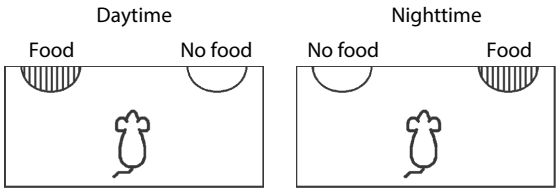


FIGURE 9.75 The time–place paradigm. In this simple time-learning paradigm, food is consistently found in one place at one time of the day and in another place at another time of the day. A starved animal quickly learns to choose the right place at the right time.

and afternoon times or early night and late night times, so that the animal must use circadian time as the discriminative stimulus. An example of results obtained in this paradigm is shown in Figure 9.76. Rats were trained to feed at one feeder early in the night and at another feeder late in the night for a whole month. The data shown refer to the average of 4 days after the one-month training. Notice that the hungry rats learned to anticipate the meals mostly at the right place at the right time (feeder approaches during the meals were omitted from the figure to facilitate visual inspection). Similar results were obtained in hamsters,[515,516] rats,[517,518] birds,[519,520] and fishes.[521,522]

Studies on rats have raised controversies that have not been fully resolved yet. It has been suggested that time–place learning occurs only under special protocols,[523] only in certain strains of rats,[524] and only if food restriction is involved.[525] One study even suggested that rats may never learn a true time–place association.[526] After the animals had learned to choose the correct place at the correct time of day (morning *versus* afternoon), the researchers started to occasionally skip either a morning or an afternoon session. In the morning sessions that followed skipped afternoon sessions, the rats continued to expect food at the morning location. However, in the afternoon sessions that followed skipped morning sessions, the rats incorrectly expected food at the morning location. This suggests that receiving food at the morning location, rather than the passage of time, was necessary for the rats to anticipate the location of food in the afternoon. In other words, the morning choices may have reflected an association between time and place, but the afternoon choices reflected a session-to-session alternation strategy. Similar results were obtained in a different laboratory.[527] You may not care much about what is going on inside a rat's head, but the issue of session-to-session alternation is quite important. If only the morning choices reflect an association between time and place, then the notion of time–place learning may not be needed at all. The morning place would be *the* place of feeding, the morning time would be the reflexive phase angle of entrainment, and the afternoon performance would reflect only the alternation of responses. In other words, time–place learning would not involve temporal learning at all. Or, as claimed by another research team, time–place learning in rats may not involve memory for time of day but only memory for time elapsed.[528] In this respect, it is worth noting that studies in both mice and golden hamsters have demonstrated time–place learning in animals with their master circadian pacemakers surgically destroyed,[529,530] which confirms that awareness of time of day is not needed for time–place learning.

The issue of learning in the circadian system can be taken a step further. There is little doubt that the ability of the circadian pacemaker to be phase-shifted by environmental stimuli such as light is innate. However, can the circadian clock *learn* to phase-shift in response to a neutral stimulus if this stimulus is experimentally associated with light? This would be a case of *classical conditioning*, or the process by which a previously neutral stimulus acquires the ability to elicit a response that is naturally elicited by a different stimulus. Direct your attention to Figure 9.77. Dogs instinctively salivate in response to the sight and smell of food but do not salivate in response to the sound of a bell. However, if the bell is presented in close temporal association with food several times, the dog will eventually salivate in response to the sound of the bell. Because food unconditionally evokes salivation, it is called an *unconditional* (or *unconditioned*) stimulus. Because the bell evokes salivation only under the condition that it has been previously paired with food, it is called a *conditional* (or *conditioned*) stimulus.

I have tried many times unsuccessfully to obtain phase shifts in response to sounds or odors previously associated

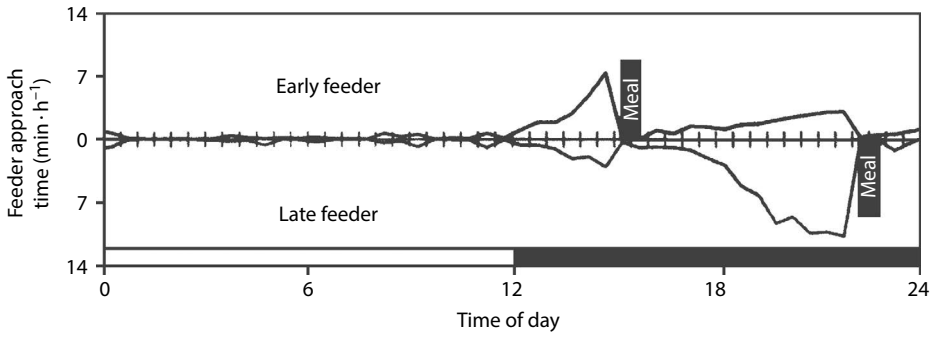


FIGURE 9.76 Time–place learning in rats. The graph shows the mean time spent by rats (*Rattus norvegicus*) close to a feeder previously associated with meals early at night and close to a feeder previously associated with meals late at night. The curves correspond to the means of 16 rats, each averaged over 4 days. Training had been going on for a month. Notice that the animals exhibit much greater activity in anticipation of the meal at the early feeder early in the night and at the late feeder late at night. (Data from Aragona, B.J. et al., *J. Biol. Rhythms*, 17, 330, 2002.)

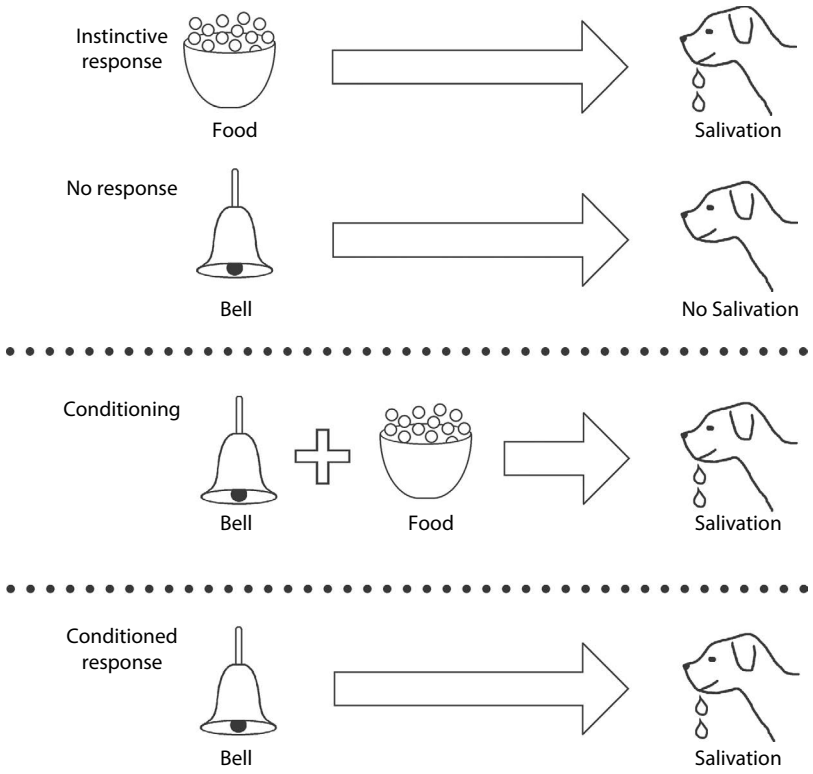


FIGURE 9.77 Classical conditioning. Associative learning by classical conditioning relies on a stimulus that unconditionally elicits a response (such as food) and an originally neutral stimulus (such as the sound of a bell). During conditioning, the neutral stimulus is paired with the unconditional stimulus. After several trials, the neutral stimulus acquires the ability to elicit the response and becomes a conditional (or conditioned) stimulus.

with phase shifts caused by light pulses in various rodent species. I assume that many other investigators also failed to demonstrate conditioning in the circadian system but did not publish their negative results. One group of investigators, however, reported that air puffs previously paired with light pulses can cause phase shifts in rats.[531] The conditioning procedure consisted of the presentation of an air puff (the conditional stimulus) contiguously with a brief pulse of dim blue light (the unconditional stimulus) for 10 consecutive days. The stimuli were presented at zeitgeber time 15 (i.e., 3 hours after lights off) to rats housed under a white light–dark cycle. After the 10 days of conditioning, the animals were kept in constant darkness (without air puffs or light pulses) for 5 days before receiving a test pulse at circadian time 15. The results are shown in Figure 9.78. As you can see, the air puff alone (without previous conditioning) evoked no phase shift. The light pulse (the unconditional stimulus) evoked a sizeable phase shift. The interesting group is, of course, the one that received the air puff previously paired with the light pulse. This group exhibited a phase shift almost as large as that exhibited by the group that received the light pulse. The air puff evidently acquired the ability to evoke a phase shift. The data clearly suggest that the phase-shifting response can be classically conditioned. The same research team also later reported success when using light as the unconditional stimulus and circadian time as the conditional stimulus.[532]

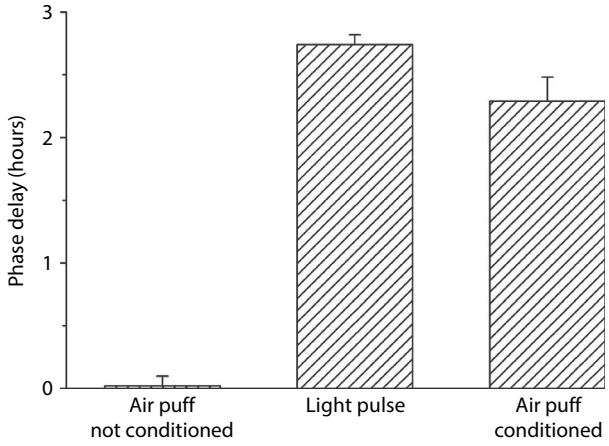


FIGURE 9.78 Classical conditioning in the circadian system. The graph shows the mean phase delays of the locomotor activity rhythms of rats (*Rattus norvegicus*) exposed to brief pulses of a neutral stimulus (an air puff), an unconditional stimulus (a light pulse), or a conditioned stimulus (an air puff previously paired with a light pulse). Each bar is the mean (± SE) of three rats. Notice that the conditioned stimulus elicited a mean phase delay almost as large as that elicited by the unconditional stimulus. (Data from Amir, S. and Stewart, J., *Nature*, 379, 542, 1996.)

The data in Figure 9.78 bother me not only because they indicate that someone else succeeded where I failed (in demonstrating classical conditioning in the circadian system) but, more importantly, because the number of animals used was extremely small (namely, three rats per group). To my relief, one of probably many research teams that failed to replicate the findings eventually published a report.[533] The authors of the original article blamed the other team's failure on the fact that they used white light as both the zeitgeber and the unconditional stimulus.[534] This is true, but both groups also used skeleton photoperiods (with white light only), and one claimed to obtain conditioning,[535] whereas the other still found nothing significant.[533] Therefore, the conditions necessary for successful conditioning of the phase-shifting response remain rather obscure.[536] The only thing that is very clear is that, 20 years after the publication of the original article,[531] no independent group has confirmed the occurrence of classical conditioning in the circadian system. It is, therefore, likely that the findings cannot be replicated.

9.3.6 Sexual Dimorphism

Physical differences between the sexes are generally small but very clear. Both men and women have a face, two arms, two legs, a heart, a stomach, two kidneys, and so on. Men have penises, scrotums, and facial hair, whereas women have vaginas, uteruses, ovaries, and breasts. When it comes to personal relationships, men tend to emphasize practical aspects, whereas women tend to emphasize romantic aspects.[537] In this final subsection of the chapter, we will look at sexual differences in the circadian system. Very little research has been systematically conducted on the topic of sexual dimorphism of circadian rhythms. We can cover all of it in two short paragraphs.

Figure 9.79 compares male and female mice on three variables. You can see that male mice are bigger than female mice but are also less active. Regarding free-running period, the two sexes do not differ.[538] Few other studies have systematically compared circadian parameters between males and females, but the findings have generally been that period and phase of circadian rhythms are *not* significantly different in males and females.[83,420,481,539–546] Not surprisingly in view of the characteristics of estrous rhythmicity discussed in Chapter 4, female rodents were found to exhibit greater variation than males in circadian phase (i.e., comparable mean but larger standard deviation).[543,545,547]

Except for a rise during proestrus, the daily rhythm of body temperature of female rats was found to be equivalent to that of males.[548] The rhythm of urinary excretion was also found to be similar in male and female rats, except that females excrete a consistently greater volume of urine.[549] In humans, the daily rhythm of melatonin secretion was found to be phase-advanced by about 40 minutes in females as compared to males,[550,551] although the difference between the sexes was much smaller in bedtime and wake-up time.[261,550–553] A study of over 2300 people from five countries found that men were generally more active than women but that they distributed their activity along the day in the same manner as women did.[432]

SUMMARY

1. Circadian rhythmicity is exhibited by many variables simultaneously, and different variables reach their daily peaks at different times of the day. Although the circadian pacemaker does not generate each and every rhythm individually, the causal connections between different rhythms are not fully known. It is known that the rhythm of body temperature is *not* caused either by the rhythm of activity or by the rhythm of feeding.
2. Circadian rhythmicity is an evolutionarily old process that is found in all domains of life today. Some organisms are diurnal, some are nocturnal, some are crepuscular, and some are arrhythmic. The circadian systems of diurnal and nocturnal organisms do not seem to differ, and the adoption of diurnal or nocturnal niches is believed to be determined by mechanisms located downstream from the pacemaker.

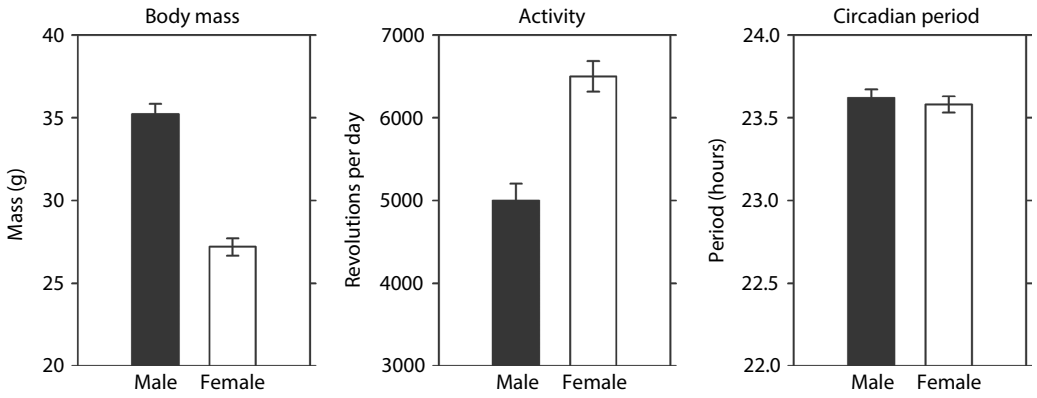


FIGURE 9.79 Sex differences in mice. The graphs show the mean body masses, mean running-wheel activity levels, and mean free-running periods in constant darkness of male and female domestic mice (*Mus musculus*). Each bar corresponds to the mean (± SE) of 24 mice. There are significant sex differences in body mass and activity level but not in circadian period. (Data from Koteja, P. et al., *J. Biol. Rhythms*, 18, 491, 2003.)

3. In many organisms, circadian rhythmicity is not present at birth. It develops during early life and often degenerates in old age. During adulthood, circadian rhythms can be modulated by infradian rhythms such as the reproductive cycle and annual rhythms. The circadian systems of males and females do not differ to a great extent.

EXERCISES

9.1 Phase relationship of multiple daily rhythms in humans. In this exercise, you will measure multiple daily rhythms simultaneously so that you can observe their phase relationship. You can be your own subject, and no expensive scientific equipment is required.

1. Ideally, you would use a friend or colleague as your subject. If this is not possible, you may record your own rhythms.
2. The more variables you can measure, the better. Advice on how to measure the rhythms of body temperature and heart rate has been previously provided in Exercises 1.2 and 5.1. Other rhythms that can be conveniently measured include blood pressure, amount of food ingested, urinary excretion, and general sleepiness.
3. For the measurement of blood pressure, you can use a standard sphygmomanometer with an inflatable cuff around your arm, which you can purchase at a regular drugstore. You may record mean arterial blood pressure (the mean of the systolic and diastolic measurements) or the two measures (systolic and diastolic) as two separate variables.
4. For the measurement of amount of food ingested, all you need to do is to keep a record of the time and weight of any food you eat. Before you eat, weigh the food with a regular kitchen scale (making sure to exclude the weight of the food container). If you don't finish the meal, weigh the unused amount and deduct it from the original figure. In case you would like to measure calorie intake (in addition to or instead of amount of food ingested), you can easily convert the weight of food items into metabolizable calories by consulting a food table. Food tables are available in nutrition textbooks and fitness magazines.
5. For the measurement of urinary volume, measure the time and volume of urine every time you need to empty your bladder. To measure the volume of urine, simply collect the urine in a calibrated beaker (a 1000 mL beaker should be appropriate for most people).
6. A simple subjective scale (0: very awake, 5: very sleepy) will suffice for the measurement of general sleepiness.
7. Another interesting variable to be recorded is reaction time (that is, how fast a person can react to the presentation of a stimulus). The CD that comes with the book contains a short program designed to measure visual reaction time. Although the program does not appear in the Circadian banner, it should have been copied to your hard drive when the software package was installed. Look for it (**Reaction.exe**) in the folder where the other programs are ("\Program Files\Circadian") and check it out. It is quite intuitive and easy to use.
8. If you or someone you know has the facilities, you may also easily measure the concentration of hormones in your saliva or urine. The measurement of salivary melatonin would be especially interesting because the rhythm of melatonin concentration peaks at night, not during the day like most other variables that you will be recording.
9. If possible, you should measure each variable every hour. If this is not possible, an interval of 2 hours (or even 3 hours) is acceptable. If you repeat the measurements over several days, you can average the hourly values to obtain smoother curves (which will reflect the *true* rhythms more closely).
10. Of course, measurements to be conducted at night while you sleep will be a problem. If you are using a friend as the subject, you may be able to conduct some of the measurements while he or she sleeps. If you are your own subject, you may occasionally use an alarm clock to briefly wake you up in the middle of the night, but you should not do this too often, otherwise you may disturb your body's clock. Food intake and urinary excretion should not occur during sleep time at all (unless the subject is a sleepwalker or a bed wetter). Sleepiness can be assumed to be maximal when the person is asleep. The reaction time is "off the chart" during sleep.
11. When you are finished collecting data, you should plot all variables on Cartesian coordinates (time in the X-axis, variable in the Y-axis) and place all plots on the same page so that you can compare them.
12. Data analysis can highlight various aspects of daily rhythmicity. First of all, you should be able to see a daily pattern of oscillation in most, if not all, variables. Second, you should be able to notice differences in phase (that is, different rhythms will peak at different times of the day). While doing this, you will experience a third aspect: the variability in waveforms. Because different rhythms have different waveforms, it is often difficult to examine their phase relationship. You may have to fit a smooth curve to some of the rhythms in order to be able to compare their acrophases.
13. As a fourth aspect of data analysis, you can speculate about the dependence of some rhythms on others. For instance, is there really a rhythm of urinary excretion, or is it just a consequence of the inhibition of urination during sleep? You can evaluate this particular question experimentally by altering your research protocol a little bit. During the last night

of data collection, simply stay up all night. You will then be able to compare the measurements of urinary excretion on a regular night with those on the night that you stayed awake. Notice that, to make this a fair comparison, you will have to withhold water intake (drinking) during the whole night that you stay awake.

9.2 Phase relationship of multiple daily rhythms in laboratory animals. In Exercise 9.1, you investigated the phase relationship of multiple daily rhythms in your own body or in the body of a friend or colleague. Of course, you were not able to use invasive research methods or to conduct the investigation under controlled environmental conditions. If you have access to a properly equipped laboratory, with approval of and supervision by an experienced animal researcher, you may conduct the present exercise.

1. The species to be used is not a critical factor. In most countries, you do not need an approved animal research protocol to conduct research on invertebrates, although there are not too many rhythms that you can record in an invertebrate. Among the vertebrates, laboratory rats are probably the most convenient species.
2. The more variables you can measure, the better. Depending on the equipment available, you may want to measure locomotor activity, food intake, fluid intake, urinary excretion, defecation, blood pressure, heart rate, oxygen consumption, blood glucose level, melatonin secretion, and any other variable that you can record.
3. If the data collection procedures are automated, you should conduct the study for at least a week, so that you can have enough data points for analysis. Some variables can be sampled every minute, whereas others cannot be sampled more often than once every hour or every 2 hours. If you cannot record any particular variable more often than every 3 or 4 hours, you might as well not record it at all.
4. If possible, you should collect data for a week while the animal is under a light–dark cycle and for another week while the animal is in constant darkness (or constant light). This will allow you to compare the relative phase angles of the various rhythms between the entrained state and the free-running state.
5. When you are finished collecting data, you should plot all variables on Cartesian coordinates (time in the X-axis, variable in the Y-axis) and place all plots on the same page so that you can compare them. To avoid spending many hours in a very tedious task, you should use a computer program to prepare the graphs. Presumably, you will have all the data on disk files so that you can easily load them into the graphics program.
6. As in Exercise 9.1, data analysis can highlight various aspects of daily rhythmicity. First of all, you should be able to see a daily pattern of oscillation in most, if not all, variables. Second, you should be able to notice differences in phase (that is, different rhythms will peak at different times of the day). While doing this, you will experience a third aspect: the variability in waveforms. Because different rhythms have different waveforms, it is often difficult to examine their phase relationship. You may have to fit a smooth curve to some of the rhythms in order to be able to compare their acrophases. Fourth, you can compare entrained rhythms with free-running rhythms. Are the relative phase angles of the various rhythms that you observed during entrainment the same as those you find in the free-running state? Does a rhythm that peaked earlier in the day than another one now peak after it?

9.3 Latin names and English names of species cited in the book. Research conducted on over 700 species of animals, plants, and microbes is discussed in this book. A list of Organisms Used is provided at the end of the book, right before the Index. The list provides the English names for all the species listed in alphabetical order of Latin name. You may find it more convenient, however, to have an electronic database that you can consult in both directions and search by specific words. This is what the program **Organism** (the first icon on the right in the Circadian banner) provides you with. The program is self-explanatory but has a Help menu item just in case you need assistance. You may want to try it out now, as it may come in handy as you keep reading this book.

SUGGESTIONS FOR FURTHER READING

Futuyma, D. J. (2013). *Evolution*, 3rd edn. Sunderland, MA: Sinauer.

A good undergraduate textbook on the biology of evolution.

Lincoln, R. J., Boxshall, G. A., and Clark, P. F. (1998). *A Dictionary of Ecology, Evolution and Systematics*, 2nd edn. New York: Cambridge University Press.

Naturally, not a book to be read from cover to cover, but a very helpful resource to have around when dealing with evolutionary issues.

Gilbert, S. F. (2013). *Developmental Biology*, 10th edn. Sunderland, MA: Sinauer.

A good undergraduate textbook on developmental biology.

Ekerdt, D. J. (Ed.) (2002). *Encyclopedia of Aging*. New York: Macmillan.

This 4-volume set contains more than 400 entries on aging-related topics accessible to high-school and college students.

WEBSITES TO EXPLORE

Ecology (Encyclopedia of Earth): http://www.eoearth.org/view/article/151932/.

Evolution Videos (Howard Hughes Medical Institute): http://www.hhmi.org/biointeractive/explore-evolution.

Paleoclimatology (U.S. National Oceanic and Atmospheric Administration): http://www.ngdc.noaa.gov/paleo/paleo.html

PBS's Evolution Web Site: http://www.pbs.org/wgbh/evolution.

Understanding Evolution (University of California Museum of Paleontology): http://evolution.berkeley.edu/.

U.S. National Institute on Aging: http://www.nia.nih.gov.

REFERENCES

1. Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N., and Scheving, L. E. (1979). Nomifensine chronopharmacology, schedule-shifts and circadian temperature rhythms in di-suprachiasmatically lesioned rats: Modeling emotional chronopathology and chronotherapy. *Chronobiologia* 6: 405–424.
2. Chia-Looi, A. and Cumming, B. G. (1972). Circadian rhythms of dark respiration, flowering, net photosynthesis, chlorophyll content, and dry weight changes in *Chenopodium rubrum*. *Canadian Journal of Botany* 50: 2219–2226.
3. Meinrath, M. and D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
4. Jilge, B. (1985). The rhythm of food and water ingestion, faeces excretion and locomotor activity in the guinea pig. *Zeitschrift für Versuchstierkunde* 27: 215–225.
5. Fischette, C. T., Edinger, H. M., and Siegel, A. (1981). Temporary desynchronization among circadian rhythms with lateral fornix ablation. *Brain Research* 229: 85–101.
6. Honnebier, M. B. O. M., Jenkins, S. L., and Nathanielsz, P. W. (1992). Circadian timekeeping during pregnancy: Endogenous phase relationships between maternal plasma hormones and the maternal body temperature rhythm in pregnant rhesus monkeys. *Endocrinology* 131: 2051–2058.
7. Robinson, E. L. and Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
8. Moore-Ede, M. C., Kass, D. A., and Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
9. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L., and Bitman, J. (1999). Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. *Domestic Animal Endocrinology* 16: 41–55.
10. Jilge, B. (1991). Restricted feeding: A nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
11. Piccione, G., Caola, G., and Refinetti, R. (2005). Daily rhythms of blood pressure, heart rate, and body temperature in fed and fasted male dogs. *Journal of Veterinary Medicine A* 52: 377–381.
12. Piccione, G., Caola, G., and Refinetti, R. (2005). Temporal relationships of 21 physiological variables in horse and sheep. *Comparative Biochemistry and Physiology A* 142: 389–396.
13. Fukuhara, C., Aguzzi, J., Bullock, N., and Tosini, G. (2005). Effect of long-term exposure to constant dim light on the circadian system of rats. *Neurosignals* 14: 117–125.
14. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
15. Scales, W. E., Vander, A. J., Brown, M. B., and Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
16. Ghata, J., Reinberg, A., Lagoguey, M., and Touitou, Y. (1977). Human circadian rhythms documented in May-June from three groups of young healthy males living respectively in Paris, Colombo and Sydney. *Chronobiologia* 4: 181–190.
17. Kräuchi, K. and Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *American Journal of Physiology* 267: R819–R829.
18. Kennaway, D. J. and Van Dorp, C. F. (1991). Free-running rhythms of melatonin, cortisol, electrolytes, and sleep in humans in Antarctica. *American Journal of Physiology* 260: R1137–R1144.
19. Kraemer, S., Danker-Hopfe, H., Dorn, H., Schmidt, A., Ehlert, I., and Herrmann, W. M. (2000). Time-of-day variations of indicators of attention: Performance, physiologic parameters, and self-assessment of sleepiness. *Biological Psychiatry* 48: 1069–1080.
20. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J., and Van Cauter, E. (1997). Sleepiness, performance, and neuroendocrine function during sleep deprivation: Effects of exposure to bright light or exercise. *Journal of Biological Rhythms* 12: 245–258.
21. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M., and Czeisler, C. A. (1992). Short-term memory, alertness and performance: A reappraisal of their relationship to body temperature. *Journal of Sleep Research* 1: 24–29.
22. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F., and Pauly, J. E. (Eds.), *Chronobiology*. Tokyo, Japan: Igaku Shoin, pp. 451–459.
23. Minami, Y., Kasukawa, T., Kakazu, Y., Iigo, M., Sugimoto, M., Ikeda, S., Yasui, A., van der Horst, G. T., Soga, T., and Ueda, H. R. (2009). Measurement of internal body time by blood metabolomics. *Proceedings of the National Academy of Sciences United States of America* 106: 9890–9895.
24. Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C., and Brown, S. A. (2012). The human circadian metabolome. *Proceedings of the National Academy of Sciences United States of America* 109: 2625–2629.
25. Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D., and Lalouette, A. (1994). Dissociation in body temperature, drinking and feeding rhythms following a light–dark cycle inversion in the rat. *Medical Science Research* 22: 53–55.
26. Refinetti, R. and Menaker, M. (1992). The circadian rhythm of body temperature of normal and tau-mutant golden hamsters. *Journal of Thermal Biology* 17: 129–133.
27. Refinetti, R. and Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
28. Pitrosky, B., Kirsch, R., Vivien-Roels, B., Georg-Bentz, I., Canguilhem, B., and Pévet, P. (1995). The photoperiodic response in Syrian hamster depends upon a melatonin-driven circadian rhythm of sensitivity to melatonin. *Journal of Neuroendocrinology* 7: 889–895.
29. Heusner, A. (1959). Variation nycthémérale de la température centrale chez le rat adapté à la neutralité thermique. *Comptes Rendus de Séances de la Société de Biologie de Strasbourg* 153: 1258–1261.

30. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
31. Aschoff, J. and von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
32. Honma, K. and Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
33. Pickard, G. E., Kahn, R., and Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiology and Behavior* 32: 763–766.
34. Kittrell, E. M. W. and Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.
35. Tapp, W. N. and Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
36. Brown, C. M. and Refinetti, R. (1996). Daily rhythms of metabolic heat production, body temperature, and locomotor activity in golden hamsters. *Journal of Thermal Biology* 21: 227–230.
37. Weinert, D. and Waterhouse, J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. *Physiology and Behavior* 63: 837–843.
38. DeCoursey, P. J., Pius, S., Sandlin, C., Wethey, D., and Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiology and Behavior* 65: 457–463.
39. Tang, I., Murakami, D. M., and Fuller, C. A. (1999). Effects of square-wave and simulated natural light-dark cycles on hamster circadian rhythms. *American Journal of Physiology* 276: R1195–R1202.
40. Weinert, D., Nevill, A., Weinandy, R., and Waterhouse, J. (2003). The development of new purification methods to assess the circadian rhythm of body temperature in Mongolian gerbils. *Chronobiology International* 20: 249–270.
41. Castillo, M. R., Hochstetler, K. J., Greene, D. M., Firmin, S. I., Tavernier, R. J., Raap, D. K., and Bult-Ito, A. (2005). Circadian rhythm of core body temperature in two laboratory mouse lines. *Physiology and Behavior* 86: 538–545.
42. Cohen, R. and Kronfeld-Schor, N. (2006). Individual variability and photic entrainment of circadian rhythms in golden spiny mice. *Physiology and Behavior* 87: 563–574.
43. Erkert, H. G. and Cramer, B. (2006). Chronobiological background to cathemerality: Circadian rhythms in *Eulemur fulvus albifrons* (Prosimii) and *Aotus azarai boliviensis* (Anthropoidea). *Folia Primatologica* 77: 87–103.
44. Hilmer, S., Algar, D., Plath, M., and Schleucher, E. (2010). Relationship between daily body temperature and activity patterns of free-ranging feral cats in Australia. *Journal of Thermal Biology* 35: 270–274.
45. Helwig, B. G., Ward, J. A., Blaha, M. D., and Leon, L. R. (2012). Effect of intraperitoneal radiotelemetry instrumentation on voluntary wheel running and surgical recovery in mice. *Journal of the American Association for Laboratory Animal Science* 51: 600–608.
46. Helwig, B. G., Ward, J. A., Blaha, M. D., and Leon, L. R. (2012). Effect of intraperitoneal radiotelemetry instrumentation on voluntary wheel running and surgical recovery in mice. *Journal of the American Association for Laboratory Animal Science* 51: 1–9.
47. Hoffmann, K., Coolen, A., Schlumbohm, C., Meerlo, P., and Fuchs, E. (2012). Remote long-term registrations of sleep–wake rhythms, core body temperature and activity in marmoset monkeys. *Behavioural Brain Research* 235: 113–123.
48. Studholme, K. M., Gompf, H. S., and Morin, L. P. (2013). Brief light stimulation during the mouse nocturnal activity phase simultaneously induces a decline in core temperature and locomotor activity followed by EEG-determined sleep. *American Journal of Physiology* 304: R459–R471.
49. Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep–wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
50. Refinetti, R. (1997). Phase relationship of the body temperature and locomotor activity rhythms in free-running and entrained rats. *Biological Rhythm Research* 28: 19–24.
51. Refinetti, R. (1999). Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species. *American Journal of Physiology* 277: R1493–R1500.
52. Eastman, C. and Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
53. Oshima, I., Yamada, H., Goto, M., Sato, K., and Ebihara, S. (1989). Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. *Journal of Comparative Physiology A* 166: 217–226.
54. Refinetti, R. and Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
55. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
56. Saltin, B., Gagge, A. P., and Stolwijk, J. A. J. (1970). Body temperature and sweat during thermal transients caused by exercise. *Journal of Applied Physiology* 28: 318–327.
57. Cabanac, M., Cunningham, D. J., and Stolwijk, J. A. J. (1971). Thermoregulatory set point during exercise: A behavioral approach. *Journal of Comparative and Physiological Psychology* 76: 94–102.
58. Webb, P. (1993). Daily activity and body temperature. *European Journal of Applied Physiology* 66: 174–177.
59. Deschenes, M. R., Kraemer, W. J., Bush, J. A., Doughty, T. A., Kim, D., Mullen, K. M., and Ramsey, K. (1998). Biorhythmic influences on functional capacity of human muscle and physiological responses. *Medicine and Science in Sports and Exercise* 30: 1399–1407.
60. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C., and Van Hoecke, J. (2001). Nycthemeral variations in core temperature and heart rate: Continuous cycling exercise versus continuous rest. *International Journal of Sports Medicine* 22: 553–557.
61. Aldemir, H., Atkinson, G., Cable, T., Edwards, B., Waterhouse, J., and Reilly, T. (2000). A comparison of the immediate effects of moderate exercise in the early morning and late afternoon on core temperature and cutaneous thermoregulatory mechanisms. *Chronobiology International* 17: 197–207.
62. Simpson, S. and Galbraith, J. J. (1906). Observations on the normal temperature of the monkey and its diurnal variation, and on the effect of changes in the daily routine on this variation. *Transactions of the Royal Society of Edinburgh* 45: 65–104.
63. Arnold, J., LeBlanc, J., Cote, J., Lalonde, J., and Richard, D. (1986). Exercise suppression of thermoregulatory thermogenesis in warm- and cold-acclimated rats. *Canadian Journal of Physiology and Pharmacology* 64: 922–926.

64. Yousef, M. K., Robertson, W. D., Dill, D. B., and Johnson, H. D. (1973). Energetic cost of running in the antelope ground squirrel *Ammospermophilus leucurus*. *Physiological Zoology* 46: 139–147.
65. Zerba, E. and Walsberg, G. E. (1992). Exercise-generated heat contributes to thermoregulation by Gambel's quail in the cold. *Journal of Experimental Biology* 171: 409–422.
66. Golombek, D. A., Ortega, G., and Cardinali, D. P. (1993). Wheel running raises body temperature and changes the daily cycle in golden hamsters. *Physiology and Behavior* 53: 1049–1054.
67. Riccio, A. P. and Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
68. Piccione, G., Caola, G., and Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
69. Marotte, H. and Timbal, J. (1981). Circadian rhythm of temperature in man: Comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
70. Gander, P. H., Connell, L. J., and Graeber, R. C. (1986). Masking of the circadian rhythm of heart rate and core temperature by the rest-activity cycle in man. *Journal of Biological Rhythms* 1: 119–135.
71. Golja, P., Eiken, O., Rodman, S., Sirok, B., and Mekjavic, I. B. (2002). Core temperature circadian rhythm during 35 days of horizontal bed rest. *Proceedings of the European Symposium on Life Science Research in Space* 8: 161–162.
72. Monk, T. H., Buysse, D. J., Reynolds, C. F., Kupfer, D. J., and Houck, P. R. (1996). Subjective alertness rhythms in elderly people. *Journal of Biological Rhythms* 11: 268–276.
73. Carrier, J. and Monk, T. H. (1997). Estimating the endogenous circadian temperature rhythm without keeping people awake. *Journal of Biological Rhythms* 12: 266–277.
74. Murray, G., Allen, N. B., and Trinder, J. (2002). Mood and the circadian system: Investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
75. Buysse, D. J., Monk, T. H., Carrier, J., and Begley, A. (2005). Circadian patterns of sleep, sleepiness, and performance in older and younger adults. *Sleep* 28: 1365–1376.
76. Bolles, R. C., Duncan, P. M., Grossen, N. E., and Matter, C. F. (1968). Relationship between activity level and body temperature in the rat. *Psychological Reports* 23: 991–994.
77. Refinetti, R. (1994). Contribution of locomotor activity to the generation of the daily rhythm of body temperature in golden hamsters. *Physiology and Behavior* 56: 829–831.
78. Gordon, C. J. and Yang, Y. (1997). Contribution of spontaneous motor activity to the 24 hour control of body temperature in male and female rats. *Journal of Thermal Biology* 22: 59–68.
79. Obál Jr., F., Rubicsek, G., Alföldi, P., Sáry, G., and Obál, F. (1985). Changes in the brain and core temperatures in relation to the various arousal states in rats in the light and dark periods of the day. *Pflügers Archiv* 404: 73–79.
80. Barrett, J., Lack, L., and Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
81. Kräuchi, K., Knoblauch, V., Wirz-Justice, A., and Cajochen, C. (2006). Challenging the sleep homeostat does not influence the thermoregulatory system in men: Evidence from a nap vs. sleep-deprivation study. *American Journal of Physiology* 290: R1052–R1061.
82. Shechter, A., Boudreau, P., Varin, F., and Boivin, D. B. (2011). Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. *Journal of Biological Rhythms* 26: 260–270.
83. Sarabia, J. A., Rol, M. A., Mendiola, P., and Madrid, J. A. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate for a new index of the circadian system. *Physiology and Behavior* 95: 570–580.
84. Heller, H. C., Graf, R., and Rautenberg, W. (1983). Circadian and arousal state influences on thermoregulation in the pigeon. *American Journal of Physiology* 245: R321–R328.
85. Abrams, R. and Hammel, H. T. (1964). Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping. *American Journal of Physiology* 206: 641–646.
86. Glotzbach, S. F. and Heller, H. C. (1976). Central nervous regulation of body temperature during sleep. *Science* 194: 537–539.
87. Glotzbach, S. F. and Heller, H. C. (1984). Changes in the thermal characteristics of hypothalamic neurons during sleep and wakefulness. *Brain Research* 309: 17–26.
88. Schmidek, W. R., Zachariassen, K. E., and Hammel, H. T. (1983). Total calorimetric measurements in the rat: Influences of the sleep-wakefulness cycle and of environmental temperature. *Brain Research* 288: 261–271.
89. Walker, J. M., Walker, L. E., Harris, D. V., and Berger, R. J. (1983). Cessation of thermoregulation during REM sleep. *American Journal of Physiology* 244: R114–R118.
90. Parmeggiani, P. L. and Rabini, C. (1967). Modificazioni delle fasi del sonno nel gatto per esposizione di breve durata a differenti temperature ambientali. *Bollettino della Società Italiana di Biologia Sperimentale* 43: 1079–1083.
91. Hendricks, J. C. (1982). Absence of shivering in the cat during paradoxical sleep without atonia. *Experimental Neurology* 75: 700–710.
92. Amoros, C., Sagot, J. C., Libert, J. P., and Candas, V. (1986). Sweat gland response to local heating during sleep in man. *Journal de Physiology* 81: 209–215.
93. Shapiro, C. M., Moore, A. T., Mitchell, D., and Yodaiken, M. L. (1974). How well does man thermoregulate during sleep? *Experientia* 30: 1279–1281.
94. Sagot, J. C., Amoros, C., Candas, V., and Libert, J. P. (1987). Sweating responses and body temperatures during nocturnal sleep in humans. *American Journal of Physiology* 252: R462–R470.
95. Muzet, A., Libert, J. P., and Candas, V. (1984). Ambient temperature and human sleep. *Experientia* 40: 425–429.
96. Dewasmes, G., Bothorel, B., Candas, V., and Libert, J. P. (1997). A short-term poikilothermic period occurs just after paradoxical sleep onset in humans: Characterization changes in sweating effector activity. *Journal of Sleep Research* 6: 252–258.
97. Tayefeh, F., Plattnet, O., Sessler, D. I., Ikeda, T., and Marder, D. (1998). Circadian changes in the sweating-to-vasoconstriction interthreshold range. *Pflügers Archiv* 435: 402–406.
98. Dijk, D. J., Cajochen, C., and Borbély, A. A. (1991). Effect of a single 3-hour exposure to bright light on core body temperature and sleep in humans. *Neuroscience Letters* 121: 59–62.
99. Myers, B. L. and Badia, P. (1993). Immediate effects of different light intensities on body temperature and alertness. *Physiology and Behavior* 54: 199–202.
100. Cagnacci, A., Soldani, R., and Yen, S. S. C. (1993). The effect of light on core body temperature is mediated by melatonin in women. *Journal of Clinical Endocrinology and Metabolism* 76: 1036–1038.
101. Lavoie, S., Paquet, J., Selmaoui, B., Rufiange, M., and Dumont, M. (2003). Vigilance levels during and after bright light exposure in the first half of the night. *Chronobiology International* 20: 1019–1038.

102. Rüger, M., Gordijn, M. C. M., Beersma, D. G. M., de Vries, B., and Daan, S. (2003). Acute and phase-shifting effects of ocular and extraocular light in human circadian physiology. *Journal of Biological Rhythms* 18: 409–419.
103. Ingram, D. L. and Legge, K. F. (1970). Variations in deep body temperature in the young unrestrained pig over the 24 hour period. *Journal of Physiology* 210: 989–998.
104. Wilson, H. R., Mather, F. B., Brigmon, R. L., Besch, E. L., Dugan, V. P., and Boulos, N. Z. (1989). Feeding time and body temperature interactions in broiler breeders. *Poultry Science* 68: 608–616.
105. Mohr, E. G. and Krzywanek, H. (1995). Endogenous oscillator and regulatory mechanisms of body temperature in sheep. *Physiology and Behavior* 57: 339–347.
106. Miyazaki, H., Yoshida, M., Samura, K., Matsumoto, H., Ikemoto, F., and Tagawa, M. (2002). Ranges of diurnal variation and the pattern of body temperature, blood pressure and heart rate in laboratory Beagle dogs. *Experimental Animals* 51: 95–98.
107. Refinetti, R. and Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.
108. Piccione, G., Caola, G., and Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
109. Bolles, R. C. and Duncan, P. M. (1969). Daily course of activity and subcutaneous body temperature in hungry and thirsty rats. *Physiology and Behavior* 4: 87–89.
110. Ingram, D. L. and Mount, L. E. (1973). The effects of food intake and fasting on 24-hourly variations in body temperature in the young pig. *Pflügers Archiv* 339: 299–304.
111. Fowler, P. A. and Racey, P. A. (1990). Daily and seasonal cycles of body temperature and aspects of heterothermy in the hedgehog *Erinaceus europaeus*. *Journal of Comparative Physiology B* 160: 299–307.
112. Hohtola, E., Hissa, R., Pyörnilä, A., Rintamäki, H., and Saarela, S. (1991). Nocturnal hypothermia in fasting Japanese quail: The effect of ambient temperature. *Physiology and Behavior* 49: 563–567.
113. Shido, O., Sakurada, S., Kohda, W., and Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
114. Rashotte, M. E., Pastukhov, I. F., Poliakov, E. L., and Henderson, R. P. (1998). Vigilance states and body temperature during the circadian cycle in fed and fasted pigeons (*Columbia livia*). *American Journal of Physiology* 275: R1690–R1702.
115. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T., and Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *Journal of Physiology* 526: 417–424.
116. Piccione, G., Caola, G., and Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
117. Piccione, G., Bertolucci, C., Foà, A., and Caola, G. (2004). Influence of fasting and exercise on the daily rhythm of serum leptin in the horse. *Chronobiology International* 21: 405–417.
118. Kalsbeek, A., Fliers, E., Romijn, J. A., la Fleur, S. E., Wortel, J., Bakker, O., Endert, E., and Buijs, R. M. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685.
119. Piccione, G., Caola, G., and Refinetti, R. (2003). Circadian rhythms of body temperature and liver function in fed and food-deprived goats. *Comparative Biochemistry and Physiology A* 134: 563–572.
120. Piccione, G., Fazio, F., Caola, G., and Refinetti, R. (2008). Daily rhythmicity of glycemia in four species of domestic animals under various feeding regimes. *Journal of Physiological Sciences* 58: 271–275.
121. Gordon, M. E. and McKeever, K. H. (2005). Diurnal variation of ghrelin, leptin, and adiponectin in Standardbred mares. *Journal of Animal Science* 83: 2365–2371.
122. Kerkhof, G. A., Van Dongen, H. P. A., and Bobbert, A. C. (1998). Absence of endogenous circadian rhythmicity in blood pressure? *American Journal of Hypertension* 11: 373–377.
123. Scheer, F. A. J. L., van Doornen, L. J. P., and Buijs, R. M. (1999). Light and diurnal cycle affect human heart rate: Possible role for the circadian pacemaker. *Journal of Biological Rhythms* 14: 202–212.
124. Viola, A. U., Simon, C., Ehrhart, J., Geny, B., Piquard, F., Muzet, A., and Brandenberger, G. (2002). Sleep processes exert a predominant influence on the 24-h profile of heart rate variability. *Journal of Biological Rhythms* 17: 539–547.
125. Hu, K., Ivanov, P. C., Hilton, M. F., Chen, Z., Ayers, R. T., Stanley, H. E., and Shea, S. A. (2004). Endogenous circadian rhythm in an index of cardiac vulnerability independent of changes in behavior. *Proceedings of the National Academy of Sciences United States of America* 101: 18223–18227.
126. Lovegrove, B. G., Heldmaier, G., and Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
127. Lovegrove, B. G. and Papenfus, M. E. (1995). Circadian activity rhythms in the solitary Cape molerat (*Georychus capensis*: Bathyergidae) with some evidence of splitting. *Physiology and Behavior* 58: 679–685.
128. Goldman, B. D., Goldman, S. L., Riccio, A. P., and Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
129. Begall, S., Daan, S., Burda, H., and Overkamp, G. J. F. (2002). Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83: 153–158.
130. Oosthuizen, M. K., Cooper, H. M., and Bennett, N. C. (2003). Circadian rhythms of locomotor activity in solitary and social species of African mole-rats (Family: Bathyergidae). *Journal of Biological Rhythms* 18: 481–490.
131. Schöttner, K., Oosthuizen, M. K., Broekman, M., and Bennett, N. C. (2006). Circadian rhythms of locomotor activity in the Lesotho mole-rat, *Cryptomys hottentotus* subspecies from Sani Pass, South Africa. *Physiology and Behavior* 89: 205–212.
132. Meyerowitz, E. M. (2002). Plants compared to animals: The broadest comparative study of development. *Science* 295: 1482–1485.
133. Jacobsen, S. B. (2003). How old is planet Earth? *Science* 300: 1513–1514.
134. Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science* 284: 2124–2128.
135. Ciccarelli, F. D., Doerks, T., von Mering, C., Creevey, C. J., Snel, B., and Bork, P. (2006). Toward automatic reconstruction of a highly resolved tree of life. *Science* 311: 1283–1287.
136. Rogers, L. A. and Greenbank, G. R. (1930). The intermittent growth of bacterial cultures. *Journal of Bacteriology* 19: 181–190.
137. Halberg, F. and Conner, R. L. (1961). Circadian organization and microbiology: Variance spectra and periodogram on behavior of Escherichia coli growing in fluid culture. *Proceedings of the Minnesota Academy of Science* 29: 227–239.

138. Kondo, T., Mori, T., Lebedeva, N. V., Aoki, S., Ishiura, M., and Golden, S. S. (1997). Circadian rhythms in rapidly dividing cyanobacteria. *Science* 275: 224–227.
139. Ouyang, Y., Andersson, C. R., Kondo, T., Golden, S. S., and Johnson, C. H. (1998). Resonating circadian clocks enhance fitness in cyanobacteria. *Proceedings of the National Academy of Sciences United States of America* 95: 8660–8664.
140. Dvomyk, V., Vinogradova, O., and Nevo, E. (2003). Origin and evolution of circadian clock genes in prokaryotes. *Proceedings of the National Academy of Sciences United States of America* 100: 2495–2500.
141. Brown, S. A., Kowalska, E., and Dallmann, R. (2012). (Re) inventing the circadian feedback loop. *Developmental Cell* 22: 477–487.
142. Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M., and Allada, R. (2002). A role for casein kinase 2 α in the *Drosophila* circadian clock. *Nature* 420: 816–820.
143. Van Gelder, R. N., Herzog, E. D., Schwartz, W. J., and Taghert, P. H. (2003). Circadian rhythms: In the loop at last. *Science* 300: 1534–1535.
144. Chang, D. C., McWatters, H. G., Williams, J. A., Gotter, A. L., Levine, J. D., and Reppert, S. M. (2003). Constructing a feedback loop with circadian clock molecules from the silkmoth, *Antheraea pernyi*. *Journal of Biological Chemistry* 278: 38149–38158.
145. Constance, C. M., Green, C. B., Tei, H., and Block, G. D. (2002). *Bulla gouldiana period* exhibits unique regulation at the mRNA and protein levels. *Journal of Biological Rhythms* 17: 413–427.
146. Edgar, R. S., Green, E. W., Zhao, Y., van Ooijen, G., Olmedo, M., Maywood, E. S., Hastings, M. H., Baliga, N. S. et al. (2012). Peroxiredoxins are conserved markers of circadian rhythms. *Nature* 485: 459–464.
147. Barnett, J. E. (1998). *Time's Pendulum: From Sundials to Atomic Clocks*. New York: Plenum.
148. Murphy, W. J., Eizirik, E., O'Brien, S. J., Madsen, O., Scally, M., Douady, C. J., Teeling, E. et al. (2001). Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 294: 2348–2351.
149. Falkowski, P. G., Katz, M. E., Milligan, A. J., Fennel, K., Cramer, B. S., Aubry, M. P., Berner, R. A., Novacek, M. J., and Zapol, W. M. (2005). The rise of oxygen over the past 205 million years and the evolution of large placental mammals. *Science* 309: 2202–2204.
150. Rokas, A., Krüger, D., and Carroll, S. B. (2005). Animal evolution and the molecular signature of radiations compressed in time. *Science* 310: 1933–1938.
151. Stringer, C. (2002). Modern human origins: Progress and prospects. *Philosophical Transactions of the Royal Society of London B* 357: 563–579.
152. Macaulay, V., Hill, C., Achilli, A., Rengo, C., Clarke, D., Meehan, W., Blackburn, J., Semino, O. et al. (2005). Single, rapid coastal settlement of Asia revealed by analysis of complete mitochondrial genomes. *Science* 308: 1034–1036.
153. Balter, M. (2011). Was North Africa the launch pad for modern human migrations? *Science* 331: 20–23.
154. Poznik, G. D., Henn, B. M., Yee, M. C., Sliwerska, E., Euskirchen, G. M., Lin, A. A., Snyder, M. et al. (2013). Sequencing Y chromosomes resolves discrepancy in time to common ancestor of males versus females. *Science* 341: 562–565.
155. Torsvik, T. H. (2003). The Rodinia jigsaw puzzle. *Science* 300: 1379–1381.
156. Halberg, F. (1953). Some physiological and clinical aspects of 24-hour periodicity. *Journal-Lancet* 73: 20–32.
157. Pittendrigh, C. S. (1967). Circadian rhythms, space research and manned space flight. *Life Sciences and Space Research* 5: 122–134.
158. Engelmann, W. (1988). Evolution and selective advantage of circadian rhythms. *Acta Physiologica Polonica* 39: 345–356.
159. Marques, M. D. and Waterhouse, J. M. (1994). Masking and the evolution of circadian rhythmicity. *Chronobiology International* 11: 146–155.
160. Roenneberg, T. and Merrow, M. (2002). Life before the clock: Modeling circadian evolution. *Journal of Biological Rhythms* 17: 495–505.
161. Rutter, J., Reick, M., and McKnight, S. L. (2002). Metabolism and the control of circadian rhythms. *Annual Review of Biochemistry* 71: 307–331.
162. Green, R. M., Tingay, S., Wang, Z. Y., and Tobin, E. M. (2002). Circadian rhythms confer a higher level of fitness to Arabidopsis plants. *Plant Physiology* 129: 576–584.
163. Michael, T. P., Salomé, P. A., Yu, H. J., Spencer, T. R., Sharp, E. L., McPeek, M. A., Alonso, J. M., Ecker, J. R., and McClung, C. R. (2003). Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* 302: 1049–1053.
164. Filipski, E., King, V. M., Li, X. M., Granda, T. G., Mormont, M. C., Liu, X. H., Claustrat, B., Hastings, M. H., and Lévi, F. (2002). Host circadian clock as a control point in tumor progression. *Journal of the National Cancer Institute* 94: 690–697.
165. Beaver, L. M., Gvakharia, B. O., Vollintine, T. S., Hege, D. M., Stanewsky, R., and Giebultowicz, J. M. (2002). Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences United States of America* 99: 2134–2139.
166. DeCoursey, P. J., Krulas, J. R., Mele, G., and Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior* 62: 1099–1108.
167. DeCoursey, P. J., Walker, J. K., and Smith, S. A. (2000). A circadian pacemaker in free-living chipmunks: Essential for survival? *Journal of Comparative Physiology A* 186: 169–180.
168. Woelfle, M. A., Ouyang, Y., Phanvijhitsiri, K., and Johnson, C. H. (2004). The adaptive value of circadian clocks: An experimental assessment in cyanobacteria. *Current Biology* 14: 1481–1486.
169. Field, K. G., Olsen, G. J., Lane, D. J., Giovannoni, S. J., Ghiselin, M. T., Raff, E. C., Pace, N. R., and Raff, R. A. (1988). Molecular phylogeny of the animal kingdom. *Science* 239: 748–753.
170. Doolittle, R. F., Feng, D. F., Tsang, S., Cho, G., and Little, E. (1996). Determining divergence times of the major kingdoms of living organisms with a protein clock. *Science* 271: 470–477.
171. Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L., and Hedges, S. B. (2001). Molecular evidence for the early colonization of land by fungi and plants. *Science* 293: 1129–1133.
172. Jablonski, D., Roy, K., and Valentine, J. W. (2006). Out of the tropics: Evolutionary dynamics of the latitudinal diversity gradient. *Science* 314: 102–106.
173. Benton, M. J. and Ayala, F. J. (2003). Dating the tree of life. *Science* 300: 1698–1700.
174. Bowen, G. J., Clyde, W. C., Koch, P. L., Ting, S., Alroy, J., Tsubamoto, T., Wang, Y., and Wang, Y. (2002). Mammalian dispersal at the Paleocene/Eocene boundary. *Science* 295: 2062–2065.

175. Bakker, R. T. (1972). Anatomical and ecological evidence of endothermy in dinosaurs. *Nature* 238: 81–85.
176. Barrick, R. E. and Showers, W. J. (1994). Thermophysiology of *Tyrannosaurus rex*: Evidence from oxygen isotopes. *Science* 265: 222–224.
177. Eagle, R. A., Tütken, T., Martin, T. S., Tripati, A. K., Fricke, H. C., Connely, M., Cifelli, R. L., and Eiler, J. M. (2011). Dinosaur body temperatures determined from isotopic (^{13}C-^{18}O) ordering in fossil biominerals. *Science* 333: 443–445.
178. Bernard, A., Lécuyer, C., Vincent, P., Amiot, R., Bardet, N., Buffetaut, E., Cuny, G. et al. (2010). Regulation of body temperature by some Mesozoic marine reptiles. *Science* 328: 13791382.
179. Thulborn, R. A. (1973). Thermoregulation in dinosaurs. *Nature* 245: 51–52.
180. Paladino, F. V., O'Connor, M. P., and Spotila, J. R. (1990). Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* 344: 858–860.
181. Ruben, J. A., Hillenius, W. J., Geist, N. R., Leitch, A., Jones, T. D., Currie, P. J., Horner, J. R., and Espe, G. (1996). The metabolic status of some late Cretaceous dinosaurs. *Science* 273: 1204–1207.
182. Dawson, T. J. (1972). Primitive mammals and patterns in the evolution of thermoregulation. In: Bligh, J. and Moore, R. E. (Eds.), *Essays on Temperature Regulation*. Amsterdam, the Netherlands: North-Holland, pp. 1–18.
183. Hillenius, W. J. and Ruben, J. A. (2004). The evolution of endothermy in terrestrial vertebrates: Who? When? Why? *Physiological and Biochemical Zoology* 77: 1019–1042.
184. Lovegrove, B. G. (2012). The evolution of endothermy in Cenozoic mammals: A plesiomorphic-apomorphic continuum. *Biological Reviews* 87: 128–162.
185. Hammel, H. T. (1983). Phylogeny of regulatory mechanisms in temperature regulation. *Journal of Thermal Biology* 8: 37–42.
186. Crowley, T. J., Short, D. A., Mengel, J. G., and North, G. R. (1986). Role of seasonality in the evolution of climate during the last 100 million years. *Science* 231: 579–584.
187. Crompton, A. W., Taylor, C. R., and Jagger, J. A. (1978). Evolution of homeothermy in mammals. *Nature* 272: 333–336.
188. May, R. M. (1988). How many species are there on Earth? *Science* 241: 1441–1449.
189. Costello, M. J., May, R. M., and Stork, N. E. (2013). Can we name Earth's species before they go extinct? *Science* 339: 413–416.
190. Sarnat, H. B. and Netsky, M. G. (1981). *Evolution of the Nervous System*, 2nd edn. New York: Oxford University Press.
191. Amend, J. P. and Shock, E. L. (1998). Energetics of amino acid synthesis in hydrothermal ecosystems. *Science* 281: 1659–1662.
192. Cody, G. D., Boctor, N. Z., Filley, T. R., Hazen, R. M., Scott, J. H., Sharma, A., and Yoder, H. S. (2000). Primordial carbonylated iron-sulfur compounds and the synthesis of pyruvate. *Science* 289: 1337–1340.
193. Martin, W. F., Sousa, F. L., and Lane, N. (2014). Energy at life's origin. *Science* 344: 1092–1093.
194. Gehring, W. and Rosbash, M. (2003). The coevolution of blue-light photoreception and circadian rhythms. *Journal of Molecular Evolution* 57: S286–S289.
195. Levy, O., Appelbaum, L., Leggat, W., Gothlif, Y., Hayward, D. C., Miller, D. J., and Hoegh-Guldberg, O. (2007). Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. *Science* 318: 467–470.
196. Cloudsley-Thompson, J. L. (1960). Adaptive functions of circadian rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 345–355.
197. Daan, S. (1981). Adaptive daily strategies in behavior. In: Aschoff, J. (Ed.), *Biological Rhythms* (*Handbook of Behavioral Neurobiology*, Vol. 4). New York: Plenum, pp. 275–298.
198. Gay, F., Valiante, S., Sciarrillo, R., de Falco, M., Laforgia, V., and Capaldo, A. (2010). Annual and daily serum aldosterone and catecholamine patterns in males of the Italian crested newt, *Triturus carnifex* (Amphibia, Urodela). *Italian Journal of Zoology* 77: 384–390.
199. Refinetti, R. (2008). The diversity of temporal niches in mammals. *Biological Rhythm Research* 39: 173–192.
200. Reebs, S. G. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries* 12: 349–371.
201. Ankel-Simons, F. and Rasmussen, D. T. (2008). Diurnality, nocturnality, and the evolution of primate visual systems. *Yearbook of Physical Anthropology* 51: 100–117.
202. Tattersall, I. (2006). The concept of cathemerality: History and definition. *Folia Primatologica* 77: 7–14.
203. Curtis, D. J. and Rasmussen, M. A. (2006). The evolution of cathemerality in primates and other mammals: A comparative and chronoecological approach. *Folia Primatologica* 77: 178–193.
204. Aschoff, J. (1966). Circadian activity pattern with two peaks. *Ecology* 47: 657–662.
205. Kas, M. J. H. and Edgar, D. M. (1998). Crepuscular rhythms of EEG sleep–wake in a hystricomorph rodent, *Octodon degus*. *Journal of Biological Rhythms* 13: 9–17.
206. Verhagen, L. A. W., Pévet, P., Saboureau, M., Sicard, B., Nesme, B., Claustrat, B., Buijs, R. M., and Kalsbeek, A. (2004). Temporal organization of the 24-h corticosterone rhythm in the diurnal murid rodent *Arvicanthis ansorgei* Thomas 1910. *Brain Research* 995: 197–204.
207. Kurumiya, S. and Kawamura, H. (1988). Circadian oscillation of the multiple unit activity in the guinea pig suprachiasmatic nucleus. *Journal of Comparative Physiology A* 162: 301–308.
208. Iigo, M. and Tabata, M. (1996). Circadian rhythms of locomotor activity in the goldfish *Carassius auratus*. *Physiology and Behavior* 60: 775–781.
209. Sánchez-Vázquez, F. J., Madrid, J. A., and Zamora, S. (1995). Circadian rhythms of feeding activity in sea bass, *Dicentrarchus labrax* L.: Dual phasing capacity of diel demand-feeding pattern. *Journal of Biological Rhythms* 10: 256–266.
210. Sharma, V. K., Lone, S. R., Mathew, D., Goel, A., and Chandrashekaran, M. K. (2004). Possible evidence for shift work schedules in the media workers of the ant species *Camponotus compressus*. *Chronobiology International* 21: 297–308.
211. Refinetti, R. (2006). Variability of diurnality in laboratory rodents. *Journal of Comparative Physiology A* 192: 701–714.
212. Oster, H., Avivi, A., Joel, A., Albrecht, U., and Nevo, E. (2002). A switch from diurnal to nocturnal activity in *S. ehrenbergi* is accompanied by an uncoupling of light input and the circadian clock. *Current Biology* 12: 1919–1922.
213. Merrill, S. B. and Mech, L. D. (2003). The usefulness of GPS telemetry to study wolf circadian and social activity. *Wildlife Society Bulletin* 31: 947–960.
214. Rattenborg, N. C., Mandt, B. H., Obermeyer, W. H., Winsauer, P. J., Huber, R., Wikelski, M., and Benca, R. M. (2004). Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLoS Biology* 2: 924–936.

215. Cochran, W. W. (1987). Orientation and other migratory behaviours of a Swainson's thrush followed for 1500 km. *Animal Behaviour* 35: 927–928.
216. Mukhin, A., Kosarev, V., and Ktitorov, P. (2005). Nocturnal life of young songbirds well before migration. *Proceedings of the Royal Society B* 272: 1535–1539.
217. Kenagy, G. J., Nespolo, R. F., Vásquez, R. A., and Bozinovic, F. (2002). Daily and seasonal limits of time and temperature to activity of degus. *Revista Chilena de Historia Natural* 75: 567–581.
218. Kas, M. J. H. and Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal–nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.
219. Ocampo-Garcés, A., Hernández, F., Mena, W., and Palacios, A. G. (2005). Wheel-running and rest activity pattern interaction in two octodontids (*Octodon degus*, *Octodon bridgesi*). *Biological Research* 38: 299–305.
220. Blanchong, J. A., McElhinny, T. L., Mahoney, M. M., and Smale, L. (1999). Nocturnal and diurnal rhythms in the unstriped Nile rate, *Arvicanthis niloticus*. *Journal of Biological Rhythms* 14: 364–377.
221. Schwartz, M. D. and Smale, L. (2005). Individual differences in rhythms of behavioral sleep and its neural substrates in Nile grass rats. *Journal of Biological Rhythms* 20: 526–537.
222. Refinetti, R. (2004). Parameters of photic resetting of the circadian system of a diurnal rodent, the Nile grass rat. *Acta Scientiae Veterinariae* 32: 1–6.
223. Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. *Physiology and Behavior* 82: 285–294.
224. Redlin, U. and Mrosovsky, N. (2004). Nocturnal activity in a diurnal rodent (*Arvicanthis niloticus*): The importance of masking. *Journal of Biological Rhythms* 19: 58–67.
225. Johnston, P. G. and Zucker, I. (1983). Lability and diversity of circadian rhythms of cotton rats, *Sigmodon hispidus*. *American Journal of Physiology* 244: R338–RR346.
226. Rieger, D., Fraunholz, C., Popp, J., Bichler, D., Dittmann, R., and Helfrich-Förster, C. (2007). The fruit fly *Drosophila melanogaster* favors dim light and times its activity peaks to early dawn and late dusk. *Journal of Biological Rhythms* 22: 387–399.
227. Chiesa, J. J., Aguzzi, J., García, J. A., Sardà, F., and de la Iglesia, H. O. (2010). Light intensity determines temporal niche switching of behavioral activity in deep-water *Nephrops norvegicus* (Crustacea: Decapoda). *Journal of Biological Rhythms* 25: 277–287.
228. Tan, C. L., Yang, Y., and Niu, K. (2013). Into the night: Camera traps reveal nocturnal activity in a presumptive diurnal primate, *Rhinopithecus brelichi*. *Primates* 54: 1–6.
229. Shkolnik, A. (1971). Diurnal activity in a small desert rodent. *International Journal of Biometeorology* 15: 115–120.
230. Kronfeld-Schor, N., Dayan, T., Elvert, R., Haim, A., Zisapel, N., and Heldmaier, G. (2001). On the use of the time axis for ecological separation: Diel rhythms as an evolutionary constraint. *American Naturalist* 158: 451–457.
231. Cohen, R., Smale, L., and Kronfeld-Schor, N. (2010). Masking and temporal niche switches in spiny mice. *Journal of Biological Rhythms* 25: 47–52.
232. Fenn, M. G. P. and Macdonald, D. W. (1995). Use of middens by red foxes: Risk reverses rhythms of rats. *Journal of Mammalogy* 76: 130–136.
233. Gattermann, R., Johnston, R. E., Yigit, N., Fritzsche, P., Larimer, S., Özkurt, S., Neumann, K. et al. (2008). Golden hamsters are nocturnal in captivity but diurnal in nature. *Biology Letters* 4: 253–255.
234. Cozzi, G., Broekhuis, F., McNutt, J. W., Turnbull, L. A., Macdonald, D. W., and Schmid, B. (2012). Fear of the dark or dinner by moonlight? Reduced temporal partitioning among Africa's large carnivores. *Ecology* 93: 2590–2599.
235. Kirk, E. C. (2006). Effects of activity pattern on eye size and orbital aperture size in primates. *Journal of Human Evolution* 51: 159–170.
236. Bobu, C., Lahmam, M., Vuillez, P., Ouarour, A., and Hicks, D. (2008). Photoreceptor organisation and phenotypic characterization in retinas of two diurnal rodent species: Potential use as experimental animal models for human vision research. *Vision Research* 48: 424–432.
237. Ringvold, A. and Reubsaet, J. L. E. (2005). Acetylcholine in the corneal epithelium of diurnal and nocturnal mammals. *Cornea* 24: 1000–1003.
238. Ringvold, A., Anderssen, E., and Kjønniksen, I. (1998). Ascorbate in the corneal epithelium of diurnal and nocturnal species. *Investigative Ophtalmology and Visual Science* 39: 2774–2777.
239. Aschoff, J. (1979). Circadian rhythms: Influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
240. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
241. Lincoln, G., Messager, S., Andersson, H., and Hazlerigg, D. (2002). Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: Evidence for an internal coincidence timer. *Proceedings of the National Academy of Sciences United States of America* 99: 13890–13895.
242. Fidler, A. E. and Gwinner, E. (2003). Comparative analysis of avian BMAL1 and CLOCK protein sequences: A search for features associated with owl nocturnal behavior. *Comparative Biochemistry and Physiology B* 136: 861–874.
243. Mrosovsky, N. (2003). Beyond the suprachiasmatic nucleus. *Chronobiology International* 20: 1–8.
244. Dardente, H., Menet, J. S., Challet, E., Tournier, B. B., Pévet, P., and Masson-Pévet, M. (2004). Daily and circadian expression of neuropeptides in the suprachiasmatic nuclei of nocturnal and diurnal rodents. *Molecular Brain Research* 124: 143–151.
245. Smale, L., Lee, T., and Nunez, A. A. (2003). Mammalian diurnality: Some facts and gaps. *Journal of Biological Rhythms* 18: 356–366.
246. Nunez, A. A., Bult, A., McElhinny, T. L., and Smale, L. (1999). Daily rhythms of Fos expression in hypothalamic targets of the suprachiasmatic nucleus in diurnal and nocturnal rodents. *Journal of Biological Rhythms* 14: 300–306.
247. Novak, C. M., Harris, J. A., Smale, L., and Nunez, A. A. (2000). Suprachiasmatic nucleus projections to the paraventricular thalamic nucleus in nocturnal rats (*Rattus norvegicus*) and diurnal Nile grass rats (*Arvicanthis niloticus*). *Brain Research* 874: 147–157.
248. Schwartz, M. D., Nunez, A. A., and Smale, L. (2004). Differences in the suprachiasmatic nucleus and lower subparaventricular zone of diurnal and nocturnal rodents. *Neuroscience* 127: 13–23.
249. Mrosovsky, N. and Hattar, S. (2005). Diurnal mice (*Mus musculus*) and other examples of temporal niche switching. *Journal of Comparative Physiology A* 191: 1011–1024.
250. Jiang, P., Franklin, K. M., Duncan, M. J., O'Hara, B. F., and Wisor, J. P. (2012). Distinct phase relationships between suprachiasmatic molecular rhythms, cerebral cortex molecular rhythms, and behavioral rhythms in early runner (CAST/EiJ) and nocturnal (C57BL/6J) mice. *Sleep* 35: 1385–1394.

251. Kas, M. J. H. and Edgar, D. M. (2000). Photic phase response curve in *Octodon degus*: Assessment as a function of activity phase preference. *American Journal of Physiology* 278: R1385–R1389.
252. Mahoney, M., Bult, A., and Smale, L. (2001). Phase response curve and light-induced Fos expression in suprachiasmatic nucleus and adjacent hypothalamus of *Arvicanthis niloticus*. *Journal of Biological Rhythms* 16: 149–162.
253. Hut, R. A., Mrosovsky, N., and Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
254. Glass, J. D., Tardif, S. D., Clements, R., and Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
255. Novak, C. M. and Albers, H. E. (2004). Novel phase-shifting effects of $GABA_A$ receptor activation in the suprachiasmatic nucleus of a diurnal rodent. *American Journal of Physiology* 286: R820–R825.
256. Novak, C. M. and Albers, H. E. (2004). Circadian phase alteration by GABA and light differs in diurnal and nocturnal rodents during the day. *Behavioral Neuroscience* 118: 498–504.
257. Chakir, I., Dumont, S., Pévet, P., Ouarour, A., Challet, E., and Vuillez, P. (2015). The circadian gene *Clock* oscillates in the suprachiasmatic nuclei of the diurnal rodent Barbary striped grass mouse, *Lemniscomys barbarus*: A general feature of diurnality? *Brain Research* 1594: 165–172.
258. Savides, T. J., Messin, S., Senger, C., and Kripke, D. F. (1986). Natural light exposure of young adults. *Physiology and Behavior* 38: 571–574.
259. Cole, R. J., Kripke, D. F., Wisbey, J., Mason, W. J., Gruen, W., Hauri, P. J., and Juarez, S. (1995). Seasonal variation in human illumination exposure at two different latitudes. *Journal of Biological Rhythms* 10: 324–334.
260. Hébert, M., Dumont, M., and Paquet, J. (1998). Seasonal and diurnal patterns of human illumination under natural conditions. *Chronobiology International* 15: 59–70.
261. Roenneberg, T., Wirz-Justice, A., and Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
262. Roenneberg, T., Allebrandt, K. V., Merrow, M., and Vetter, C. (2012). Social jet lag and obesity. *Current Biology* 22: 939–943.
263. Pratt, B. L. and Goldman, B. D. (1986). Activity rhythms and photoperiodism of Syrian hamsters in a simulated burrow system. *Physiology and Behavior* 36: 83–89.
264. Boulos, Z., Macchi, M., Houpt, T. A., and Terman, M. (1996). Photic entrainment in hamsters: Effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
265. Boulos, Z., Macchi, M., and Terman, M. (1996). Twilight transitions promote circadian entrainment to lengthening light-dark cycles. *American Journal of Physiology* 271: R813–R818.
266. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
267. Marimuthu, G., Rajan, S., and Chandrashekaran, M. K. (1981). Social entrainment of the circadian rhythm in the flight activity of the microchiropteran bat *Hipposideros speoris*. *Behavioral Ecology and Sociobiology* 8: 147–150.
268. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1996). Daily restricted feeding effects on the circadian activity rhythms of the stripe-faced dunnart, *Sminthopsis macroura*. *Journal of Biological Rhythms* 11: 188–195.
269. Hut, R. A., van Oort, B. E. H., and Daan, S. (1999). Natural entrainment without dawn and dusk: The case of the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 290–299.
270. Everts, L. G., Strijkstra, A. M., Hut, R. A., Hoffmann, I. E., and Millesi, E. (2004). Seasonal variation in daily activity patterns of free-ranging European ground squirrels (*Spermophilus citellus*). *Chronobiology International* 21: 57–71.
271. Blanchong, J. A. and Smale, L. (2000). Temporal patterns of activity of the unstriped Nile rat, *Arvicanthis niloticus*. *Journal of Mammalogy* 81: 595–599.
272. Usui, S., Takahashi, Y., and Okazaki, T. (2000). Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. *American Journal of Physiology* 278: R1148–R1156.
273. Boulos, Z., Macchi, M. M., and Terman, M. (2002). Twilights widen the range of photic entrainment in hamsters. *Journal of Biological Rhythms* 17: 353–363.
274. Thor, D. H. and Hoats, D. L. (1968). A circadian variable in self-exposure to light by the rat. *Psychonomic Science* 12: 1–2.
275. Fishman, R. and Roffwarg, H. P. (1970). Free choice of lighting and sleep-waking behavior in the laboratory rat. *Psychophysiology* 7: 304–305.
276. Refinetti, R. (1997). Circadian rhythm of self-exposure to light in the golden hamster. *Behavioural Processes* 40: 107–111.
277. Sevenster, P., Feuth de Bruijn, E., and Huisman, J. J. (1995). Temporal structure in stickleback behaviour. *Behaviour* 132: 1267–1284.
278. Czeisler, C. A., Duffy, J. F, Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M. et al. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177–2181.
279. Krebs, J. R. and Davies, N. B. (1993). *An Introduction to Behavioural Ecology*, 3rd edn. London, U.K.: Blackwell Scientific.
280. Adair, E. R. and Wright, B. A. (1976). Behavioral thermoregulation in the squirrel monkey when response effort is varied. *Journal of Comparative and Physiological Psychology* 90: 179–184.
281. Refinetti, R. (1982). Custo da resposta termoregulatória de pressão à barra. *Ciência e Cultura* 34(S): 896–897.
282. Refinetti, R. and Carlisle, H. J. (1986). Complementary nature of heat production and heat intake during behavioral thermoregulation in the rat. *Behavioral and Neural Biology* 46: 64–70.
283. Rashotte, M. E. and Henderson, D. (1988). Coping with rising food costs in a closed economy: Feeding behavior and nocturnal hypothermia in pigeons. *Journal of the Experimental Analysis of Behavior* 50: 441–456.
284. Collier, G. H., Johnson, D. F., Naveira, J., and Cybulski, K. A. (1989). Ambient temperature and food costs: Effects on behavior patterns in rats. *American Journal of Physiology* 257: R1328–R1334.
285. Sherwin, C. M. and Nicol, C. J. (1996). Reorganization of behaviour in laboratory mice, *Mus musculus*, with varying cost of access to resources. *Animal Behaviour* 51: 1087–1093.
286. Sullivan, C. M. and Fisher, K. C. (1954). The effects of light on temperature selection in speckled trout *Salvelinus fontinalis* (Mitchill). *Biological Bulletin* 107: 278–288.
287. Refinetti, R. (1984). Behavioral temperature regulation in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 47: 29–43.
288. Rautenberg, W., May, B., and Arabin, G. (1980). Behavioral and autonomic temperature regulation in competition with food intake and water balance of pigeons. *Pflügers Archiv* 384: 253–260.

289. Munson, B. H., McCormick, J. H., and Collins, H. L. (1980). Influence of thermal challenge on conditioned feeding forays of juvenile rainbow trout. *Transactions of the American Fisheries Society* 109: 116–121.
290. Cabanac, M. and Johnson, K. G. (1983). Analysis of a conflict between palatability and cold exposure in rats. *Physiology and Behavior* 31: 249–253.
291. Refinetti, R. (1983). Conflito entre alimentação e termorregulação no peixinho dourado. *Ciência e Cultura* 35(S): 820.
292. Balasko, M. and Cabanac, M. (1998). Behavior of juvenile lizards (*Iguana iguana*) in a conflict between temperature regulation and palatable food. *Brain, Behavior and Evolution* 52: 257–262.
293. Reebs, S. G. and Maillet, D. (2003). Effect of cage enrichment on the daily use of running wheels by Syrian hamsters. *Chronobiology International* 20: 9–20.
294. Geiser, F., Holloway, J. C., and Körtner, G. (2007). Thermal biology, torpor and behaviour in sugar gliders: A laboratory-field comparison. *Journal of Comparative Physiology B* 177: 495–501.
295. Hut, R. A., Pilorz, V., Boerema, A. S., Strijstra, A. M., and Daan, S. (2011). Working for food shifts nocturnal mouse activity into the day. *PLOS ONE* 6: e17527.
296. Van der Vinne, V., Riede, S. J., Gorter, J. A., Eijer, W. G., Sellix, M. T., Menaker, M., Daan, S., Pilorz, V., and Hut, R. A. (2014). Cold and hunger induce diurnality in a nocturnal mammal. *Proceedings of the National Academy of Sciences United States of America* 111: 15256–15260.
297. Refinetti, R. and Horvath, S. M. (1989). Thermopreferendum of the rat: Inter- and intra-subject variability. *Behavioral and Neural Biology* 52: 87–94.
298. Refinetti, R. (1989). Magnitude estimation of warmth: Intra- and intersubject variability. *Perception and Psychophysics* 46: 81–84.
299. Rising, R., Keys, A., Ravussin, E., and Bogardus, C. (1992). Concomitant interindividual variation in body temperature and metabolic rate. *American Journal of Physiology* 263: E730–E734.
300. van Vonderen, I. K., Kooistra, H. S., and Rijnberk, A. (1997). Intra- and interindividual variation in urine osmolality and urine specific gravity in healthy pet dogs of various ages. *Journal of Veterinary Internal Medicine* 11: 30–35.
301. Gidal, B. E., Radulovic, L. L., Kruger, S., Rutecki, P., Pitterle, M., and Bockbrader, H. N. (2000). Inter- and intra-subject variability in gabapentin absorption and absolute bioavailability. *Epilepsy Research* 40: 123–127.
302. Symanski, E., Bergamaschi, E., and Mutti, A. (2001). Inter- and intra-individual sources of variability in levels of urinary styrene metabolites. *International Archives of Occupational and Environmental Health* 74: 336–344.
303. Eckburg, P. B., Bik, E. M., Bernstein, C. N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S. R., Nelson, K. E., and Relman, D. A. (2005). Diversity of the human intestinal microbial flora. *Science* 308: 1635–1638.
304. Costello, E. K., Lauber, C. L., Hamady, M., Fierer, N., Gordon, J. I., and Knight, R. (2009). Bacterial community variation in human body habitats across space and time. *Science* 326: 1694–1697.
305. Refinetti, R. and Piccione, G. (2005). Intra- and inter-individual variability in the circadian rhythm of body temperature of rats, squirrels, dogs, and horses. *Journal of Thermal Biology* 30: 139–146.
306. Romeijn, N. and Van Someren, E. J. W. (2011). Correlated fluctuations of daytime skin temperature and vigilance. *Journal of Biological Rhythms* 26: 68–77.
307. Barassin, S., Saboureau, M., Kalsbeek, A., Bothorel, B., Vivien-Roels, B., Malan, A. Buijs, R. M., Guardiola-Lemaitre, B., and Pévet, P. (1999). Interindividual differences in the pattern of melatonin secretion of the Wistar rat. *Journal of Pineal Research* 27: 193–201.
308. Selmaoui, B. and Touitou, Y. (2003). Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: A study of three different 24-h cycles over six weeks. *Life Sciences* 73: 3339–3349.
309. Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., and Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: A replication of flattened cycles for some individuals. *Psychoneuroendocrinology* 26: 295–306.
310. DeCoursey, P. (1960). Phase control of activity in a rodent. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 49–55.
311. Eskin, A. (1971). Some properties of the system controlling the circadian activity rhythm of sparrows. In: Menaker, M. (Ed.), *Biochronometry*. Washington, DC: National Academy of Sciences, pp. 55–80.
312. Pohl, H. (1983). Light pulses entrain the circadian activity rhythm of a diurnal rodent (*Ammospermophilus leucurus*). *Comparative Biochemistry and Physiology B* 76: 723–729.
313. Sharma, V. K. (1996). Light-induced phase response curves of the circadian activity rhythm in individual field mice, *Mus booduga*. *Chronobiology International* 13: 401–409.
314. Davis, F. C. and Menaker, M. (1981). Development of the mouse circadian pacemaker: Independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
315. Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.
316. Duffield, G. E. and Ebling, F. J. P. (1998). Maternal entrainment of the developing circadian system in the Siberian hamster (*Phodopus sungorus*). *Journal of Biological Rhythms* 13: 315–329.
317. Canal-Corretger, M. M., Cambras, T., Vilaplana, J., and Díez-Noguera, A. (2000). Bright light during lactation alters the functioning of the circadian system of adult rats. *American Journal of Physiology* 278: R201–R208.
318. Yamazaki, S., Alones, V., and Menaker, M. (2002). Interaction of the retina with suprachiasmatic pacemakers in the control of circadian behavior. *Journal of Biological Rhythms* 17: 315–329.
319. Canal-Corretger, M. M., Cambras, T., and Díez-Noguera, A. (2003). Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiology International* 20: 21–35.
320. Aton, S. J., Block, G. D., Tei, H., Yamazaki, S., and Herzog, E. D. (2004). Plasticity of circadian behavior and the suprachiasmatic nucleus following exposure to non-24-hour light cycles. *Journal of Biological Rhythms* 19: 198–207.
321. Tomioka, K., Uwozumi, K., and Matsumoto, N. (1997). Light cycles given during development affect freerunning period of circadian locomotor rhythm of *period* mutants in *Drosophila melanogaster*. *Journal of Insect Physiology* 43: 297–305.
322. Sheeba, V., Chandrashekaran, M. K., Joshi, A., and Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *Journal of Insect Physiology* 48: 25–32.
323. Weinert, D. (2005). Ontogenetic development of the mammalian circadian system. *Chronobiology International* 22: 179–205.
324. Brooks, E. and Canal, M. M. (2013). Development of circadian rhythms: Role of postnatal light environment. *Neuroscience and Biobehavioral Reviews* 37: 551–560.

325. Attanasio, A., Rager, K., and Gupta, D. (1986). Ontogeny of circadian rhythmicity for melatonin, serotonin, and N-acetylserotonin in humans. *Journal of Pineal Research* 3: 251–256.
326. Ardura, J., Gutierrez, R., Andres, J., and Agapito, T. (2003). Emergence and evolution of the circadian rhythm of melatonin in children. *Hormone Research* 59: 66–72.
327. Kleitman, N., Titelbaum, S., and Hoffmann, H. (1937). The establishment of the diurnal temperature cycle. *American Journal of Physiology* 119: 48–54.
328. Abe, K. and Fukui, S. (1979). The individual development of circadian temperature rhythm in infants. *Journal of Interdisciplinary Cycle Research* 10: 227–232.
329. Lodemore, M., Petersen, S. A., and Wailoo, M. P. (1991). Development of night time temperature rhythms over the first six months of life. *Archives of Disease in Childhood* 66: 521–524.
330. Updike, P. A., Accurso, F. J., and Jones, R. H. (1985). Physiologic circadian rhythmicity in preterm infants. *Nursing Research* 34: 160–163.
331. Rivkees, S. A., Mayes, L., Jacobs, H., and Gross, I. (2004). Rest-activity patterns of premature infants are regulated by cycled lighting. *Pediatrics* 113: 833–839.
332. Korte, J., Hoehn, T., and Siegmund, R. (2004). Actigraphic recordings of activity-rest rhythms of neonates born by different delivery modes. *Chronobiology International* 21: 95–106.
333. Nishihara, K., Horiuchi, S., Eto, H., and Uchida, S. (2002). The development of infants' circadian rest-activity rhythm and mothers' rhythm. *Physiology and Behavior* 77: 91–98.
334. Sievers, E., Oldigs, H. D., Santer, R., and Schaub, J. (2002). Feeding patterns in breast-fed and formula-fed infants. *Annals of Nutrition and Metabolism* 46: 243–248.
335. Wang, Z., Sun, X., Cornélissen, G., Wu, J., Meng, L., Cai, D., Xue, Z., and Halberg, F. (1993). Doppler flowmeter-assessed circadian rhythms in neonatal cardiac function, family history, and intrauterine growth retardation. *American Journal of Perinatology* 10: 119–125.
336. Ardura, J., Andrés, J., Aldana, J., Revilla, M. A., and Aragón, M. P. (1997). Heart rate biorhythm changes during the first three months of life. *Biology of the Neonate* 72: 94–101.
337. Dimitriou, G., Greenough, A., Kavvadia, V., and Mantagos, S. (1999). Blood pressure rhythms during the perinatal period in very immature, extremely low birthweight neonates. *Early Human Development* 56: 49–56.
338. Marcun, V. and Gregoric, A. (2005). Twenty-four-hour ambulatory blood pressure monitoring in infants and toddlers. *Pediatric Nephrology* 20: 798–802.
339. Serón-Ferré, M., Riffo, R., Valenzuela, G. J., and Germain, A. M. (2001). Twenty-four-hour pattern of cortisol in the human fetus at term. *American Journal of Obstetrics and Gynecology* 184: 1278–1283.
340. Ivars, K., Nelson, N., Theodorsson, A., Theodorsson, E., Ström, J. O., and Mörelius, E. (2015). Development of salivary cortisol circadian rhythm and reference intervals in full-term infants. *PLOS ONE* 10: e0129502.
341. Schmidt, I., Barone, A., and Carlisle, H. J. (1986). Diurnal cycle of core temperature in huddling, week-old rat pups. *Physiology and Behavior* 37: 105–109.
342. Spiers, D. E. (1988). Nocturnal shifts in thermal and metabolic responses of the immature rat. *Journal of Applied Physiology* 64: 2119–2124.
343. Nuesslein, B. and Schmidt, I. (1990). Development of circadian cycle of core temperature in juvenile rats. *American Journal of Physiology* 259: R270–R276.
344. Nuesslein-Hildesheim, B., Imai-Matsumura, K., Döring, H., and Schmidt, I. (1995). Pronounced juvenile circadian core temperature rhythms exist in several strains of rats but not in rabbits. *Journal of Comparative Physiology B* 165: 13–17.
345. Imai-Matsumura, K., Kaul, R., and Schmidt, I. (1995). Juvenile circadian core temperature rhythm in Wistar and Lean (Fa/–) Zucker rat pups. *Physiology and Behavior* 57: 135–139.
346. Seifert, E. L. and Mortola, J. P. (2000). Light-dark differences in the effects of ambient temperature on gaseous metabolism in newborn rats. *Journal of Applied Physiology* 88: 1853–1858.
347. Kittrell, E. M. W. and Satinoff, E. (1986). Development of the circadian rhythm of body temperature in rats. *Physiology and Behavior* 38: 99–104.
348. Allen, C. and Kendall, J. W. (1967). Maturation of the circadian rhythm of plasma corticosterone in the rat. *Endocrinology* 80: 926–930.
349. Nuesslein-Hildesheim, B. and Schmidt, I. (1994). Is the circadian core temperature rhythm of juvenile rats due to a periodic blockade of thermoregulatory thermogenesis? *Pflügers Archiv* 427: 450–454.
350. Jilge, B., Kuhnt, B., Landerer, W., and Rest, S. (2000). Circadian thermoregulation in suckling rabbit pups. *Journal of Biological Rhythms* 15: 329–335.
351. Jilge, B., Kuhnt, B., Landerer, W., and Rest, S. (2001). Circadian temperature rhythms in rabbit pups and in their does. *Laboratory Animals* 35: 364–373.
352. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
353. Moore, D., Angel, J. E., Cheeseman, I. M., Fahrbach, S. E., and Robinson, G. E. (1998). Timekeeping in the honey bee colony: Integration of circadian rhythms and division of labor. *Behavioral Ecology and Sociobiology* 43: 147–160.
354. Bloch, G. and Robinson, G. E. (2001). Reversal of honeybee behavioural rhythms. *Nature* 410: 1048.
355. Piccione, G., Caola, G., and Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
356. Macaulay, A. S., Hahn, G. L., Clark, D. H., and Sisson, D. V. (1995). Comparison of calf housing types and tympanic temperature rhythms in Holstein calves. *Journal of Dairy Science* 78: 856–862.
357. Piccione, G., Caola, G., and Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.
358. Piccione, G., Fazio, F., Giudice, E., and Refinetti, R. (2009). Body size and the daily rhythm of body temperature in dogs. *Journal of Thermal Biology* 34: 171–175.
359. Piccione, G., Caola, G., and Refinetti, R. (2007). Annual rhythmicity and maturation of physiological parameters in goats. *Research in Veterinary Science* 83: 239–243.
360. Aarseth, J. J., Van't Hof, T. J., and Stokkan, K. A. (2003). Melatonin is rhythmic in newborn seals exposed to continuous light. *Journal of Comparative Physiology B* 173: 37–42.
361. Tamarkin, L., Reppert, S. M., Orloff, D. J., Klein, D. C., Yellon, S. M., and Goldman, B. D. (1980). Ontogeny of the pineal melatonin rhythm in the Syrian (*Mesocricetus auratus*) and Siberian (*Phodopus sungorus*) hamsters and in the rat. *Endocrinology* 107: 1061–1064.
362. Kilmer, D. M., Sharp, D. C., Berglund, L. A., Grubaugh, W., McDowell, K. J., and Peck, L. S. (1982). Melatonin rhythms in Pony mares and foals. *Journal of Reproduction and Fertility Supplement* 32: 303–307.

363. Fuchs, J. L. and Moore, R. Y. (1980). Development of circadian rhythmicity and light responsiveness in the rat suprachiasmatic nucleus: A study using the 2-deoxy[1–^{14}C]glucose method. *Proceedings of the National Academy of Sciences United States of America* 77: 1204–1208.
364. Reppert, S. M. and Schwartz, W. J. (1984). The suprachiasmatic nuclei of the fetal rat: Characterization of a functional circadian clock using ^{14}C-labeled deoxyglucose. *Journal of Neuroscience* 4: 1677–1682.
365. Bendová, Z., Sumová, A., and Illnerová, H. (2004). Development of circadian rhythmicity and photoperiodic response in subdivisions of the rat suprachiasmatic nucleus. *Developmental Brain Research* 148: 105–112.
366. Shibata, S. and Moore, R. Y. (1987). Development of neuronal activity in the rat suprachiasmatic nucleus. *Brain Research* 431: 311–315.
367. Houghton, D. C., Young, I. R., and McMillen, I. C. (1995). Evidence for hypothalamic control of the diurnal rhythms in prolactin and melatonin in the fetal sheep during late gestation. *Endocrinology* 136: 218–223.
368. Parraguez, V. H., Valenzuela, G. J., Vergara, M., Ducsay, C. A., Yellon, S. M., and Serón-Ferré, M. (1996). Effect of constant light on fetal and maternal prolactin rhythms in sheep. *Endocrinology* 137: 2355–2361.
369. Delaunay, F., Thisse, C., Marchand, O., Laudet, V., and Thisse, B. (2000). An inherited functional circadian clock in zebrafish embryos. *Science* 289: 297–300.
370. Shinkoda, H., Matsumoto, K., Park, Y. M., and Nagashima, H. (2000). Sleep-wake habits of schoolchildren according to grade. *Psychiatry and Clinical Neurosciences* 54: 287–289.
371. Kim, S., Dueker, G. L., Hasher, L., and Goldstein, D. (2002). Children's time of day preference: Age, gender and ethnic differences. *Personality and Individual Differences* 33: 1083–1090.
372. Giannotti, F., Cortesi, F., Sebastiani, T., and Ottaviano, S. (2002). Circadian preference, sleep and daytime behaviour in adolescence. *Journal of Sleep Research* 11: 191–199.
373. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K., and Shiraishi, T. (1988). Disruption of light-dark cycle of feeding and drinking behavior, and ambulatory activity induced by development of obesity in the Zucker rat. *International Journal of Obesity* 12: 481–490.
374. Honegger, H. W. (1976). Locomotor activity in *Uca crenulata*, and the response to two Zeitgebers, light-dark and tides. In: DeCoursey, P. J. (Ed.), *Biological Rhythms in the Marine Environment*. Columbia, SC: University of South Carolina Press, pp. 93–102.
375. de la Iglesia, H. O., Rodríguez, E. M., and Dezi, R. E. (1994). Burrow plugging in the crab *Uca uruguayensis* and its synchronization with photoperiod and tides. *Physiology and Behavior* 55: 913–919.
376. Wikelski, M. and Hau, M. (1995). Is there an endogenous tidal foraging rhythm in marine iguanas? *Journal of Biological Rhythms* 10: 335–350.
377. Akiyama, T. (2004). Entrainment of the circatidal swimming activity rhythm in the cumacean *Dimorphostylis asiatica* (Crustacea) to 12.5-hour hydrostatic pressure cycles. *Zoological Science* 21: 29–38.
378. Al-Adhub, A. H. Y. and Naylor, E. (1975). Emergence rhythms and tidal migrations in the brown shrimp *Crangon crangon* (L.). *Journal of the Marine Biological Association of the United Kingdom* 55: 801–810.
379. Barlow, R. B., Powers, M. K., Howard, H., and Kass, L. (1986). Migration of *Limulus* for mating: Relation to lunar phase, tide height, and sunlight. *Biological Bulletin* 171: 310–329.
380. Chabot, C. C., Skinner, S. J., and Watson, W. H. (2008). Rhythms of locomotion expressed by *Limulus polyphemus*, the American horseshoe crab: I. Synchronization by artificial tides. *Biological Bulletin* 215: 34–45.
381. Zhang, L., Hastings, M. H., Green, E. W., Tauber, E., Sladek, M., Webster, S. G., Kyriacou, C. P., and Wilcockson, D. C. (2013). Dissociation of circadian and circatidal timekeeping in the marine crustacean *Eurydice pulchra*. *Current Biology* 23: 1863–1873.
382. Palmer, J. D. (1967). Daily and tidal components in the persistent rhythmic activity of the crab, *Sesarma*. *Nature* 215: 64–66.
383. Cummings, S. M. and Morgan, E. (2001). Time-keeping system of the eel pout, *Zoarces viviparus*. *Chronobiology International* 18: 27–46.
384. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
385. Watson, W. H., Bedford, L., and Chabot, C. C. (2008). Rhythms of locomotion expressed by *Limulus polyphemus*, the American horseshoe crab: II. Relationship to circadian rhythms of visual sensitivity. *Biological Bulletin* 215: 46–56.
386. Alleva, J. J., Waleski, M. V., and Alleva, F. R. (1971). A biological clock controlling the estrous cycle of the hamster. *Endocrinology* 88: 1368–1379.
387. Fitzgerald, K. M. and Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences United States of America* 73: 2923–2927.
388. Stetson, M. H. and Gibson, J. T. (1977). The estrous cycle in golden hamsters: A circadian pacemaker times preovulatory gonadotropin release. *Journal of Experimental Zoology* 201: 289–294.
389. Sridaran, R. and McCormack, C. E. (1980). Parallel effects of light signals on the circadian rhythms of running activity and ovulation in rats. *Journal of Endocrinology* 85: 111–120.
390. Carmichael, M. S., Nelson, R. J., and Zucker, I. (1981). Hamster activity and estrous cycles: Control by a single versus multiple circadian oscillator(s). *Proceedings of the National Academy of Sciences United States of America* 78: 7830–7834.
391. Swann, J. and Turek, F. W. (1982). Cycle of lordosis behavior in female hamsters whose circadian activity rhythm has split into two components. *American Journal of Physiology* 243: R112–R118.
392. Stetson, M. H. and Watson-Whitmyre, M. (1976). Nucleus suprachiasmaticus: The biological clock in the hamster? *Science* 191: 197–199.
393. Dunn, J. D., Castro, A. J., and McNulty, J. A. (1977). Effect of suprachiasmatic ablation on the daily temperature rhythm. *Neuroscience Letters* 6: 345–348.
394. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N., and Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
395. Miller, B. H., Olson, S. L., Turek, F. W., Levine, J. E., Horton, T. H., and Takahashi, J. S. (2004). Circadian *Clock* mutation disrupts estrous cyclicity and maintenance of pregnancy. *Current Biology* 14: 1367–1373.
396. Refinetti, R. and Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.
397. Neill, J. D. (1972). Sexual differences in the hypothalamic regulation of prolactin secretion. *Endocrinology* 90: 1154–1159.

398. Seegal, R. F. and Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. *Biology of Reproduction* 12: 223–231.
399. Stetson, M. H., Watson-Whitmyre, M., DiPinto, M. N., and Smith, S. G. (1981). Daily luteinizing hormone release in ovariectomized hamsters: Effect of barbiturate blockade. *Biology of Reproduction* 24: 139–144.
400. Swann, J. M. and Turek, F. W. (1985). Multiple circadian oscillators regulate the timing of behavioral and endocrine rhythms in female golden hamsters. *Science* 228: 898–900.
401. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M., and Loudon, A. S. I. (1999). Free running circadian rhythms of melatonin, luteinizing hormone, and cortisol in Syrian hamsters bearing the circadian *tau* mutation. *Endocrinology* 140: 758–764.
402. Palm, I. F., van der Beek, E. M., Swarts, H. J. M., van der Vliet, J., Wiegant, V. M., Buijs, R. M., and Kalsbeek, A. (2001). Control of the estradiol-induced prolactin surge by the suprachiasmatic nucleus. *Endocrinology* 142: 2296–2302.
403. Häster, L. and Erkert, H. G. (1993). Alteration of circadian period length does not influence the ovarian cycle length in common marmosets, *Callithrix j. jacchus* (Primates). *Chronobiology International* 10: 165–175.
404. Rauth-Widmann, B., Fuchs, E., and Erkert, H. G. (1996). Infradian alteration of circadian rhythms in owl monkeys (*Aotus lemurinus griseimembra*): An effect of estrous? *Physiology and Behavior* 59: 11–18.
405. Houdelier, C., Guyomarc'h, C., Lumineau, S., and Richard, J. P. (2002). Circadian rhythms of oviposition and feeding activity in Japanese quail: Effects of cyclic administration of melatonin. *Chronobiology International* 19: 1107–1119.
406. Zivkovic, B. D., Underwood, H., Steele, C. T., and Edmonds, K. (1999). Formal properties of the circadian and photoperiodic systems of Japanese quail: Phase response curve and effects of T-cycles. *Journal of Biological Rhythms* 14: 378–390.
407. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
408. Bobr, L. W. and Sheldon, B. L. (1977). Analysis of ovulation-oviposition patterns in the domestic fowl by telemetry measurement of deep body temperature. *Australian Journal of Biological Sciences* 30: 243–257.
409. Kadono, H., Besch, E. L., and Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
410. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W., and Bacon, W. L. (2000). Circadian rhythm of the preovulatory surge of luteinizing hormone and its relationships to rhythms of body temperature and locomotor activity in turkey hens. *Biology of Reproduction* 62: 1452–1458.
411. Hayes, S. R. (1976). Daily activity and body temperature of the southern woodchuck, *Marmota monax monax*, in northwestern Arkansas. *Journal of Mammalogy* 57: 291–299.
412. Bakko, E. B., Porter, W. P., and Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
413. Halle, S. (1995). Diel pattern of locomotor activity in populations of root voles, *Microtus oeconomus*. *Journal of Biological Rhythms* 10: 211–224.
414. Scheibe, K. M., Berher, A., Langbein, J., Streich, W. J., and Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biological Rhythm Research* 30: 216–233.
415. Rezende, E. L., Cortés, A., Bacigalupe, L. D., Nespolo, R. F., and Bozinovic, F. (2003). Ambient temperature limits aboveground activity of the subterranean rodent *Spalacopus cyanus*. *Journal of Arid Environments* 55: 63–74.
416. Berger, A., Scheibe, K. M., Michaelis, S., and Streich, W. J. (2003). Evaluation of living conditions of free-ranging animals by automated chronobiological analysis of behavior. *Behavior Research Methods, Instruments, and Computers* 35: 458–466.
417. Monecke, S. and Wollnik, F. (2005). Seasonal variations in circadian rhythms coincide with a phase of sensitivity to short photoperiods in the European hamster. *Journal of Comparative Physiology B* 175: 167–183.
418. Fernandez-Duque, E. and Erkert, H. G. (2006). Cathemerality and lunar periodicity of activity rhythms in owl monkeys of the Argentinian Chaco. *Folia Primatologica* 77: 123–138.
419. Ware, J. V., Nelson, O. L., Robbins, C. T., and Jansen, H. T. (2012). Temporal organization of activity in the brown bear (*Ursus arctos*): Roles of circadian rhythms, light, and food entrainment. *American Journal of Physiology* 303: R890–R902.
420. Ensing, E. P., Ciuti, S., de Wijs, F. A. L. M., Lentferink, D. H., ten Hoedt, A., Boyce, M. S., and Hut, R. A. (2014). GPS based daily activity patterns in European red deer and North American elk (*Cervus elaphus*): Indication for a weak circadian clock in ungulates. *PLOS ONE* 9: e106997.
421. Haim, A., Yedidia, I., Haim, D., and Zisapel, N. (1994). Photoperiodicity in daily rhythms of body temperature, food and energy intake of the golden spiny mouse (*Acomys russatus*). *Israel Journal of Zoology* 40: 145–150.
422. Basco, P. S., Rashotte, M. E., and Stephan, F. K. (1996). Photoperiod duration and energy balance in the pigeon. *Physiology and Behavior* 60: 151–159.
423. Schilling, A., Richard, J. P., and Servière, J. (1999). Duration of activity and period of circadian activity-rest rhythm in a photoperiod-dependent primate, *Microcebus murinus*. *Comptes Rendus de l'Académie des Sciences* 322: 759–770.
424. Anchordoquy, H. C. and Lynch, G. R. (2000). Timing of testicular recrudescence in Siberian hamsters is unaffected by pinealectomy or long-day photoperiod after 9 weeks in short days. *Journal of Biological Rhythms* 15: 406–416.
425. Perret, M. and Aujard, F. (2001). Daily hypothermia and torpor in a tropical primate: Synchronization by 24-h light-dark cycle. *American Journal of Physiology* 281: R1925–R1933.
426. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
427. Haritou, S. J. A., Zylstra, R., Ralli, C., Turner, S., and Tortonese, D. J. (2008). Seasonal changes in circadian peripheral plasma concentrations of melatonin, serotonin, dopamine and cortisol in aged horses with Cushing's disease under natural photoperiod. *Journal of Neuroendocrinology* 20: 988–996.
428. Strand, J. E. T., Aarseth, J. J., Hanebrekke, T. L., and Jørgensen, E. H. (2008). Keeping track of time under ice and snow in a sub-arctic lake: Plasma melatonin rhythms in Arctic charr overwintering under natural conditions. *Journal of Pineal Research* 44: 227–233.
429. Kowalczyk, R., Jedrzejewska, B., and Zalewski, A. (2003). Annual and circadian activity patterns of badgers (*Meles meles*) in Bialowieza Primeval Forest (eastern Poland) compared with other Palaearctic populations. *Journal of Biogeography* 30: 463–472.
430. Binkley, S., Tome, M. B., Crawford, D., and Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.

431. Yoneyama, S., Hashimoto, S., and Honma, K. (1999). Seasonal changes of human circadian rhythms in Antarctica. *American Journal of Physiology* 277: R1091–R1097.
432. Sani, M., Refinetti, R., Jean-Louis, G., Pandi-Perumal, S. R., Durazo-Arvizu, R. A., Dugas, L. R., Kafensztok, R. et al. (2015). Daily activity patterns of 2316 men and women from five countries differing in socioeconomic development. *Chronobiology International* 32: 650–656.
433. Menaker, M. (1961). The free running period of the bat clock: Seasonal variations at low body temperature. *Journal of Cellular and Comparative Physiology* 57: 81–86.
434. Mrosovsky, N., Boshes, M., Hallonquist, J. D., and Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.
435. Cisse, F., Martineaud, R., and Martineaud, J. P. (1991). Circadian cycles of central temperature in hot climate in man. *Archives Internationales de Physiologie, de Biochimie et de Biophysique* 99: 155–159.
436. Honma, K., Honma, S., Kohsaka, M., and Fukuda, N. (1992). Seasonal variation in the human circadian rhythm: Dissociation between sleep and temperature rhythm. *American Journal of Physiology* 262: R885–R891.
437. Grahn, D. A., Miller, J. D., Houng, V. S., and Heller, H. C. (1994). Persistence of circadian rhythmicity in hibernating ground squirrels. *American Journal of Physiology* 266: R1251–R1258.
438. Wollnik, F. and Schmidt, B. (1995). Seasonal and daily rhythms of body temperature in the European hamster (*Cricetus cricetus*) under semi-natural conditions. *Journal of Comparative Physiology B* 165: 171–182.
439. Lee, T. M. and Zucker, I. (1995). Seasonal variations in circadian rhythms persist in gonadectomized golden-mantled ground squirrels. *Journal of Biological Rhythms* 10: 188–195.
440. van Houwelingen, C. A. J. and Beersma, D. G. M. (2001). Seasonal changes in 24-h patterns of suicide rates: A study on train suicides in The Netherlands. *Journal of Affective Disorders* 66: 215–223.
441. Bertolucci, C., Foà, A., and Van't Hof, T. J. (2002). Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. *Hormones and Behavior* 41: 414–419.
442. van Oort, B. E. H., Tyler, N. J. C., Gerkema, M. P., Folkow, L., and Stokkan, K. A. (2007). Where clocks are redundant: Weak circadian mechanisms in reindeer living under polar photic conditions. *Naturwissenschaften* 94: 183–194.
443. Bovet, J. and Oertli, E. F. (1974). Free-running circadian activity rhythms in free-living beaver (*Castor canadensis*). *Journal of Comparative Physiology* 92: 1–10.
444. Lancia, R. A., Dodge, W. E., and Larson, J. S. (1982). Winter activity patterns of two radio-marked beaver colonies. *Journal of Mammalogy* 63: 598–606.
445. Dyck, A. P. and MacArthur, R. A. (1992). Seasonal patterns of body temperature in free-ranging beaver (*Castor canadensis*). *Canadian Journal of Zoology* 70: 1668–1672.
446. Bartell, P. A. and Gwinner, E. (2005). A separate circadian oscillator controls nocturnal migratory restlessness in the songbird *Sylvia borin*. *Journal of Biological Rhythms* 20: 538–549.
447. Waßmer, T. and Wollnik, F. (1997). Timing of torpor bouts during hibernation in European hamsters (*Cricetus cricetus* L.). *Journal of Comparative Physiology B* 167: 270–279.
448. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
449. Körtner, G., Song, X., and Geiser, F. (1998). Rhythmicity of torpor in a marsupial hibernator, the mountain pygmy-possum (*Burramys parvus*), under natural and laboratory conditions. *Journal of Comparative Physiology B* 168: 631–638.
450. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
451. Hut, R. A., Barnes, B. M., and Daan, S. (2002). Body temperature patterns before, during, and after semi-natural hibernation in the European ground squirrel. *Journal of Comparative Physiology B* 172: 47–58.
452. Canguilhem, B., Malan, A., Masson-Pévet, M., Nobelis, P., Kirsch, R., Pévet, P., and Le Minor, J. (1994). Search for rhythmicity during hibernation in the European hamster. *Journal of Comparative Physiology B* 163: 690–698.
453. Oklejewicz, M., Daan, S., and Strijkstra, A. M. (2001). Temporal organisation of hibernation in wild-type and *tau* mutant Syrian hamsters. *Journal of Comparative Physiology B* 171: 431–439.
454. Larkin, J. E., Franken, P., and Heller, H. C. (2002). Loss of circadian organization of sleep and wakefulness during hibernation. *American Journal of Physiology* 282: R1086–R1095.
455. Gür, M. K., Refinetti, R., and Gür, H. (2009). Daily rhythmicity and hibernation in the Anatolian ground squirrel under natural and laboratory conditions. *Journal of Comparative Physiology B* 179: 155–164.
456. Øivind, T., Blake, J., Edgar, D. M., Grahn, D. A., Heller, H. C., and Barnes, B. M. (2011). Hibernation in black bears: Independence of metabolic suppression from body temperature. *Science* 331: 906–909.
457. Williams, C. T., Sheriff, M. J., Schmutz, J. A., Kohl, F., Øivind, T., Buck, C. L., and Barnes, B. M. (2011). Data logging of body temperatures provides precise information on phenology of reproductive events in a free-ling Arctic hibernator. *Journal of Comparative Physiology B* 181: 1101–1109.
458. Williams, C. T., Barnes, B. M., and Buck, C. L. (2012). Daily body temperature rhythms persist under the midnight sun but are absent during hibernation in free-living Arctic ground squirrels. *Biology Letters* 8: 31–34.
459. Kilduff, T. S., Miller, J. D., Radeke, C. M., Sharp, F. R., and Heller, H. C. (1990). ^{14}C-2-deoxyglucose uptake in the ground squirrel brain during entrance to and arousal from hibernation. *Journal of Neuroscience* 10: 2463–2475.
460. Vaupel, J. W. and Loichinger, E. (2006). Redistributing work in aging Europe. *Science* 312: 1911–1913.
461. Hayflick, L. (1989). Antecedents of cell aging research. *Experimental Gerontology* 24: 355–365.
462. Green, D. R., Galluzzi, L., and Kroemer, G. (2011). Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 333: 1109–1112.
463. Brock, M. A. (1985). Biological clocks and aging. *Review of Biological Research in Aging* 2: 445–462.
464. Adan, A. (1997). Diferencias individuales en las variaciones diurnas fisiologicas y comportamentales. *Revista Latinoamericana de Psicologia* 29: 81–114.
465. Turek, F. W., Scarbrough, K., Penev, P., Labyak, S., Valentinuzzi, V. S., and Van Reeth, O. (2001). Aging of the mammalian circadian system. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 291–317.
466. Vitiello, M. V., Smallwood, R. G., Avery, D. H., Pascualy, R. A., Martin, D. C., and Prinz, P. N. (1986). Circadian temperature rhythms in young adult and aged men. *Neurobiology of Aging* 7: 97–100.

467. Touitou, Y., Reinberg, A., Bogdan, A., Auzéby, A., Beck, H., and Touitou, C. (1986). Age-related changes in both circadian and seasonal rhythms of rectal temperature with special reference to senile dementia of Alzheimer type. *Gerontology* 32: 110–118.
468. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T., and Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
469. Yunis, E. J., Fernandes, G., Nelson, W., and Halberg, F. (1974). Circadian temperature rhythms and aging in rodents. In: Scheving, L. E., Halberg, F., and Pauly, J. E. (Eds.), *Chronobiology*. Tokyo, Japan: Igaku Shoin, pp. 358–363.
470. Sacher, G. A. and Duffy, P. H. (1978). Age changes in rhythms of energy metabolism, activity, and body temperature in *Mus* and *Peromyscus*. In: Samis, H. V. and Capobianco, S. (Eds.), *Aging and Biological Rhythms*. New York: Plenum, pp. 105–124.
471. Halberg, J., Halberg, E., Regal, P., and Halberg, F. (1981). Changes with age characterize circadian rhythm in telemetered core temperature of stroke-prone rats. *Journal of Gerontology* 36: 28–30.
472. Refinetti, R., Ma, H., and Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
473. Koster-van-Hoffen, G. C., Mirmiran, M., Bos, N. P. A., Witting, W., Delagrande, P., and Guardiola-Lemaitre, B. (1993). Effects of a novel melatonin analog on circadian rhythms of body temperature and activity in young, middle-aged, and old rats. *Neurobiology of Aging* 14: 565–569.
474. Li, H. and Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
475. McDonald, R. B., Hoban-Higgins, T. M., Ruhe, R. C., Fuller, C. A., and Horwitz, B. A. (1999). Alterations in endogenous circadian rhythm of core temperature in senescent Fischer 344 rats. *American Journal of Physiology* 276: R824–R830.
476. Irizarry, R. A., Tankersley, C., Frank, R., and Flanders, S. (2001). Assessing homeostasis through circadian patterns. *Biometrics* 57: 1228–1237.
477. Benstaali, C., Bogdan, A., and Touitou, Y. (2002). Effect of a short photoperiod on circadian rhythms of body temperature and motor activity in old rats. *Pflügers Archiv* 444: 73–79.
478. Frank, R. and Tankersley, C. (2002). Air pollution and daily mortality: A hypothesis concerning the role of impaired homeostasis. *Environmental Health Perspectives* 110: 61–65.
479. Bourlière, F. (1947). Quelques caractéristiques physiologiques de la sénescence chez le rat. *Revue Canadienne de Biologie* 6: 245–259.
480. Sharma, V. K. and Chandrashekaran, M. K. (1998). Age-dependent modulation of circadian parameters in the field mouse *Mus booduga*. *Journal of Experimental Zoology* 280: 321–326.
481. Duffy, J. F., Viswanathan, N., and Davis, F. C. (1999). Free-running circadian period does not shorten with age in female Syrian hamsters. *Neuroscience Letters* 271: 77–80.
482. Davis, F. C. and Viswanathan, N. (1998). Stability of circadian timing with age in Syrian hamsters. *American Journal of Physiology* 275: R960–R968.
483. Weinert, D. and Waterhouse, J. (1999). Daily activity and body temperature rhythms do not change simultaneously with age in laboratory mice. *Physiology and Behavior* 66: 605–612.
484. Siwak, C. T., Tapp, P. D., Zicker, S. C., Murphey, H. L., Muggenburg, B. A., Head, E., and Cotman, C. W. (2003). Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience* 117: 813–824.
485. Pang, K. C. H., Miller, J. P., and McAuley, J. D. (2004). Circadian rhythms in SAMP8: A longitudinal study of the effects of age and experience. *Neurobiology of Aging* 25: 111–123.
486. Tankersley, C. G., Irizarry, R., Flanders, S. E., Rabold, R., and Frank, R. (2003). Unstable heart rate and temperature regulation predict mortality in AKR/J mice. *American Journal of Physiology* 284: R742–R750.
487. Kolker, D. E., Vitaterna, M. H., Fruechte, E. M., Takahashi, J. S., and Turek, F. W. (2004). Effects of age on circadian rhythms are similar in wild-type and heterozygous *Clock* mutant mice. *Neurobiology of Aging* 25: 517–523.
488. Kopp, C., Ressel, V., Wigger, E., and Tobler, I. (2006). Influence of estrous cycle and ageing on activity patterns in two inbred mouse strains. *Behavioural Brain Research* 167: 165–174.
489. Farjnia, S., Michel, S., Deboer, T., van der Leest, H. T., Houben, T., Rohling, J. H. T., Ramkisoensing, A., Yasenkov, R., and Meijer, J. H. (2012). Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. *Journal of Neuroscience* 32: 5891–5899.
490. Atwood, C. S., James, I. R., Keil, U., Roberts, N. K., and Hartmann, P. E. (1991). Circadian changes in salivary constituents and conductivity in women and men. *Chronobiologia* 18: 125–140.
491. O'Sullivan, C., Duggan, J., Atkins, N., and O'Brien, E. (2003). Twenty-four-hour ambulatory blood pressure in community-dwelling elderly men and women aged 60–102 years. *Journal of Hypertension* 21: 1641–1647.
492. Takeuchi, T. and Harada, E. (2002). Age-related changes in sleep-wake rhythm in dog. *Behavioural Brain Research* 136: 193–199.
493. Gupta, S. K., Lindemulder, E. A., and Sathyan, G. (2000). Modeling of circadian testosterone in healthy men and hypogonadal men. *Journal of Clinical Pharmacology* 40: 731–738.
494. Zhao, Z. Y., Xie, Y., Fu, Y. R., Bogdan, A., and Touitou, Y. (2002). Aging and the circadian rhythm of melatonin: A cross-sectional study of Chinese subjects 30–110 yr of age. *Chronobiology International* 19: 1171–1182.
495. Winocur, G. and Hasher, L. (2004). Age and time-of-day effects on learning and memory in a non-matching-to-sample test. *Neurobiology of Aging* 25: 1107–1115.
496. Hurd, M. W. and Ralph, M. R. (1998). The significance of circadian organization for longevity in the golden hamster. *Journal of Biological Rhythms* 13: 430–436.
497. May, C. P., Hasher, L., and Stoltzfus, E. R. (1993). Optimal time of day and the magnitude of age differences in memory. *Psychological Science* 4: 326–330.
498. Baehr, E. K., Revelle, W., and Eastman, C. I. (2000). Individual differences in the phase and amplitude of the human circadian temperature rhythm with an emphasis on morningness-eveningness. *Journal of Sleep Research* 9: 117–127.
499. Klerman, E. B., Duffy, J. F., Dijk, D. J., and Czeisler, C. A. (2001). Circadian phase resetting in older people by ocular bright light exposure. *Journal of Investigative Medicine* 49: 30–40.
500. Robilliard, D. L., Archer, S. N., Arendt, J., Lockley, S. W., Hack, L. M., English, J., Leger, D. et al. (2002). The 3111 *Clock* gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. *Journal of Sleep Research* 11: 305–312.
501. Zavada, A., Gordijn, M. C. M., Beersma, D. G. M., Daan, S., and Roenneberg, T. (2005). Comparison of the Munich chronotype questionnaire with the Horne-Östberg's morningness–eveningness score. *Chronobiology International* 22: 267–278.

502. Pittendrigh, C. S. and Daan, S. (1974). Circadian oscillations in rodents: A systematic increase of their frequency with age. *Science* 186: 548–550.
503. Morin, L. P. (1988). Age-related changes in hamster circadian period, entrainment, and rhythm splitting. *Journal of Biological Rhythms* 3: 237–248.
504. Rosenberg, R. S., Zee, P. C., and Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.
505. Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *Journal of Biological Rhythms* 8: 189–197.
506. Viswanathan, N. and Davis, F. C. (1995). Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. *Brain Research* 686: 10–16.
507. Oklejewicz, M., Hut, R. A., Daan, S., Loudon, A. S. I., and Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.
508. Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H., and Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *Journal of Biological Rhythms* 18: 159–169.
509. Witting, W., Mirmiran, M., Bos, N. P. A., and Swaab, D. F. (1994). The effect of old age on the free-running period of circadian rhythms in rat. *Chronobiology International* 11: 103–112.
510. Possidente, B., McEldowney, S., and Pabon, A. (1995). Aging lengthens circadian period for wheel-running activity in C57BL mice. *Physiology and Behavior* 57: 575–579.
511. Mayeda, A. R., Hofstetter, J. R., and Possidente, B. (1997). Aging lengthens TauDD in C57BL/6J, DBA/2J, and SWR male mice (*Mus musculus*). *Chronobiology International* 14: 19–23.
512. Pohl, H. (1993). Does aging affect the period of the circadian pacemaker in vertebrates? *Naturwissenschaften* 80: 478–481.
513. Rappold, I. and Erkert, H. G. (1994). Re-entrainment, phase-response and range of entrainment of circadian rhythms in owl monkeys (*Aotus lemurinus g.*) of different age. *Biological Rhythm Research* 25: 133–152.
514. Moore, D. (2001). Honey bee circadian clocks: Behavioral control from individual workers to whole-colony rhythms. *Journal of Insect Physiology* 47: 843–857.
515. Ralph, M. R., Ko, C. H., Antoniadia, E. A., Seco, P., Irani, F., Presta, C., and McDonald, R. J. (2002). The significance of circadian phase for performance on a reward-based learning task in hamsters. *Behavioural Brain Research* 136: 179–184.
516. Cain, S. W., Chou, T., and Ralph, M. R. (2004). Circadian modulation of performance on an aversion-based place learning task in hamsters. *Behavioural Brain Research* 150: 201–205.
517. Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M., and Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research* 739: 12–18.
518. Aragona, B. J., Curtis, J. T., Davidson, A. J., Wang, Z., and Stephan, F. K. (2002). Behavioral and neurochemical investigation of circadian time-place learning in the rat. *Journal of Biological Rhythms* 17: 330–344.
519. Biebach, H., Gordijn, M., and Krebs, J. (1989). Time-place learning by garden warblers, *Sylvia borin*. *Animal Behaviour* 37: 353–360.
520. Saksida, L. M. and Wilkie, D. M. (1994). Time-of-day discrimination by pigeons, *Columba livia*. *Animal Learning and Behavior* 22: 143–154.
521. Reebs, S. G. (1996). Time-place learning in golden shiners (Pisces: Cyprinidae). *Behavioural Processes* 36: 253–262.
522. Reebs, S. G. (1999). Time-place learning based on food but not on predation risk in a fish, the inanga (*Galaxias maculates*). *Ethology* 105: 361–371.
523. Thorpe, C. M., Bates, M. E., and Wilkie, D. M. (2003). Rats have trouble associating all three parts of the time-place-event memory code. *Behavioural Processes* 63: 95–110.
524. Cain, S. W., Ko, C. H., Chalmers, J. A., and Ralph, M. R. (2004). Time of day modulation of conditioned place preference in rats depends on the strain of rat used. *Neurobiology of Learning and Memory* 81: 217–220.
525. Lukoyanov, N. V., Pereira, P. A., Mesquita, R. M., and Andrade, J. P. (2002). Restricted feeding facilitates time-place learning in adult rats. *Behavioural Brain Research* 134: 283–290.
526. Carr, J. A. R. and Wilkie, D. M. (1999). Rats are reluctant to use circadian timing in a daily time-place task. *Behavioural Processes* 44: 287–299.
527. Pizzo, M. J. and Crystal, J. D. (2004). Evidence for an alternation strategy in time-place learning. *Behavioural Processes* 67: 533–537.
528. Roberts, W. A., Feeney, M. C., MacPherson, K., Petter, M., McMillan, N., and Musolino, E. (2008). Episodic-like memory in rats: Is it based on when or how long ago? *Science* 320: 113–115.
529. Mulder, C. K., Papantoniou, C., Gerkema, M. P., and van der Zee, E. A. (2014). Neither the SCN nor the adrenals are required for circadian time-place learning in mice. *Chronobiology International* 31: 1075–1092.
530. Cain, S. W., Yoon, J., Shrestha, T. C., and Ralph, M. R. (2014). Retention of a 24-hour time memory in Syrian hamsters carrying the 20-hour short circadian period mutation in casein kinase 1 ε. *Neurobiology of Learning and Memory* 114: 171–177.
531. Amir, S. and Stewart, J. (1996). Resetting of the circadian clock by a conditioned stimulus. *Nature* 379: 542–545.
532. Arvanitogiannis, A. and Amir, S. (2001). A novel, associative process modulating photic resetting of the circadian clock. *Neuroscience* 104: 615–618.
533. De Groot, M. H. M. and Rusak, B. (2000). Responses of the circadian system of rats to conditioned and unconditioned stimuli. *Journal of Biological Rhythms* 15: 277–291.
534. Arvanitogiannis, A., Stewart, J., and Amir, S. (2000). Conditioned stimulus control in the circadian system: Two tales tell one story. *Journal of Biological Rhythms* 15: 292–293.
535. Arvanitogiannis, A. and Amir, S. (1999). Conditioned stimulus control in the rat circadian system depends on clock resetting during conditioning. *Behavioral Neuroscience* 113: 1297–1300.
536. Rusak, B. and de Groot, M. H. M. (2000). Requirements for conditioning of the rat circadian system remain elusive. *Journal of Biological Rhythms* 15: 294–295.
537. Buss, D. M. (1989). Sex differences in human mate preferences: Evolutionary hypotheses tested in 37 cultures. *Behavioral and Brain Sciences* 12: 1–49.
538. Koteja, P., Swallow, J. G., Carter, P. A., and Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.
539. Zucker, I., Fitzgerald, K. M., and Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.

540. Weber, E. T. and Hohn, V. M. (2005). Circadian activity rhythms in the spiny mouse, *Acomys cahirinus*. *Physiology and Behavior* 86: 427–433.
541. Pezuk, P., Mohawk, J. A., Yoshikawa, T., Sellix, M. T., and Menaker, M. (2010). Circadian organization is governed by extra-SCN pacemakers. *Journal of Biological Rhythms* 25: 432–441.
542. Eastman, C. I., Molina, T. A., Dziepak, M. E., and Smith, M. R. (2012). Blacks (African Americans) have shorter free-running circadian periods than Whites (Caucasian Americans). *Chronobiology International* 29: 1072–1077.
543. Bittman, E. L. (2012). Does the precision of a biological clock depend upon its period? Effects of the duper and tau mutations in Syrian hamsters. *PLOS ONE* 7: e36119.
544. Wolff, G., Duncan, M. J., and Esser, K. A. (2013). Chronic phase advance alters circadian physiological rhythms and peripheral molecular clocks. *Journal of Applied Physiology* 115: 373–382.
545. Kuljis, D. A., Loh, D. H., Truong, D., Vosko, A. M., Ong, M. L., McClusky, R., Arnold, A. P., and Colwell, C. S. (2013). Gonadal- and sex-chromosome-dependent sex differences in the circadian system. *Endocrinology* 154: 1501–1512.
546. Stryjek, R., Modlinska, K., Turlejski, K., and Pisula, W. (2013). Circadian rhythm of outside-nest activity in wild (WWCPS), albino and pigmented laboratory rats. *PLOS ONE* 8: e66055.
547. Daan, S. and Oklejewicz, M. (2003). The precision of circadian clocks: Assessment and analysis in Syrian hamsters. *Chronobiology International* 20: 209–221.
548. Yang, Y. and Gordon, C. J. (1996). Ambient temperature limits and stability of temperature regulation in telemetered male and female rats. *Journal of Thermal Biology* 21: 353–363.
549. Schmidt, F., Yoshimura, Y., Ni, R. X., Kneesel, S., and Constantinou, C. E. (2001). Influence of gender on the diurnal variation of urine production and micturition characteristics of the rat. *Neurourology and Urodynamics* 20: 287–295.
550. Cain, S. W., Dennison, C. F., Zeitzer, J. M., Guzik, A. M., Khalsa, S. B. S., Santhi, N., Schoen, M. W., Czeisler, C. A., and Duffy, J. F. (2010). Sex differences in phase angle of entrainment and melatonin amplitude in humans. *Journal of Biological Rhythms* 25: 288–296.
551. Van Reen, E., Sharkey, K. M., Roane, B. M., Barker, D., Seifer, R., Raffray, T., Bond, T. L., and Carskadon, M. A. (2013). Sex of college students moderates associations among bedtime, time in bed, and circadian phase angle. *Journal of Biological Rhythms* 28: 425–431.
552. Campbell, S. S., Gillin, J. C., Kripke, D. F., Erikson, P., and Clopton, P. (1989). Gender differences in the circadian temperature rhythms of healthy elderly subjects: Relationships to sleep quality. *Sleep* 12: 529–536.
553. Adan, A. and Natale, V. (2002). Gender differences in morningness-eveningness preference. *Chronobiology International* 19: 709–720.
554. Hoban, T. M., Levine, A. H., Shane, R. B., and Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
555. Moritz, R. F. A. and Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.
556. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P., and Challet, E. (2003). Circadian profile and photic regulation of clock genes in the suprachiasmatic nucleus of a diurnal mammal, *Arvicanthis ansorgei*. *Neuroscience* 116: 583–591.
557. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S., and Tabata, M. (1997). Feeding entrainment of locomotor activity rhythms in the goldfish is mediated by a feeding-entrainable circadian oscillator. *Journal of Comparative Physiology A* 181: 121–132.
558. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G., and Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
559. Büttner, D. (1992). Social influences on the circadian rhythm of locomotor activity and food intake of guinea pigs. *Journal of Interdisciplinary Cycle Research* 23: 100–112.
560. Tavernier, R. J., Largen, A. L., and Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
561. Oshima, I. and Ebihara, S. (1988). The measurement and analysis of circadian locomotor activity and body temperature rhythms by a computer-based system. *Physiology and Behavior* 43: 115–119.
562. Guyomarc'h, C., Lumineau, S., and Richard, J. P. (1998). Circadian rhythm of activity in Japanese quail in constant darkness: Variability of clarity and possibility of selection. *Chronobiology International* 15: 219–230.
563. Lumineau, S. and Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
564. Hurd, M. W., Debruyne, J., Straume, M., and Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiology and Behavior* 65: 465–472.
565. Kennedy, G. A., Coleman, G. J., and Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
566. Lowe, C. H., Hinds, D. S., Lardner, P. J., and Justice, K. E. (1967). Natural free-running period in vertebrate animal populations. *Science* 156: 531–534.
567. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T., and Tomioka, K. (2004). *Drosophila cry^b* mutation reveals two circadian clocks that drive locomotor rhythm and have different responsiveness to light. *Journal of Insect Physiology* 50: 479–488.
568. Honma, S. and Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
569. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., and Johnson, R. F. (1987). A two-peak circadian system in body temperature and activity in the domestic cat, *Felis catus*. *Journal of Thermal Biology* 12: 27–37.
570. Rajaratnam, S. M. W. and Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
571. Refinetti, R. and Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.
572. Koilraj, A. J., Sharma, V. K., Marimuthu, G., and Chandrashekaran, M. K. (2000). Presence of circadian rhythms in the locomotor activity of a cave-dwelling millipede *Glyphiulus cavernicolus sulu* (Cambalidae: Spirostreptida). *Chronobiology International* 17: 757–765.
573. Chandrashekaran, M. K., Marimuthu, G., and Geetha, L. (1997). Correlations between sleep and wake in internally synchronized and desynchronized circadian rhythms in humans under prolonged isolation. *Journal of Biological Rhythms* 12: 26–33.

574. Reinberg, A., Halberg, F., Ghata, J., and Siffre, M. (1966). Spectre thermique (rythmes de la température rectale) d'une femme adulte avant, pendant et après son isolement souterrain de trois mois. *Comptes Rendus de l'Académie des Sciences* 262: 782–785.
575. Pollak, C. P. and Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
576. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C., and Knauer, R. S. (1980). Human sleep: Its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
577. Green, J., Pollak, C. P., and Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
578. Steel, G. D., Callaway, M., Suedfeld, P., and Palinkas, L. (1995). Human sleep–wake cycles in the high Arctic: Effects of unusual photoperiodicity in a natural setting. *Biological Rhythm Research* 26: 582–592.
579. Aschoff, J. (1994). The timing of defecation within sleep–wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
580. Campbell, S. S., Dawson, D., and Zulley, J. (1993). When the human circadian system is caught napping: Evidence for endogenous rhythms close to 24 hours. *Sleep* 16: 638–640.
581. Aschoff, J. (1994). Naps as integral parts of the wake time within the human sleep–wake cycle. *Journal of Biological Rhythms* 9: 145–155.
582. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A., and Dijk, D. J. (1999). Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *American Journal of Physiology* 277: R1152–R1163.
583. Wever, R. A. (1989). Light effects on human circadian rhythms: A review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
584. Honma, K., Honma, S., and Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
585. Lockley, S. W., Skene, D. J., Butler, L. J., and Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 22: 616–623.
586. Endo, T., Honma, S., Hashimoto, S., and Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
587. Sack, R. L., Brandes, R. W., Kendall, A. R., and Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
588. Hack, L. M., Lockley, S. W., Arendt, J., and Skene, D. J. (2003). The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects. *Journal of Biological Rhythms* 18: 420–429.
589. Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. *Journal of Comparative Physiology A* 179: 135–142.
590. Tosini, G. and Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *Journal of Biological Rhythms* 10: 248–255.
591. Bartell, P. A., Miranda-Anaya, M., and Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
592. Page, T. L., Mans, C., and Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.
593. Ortega-Escobar, J. (2002). Circadian rhythms of locomotor activity in *Lycosa tarentula* (Araneae, Lycosidae) and the pathways of ocular entrainment. *Biological Rhythm Research* 33: 561–576.
594. Tokura, H. and Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
595. Shimomura, K., Nelson, D. E., Ihara, N. L., and Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
596. Weinert, D., Fritzche, P., and Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
597. Ralph, M. R. and Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
598. Refinetti, R. and Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
599. Daan, S. and Pittendrigh, C. S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents. II. The variability of phase response curves. *Journal of Comparative Physiology* 106: 253–266.
600. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
601. Refinetti, R., Nelson, D. E., and Menaker, M. (1992). Social stimuli fail to act as entraining agents of circadian rhythms in the golden hamster. *Journal of Comparative Physiology A* 170: 181–187.
602. Perret, M., Aujard, F., Séguy, M., and Schilling, A. (2003). Olfactory bulbectomy modifies photic entrainment and circadian rhythms of body temperature and locomotor activity in a nocturnal primate. *Journal of Biological Rhythms* 18: 392–401.
603. Gerkema, M. P., Groos, G. A., and Daan, S. (1990). Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. *Journal of Biological Rhythms* 5: 81–95.
604. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
605. Edgar, D. M., Kilduff, T. S., Martin, C. E., and Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior* 50: 373–378.
606. Edgar, D. M., Martin, C. E., and Dement, W. C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *Journal of Biological Rhythms* 6: 185–199.
607. Von Gall, C., Duffield, G. E., Hastings, M. H., Kopp, M. D. A., Dehghani, F., Korf, H. W., and Stehle, J. H. (1998). CREB in the mouse SCN: A molecular interface coding the phase-adjusting stimuli light, glutamate, PACAP, and melatonin for clockwork access. *Journal of Neuroscience* 18: 10389–10397.
608. Abe, H., Honma, S., Honma, K., Suzuki, T., and Ebihara, S. (1999). Functional diversities of two activity components of circadian rhythm in genetical splitting mice (CS strain). *Journal of Comparative Physiology A* 184: 243–251.

609. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S., and Honma, K. (2001). Clock gene expressions in the suprachiasmatic nucleus and other areas of the brain during rhythm splitting in CS mice. *Molecular Brain Research* 87: 92–99.
610. Edgar, D. M. and Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
611. Benloucif, S. and Dubocovich, M. L. (1996). Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN mouse. *Journal of Biological Rhythms* 11: 113–125.
612. Labyak, S. E. and Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
613. Lee, T. M. and Labyak, S. E. (1997). Free-running rhythms and light- and dark-pulse phase response curves for diurnal *Octodon degus* (Rodentia). *American Journal of Physiology* 273: R278–R286.
614. Kennedy, G. A., Hudson, R., and Armstrong, S. M. (1994). Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiology and Behavior* 55: 385–389.
615. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences United States of America* 59: 414–421.
616. Hau, M. and Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
617. Lindberg, R. G. and Hayden, P. (1974). Thermoperiodic entrainment of arousal from torpor in the little pocket mouse, *Perognathus longimembris*. *Chronobiologia* 1: 356–361.
618. Puchalski, W. and Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: Effects of photoperiodic pretreatment and artificial selection. *American Journal of Physiology* 261: R670–R676.
619. Innicenti, A., Minutini, L., and Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
620. Hamasaka, Y., Watari, Y., Arai, T., Numata, H., and Shiga, S. (2001). Retinal and extraretinal pathways for entrainment of the circadian activity rhythm in the blow fly, *Protophormia terraenovae*. *Journal of Insect Physiology* 47: 867–875.
621. Borbély, A. A. and Neuhaus, H. U. (1978). Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. *Journal of Comparative Physiology* 128: 37–46.
622. Ikeda, M. and Inoué, S. (1998). Simultaneous recording of circadian rhythms of brain and intraperitoneal temperatures and locomotor and drinking activities in the rat. *Biological Rhythm Research* 29: 142–150.
623. Francis, A. J. P. and Coleman, G. J. (1988). The effect of ambient temperature cycles upon circadian running and drinking activity in male and female laboratory rats. *Physiology and Behavior* 43: 471–477.
624. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S., and Takahashi, K. (1988). Free-access to a running wheel shortens the period of free-running rhythm in blinded rats. *Physiology and Behavior* 42: 87–91.
625. Summer, T. L., Ferraro, J. S., and McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
626. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
627. Stokes, M. A., Kent, S., and Armstrong, S. M. (1999). The effect of multiple pulses of light on the circadian phase response of the rat. *Journal of Biological Rhythms* 14: 172–184.
628. Canal-Corretger, M. M., Vilaplana, J., Cambras, T., and Díez-Noguera, A. (2001). Functioning of the rat circadian system is modified by light applied in critical postnatal days. *American Journal of Physiology* 280: R1023–R1030.
629. Honma, K., von Goetz, C., and Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.
630. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M., and Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.
631. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M., and Daan, S. (1997). Stress-induced changes in circadian rhythms of body temperature and activity in rats are not caused by pacemaker changes. *Journal of Biological Rhythms* 12: 80–92.
632. Fuller, C. A. and Edgar, D. M. (1986). Effects of light intensity on the circadian temperature and feeding rhythms in the squirrel monkey. *Physiology and Behavior* 36: 687–691.
633. Underwood, H. (1983). Circadian pacemakers in lizards: Phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
634. King, V. M., Bentley, G. E., and Follett, B. K. (1997). A direct comparison of photoperiodic time measurement and the circadian system in European starlings and Japanese quail. *Journal of Biological Rhythms* 12: 431–442.
635. Ebihara, S. and Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
636. Kramm, K. R. and Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.
637. Herrero, M. J., Madrid, J. A., and Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
638. Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A., and Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740.
639. Rothenfluh, A., Abodeely, M., Price, J. L., and Young, M. W. (2000). Isolation and analysis of six *timeless* alleles that cause short- or long-period circadian rhythms in Drosophila. *Genetics* 156: 665–675.
640. Comolli, J., Taylor, W., and Hastings, J. W. (1994). An inhibitor of protein phosphorylation stops the circadian oscillator and blocks light-induced phase shifting in *Gonyaulax polyedra*. *Journal of Biological Rhythms* 9: 13–26.
641. Morse, D., Hastings, J. W., and Roenneberg, T. (1994). Different phase responses of the two circadian oscillators in *Gonyaulax*. *Journal of Biological Rhythms* 9: 263–274.
642. Khalsa, S. B. S., Jewett, M. E., Cajochen, C., and Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.

643. Jewett, M. E., Kronauer, R. E., and Czeisler, C. A. (1994). Phase-amplitude resetting of the human circadian pacemaker via bright light: A further analysis. *Journal of Biological Rhythms* 9: 295–314.
644. Dawson, D., Lack, L., and Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102.
645. Honma, K. and Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167–168.
646. Christian, C. A. and Harrington, M. E. (2002). Three days of novel wheel access diminishes light-induced phase delays in vivo with no effect on *per1* induction by light. *Chronobiology International* 19: 671–682.
647. Grosse, J., Loudon, A. S. I., and Hastings, M. H. (1995). Behavioural and cellular responses to light of the circadian system of *tau* mutant and wild-type Syrian hamsters. *Neuroscience* 65: 587–597.
648. Meijer, J. H. and De Vries, M. J. (1995). Light-induced phase shifts in onset and offset of running-wheel activity in the Syrian hamster. *Journal of Biological Rhythms* 10: 4–16.
649. Takahashi, J. S., DeCoursey, P. J., Mauman, L., and Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.
650. Shimomura, K. and Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
651. Sharma, V. K., Chandrashekaran, M. K., and Nongkynrih, P. (1997). Daylight and artificial light phase response curves for the circadian rhythm in locomotor activity of the field mouse *Mus booduga*. *Biological Rhythm Research* 28: 39–49.
652. Shrama, V. K., Chidambaram, R., and Yadunandam, A. K. (2003). Melatonin enhances the sensitivity of circadian pacemakers to light in the nocturnal field mouse *Mus booduga*. *Journal of Experimental Zoology* 297A: 160–168.
653. Barbacka-Surowiak, G. (2000). Is the PRC for dark pulses in LL a mirror image of the PRC for light pulses in DD in mice? *Biological Rhythm Research* 31: 531–544.
654. Kilduff, T. S., Vugrinic, C., Lee, S. L., Milbrandt, J. D., Mikkelsen, J. D., O'Hara, B. F., and Heller, H. C. (1998). Characterization of the circadian system of *NGFI-A* and *NGFI-A/NGFI-B* deficient mice. *Journal of Biological Rhythms* 13: 347–357.
655. Yoshimura, T., Nishio, M., Goto, M., and Ebihara, S. (1994). Differences in circadian photosensitivity between retinally degenerate CBA/J mice (*rd/rd*) and normal CBA/M mice (+/+). *Journal of Biological Rhythms* 9: 51–60.
656. Van den Pol, A. N., Cao, V., and Heller, H. C. (1998). Circadian system of mice integrates brief light stimuli. *American Journal of Physiology* 275: R654–R657.
657. Sharma, V. K. and Daan, S. (2002). Circadian phase and period responses to light stimuli in two nocturnal rodents. *Chronobiology International* 19: 659–670.
658. Bauer, M. S. (1992). Irradiance responsivity and unequivocal type-1 phase responsivity of rat circadian activity rhythms. *American Journal of Physiology* 263: R1110–R1114.
659. Saunders, D. S. (1978). An experimental and theoretical analysis of photoperiodic induction in the flesh-fly, *Sarcophaga argyrostoma*. *Journal of Comparative Physiology* 124: 75–95.
660. Meijer, J. H., Daan, S., Overkamp, G. J. F., and Hermann, P. M. (1990). The two-oscillator circadian system of tree shrews (*Tupaia belangeri*) and its response to light and dark pulses. *Journal of Biological Rhythms* 5: 1–16.

This page intentionally left blank

10 Homeostasis and Circadian Rhythmicity

10.1 TEMPERATURE REGULATION

The concept of *homeostasis* is perhaps the most important concept in physiology.[1] From its origins in the work of Claude Bernard (Figure 10.1) in the 1800s,[2] this concept has been refined over decades[3,4] and is considered to be an essential part of cultural literacy in our days.[1,5] As defined by the Commission for Thermal Physiology of the International Union of Physiological Sciences, homeostasis characterizes "the relative constancy of physico-chemical properties of the internal environment of an organism as being maintained by regulation."[6]

Although homeostasis applies to numerous processes in the body—such as those required for the constancy of proper electrolyte balance, sufficient supply of nutrients, and adequate secretion of hormones—temperature regulation is possibly the most intuitive version of homeostasis. Consider Figure 10.2. In order to keep your house warm in the winter, you need to have a temperature sensor in the house, which sends a signal to a thermostat, which controls a heater (Panel A). To understand temperature regulation in a living organism, we can draw essentially the same diagram with just a small rearrangement of the components to make the terms more explicit (Panel B). Thus, the organism must have one or more temperature receptors, which send a signal to the thermostat, which compares its own setting with the incoming signal and determines whether the heat-producing effector organs should be activated or not. If the temperature of the organism is below the thermostat's setting, then the effector organs are activated, which raises the organism's temperature, which eventually reduces the error signal and causes the thermostat to deactivate the effector organs. The whole cycle is repeated as needed.

Just as old as the notion of homeostasis is the observation that physiological variables are not maintained constant in the absence of environmental perturbations but instead display regular circadian rhythmicity (see Chapter 1—and, as a matter of fact, all of the preceding chapters in this book). Although circadian rhythmicity clearly interferes with the constancy of the internal environment, only during the last 20 years or so has systematic attention been given to the integration of homeostatic and circadian processes of physiological regulation. In the particular case of the regulation of body temperature (T_b), thermal physiologists and circadian physiologists essentially ignored each other's work for most of the twentieth century and beginning of the twenty-first century. Therefore, I will first review the accumulated knowledge on the homeostatic control of T_b (the process of *temperature regulation*), and only afterward will I relate it to the accumulated knowledge on the *circadian* control of T_b.

10.1.1 Homeostatic Control of Body Temperature

It was already pointed out in Chapter 6 that the basic process of life is a series of chemical reactions catalyzed by enzymes. The rate of the reactions is temperature dependent, so that a temperature change of 10°C causes approximately a twofold change in the reaction rate.[7,8] Consequently, a temperature fall of 10°C slows down the functioning of the body to half of what it was earlier, and a temperature rise of 10°C speeds up the functioning of the body to twice of what it previously was. Considering that the mean air temperature on Earth varies from much below freezing in the polar regions to 50°C or higher in tropical deserts and that large temperature fluctuations occur on a daily and seasonal basis in many regions, it is evident that organisms must adapt to the thermal environment in order to live independent lives (Figure 10.3). Adaptation of organisms to the thermal environment may be accomplished in two basic ways: by increased *tolerance* to temperature variations or by active *regulation* of T_b. Most organisms do not regulate T_b—or regulate it in a limited fashion—but are very well adapted to the thermal environment. For instance, many Antarctic fishes spend their whole life cycle at subfreezing T_b,[9] whereas worms living in deep-sea hydrothermal vents thrive at temperatures between 40°C and 50°C.[10] Some species in the Archaea domain survive and grow at temperatures up to 120°C.[11] Lower vertebrates (reptiles, amphibians, and fishes) make use of antifreeze proteins to avoid freezing in the winter.[12,13]

Traditionally, organisms that do not regulate T_b (and, therefore, are usually cold to the touch) are called "cold blooded," whereas organisms that regulate T_b (and, therefore, are usually warm to the touch) are called "warm blooded." When you were in kindergarten, you were probably told that frogs are cold-blooded animals. However, if you lived in a very warm place (with ambient temperature occasionally exceeding 33°C or 91°F), you may have noticed that frogs actually felt warm to the touch. This was not because frogs miraculously became warm blooded on hot days but because the terminology was inappropriate. Thermal physiologists refer to organisms whose temperature varies with that of the environment as "poikilothermic" organisms; conversely, they refer to organisms whose temperature is regulated at a relatively constant level despite variations in ambient temperature as "homeothermic" organisms. Thus, frogs are poikilothermic regardless of whether they feel cold or warm to the touch. Because the temperature of the skin of the human hand is approximately 33°C,

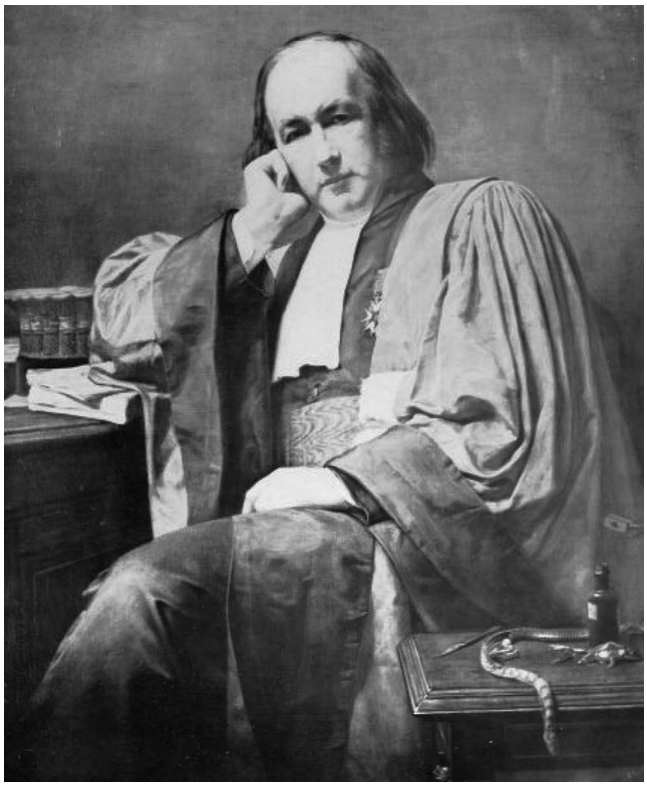


FIGURE 10.1 Claude Bernard (1813–1878). This French physiologist developed the concept of homeostasis. (Courtesy of Bibliothèque Interuniversitaire de Médecine, Paris, France.)

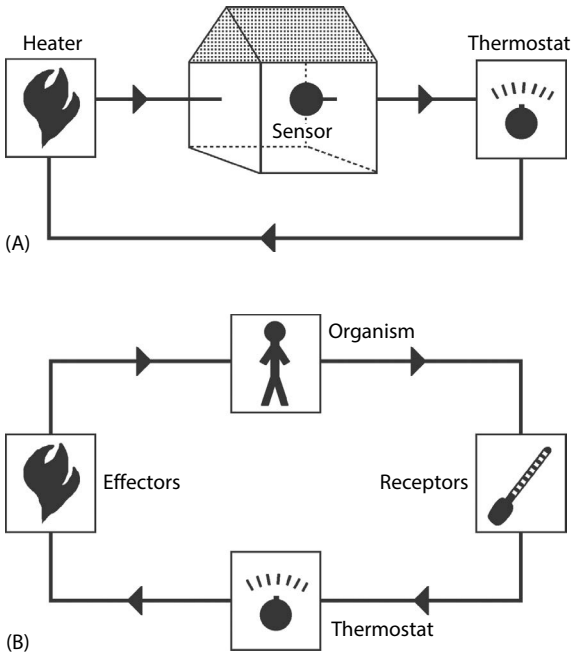


FIGURE 10.2 Homeostasis. The concept of homeostasis (regulation by negative feedback) is illustrated in a diagram of the heating system of a house (A) and in a diagram of the thermoregulatory system of organisms (B).



FIGURE 10.3 A cold tiger. Animals living in cold environments must be able to either function at low body core temperatures or to regulate body temperature actively at a level above ambient temperature. (Copyright ArtToday, Tucson, AZ.)

objects feel warm to the touch when their temperature exceeds 33°C.[14–18] It just happens that frogs are usually not found in places where ambient temperature exceeds 33°C. A dog, which is homeothermic and has a T_b similar to ours, would feel cold to the touch if we had a very high fever. Core temperature is generally regulated around 35°C in marsupial mammals, around 37.5°C in placental mammals, and around 41°C in birds (see Table 5.3). It has been suggested that the core temperature of terrestrial homeotherms must be set within the range of 35°C–45°C because only within this range is there a proper balance between the costs of energy conservation in the cold and the costs of water conservation in the heat.[19]

Many years after kindergarten, you must have also learned that things in this world are rarely as simple as they first appear to be. This is definitely true regarding the distinction between poikilothermy and homeothermy. The distinction works well if we keep organisms in small containers at constant temperatures in the laboratory, but it breaks down very quickly if we allow organisms to move freely in their natural environments. Direct your attention to Figure 10.4. Panel A shows that the T_b of an alligator housed in a chamber in the laboratory follows ambient temperature very closely. Thus, the alligator is a poikilotherm. However, Panel B shows that in the field—with freedom to warm itself up by basking in the Sun and to cool itself down by going into the water—the alligator maintains a constant T_b independently of air temperature. So, now the alligator is a homeotherm. When allowed to use behavioral responses, the alligator maintains its T_b almost as precisely as a *true* homeotherm—the mouse (Panel C). Evidently, poikilothermic organisms can exhibit sophisticated temperature regulation through behavioral means. Thus, the distinction between poikilothermy and homeothermy is too rough for most uses. A more precise distinction must be made to account for thermoregulatory differences limited to autonomic processes. Organisms that can generate enough heat endogenously to warm up their bodies above ambient temperature without resorting to behavior are called "endothermic" organisms;

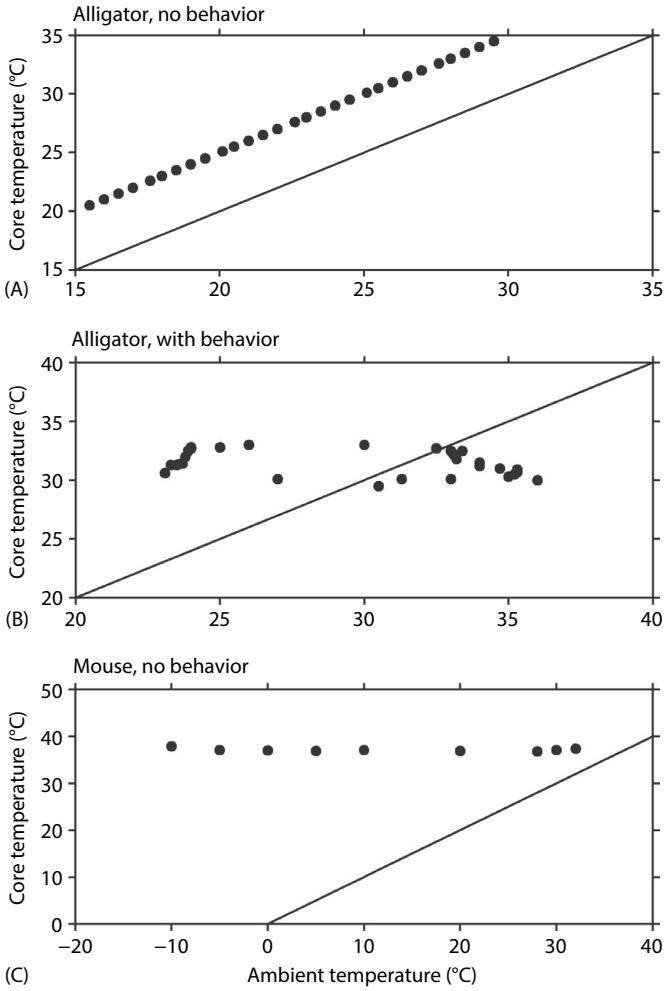


FIGURE 10.4 Poikilotherms, homeotherms, and other therms. The body core temperature of an alligator held in a chamber in the laboratory closely follows air temperature (A). However, if the alligator is allowed to thermoregulate behaviorally, its core body temperature is much less dependent on air temperature (B). In mice, body core temperature is independent of ambient temperature (within limits) even in the absence of behavioral responses (C). In all three graphs, the diagonal lines indicate identity between core temperature and ambient temperature. (Data from Smith, E.N., *Physiol. Zool.*, 48, 177, 1975; Oufara, S. et al., *Am. J. Physiol.*, 253, R39, 1987.)

conversely, organisms that require external sources to warm up their bodies above ambient temperature are called "ectothermic" organisms. The resting metabolic rate (MR) of endothermic animals is 4–5 times higher than that of ectothermic animals of similar body size tested at the same T_b, and this seems to result from the facts that endothermic animals have relatively larger internal organs, that the organs have a greater proportion of mitochondria (the cell organelles responsible for metabolic oxidation), and that the mitochondria have a greater relative membrane surface.[20,21]

Mammals and birds are generally endothermic, whereas all other life forms on Earth are ectothermic. There are several exceptions, however. Several species of tuna fish can maintain T_b relatively constant at 25°C while swimming in water ranging from 3°C to 31°C.[22–25] Leatherback turtles maintain T_b several degrees above the temperature of the sea water in which they live.[26] Some beetles can maintain a constant thoracic temperature of 38°C while air temperature varies from 13°C to 24°C.[27] More impressively, several groups of *plants* can raise metabolic heat production when blooming, thus causing dramatic elevations in the temperature of the inflorescence. For the eastern skunk cabbage, temperatures of 15°C–20°C were recorded in plants kept at ambient temperatures from −15°C to 5°C.[28] For the philodendron, temperatures of 38°C–46°C were recorded in plants kept at ambient temperatures from 4°C to 39°C.[29,30] For the sacred lotus, temperatures of 30°C–35°C were recorded in plants kept at ambient temperatures from 10°C to 30°C.[31,32] Early in the 1970s, the chemical responsible for the increased thermogenesis (the *calorigen*) was isolated in voodoo lilies,[33] and the isolate was later identified as salicylic acid.[34] It is ironic that the substance that produces a *fever* in plants is a major antipyretic in mammals (salicylic acid being the active ingredient in aspirin).[35]

Exceptions exist also among mammals and birds. As discussed in Chapter 4, hibernators in the hibernating state allow T_b to fall along with ambient temperature and do not take corrective measures unless T_b approaches 0°C. Because these animals are poikilothermic during part of the year and homeothermic during the rest of the year, they are called "heterothermic" animals. Round-tailed ground squirrels (*Xerospermophilus tereticaudus*) exhibit a different form of heterothermy: even when fully active, they allow T_b to fall more than 5°C when briefly exposed to a moderately cold environment.[36,37] Thirteen-lined ground squirrels (Figure 10.5) also allow T_b to fluctuate with ambient temperature during the nonhibernating season.[38] Even the designation of *poikilothermic* may be justified for *mole rats*. The Mashona mole rat (*Cryptomys hottentotus*) can maintain a stable, low T_b of 33°C only in the narrow range of ambient temperatures between 26°C and 32°C.[39] The naked mole rat (*Heterocephalus glaber*) is even worse: its T_b follows ambient temperature throughout the range from 12°C to 37°C.[40]



FIGURE 10.5 A heterothermic animal. The 13-lined ground squirrel (*Ictidomys tridecemlineatus*) is a facultative homeotherm. It often allows its body temperature to fluctuate in cold environments, and it hibernates in the winter. (Photo by Mark F. Wallner. All rights reserved.)

In contrast, a true homeotherm can maintain T_b within a very narrow range despite large fluctuations in ambient temperature. For instance, throughout a whole year in an outdoor paddock, a sheep (*Ovis aries*) maintained its core temperature between 37°C and 39°C while ambient temperature varied from −10°C to 20°C.[41]

Engineering models have often been used to describe homeothermic temperature regulation. A very simple model is diagrammed in Panel A of Figure 10.6. This is the same diagram that we saw in Figure 10.2. A more detailed model is diagrammed in Panel B. One alteration is that the single receptors box was replaced by two boxes: one for cold receptors and another for warm receptors. This topic will be discussed further in Chapter 11. For now, suffice it to say that, unlike manmade thermometers, biological temperature receptors are segmented. They break down the temperature continuum into two segments: the cold segment and the warm segment. The cold segment lies below neutrality and the warm segment lies above neutrality, although *neutrality* is a relative concept. The single effectors box was also replaced by two boxes: one for heat loss mechanisms and another for heat production mechanisms. You can think of these as an air-conditioning unit and a heater unit, respectively. Perhaps the most important alteration in the diagram is the inclusion of the reference value box.

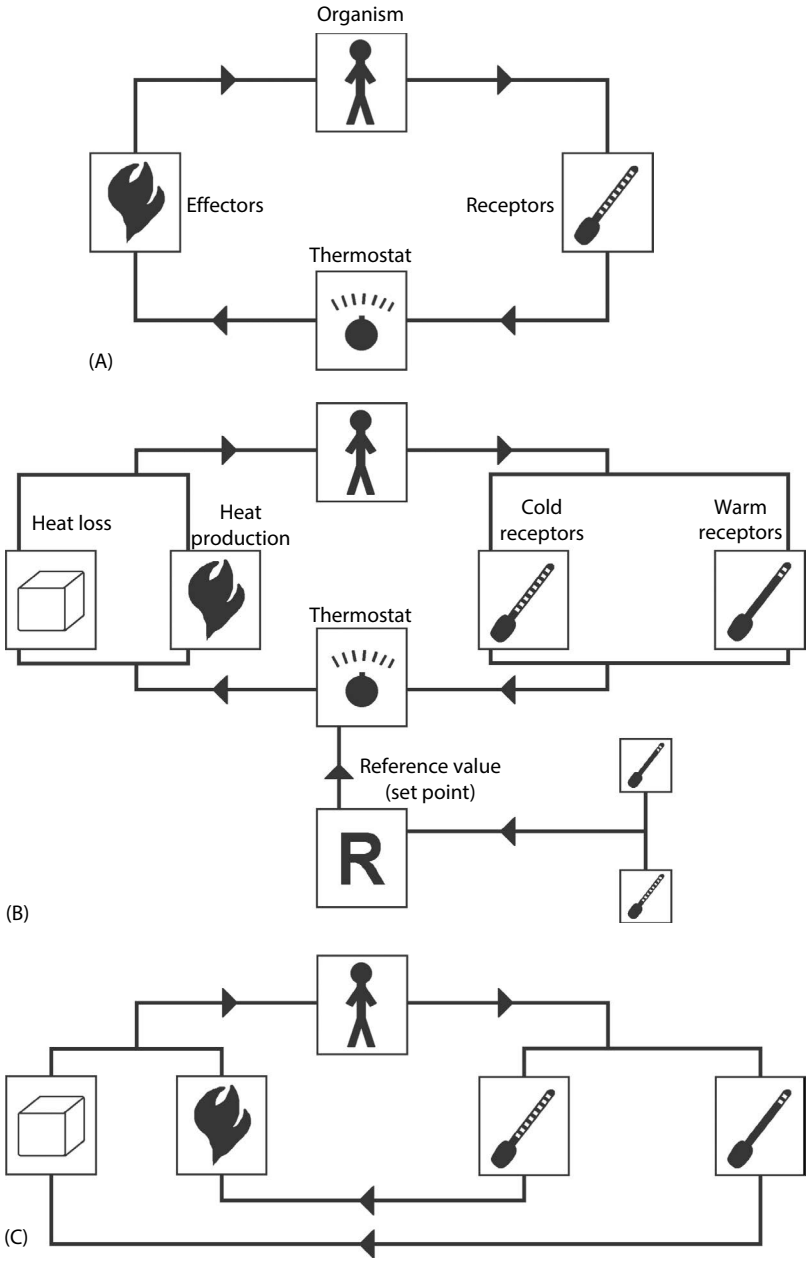


FIGURE 10.6 Homeostatic control of body temperature. The generic model of homeostasis (A) has been applied to body temperature regulation in a version that includes a central set point (B) and one that does not (C). (Adapted from Refinetti, R., *Manuscrito*, 11, 47, 1988.)

Notice that this box is outside the main loop. It provides the *set point* for the thermostat. The set point is "a fixed or constant input, established by means external to and independent of the automatic control system, which sets the ideal value of the controlled variable."[42] The controlled variable here is, of course, T_b. The thermostat must compare the input from the cold and warm receptors with the input from the reference value (the set point) and then activate the heat loss or heat production mechanisms as needed. It became obvious in the early 1960s that the constancy of the set point did not hold true in real animals. It was realized that the set point was *adjustable*, going up when skin temperature goes down, and going down when skin temperature goes up.[43] This is why the diagram includes an input from additional receptors to the reference value box. The main receptors are assumed to measure core temperature, which is the regulated variable. The additional receptors measure skin temperature.

It would be an understatement to say that the set point concept is an important concept in thermal physiology. To a large extent, the concept of set point derives its appeal from the convenient way in which it explains the complex physiological processes involved in the phenomenon of *fever*. During the rising phase of fever, various thermoregulatory responses are activated to increase heat production and reduce heat loss (thus leading to a temperature elevation), and various responses are also activated later to reduce heat gain and increase heat loss (leading to a return of temperature to its initial, nonfebrile level). A simple and coherent explanation for these physiological alterations is that the set point is elevated during the rising phase and returned to normal during the later phase. In support of this interpretation is the finding that the temperature attained during a fever is proportional to the dose of pyrogenic agents injected.[44–49] The hypothesis of a regulated change in set point is strengthened by the fact that, during the rising phase of fever, animals prefer higher external temperatures than usual (which facilitates body heat conservation), whereas in the later phase they prefer lower temperatures than usual (which facilitates heat loss).[50–54] Even in ectothermic animals, which cannot produce a fever by autonomic means, higher temperatures are selected when the animals are infected with pyrogenic substances.[55–59]

Despite the weight of tradition and compatibility with engineering models, the set point concept has not been without critics. The theoretical necessity of a central controller has been questioned by thermal physiologists who proposed an alternative view that could be called an "accelerator model."[60] As diagrammed in Panel C of Figure 10.6, it is proposed that heat loss mechanisms respond directly to signals from warm receptors, whereas heat production mechanisms respond directly to signals from cold receptors.[61–64] Thus, when temperature is elevated above thermoneutrality, heat loss mechanisms are activated; when temperature falls below thermoneutrality, heat production mechanisms are activated. The warmer it is, the more activated are the mechanisms of heat loss; the colder it is, the more activated are the mechanisms of heat production and conservation—hence the term *accelerator*. The activation threshold and the response characteristics of the system are determined by the properties of the receptors. There is no need for a master reference-signal generator (set point).

Whether there is an explicit set point for T_b or not, the thermoregulatory system must manage the flow of energy through the body. Animals produce heat endogenously by breaking down the food that they eat, and they lose heat to the environment (or sometimes gain heat from the environment) through radiation, conduction, and convection (Figure 10.7). Both endotherms and ectotherms have a vast repertoire of autonomic and behavioral responses that can be used in the control of T_b. These responses may be employed on three basic time scales: as short-term responses, as seasonal acclimatization, or as part of the evolutionary process.[65,66] The major thermoregulatory responses are listed in Table 10.1. All of these responses can be used on a short-term time scale, most of them can be modified by acclimatization, and some of

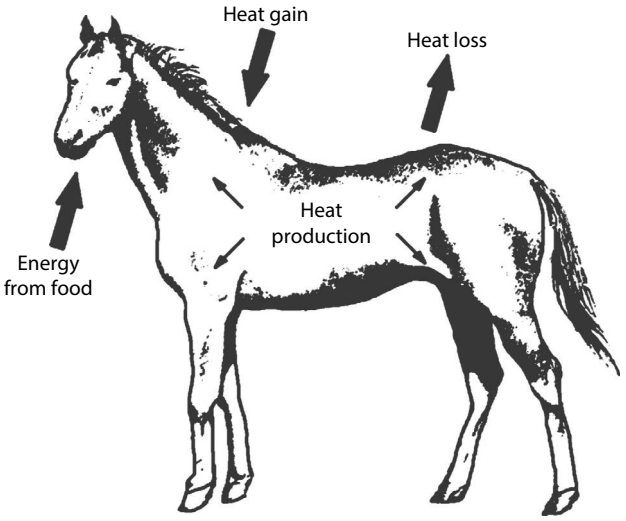


FIGURE 10.7 Heat exchange. Living beings exchange heat with the environment through radiation, conduction, and convection. In addition, they produce heat endogenously by breaking down the food that they eat.

TABLE 10.1
Thermoregulatory Responses

Autonomic Responses	Behavioral Responses
Shivering	Relocation
Nonshivering thermogenesis	Postural adjustment
Vasomotion	Huddling
Evaporation	Modulation of food intake
Insulation	Modulation of activity level
Torpor	Building of microenvironments
	Learning

Sources: Cossins, A.R. and Bower, K., *Temperature Biology of Animals*, Chapman and Hall, London, U.K., 1987; Cabanac, M., The place of behavior in physiology, in: Fregley, M.J. and Blatteis, C.M., eds., *Handbook of Physiology—Section 4: Environmental Physiology*, Vol. 2, Oxford University Press, New York, 1996, pp. 1523–1536.

them have acquired a more prominent role in different species along the evolutionary process.

Shivering is perhaps the most universal autonomic response aimed at elevating heat production. It is a widespread mechanism of thermogenesis used to prevent the fall of T_b in a cold environment. It consists of small-amplitude, high-frequency contractions of skeletal muscles. It is employed both by birds[67–75] and by mammals,[76–91] including humans.[92–94] An example is shown in Figure 10.8. King penguin chicks (*Aptenodytes patagonicus*) were studied in a research station in Antarctica. The top graph (A) shows that the penguins elevate MR to increase heat production as ambient temperature is lowered to −20°C. The middle graph (B) shows that the elevation of MR is successful in preventing a fall in T_b. The bottom panel (C) provides the integrated EMG activity data. EMG stands for *electromyogram*—the measurement of electrical activity of muscle cells. Notice how closely the EMG activity follows the elevation of MR, which indicates that shivering is responsible for the elevated heat production.[67] Inactivation of shivering by blockade of neuromuscular synapses prevents the elevation of MR.[84,95]

Forms of biological thermogenesis other than shivering are collectively called "nonshivering thermogenesis" (NST). This involves obligatory as well as adaptive mechanisms.[6] Birds seem to rely primarily on shivering and, if they exhibit thermoregulatory NST, the muscles themselves are the probable source.[67,71,72,74,96–99] Mammals, on the other hand, use NST extensively in response to cold stress, and the capacity

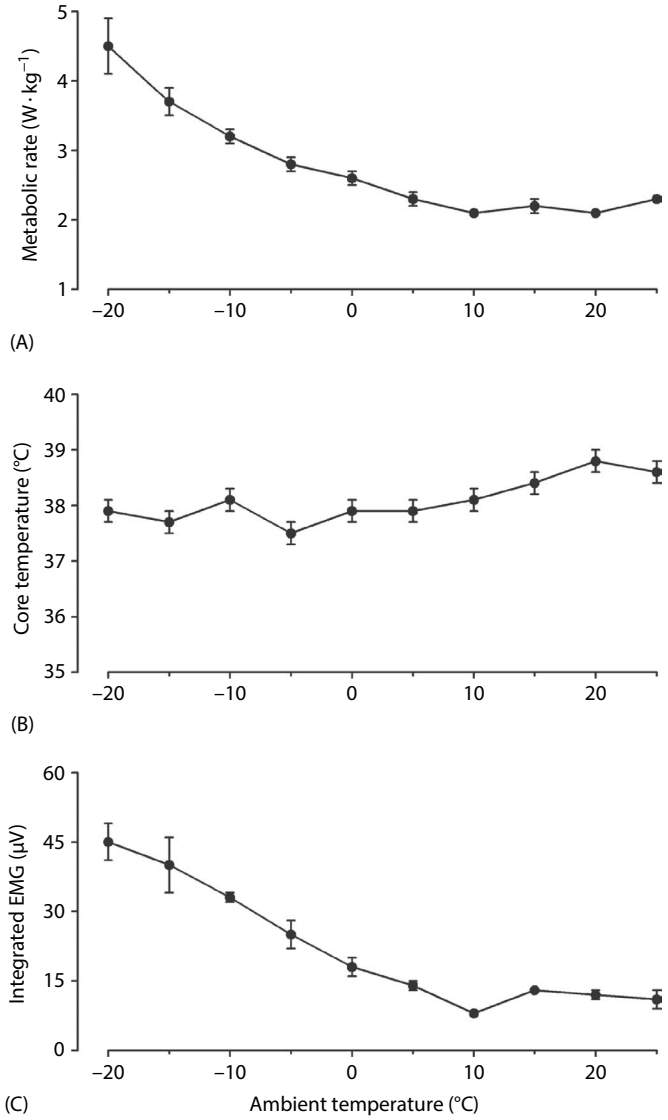


FIGURE 10.8 Autonomic temperature regulation: shivering. Birds and mammals have well-developed autonomic mechanisms of temperature regulation. These graphs show mean data (± SE) for 8 young king penguins (*Aptenodytes patagonicus*) studied at a research station in Antarctica. Thermoregulatory thermogenesis is evident in the elevation of metabolic rate at lower ambient temperatures (Panel A), which allows body temperature to remain constant despite the large variation in the temperature of the environment (Panel B). As is characteristic of birds (particularly in a non-cold-acclimated state), most of the thermoregulatory heat production derives from muscle contraction in the form of shivering (Panel C). EMG, electromyogram. (Data from Duchamp, C. et al., *Am. J. Physiol.*, 257, R744, 1989.)

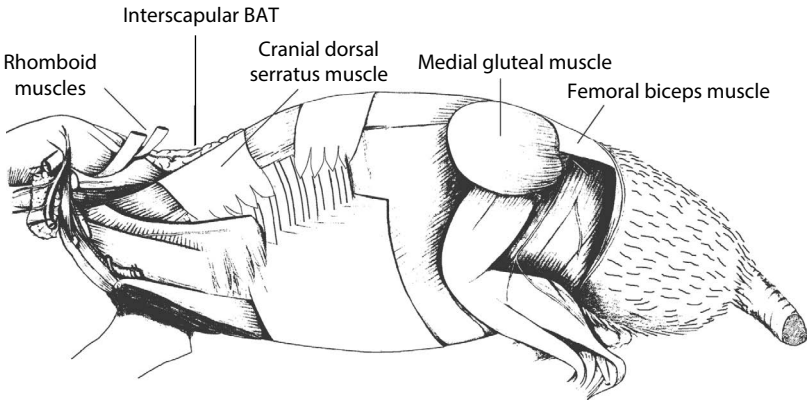


FIGURE 10.9 A different type of fat, a different type of bat. Brown adipose tissue (BAT), also known as brown fat, is a form of fat different from the ordinary white fat. It is a highly thermogenic tissue believed to play an important role in cold-induced thermogenesis in mammals. The diagram indicates the location of interscapular BAT in the rat. (Adapted from Popesko, P. et al., *A Colour Atlas of Small Laboratory Animals*, Vol. 2, Wolfe Publishing, London, U.K., 1992.)

to use NST is strongly affected by acclimation or acclimatization.[78,84–87,90,100–123] Mammalian thermoregulatory NST often relies on the activation of a specialized tissue, brown adipose tissue (BAT).[124–135] BAT is found in several depots in the mammalian body.[136] Figure 10.9 shows the location of interscapular BAT in the rat.

Vasomotion is a thermoregulatory response used both to reduce heat loss in cold environments and to increase heat loss in hot environments. When an organism is hot, it can lose heat to the environment by *vasodilation* (dilation of blood vessels close to the surface of the body). Conversely, when an organism is cold, it can reduce heat loss to the environment by *vasoconstriction* (constriction of peripheral blood vessels). These mechanisms operate on the principle that heat is generated deep inside the body and that the body shell serves as thermal insulation. By allowing warm blood to flow close to the skin, endotherms can increase the rate of heat loss; conversely, they can reduce the rate of heat loss by reducing blood flow to the periphery of the body.[113,137–153] Although vasomotion is a short-term response, some animals have evolved sophisticated systems of vascular heat exchange that allow them to optimize heat loss or heat conservation. For example, countercurrent heat exchangers are used to cool the brains of running antelopes[154] and heat-stressed camels,[155] to reduce heat loss from the fins of dolphins[156] and the tongue of gray whales swimming in cold waters,[157] to help toucans lose excessive heat through their beaks,[158] and to enable homeothermy in bluefin tuna.[159]

Evaporation of water from a body extracts heat from it. Thus, if fresh water is available for later replacement, organisms can actively expel water from the body to cool off their bodies in a hot environment. When air temperature exceeds T_b, heat loss by vasodilation is not feasible, and evaporation becomes the primary mechanism of heat loss. Some animals make use of sweating,[160–167] others make use of panting,[168–176] and some spread saliva over their fur.[177–183] Whereas sweating and panting are true autonomic responses, saliva spreading (employed mostly by rodents) requires the behavioral sequence of *grooming* (saliva spreading).

Changes in *insulation* provide an additional mechanism for the control of heat flow out of and into the body. In the short term, piloerection (the involuntary bristling of hairs or ruffling of feathers—a remnant of which is the phenomenon of *goose bumps* in humans) can increase the thermal insulation of the body by creating an air blanket around the body and, therefore, can serve as a mechanism of heat conservation in a cold environment.[173,184–186] That fur and feather coats are indeed effective is demonstrated by increased heat loss from animals that are experimentally shorn or plucked.[79,187–191] On the seasonal scale, some animals undergo molting twice a year, which provides large changes in insulation according to the seasonal needs.[192–204] On the evolutionary scale, animals living in cold environments have evolved thick fur coats, vascular heat exchangers, subcutaneous fat deposits, and other strategies to conserve body heat.[205,206]

As mentioned in Chapter 9, large body size alone can serve as an effective means of reducing heat loss.[207] The simple physical explanation of this phenomenon is diagrammed in Figure 10.10. According to Bergmann's rule, enunciated

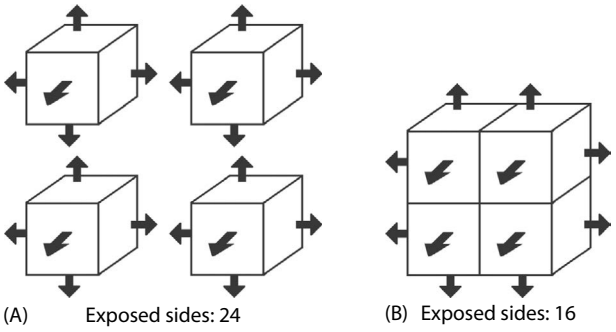


FIGURE 10.10 The deal about body size. Body size has consequences for insulation because it affects the surface–volume ratio of organisms. The two diagrams in this figure have the same volume (4 cubes) but different surface areas exposed to the environment. If all cubes generate the same amount of heat, then small organisms made up of one cube (Panel A) will lose more heat than the large organism made up of four cubes (Panel B).

in 1847, mammals and birds increase in size toward the colder portion of the temperature range that they occupy in their natural habitats.[208] Allen's rule states that animals adapted to colder environments have smaller limbs and shorter protruding body parts.[209] The rationale is that larger body size and smaller extremities provide less relative surface area for heat loss and, therefore, constitute adaptive strategies for life in a cold environment. Per Fredrik Scholander, a prominent twentieth-century physiologist, argued that Bergmann's rule and Allen's rule are unlikely to have much physiological significance because insulation in the form of fur and feather coats is the major determinant of differences in heat loss between tropical and arctic animals.[210] Of course, Bergmann's rule and Allen's rule may still be meaningful within individual species.[211] Pigs raised in the cold were found to have shorter tails and smaller ears than their littermates raised at thermoneutrality[212] and, likewise, mice raised in the cold were found to have shorter tails and smaller feet than mice raised at thermoneutrality.[213,214] However, it is possible that reduced blood flow due to vasoconstriction prevented the extremities from developing properly.[212] If so, the phenomenon may involve a developmental inhibition rather than an inherited adaptive trait.

Torpor (including hibernation) is not truly a thermoregulatory response because it allows T_b to deviate from the normothermic level. However, it is a potent mechanism of energy conservation, and its status is monitored by the thermoregulatory system. We discussed hibernation in Chapter 4 and will discuss daily torpor in detail later in this chapter. The data shown in Figure 10.11 demonstrate that hibernation is a regulated process and not just dormancy imposed by low ambient temperature. Eight arctic ground squirrels (*Urocitellus parryii*) were studied in the laboratory during the hibernating season. The graph shows the mean MR and the mean T_b of the animals at different ambient temperatures. Notice that T_b falls along with ambient temperature only down to 0°C. At temperatures below 0°C, MR is increased and T_b is prevented from falling.[215] Thus, although the animals allow their T_b to fall drastically during hibernation, they do not give up temperature regulation altogether.[216,217]

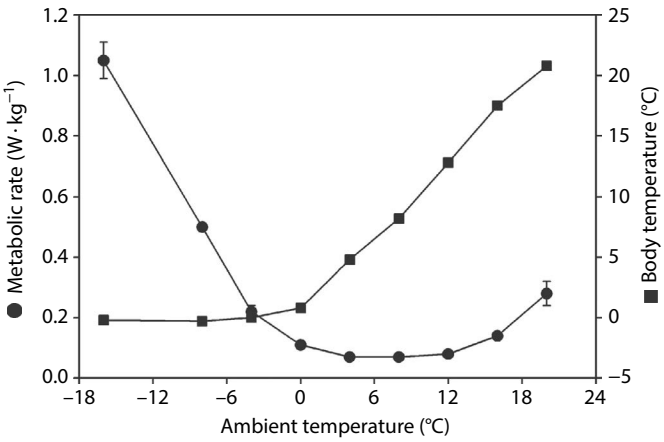


FIGURE 10.11 Hibernation is a regulated process. The graph shows the mean metabolic rate (± SE) and mean body core temperature (± SE) of 8 Arctic ground squirrels (*Urocitellus parryii*) hibernating in the laboratory at different ambient temperatures. Notice that body temperature (T_b) is allowed to fall with ambient temperature only down to 0°C. At ambient temperatures below 0°C, T_b is maintained constant as a result of an increase in metabolic heat production. (Data from Buck, C.L. and Barnes, B.M., *Am. J. Physiol.*, 279, R255, 2000.)

Let us now proceed to behavioral responses. Because both endotherms and ectotherms make ample use of behavior, these responses are much more widely used in the animal kingdom than any of the autonomic responses. The behavioral response of *relocation* is the most widely used of all thermoregulatory responses. It refers to the movement of an organism from a location whose temperature is too low or too high to a location whose temperature is closer to thermoneutrality. This is usually a very simple response, involving locomotion from a microenvironment that is too cold or too hot to a more comfortable microenvironment, but sometimes, it involves more sophisticated strategies. An example of relocation that is not common among terrestrial mammals is shown in Figure 10.12. About a third of Japanese macaques (*Macaca fuscata*) living in the wild exhibit the thermoregulatory behavior of bathing in a hot spring during the winter.[218] At the opposite end of environmental harshness, Koala bears (*Phascolarctos cinereus*) living in Australia in the summer must deal with very hot days and often find relief by selecting cooler microenvironments.[219] The Koala bear shown in Figure 10.13 selected a cool tree limb on which to stretch and cool off through conductive heat loss.

On the scale of seconds or hours, researchers have documented thermoregulatory behavior in a variety of animals, including insects,[220–224] arachnids,[58,225] crustaceans,[226–235] fishes,[55,236–249] amphibians,[57,250–252] reptiles,[253–278] birds,[279,280] and mammals.[52,281–309] As a matter of fact, relocation along a temperature gradient has been documented even in unicellular organisms[310,311] and human spermatozoa.[312]



FIGURE 10.12 In the hot tub. This live-camera shot of the Jigokudani Monkey Park (in Nagano, Japan) shows Japanese macaques (*Macaca fuscata*) fending off a cold winter day by bathing in a natural hot tub. (Courtesy of Livecam of Jigokudani Monkey Park, Nagano, Japan, www.jigokudani-yaenkoen.co.jp.)



FIGURE 10.13 Ghost on the tree. In this thermal photograph, objects with higher temperatures appear in lighter shades of gray. A hot Koala bear (*Phascolarctos cinereus*) in the Australian summer appears as a white ghost stretched over a cool tree limb to facilitate heat dissipation. (Photo courtesy of Natalie Jane Briscoe.)

Relocation on a scale of weeks or months is called "migration" (the long-distance relocation associated with the seasons) and can be observed in both endothermic and ectothermic animals.[24,313–316] As mentioned in Chapter 4, migration is probably related to seasonal variations in food availability to a much greater extent than to changes in ambient temperature.[317,318] Thus, migration is not a thermoregulatory response in the strict sense of the term.

Postural adjustment can be used for heat conservation (as in the case of a cold person curling up to reduce the area of body surface exposed to the environment), for heat loss (as in the case of rats sprawling their bodies when exposed to very warm environments), or even for heat gain (as in the case of a lizard that adjusts its posture to maximize exposure to the Sun while basking).[172,173,180,182,219,222,319–333]

Huddling is a potent mechanism of heat conservation.[296,305,327,328,334–349] When many individuals huddle together, they reduce the area of body surface exposed to the environment without reducing their endogenous thermogenic capacity. Rodent pups, which have inadequate thermogenic capacity, often huddle to avoid hypothermia. Adult penguins, which have adequate thermogenic capacity but live in a very cold environment, also resort to huddling. Figure 10.14 shows a huddle of emperor penguin chicks (*Aptenodytes forsteri*) in Antarctica. Especially interesting is the case of honeybees. Bees are ectothermic (except perhaps during flight),[350] but by clustering together in groups of thousands of individuals, they essentially create a "big bee" that functions as an endothermic unit.[351–353] A large cluster of bees was reported to maintain a core temperature of 34°C as ambient temperature was lowered all the way down to −80°C![353]

Modulation of food intake is an auxiliary thermoregulatory response, in the sense that increased food intake is a requirement for the maintenance of elevated thermogenesis in a cold environment. Thus, within limits, animals eat more

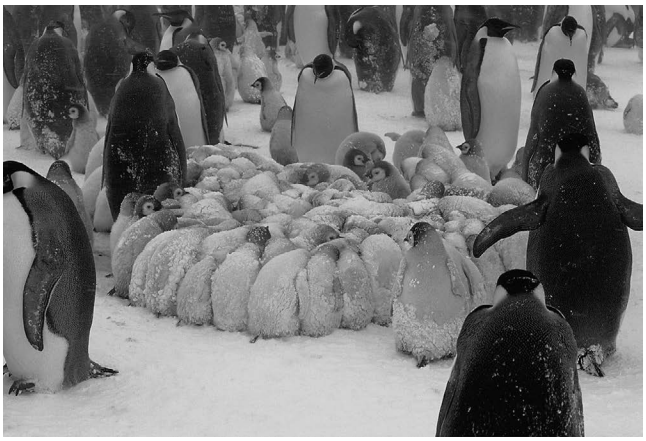


FIGURE 10.14 All together now. Huddling is one of many behavioral mechanisms of temperature regulation. In this picture taken in Antarctica, emperor penguin chicks (*Aptenodytes forsteri*) engage in huddling to prevent heat loss to the frigid environment. (Photo by Frank Todd, Copyright Alexander Photography.)

at lower ambient temperatures,[292,354–372] and fasting animals allow their T_b to fall even in a thermoneutral environment.[67,297,373–380] Curiously, low temperature during a meal (without long-term cold exposure) is sufficient to elicit greater food consumption.[381–384] Presumably, this is an anticipatory response to prolonged cold exposure.

Modulation of activity level as a thermoregulatory response has not been extensively studied, and its effectiveness remains controversial. Physical exercise is a thermogenic activity and, therefore, could be used as a thermoregulatory response in the cold. On the other hand, the increase in heat loss caused by the greater exposure to the environment might offset the increased thermogenesis. Some studies suggest that heat from exercise can replace cold-induced thermogenesis in the maintenance of T_b,[385–390]

but others do not.[371,372,391–393] Also, although some studies have indicated that animals will voluntarily increase activity in the cold,[394–396] others found no evidence that this occurs.[356,371,397,398]

Building of microenvironments is a very effective thermoregulatory response, although, of course, the thermoregulatory benefits cannot always be distinguished from the safety benefits (i.e., defense from predators). Humans are by far the most proficient animals in the building of microenvironments. There is evidence that, as early as 77,000 years ago, humans used to line their rock shelters with plant bedding.[399] Current construction of human buildings takes into consideration indoor air quality (ventilation), thermal conditions (heating and air-conditioning), visual and acoustic conditions, electromagnetic fields, static electricity, and vibration.[400] The design of thermal conditions takes into consideration the location and orientation of the building, the materials, the external color, and the floor plan.[401] Many other animals build nests, often in burrows deep in the ground, to shelter themselves from environmental cold or heat (Figure 10.15). The use or construction of microenvironments has been studied in numerous species, including mollusks and crustaceans,[227,402] insects,[222,403–406] reptiles and birds,[75,258,264,407,408] rodents,[292,338,342,347,368,409–429] and other mammals.[321,430–436]

Although the thermoregulatory responses that we have discussed so far are reflexive or instinctual, many responses can be acquired by *learning*. Humans learn how to knit sweaters, how to weave blankets, how to build energy-efficient homes, and so on. In laboratory settings, various species of animals have been taught to perform an instrumental task to obtain temporary relief from a cold or hot environment (operant conditioning). These include snails,[437] goldfish,[438] several species of birds,[439–445] mouse,[446–448] rat,[115,378,449–467] dog,[50] pig,[468–470] and squirrel monkey.[471–473]

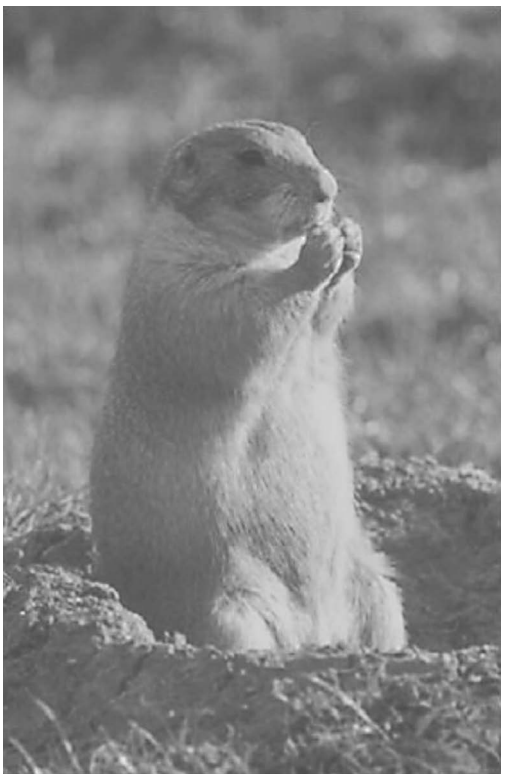


FIGURE 10.15 A prairie dog leaving its burrow. Behavioral thermoregulation in prairie dogs (*Cynomys ludovicianus*), as in many other animals, involves the use of microhabitats such as burrows and nests. (Courtesy of Northern Prairie Wildlife Research Center, United States Department of the Interior, Washington, DC.)

After this long journey through six autonomic responses and seven behavioral responses, we can now leave Table 10.1. However, a few more words are needed about how autonomic and behavioral responses are integrated. Although individual thermoregulatory responses have been studied in great detail, few investigators have examined the coordination of multiple responses, particularly across the autonomic-behavioral divide. The two classes of responses do seem to be integrated for the benefit of temperature homeostasis, as exemplified in Figure 10.16. Rats were trained to press a lever to obtain brief pulses of heat while kept in a cold environment (the behavioral response). At the same time, their oxygen consumption was monitored, so that heat production could be determined (the autonomic response). The amount of heat obtained through the behavioral response (heat intake) was calculated[474] and compared with the amount of heat obtained autonomically (heat production). Notice that when the force required to depress the lever was increased from 0.4 to 0.9 N, the rats reduced the behavioral intake of heat. At the same time, they increased heat production, so that the change in T_b during the sessions was not significantly affected. It is important to point out that 0.9 N is not the upper limit of muscular exertion for rats. When thirsty rats are required to lever-press for water, they respond at rates five times higher than when they lever-press for heat in the cold.[475] Thus, the trade-off between heat intake and heat production shown in Figure 10.16 reflects a true assessment of the costs and benefits of the two thermoregulatory responses. The two responses complement each other in the task of defending the stability of T_b.[476] Similar trade-offs between autonomic and behavioral responses were observed in other studies on birds and mammals exposed to cold[473,477,478] or heat stress.[284,307,479]

10.1.2 Circadian Control of Body Temperature

A considerable amount of information has been provided about the circadian control of T_b in previous chapters. The phenomenology of the T_b rhythm was covered in Chapter 5, and its relationship with other rhythms was discussed in Chapter 9. Two literature review articles cover part of this material.[480,481] A few additional pieces of information are needed here before we can proceed to the main issue of understanding how homeostasis and circadian rhythmicity are integrated in the control of T_b.

One aspect to be considered is the relationship between the body size of a species and the parameters of its circadian rhythm of T_b (CRT). In one of his many excellent

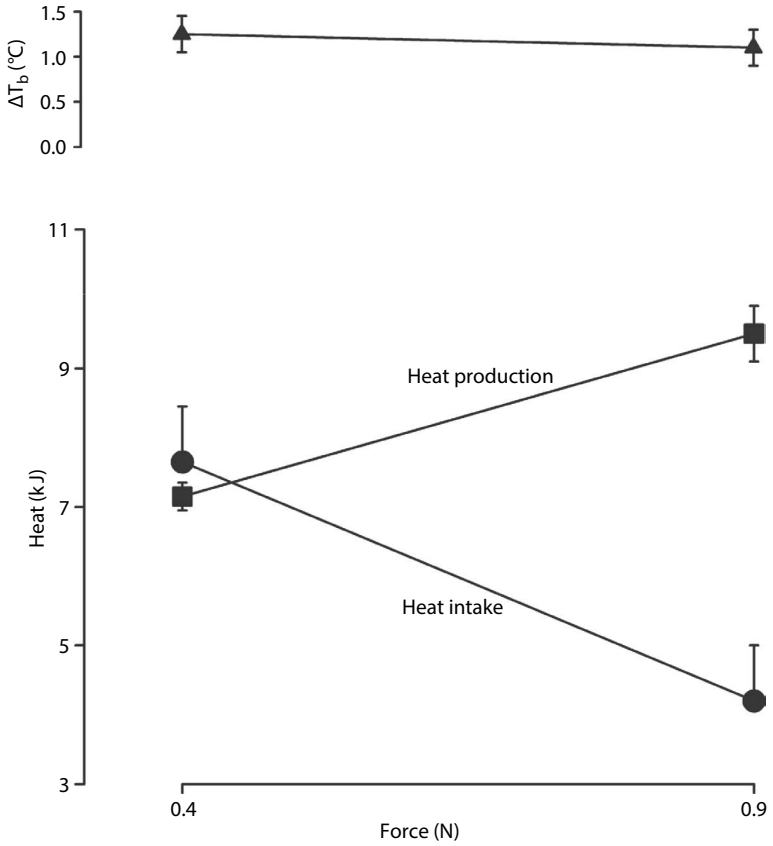


FIGURE 10.16 Trade-off of autonomic and behavioral responses. Rats were kept in the cold (3°C) for 30-minute sessions and could obtain 3-second heat rewards each time they pressed a lever. Metabolic heat production and number of lever presses were recorded. The graphs show heat production, heat intake, and the change in body temperature (T_b) when the force required to depress the lever was either 0.4 or 0.9 N. Heat production and heat intake complemented each other to maintain T_b. (Data from Refinetti, R. and Carlisle, H.J., *Behav. Neural Biol.*, 46, 64, 1986.)

review articles, Aschoff pointed out that the amplitude of the temperature rhythm is 3–6 times smaller in large animals than in small animals in the body weight range from 10 g to 1 kg.[482] Based on the data from 141 independent studies in various laboratories, I can confirm that the amplitude is about 3 times smaller in large animals in the body weight range from 10 g to 1000 kg, and up to 6 times smaller in the restricted body weight range from 10 g to 1 kg (Figure 10.17, Panel B). Presumably, large bodies buffer the effects of the oscillations in heat production and heat loss responsible for the T_b rhythm. Curiously, body size also has an effect on the mean level of the temperature rhythm (Figure 10.17, Panel A). Animals in the 1000 kg range have, on average, T_b 0.7°C higher than the T_b of animals in the 10 g range. Again, this is presumably due to the greater thermal inertia of large animals.

The data in Figure 10.17 not only highlight the relationship between body mass and T_b across various species but also emphasize the existence of great interspecies variability. When examined within small taxonomic groups, the relationship between T_b and body size may or may not follow the trend described in Figure 10.17.[483] As one example, my research collaborators and I examined the relationship in dogs. We chose dogs because there is a large variation in body size between different breeds of a single species, ranging from 2 kg Yorkshire Terriers to 80 kg Great Danes. We found a strong inverse correlation between temperature and body size among 115 adult dogs from 19 different breeds. Notice that this is the opposite of the trend described in Figure 10.17, as we found larger dogs to have lower T_b than smaller dogs.[484] Headline news: "Big dogs are cool!"

A recent literature survey based on 125 independent studies in mammals has also confirmed Aschoff's prediction.[485] The amplitude of the T_b rhythm was found to be smaller and the mean level to be higher in large animals than in small animals. However, I must mention that a compilation of studies in the 1950s based on 56 mammals (between 10 g and 2000 kg) failed to find a significant correlation between body size and mean T_b.[486] I attribute the failure to the smaller sample size and to the fact that pre-1950 studies did not measure T_b telemetrically over full circadian cycles (instead, measurements were conducted occasionally with rectal probes, which could easily overestimate the T_b of small animals).[487–491] A recent compilation of 267 studies in animals weighing under 1 kg did not identify a correlation between body size and T_b either.[492] I do not see this as a conflict, however, because the data in Figure 10.17 (Panel A) do not evince a correlation for

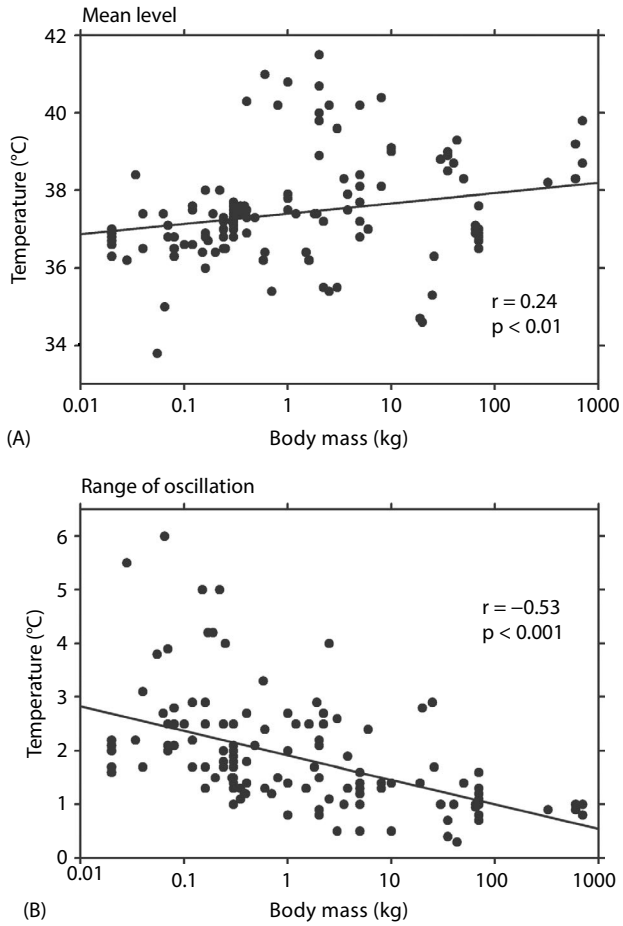


FIGURE 10.17 Mean level and amplitude of body temperature (T_b) rhythm vary with body size across species. The graph in Panel A shows the relationship between the mean level of the daily rhythm of T_b of a species and the body size of the species, as reported for various species of birds and mammals in the literature. The graph in Panel B shows the relationship between the daily range of oscillation of the temperature rhythm and body size. Some species are represented more than once. In both graphs, the abscissa is scaled logarithmically. Notice that both the mean level and the range of oscillation of the temperature rhythm are significantly correlated with body size, although in opposite directions. (Data from References 38, 41, 298, 300–302, 302, 321, 325, 327, 366, 376, 378, 395, 436, 488, 489, 494, 500, 512, 518, 529, 532, 534, 543, 546, 547, 581, 595, 598, 620–623, 647, 648, 667, 670, 701, 713–718, 814–894.)

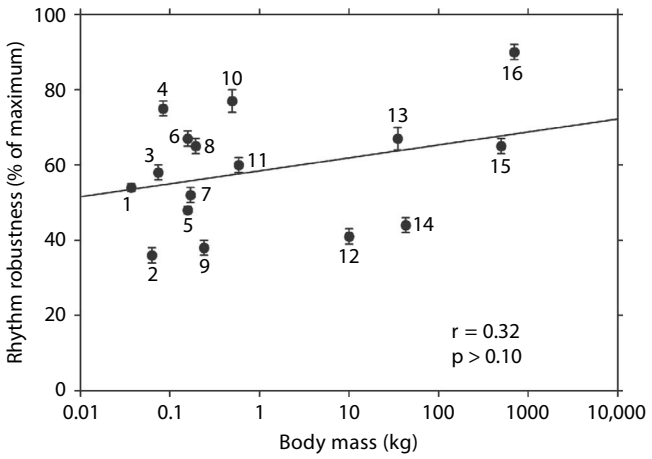


FIGURE 10.18 Does rhythm robustness depend on body size? The graph shows the relationship between the robustness of the daily rhythms of body temperature of 16 mammalian species and their average body sizes. The abscissa is scaled logarithmically. Although there is some correlation between the two variables (r = 0.32), the correlation is not statistically significant (because of the small sample size as compared to that in Figure 10.17). 1, Siberian hamster; 2, Mongolian gerbil; 3, flying squirrel; 4, fat-tailed gerbil; 5, golden hamster; 6, rat (Long-Evans); 7, thirteen-lined ground squirrel; 8, tree shrew; 9, degu; 10, rat (Sprague-Dawley); 11, Richardson's ground squirrel; 12, dog (Beagle); 13, goat; 14, sheep; 15, horse; 16, bovine. (Data from Refinetti, R., *Comp. Biochem. Physiol. A*, 119, 295, 1998; Refinetti, R., *Am. J. Physiol.*, 277, R1493, 1999; Refinetti, R. and Piccione, G., *J. Vet. Med. Sci.*, 65, 935, 2003; Piccione, G. et al., *Chronobiol. Int.*, 19, 531, 2002; Piccione, G. et al., *Biol. Rhythm Res.*, 33, 113, 2002; Piccione, G. et al., *BMC Physiol.*, 3, art. 7, 2003.)

the subgroup of animals under 1 kg. The effect is obviously dependent on large differences in body size.

Regarding the robustness of the temperature rhythm, the paucity of available data prevents any definite statement. Data from 16 mammalian species are shown in Figure 10.18. Rhythm robustness seems to increase as body size increases—with a moderate correlation of 0.32. However, due to the small number of data points, the correlation is not statistically significant.

Another aspect to be considered is the effect of ambient temperature on the T_b rhythm. Most studies in circadian physiology are conducted under thermoneutral conditions in the laboratory or under uncontrolled conditions in the field. However, several laboratory studies have addressed the issue with controlled changes in ambient temperature. An example is shown in Figure 10.19. A tree shrew (*Tupaia belangeri*) was maintained under several ambient temperatures for several days, and its T_b was monitored by telemetry. Notice that the amplitude of the rhythm is much greater at low ambient temperatures than at high ambient temperatures. Although these results are representative of those obtained in other tree shrews in the same study, no effect of ambient temperature (in the 14°C–32°C range) was found on the T_b rhythms of rats, golden hamsters, or fat-tailed gerbils.[493] Greater rhythm amplitude in the cold was observed in studies on squirrel monkeys,[494] thirteen-lined ground squirrels,[38] sheep,[495] pigeons,[73] mousebirds,[496] and sunbirds.[497] On the other hand, no effect of ambient temperature on the amplitude of the T_b rhythm was found in rats[396,498] or mouse lemurs.[395] Genuine species differences may be responsible for the conflicting results.

The fact that the amplitude of the temperature rhythm may be different at different ambient temperatures naturally raises the question of what the *normal* amplitude is. Of course, this is a senseless question. What is normal in the cold need not be normal in the heat. If one wished to force the notion of *normal*, the best one could do would be to call *normal* the amplitude observed when the organism is kept

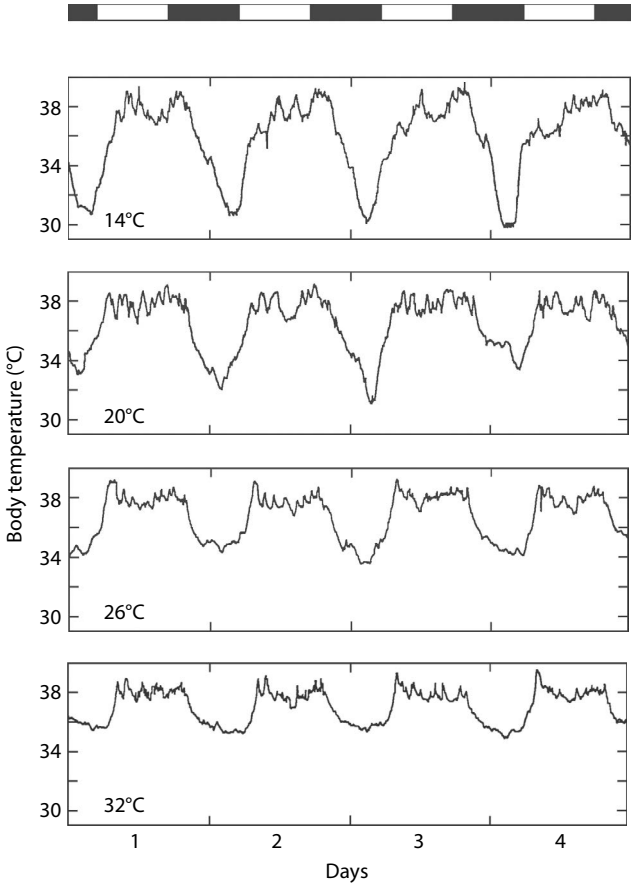


FIGURE 10.19 How ambient temperature affects the rhythm of body core temperature. The graphs show 4-day segments of the records of body temperature of a tree shrew (*Tupaia belangeri*) recorded by telemetry in 6-minute intervals while the animal was housed under four different ambient temperatures. A 24-hour light–dark cycle (LD 12:12) was present in all four conditions. Within the range tested, the amplitude of the daily rhythm decreased as ambient temperature increased. (Adapted from Refinetti, R., *J. Therm. Biol.*, 22, 281, 1997.)

at thermoneutrality. The thermoneutral zone varies from species to species. Thermal physiologists define the thermoneutral zone as "the range of ambient temperatures at which temperature regulation is achieved only by control of sensible heat loss (i.e., without regulatory changes in metabolic heat production or evaporative heat loss)."[6] For sedentary laboratory rodents, the thermoneutral zone usually ranges from about 25°C to about 32°C.[499]

Whatever the *normal* amplitude of the T_b rhythm is, one may wonder whether the oscillation is symmetrical about a neutral level or is caused by an elevation or a lowering of temperature. In order to find out, we must study organisms that do not exhibit circadian rhythmicity of T_b. One way to do this is to examine the temporal pattern of T_b of newborn animals as they develop the temperature rhythm. Data from young calves (*Bos taurus*) maintained under a natural photoperiod are shown in Figure 10.20. As previously discussed in Chapter 9, a daily rhythm of T_b is first observed on the ninth day after birth in this species. Notice that rhythmicity is established by development of oscillations both above and below the original flat level—that is, not by the development of exclusively hyperthermic excursions or exclusively hypothermic excursions.[500] A similar pattern was observed in rats,[501] but not in lambs and foals.[502] In lambs and foals, rhythmicity was found to develop by the rising of diurnal temperatures without a decline in nocturnal temperatures.

Another way to study organisms that do not exhibit circadian rhythmicity of T_b is to surgically destroy their master circadian pacemakers. Figure 10.21 shows that, regardless of the ambient temperature at which the animals are housed, the T_b rhythm of golden hamsters with ablated pacemakers (SCNX) has the same mean level as the rhythm of normal hamsters (control).[503] This again suggests that rhythmicity is established by development of oscillations both above and below a *neutral* level, not by the development of exclusively hyperthermic excursions or exclusively hypothermic excursions. Similar observations were made in other studies on hamsters[504] and ground squirrels.[505] In rats, one study found equivalent mean temperatures in rhythmic and arrhythmic

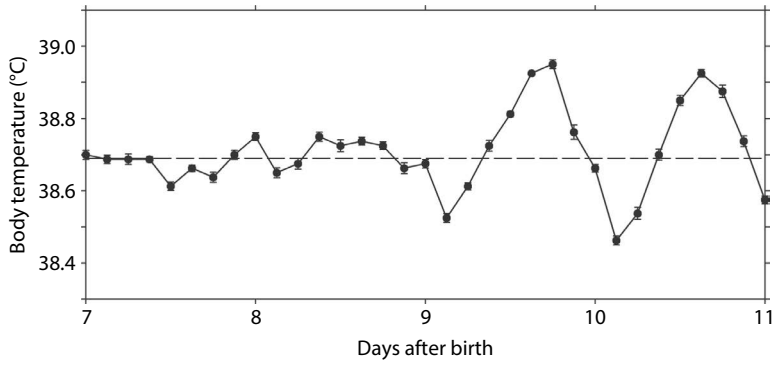


FIGURE 10.20 Developing the rhythm of body temperature (T_b). The graph shows the mean rectal temperature (± SE) of 8 newborn calves (*Bos taurus*) recorded at 3-hour intervals during the second week of life, when the daily rhythm of T_b develops in this species. The rhythm is established by oscillations both below and above the daily mean. (Data from Piccione, G. et al., *BMC Physiol.*, 3, art. 7, 2003.)

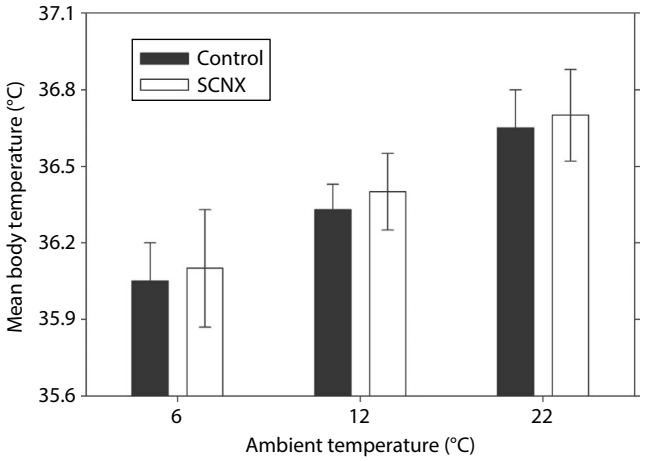


FIGURE 10.21 Disrupting the rhythm of body temperature (T_b). When the T_b rhythm is eliminated as a consequence of surgical ablation of the circadian clock in the brain, the mean level of T_b is not affected—which indicates that the rhythm was maintained by oscillations both below and above the daily mean. In this graph, bars correspond to mean values (± SE) for groups of golden hamsters (*Mesocricetus auratus*) maintained under three different ambient temperatures. Twelve brain-lesioned (SCNX) and 12 control hamsters were tested. (Data from Refinetti, R., *Brain Res. Bull.*, 36, 81, 1995.)

animals,[506] one found the mean temperature to be higher in arrhythmic animals,[507] and a third one found the mean temperature to be slightly lower in arrhythmic animals.[508] Until further studies are conducted, one can provisionally conclude that rhythmicity is established by the development of oscillations both above and below a neutral level.

10.1.3 Integration of Homeostasis and Circadian Rhythmicity

The scene is now set for a major conflict. Clearly, the homeostatic control of T_b has the goal of ensuring stability—that is, of preventing deviations from an ideal set point. On the other hand, the circadian control of T_b imposes a persistent oscillation in T_b. Somehow, these two antithetic processes must be integrated.

Thermal physiologists (including Aschoff, who was a thermal physiologist before migrating to circadian physiology) have generally assumed that the circadian oscillation in T_b is primarily under homeostatic control and is secondarily modulated by the circadian system through a daily oscillation in the thermoregulatory set point.[509–512] This assumption is diagrammed in the Panel A of Figure 10.22. According to this view, the circadian pacemaker acts on the thermoregulatory thermostat so that the set point is elevated during subjective day and lowered during subjective night in diurnal animals (or lowered during subjective day and elevated during subjective night in nocturnal animals). However, from the viewpoint of the circadian physiologist, it is more sensible to assume that the circadian oscillation in T_b is primarily under circadian control, bypassing the thermoregulatory set point, and is secondarily modulated

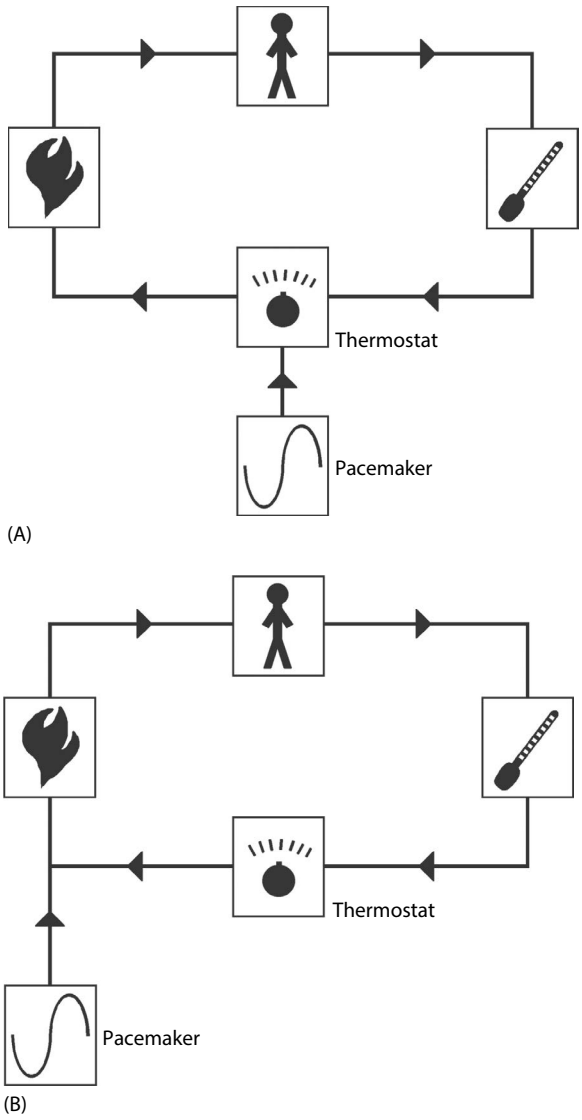


FIGURE 10.22 Change in set point or independent control? Integration of the homeostatic mechanism and the circadian mechanism that control body temperature can, in principle, be achieved either by circadian modulation of the thermostat (A) or by independent action of the two mechanisms on effector organs (B).

by the thermoregulatory system (Panel B in Figure 10.22). Which of these two hypotheses is the correct one?

One problem with the traditional hypothesis (that the set point oscillates through the circadian cycle) is, of course, the notion of set point itself. As discussed earlier, there is disagreement among thermal physiologists about the existence of a central reference-signal generator (Figure 10.6). Even if there is a central reference-signal generator, there is extensive evidence that thermoregulatory responses can be integrated at multiple levels of the neural axis.[513] The thermoregulatory system can be thought of as an assemblage of hierarchically arranged integrating units.[514,515] Therefore, circadian modulation of the set point would have to involve modulation of multiple set points, some of which probably do not even exist.

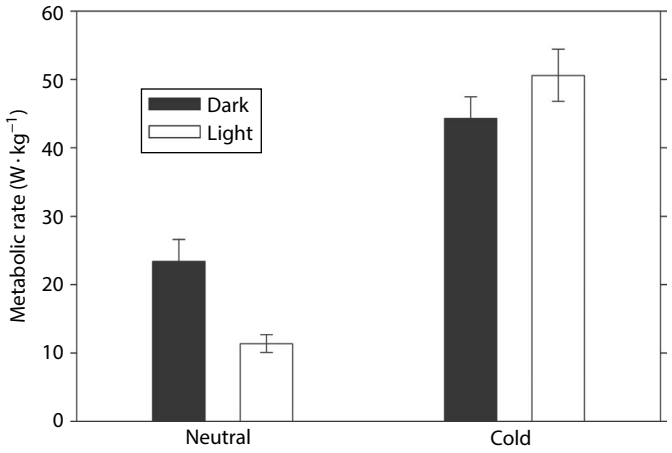


FIGURE 10.23 Autonomic thermoregulatory response opposes body temperature (T_b) rhythm. The graph shows the mean metabolic rates (± SE) of 10 golden hamsters (*Mesocricetus auratus*) measured in a neutral (24°C) and a cold (−10°C) environment during the dark and the light phases of the prevailing light–dark cycle. Notice that cold-induced thermogenesis (i.e., the difference between measurements in the cold and measurements in the neutral environment) is greater during the light phase (when T_b is lower in this nocturnal species). (Data from Watts, R.H. and Refinetti, R., *Biol. Rhythm Res.*, 27, 87, 1996.)

Assuming that there is a master thermoregulatory set point, the set-point-change hypothesis still needs to explain why, as we have just seen, the amplitude of the temperature rhythm is smaller in larger animals (Figure 10.17, Panel B). If the set point is changed on a circadian basis, one would expect it to be changed proportionally for each species. There is no reason why the set point should be changed less in large animals than in small animals. It is more likely that the set point does not change at all and that the reduced amplitude in large animals is due to their greater thermal inertia. In an analogous situation, thermal physiologists have recognized that the elevation of T_b that accompanies physical exercise is due not to an elevation of the set point but to a limitation of heat loss mechanisms.[516,517]

Also inconsistent with the set-point-change hypothesis is the increased amplitude of the temperature rhythm that is observed in some species exposed to low ambient temperatures (Figure 10.19). If the set point is changed on a circadian basis, then the set point should be defended at any ambient temperature within the capabilities of the thermoregulatory system. It does not make sense to suggest that the daily oscillation of the set point is greater when ambient temperature is lower.

None of the preceding arguments is a *coup de grâce*. To truly judge whether there is circadian modulation of the thermoregulatory set point, we must actually measure the set point. One way to do this is to measure thermoregulatory responses to environmental stress at different circadian times. If, for instance, a greater thermogenic response is evoked by the same thermal stimulus during the circadian phase when T_b is high than during the circadian phase when T_b is low, then it will be reasonable to assume that the elevated T_b resulted from an elevation of the set point. That is, if the set point is elevated, one would expect the thermoregulatory system to defend the elevated level—in our example, by generating more metabolic heat than when the set point is lower. The results from one study that utilized this approach are shown in Figure 10.23. Golden hamsters were tested in a thermoneutral environment (24°C) and a cold environment (−10°C) during the light phase and the dark phase of the light–dark cycle. In the neutral environment, MR was higher in the dark phase than in the light phase, which is what one would expect to find in a nocturnal animal. However, in the cold environment, MR was not higher in the dark phase—as a matter of fact, it was lower in the dark phase than in the light phase.[518] Thus, cold-induced thermogenesis (the difference between the MRs in the cold environment and in the neutral environment) was much greater during the light phase. Now, in nocturnal animals like the hamster, T_b is lower during the light phase. This means that cold-induced thermogenesis was higher when T_b was lower—the opposite of what the set-point-change hypothesis predicted.

Similar results to those obtained in golden hamsters were obtained in fat-tailed gerbils.[298] However, several studies involving autonomic thermoregulatory responses of heat gain and heat loss yielded results supporting the set-point-change hypothesis in rats,[144] pigeons,[519,520] and humans.[167,521–523] The discrepancy of results is annoying, but it makes one wonder if the experimental approach used in all these studies is a reliable one. No researcher questions the existence of a CRT. Also, it is a physical necessity that changes in T_b result from changes in the balance between heat production and heat loss. Thus, circadian changes in body functions related to heat production and heat loss must take place—and they must take place regardless of whether they are produced by the thermoregulatory system or by the circadian system. In other words, the measurement of autonomic thermoregulatory responses at different times of the day does not really tell us anything about the state of the set point. It tells us only that heat production and heat loss are changing. In order to measure the state of the set point, we need a variable that is not normally required for the production of the T_b rhythm but that, at the same time, does reflect the operation of the

set point. We saw a little earlier that autonomic and behavioral thermoregulatory responses can complement each other in the homeostatic control of T_b, and we know that the T_b rhythm of endotherms does not require behavioral responses. Therefore, the use of behavioral responses can provide us with a reliable measure of the state of the set point.

In 1978, Herbert Hensel—possibly the most influential thermal physiologist of the twentieth century—studied the thermal sensation evoked by warming of the hand of human subjects at different times of the day and noticed that warm stimuli were perceived as more pleasant during the circadian phase of low T_b than during the phase of high temperature.[524] The following year, Harry Carlisle—another prominent thermal physiologist who many years later would be my adviser in graduate school—noticed that rats exposed to the cold would press a lever for heat more vigorously during the phase of low T_b than during the phase of high temperature.[525] Research in many other laboratories over the years, using a variety of behavioral research techniques, has documented that higher ambient temperatures are preferred during the phase of low T_b, and lower ambient temperatures are preferred during the phase of high T_b, in rats,[286,299,300,379,526–528] mice,[296] golden hamsters,[299,529,530] Siberian hamsters,[531] fat-tailed gerbils,[298] degus,[301] stripe-faced dunnarts,[532] tree shrews,[302] flying squirrels,[302] mouse lemurs,[533] and humans.[534–538] An example is given in Figure 10.24. Fat-tailed gerbils were housed in a temperature gradient such as that described in Figure 2.32 (Chapter 2). Their preferred ambient temperature and their T_b were recorded at 6-minute intervals for 10 or more days. Notice that, as expected for a nocturnal animal, T_b is high during the night and low during the day. Notice also that the rhythm of behavioral temperature selection is 180° out of phase with the rhythm of T_b. Clearly, higher environmental temperatures are selected when T_b is low, and vice versa. Thus, the oscillation of the set point cannot possibly be responsible for the temperature rhythm. As a matter of fact, there is no reason to assume that the set point oscillates at all.[539,540] As T_b oscillates, the animals behaviorally counteract the oscillation to defend the unaltered set point. The thermoregulatory system actually opposes the oscillation of T_b imposed by the circadian system (Figure 10.25).

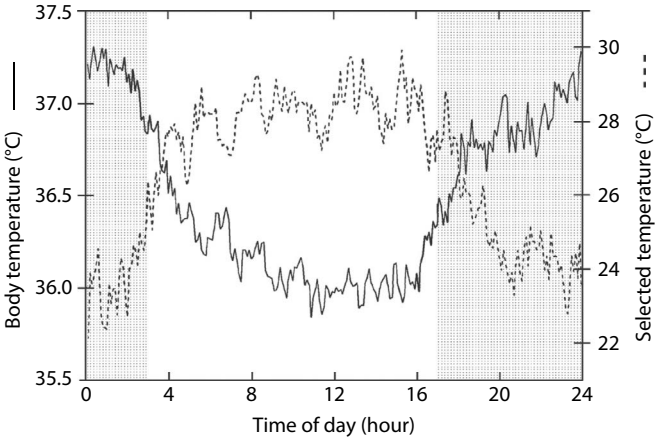


FIGURE 10.24 Rhythm of temperature selection is 180° out of phase with rhythm of body temperature (T_b). The graph shows the average rhythm of T_b and the average rhythm of selected ambient temperature of fat-tailed gerbils (*Pachyuromys duprasi*) maintained in a temperature gradient under a 24-hour light–dark cycle (as indicated by the shading). The average rhythms are derived from 5 gerbils, each studied over 10 consecutive days. Higher ambient temperatures were selected when T_b was low, and lower ambient temperatures were selected when T_b was high. (Adapted from Refinetti, R., *Comp. Biochem. Physiol. A*, 119, 295, 1998.)

Evidently, the thermoregulatory system's opposition to the circadian oscillation of T_b is not entirely successful, as witnessed by the very existence of the rhythm. However, the amplitude of the temperature rhythm is effectively reduced by the action of the thermoregulatory system. This has been shown in two ways. One way was by comparing the amplitude of the rhythm in animals maintained in a constant-temperature environment with the amplitude in animals allowed to continually select their environmental temperature in a gradient. The amplitude of the T_b rhythm was reduced in tree shrews and flying squirrels allowed to select their environmental temperature.[302] The other way was by impairing the thermoregulatory system through surgical ablation of the main thermoregulatory center in the preoptic area of the brain. The amplitude of the T_b rhythm was greatly enhanced in rats and golden hamsters with preoptic lesions.[504,541,542]


The Body Times

May 25, 1995 50 cents

WAR

Thermoregulatory System Declares War Against Circadian System

The two can no longer live together

By Liv Ordie

It was revealed today that the thermoregulatory system will no longer tolerate the disruption of homeostasis caused by the circadian system. "Enough is enough" uttered the offended party. From now on, "we will fight every attempt to interfere with the constancy of core temperature."

The relationship between the two system has been difficult since the birth of the thermoregulatory system. Witnesses say that the circadian system has never accepted the "new" member of the body family and has no regard for homeostasis. The thermoregulatory system claims that its existence is essential for survival of birds and mammals and that "only an idiot would not be able to

Other news:

The stomach will no longer contribute to acid reflux. This is due to the fact that the brain ordered the muscles to cause the ingestion of anti-acids.

The immune system has announced that there is no sign of infection by foreign pathogens at this time.

FIGURE 10.25 War! The circadian rhythm of body temperature is characterized by a conflict between the thermoregulatory system and the circadian system, as announced in this fictitious newspaper headline.

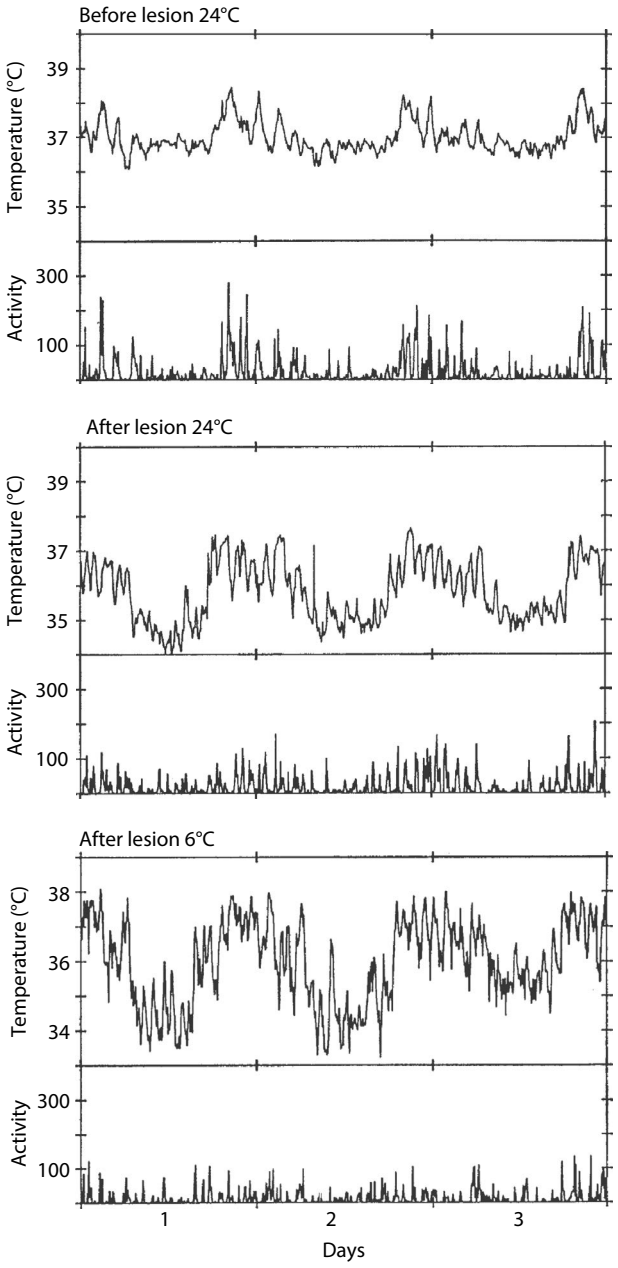


FIGURE 10.26 Disrupting the thermoregulatory system. When the thermoregulatory system is disrupted as a consequence of surgical ablation of the main thermostat in the brain, the amplitude of the body temperature (T_b) rhythm is increased—which indicates that prior to the ablation, the amplitude of the rhythm was reduced by the thermoregulatory system. These graphs show 3-day segments of the T_b rhythm of a golden hamster (*Mesocricetus auratus*) before and after surgical ablation of the preoptic and anterior hypothalamic areas. Exposure to a cold environment (6°C) further enhanced the amplitude of the rhythm. (Adapted from Osborne, A.R. and Refinetti, R., *NeuroReport*, 6, 2187, 1995.)

Figure 10.26 shows that the amplitude of the T_b rhythm of a golden hamster kept in a thermoneutral environment (24°C) is doubled after its preoptic area is destroyed. The amplitude is enhanced further if the animal is placed in a cold environment (6°C). Clearly, ablation of the preoptic area releases the

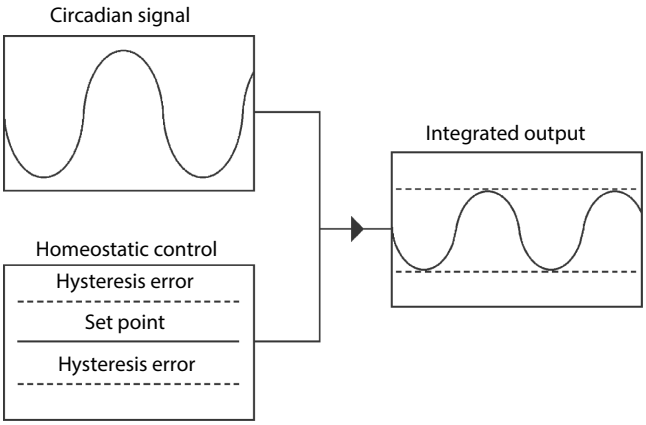


FIGURE 10.27 How the homeostatic and circadian mechanisms are integrated for the generation of the body temperature rhythm. The circadian system generates a signal for rhythmicity with relatively large amplitude. At the same time, but independently, the homeostatic system sets the limits of allowable variation around the set point. The integrated output is an oscillation with reduced amplitude.

circadian oscillation of T_b from inhibitory control. Thus, the thermoregulatory center in the preoptic area of unlesioned animals restricts the oscillation of T_b to an acceptable range. The logic of the regulatory process is diagrammed in Figure 10.27. The circadian system generates an oscillatory signal that is communicated to the organs responsible for heat production and heat loss. At the same time, the thermoregulatory system generates a set point that, like most control systems, has a margin of hysteresis error. The integrated output is an oscillation whose amplitude is restricted to the boundaries of hysteresis error.

The specifics of the mechanisms of heat production and heat loss necessary for the generation of the T_b rhythm are not intuitive and may confuse the inattentive reader. Consider Figure 10.28. Shown are 3-day segments of the records of T_b, metabolic heat production, and dry heat loss of a laboratory rat kept in constant darkness at 24°C. Notice that the oscillation of T_b parallels the oscillation of heat production. Thus, the oscillation of heat production could potentially explain the oscillation of T_b. However, notice also that heat loss parallels heat production. That is, although heat production is high when T_b is high, heat loss is also high. All three variables oscillate together, which is counterintuitive. Because a change in T_b must result from an imbalance between heat production and heat loss, one is tempted to conclude that animal physiology violates the laws of thermodynamics!

Of course, one cannot violate physical laws. The appearance of law violation comes from our presumption that a great amount of heat is needed to generate the T_b rhythm. In actuality, very little heat is needed for it. Figure 10.29 shows the fractions of the total daily energetic budget of a rat associated with essential life processes, with the maintenance of homeothermy, and with the CRT in a thermoneutral as well as in a cold environment. Most of the energy used (which is directly

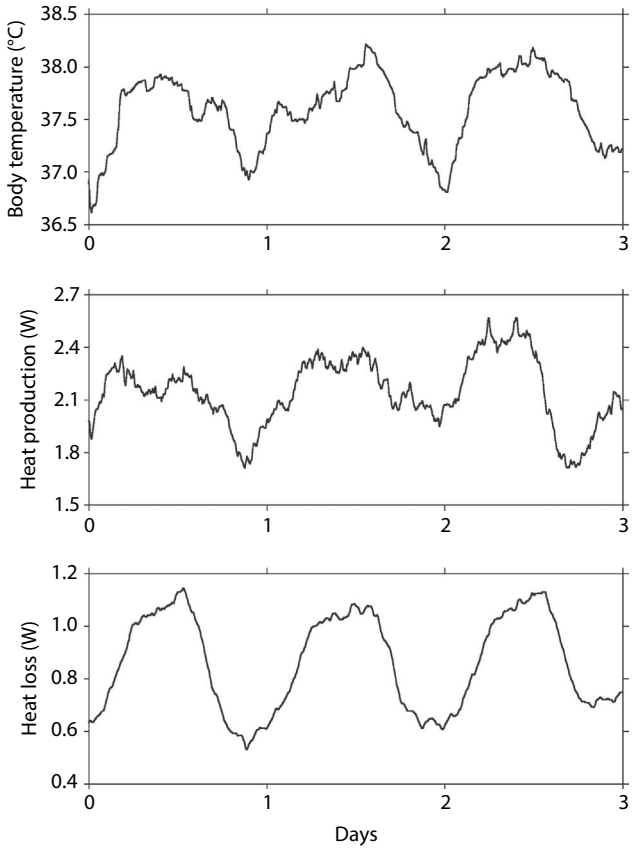


FIGURE 10.28 Matching gains and losses. The graphs show 3-day segments of the records of body temperature (T_b, measured by telemetry), heat production (computed from measurements of oxygen consumption), and heat loss (measured by direct calorimetry) of a laboratory rat (*Rattus norvegicus*) maintained in constant darkness. The absolute values of heat loss are relatively low because they do not include convective and evaporative losses. Notice that the rhythm of T_b parallels the rhythm of heat production but that the rhythm of heat loss does it also. (Adapted from Refinetti, R., *Exp. Physiol.*, 88, 423, 2003.)

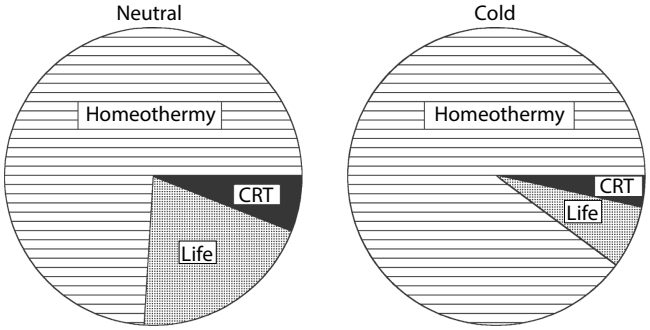


FIGURE 10.29 What is the cost of the body temperature (T_b) rhythm? These pie charts indicate the fractions of the total daily energetic budget of a rat associated with essential life processes, with the maintenance of homeothermy, and with the CRT in a thermoneutral (24°C) as well as in a cold (−7°C) environment. Values of total energy expenditure were obtained from actual measurements of oxygen consumption in rats. The fraction of essential life processes was computed by comparison with the energy expenditure of a poikilotherm (reptile) of same body size at 37°C. The fractions of maintenance of homeothermy and of rhythm generation were computed in proportion to the theoretical values of heating calculated by $Q = m \cdot c \cdot \Delta T$ (where Q is heat, m is mass, c is specific heat, and ΔT is the variation in temperature). Generation of the CRT requires 6% of the daily budget at thermoneutrality and 3% in the cold. (Data from Templeton, J.R., Reptiles, in: Whittow, G.C., ed., *Comparative Physiology of Thermoregulation*, Vol. 1, Academic Press, New York, 1970, pp. 167–221; Refinetti, R., *Exp. Physiol.*, 88, 423, 2003; Refinetti, R. and Carlisle, H.J., *Behav. Neural Biol.*, 46, 64, 1986; Refinetti, R., *Int. J. Biometeorol.*, 34, 24, 1990.)

proportional to heat production) is associated with the maintenance of homeothermy. The T_b rhythm accounts for only 6% of the energy expended at thermoneutrality and only 3% of the energy expended in the cold.[398,543] Thus, most of the daily oscillation in heat production and heat loss has nothing to do with the T_b rhythm.

Regarding the temporal coordination of the mechanisms of heat production and heat loss necessary for the generation of the T_b rhythm, Aschoff reasoned that both heat production and heat loss needed to oscillate and that the oscillation of heat loss needed to lag behind the oscillation of heat production.[544] This has indeed been observed in rats,[543,545] squirrel monkeys,[512,546] and humans.[547] An example is shown in Figure 10.30. Cosine waves were fitted to the raw data of T_b, heat production, and heat loss of a rat (Panels A, B, and C). The vertical dashed lines indicate the acrophases of the rhythms. Notice that heat production leads T_b by 1.3 hours, whereas heat loss trails T_b by 0.9 hour. If *heat balance* is calculated (Panel D), a phase difference of 6 hours is found. This phase difference is presumably due to thermal inertia of the body and should be different in animals of different body sizes.[482]

Because simultaneous measurement of heat production, heat loss, and T_b is technically difficult, various researchers have estimated the activation of heat loss processes by measuring peripheral skin temperature (because peripheral skin temperature reflects peripheral vasodilation, which is a common heat loss response, as described earlier). Rise in peripheral skin temperature has consistently been found to precede a fall in core temperature by several hours in humans[547–551] and other animals.[495,546]

Finally, one may wonder why should there be a CRT. The answer is: for the same reason that there is circadian rhythmicity in general. We saw in Chapter 9 that circadian rhythmicity is an evolutionarily old trait that most likely existed before the appearance of the first animals. Seeking warm temperatures during (or in anticipation of) the active phase of the circadian cycle would have provided an advantage to the original ectothermic animals whose ability to perform bodily functions was extremely dependent on ambient temperature.[552] Because homeothermy is found today only in mammals and birds (and a few fishes), it must have appeared much later than circadian rhythmicity. As a matter of fact, endothermic homeothermy likely evolved from an ancestral form of heterothermy.[553,554] Thus, in homeotherms, the new "drive" to maintain homeostasis conflicts with the old "drive" to oscillate T_b—and this

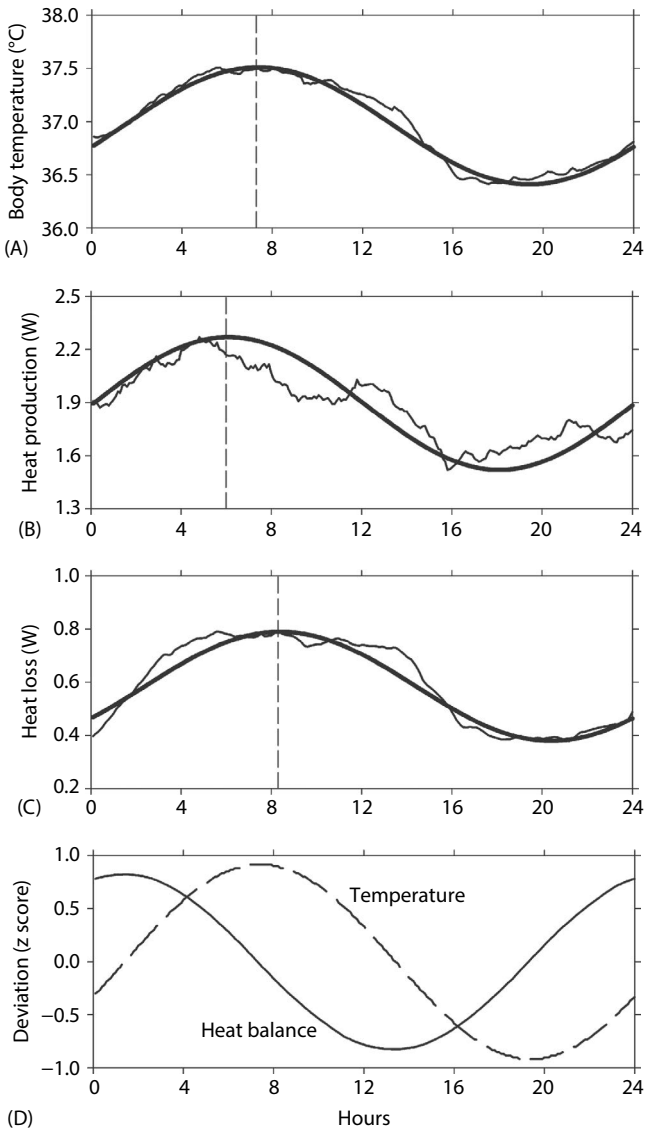


FIGURE 10.30 Thermal inertia and the body temperature (T_b) rhythm. The graphs provide a close look at the records of T_b (A), heat production (B), and heat loss (C) of a laboratory rat maintained in constant darkness (*Cf.* Figure 10.28). Thin lines correspond to actual data. Thick lines are cosine waves fit to the data. Dashed vertical lines indicate the acrophases of the rhythms. The last graph at the bottom (D) compares the T_b rhythm with the heat-balance rhythm (i.e., the difference between the normalized values of heat production and heat loss). Notice that the heat-balance rhythm leads the temperature rhythm by 6 hours. (Adapted from Refinetti, R., *Exp. Physiol.*, 88, 423, 2003.)

explains the opposition between the thermoregulatory system and the circadian system in the control of T_b.

A remaining question is, of course, why the *old drive* to oscillate T_b was retained during evolution. A possible reason to retain rhythmicity of T_b in homeotherms is the ability to use T_b as an internal nonphotic zeitgeber for the entrainment of multiple slave pacemakers distributed all over the body.[555–560] Presumably, environmental light would affect the master circadian pacemaker in the brain, which would modulate the T_b rhythm, which would then nonphotically modulate peripheral clocks. If this is true, then animals that display T_b rhythms with greater amplitude should show easier (perhaps faster) resynchronization of peripheral clocks after a shift of the light–dark cycle. This idea could be tested. As mentioned earlier, rats and golden hamsters with preoptic lesions have T_b rhythms with exaggerated amplitude[504,541,542] and should, therefore, exhibit faster resynchronization of peripheral organs after a shift of the light–dark cycle. A major problem with the hypothesis of T_b serving as an entraining agent of peripheral organs is that T_b is an unreliable variable in the majority of living beings—because most living beings are poikilothermic. Only in homeotherms, whose T_b are independent of ambient temperature, would the T_b rhythm be a reliable means to synchronize peripheral organs. Yet, the *old drive* to oscillate T_b has been retained in both homeothermic and poikilothermic animals.

Alternatively, it has been suggested that increased heat loss through peripheral vasodilation during the inactive phase may have an immunological host-defense function—because increased blood flow to the skin provides greater transport of leukocytes to this first-defense area of the body.[515] This idea is sensible but requires experimental testing.

Energy savings—in a smaller but similar fashion to that attained in hibernators—might be another advantage of the T_b rhythm. However, as we saw earlier (Figure 10.29), the fraction of energy expenditure associated with the T_b rhythm is rather small and unlikely to be of evolutionary significance. For example, an adult human may save about 260 kJ (63 kcal) per day by having a 1°C reduction in T_b at night (assuming a mean MR of 90 W and a Q_{10} of 2). Eating an extra apple a day would be sufficient to offset the costs of maintaining T_b at 37.0°C all day long. One might say that "an apple a day keeps nocturnal hypothermia away."

Preservation of the T_b rhythm as a vestigial function remains the most likely hypothesis for the presence of a CRT in extant homeothermic species.

10.2 SLEEP, FEEDING, AND ENERGY EXPENDITURE

Other variables besides body temperature are homeostatically controlled and exhibit circadian rhythmicity. Thus, conflicts between homeostasis and circadian rhythmicity can potentially exist in the control of these variables. In this section, we will examine the integration of homeostasis and circadian rhythmicity in the control of sleep, feeding, and energy expenditure.

10.2.1 Homeostatic and Circadian Control of Sleep

Humans, like most animals, are not awake and active 24 hours a day. Periods of wakefulness are interspersed with periods of sleep (Figure 10.31). If you ever stayed up all night studying for an exam or, more likely, partying with friends, you know that sleep is homeostatically controlled. The longer you stay awake, the stronger is your urge to go to sleep and the less capable are you of performing various tasks. The effects of sleep deprivation on human performance are illustrated in Figure 10.32.

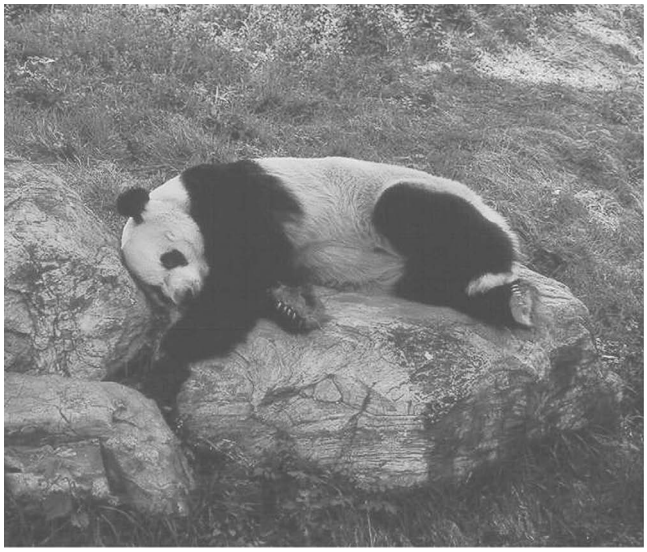


FIGURE 10.31 A sleeping panda. Most mammals and birds sleep at least a few hours each day. (Copyright ArtToday, Tucson, AZ.)

Performance was evaluated by a psychomotor vigilance test, which is a reaction time task (i.e., one must respond to a stimulus as fast as possible). Reaction times greater than half a second were considered response lapses, and the number of lapses is plotted against the number of days of sleep restriction (A) or the accumulated number of sleep hours missed (B). As denoted by the different symbols, some subjects were totally deprived of sleep for 3 days (0 hours of sleep), whereas others were allowed to sleep 4, 6, or 8 hours per night for 14 days. As shown in Panel A, the less sleep one gets, the more performance lapses one commits. Panel B shows that, regardless of how sleep deprivation is attained (i.e., by many hours in a few nights or by a few hours over many nights), one's performance gets worse as a function of the accumulated hours of wakefulness. Thus, the greater the number of sleep hours missed, the greater is the number of performance lapses. (Technically, the relationship is true for the accumulated number of wake hours above the normal value of 15.8 hours per day, not for the accumulated number of sleep hours missed.[561] The difference is subtle but mathematically meaningful.)

We do not need a concept of set point for sleep because there is theoretically an absolute zero for sleepiness, so that sleepiness can only go above this zero level. However, a homeostatic component is clearly present in the control of sleep. Laboratory studies involving polysomnographic recording (EEG) have consistently documented an increase in slow-wave sleep (as well as REM sleep) after an interval of sleep deprivation.[562–567] This has been documented also in animals devoid of circadian rhythmicity by surgical ablation of the master circadian pacemaker,[568–570] by deactivation of the pacemaker through genetic engineering,[571] and by the bizarre elimination of rhythmicity in Siberian hamsters submitted to a phase shift of the light–dark cycle.[572] Figure 10.33 shows an example of *sleep rebound* after sleep deprivation in a rat whose pacemaker had been previously ablated.

What exactly causes sleep is still not fully known, but a major candidate is the accumulation of *adenosine* during wake hours.[573,574] Adenosine is the *A* in ATP (adenosine triphosphate), the primary energy currency of cells. The greater the breakdown of ATP, the greater is the accumulation of adenosine. If you are very active in any given day or if you are relatively inactive but stay up for more hours than usual, you will break down more ATP and, consequently, will accumulate more adenosine and will feel sleepier. When you sleep, adenosine is metabolized and excreted at a higher rate than it is produced and, consequently, your vigilance is restored. Thus, sleep is a *restorative* process, and this is so whether the sleep-inducing substance is really adenosine or some other metabolite that accumulates in the awake nervous system.[575]

If you really stayed up all night working or partying at least once, you probably noticed that sleep is more than just a

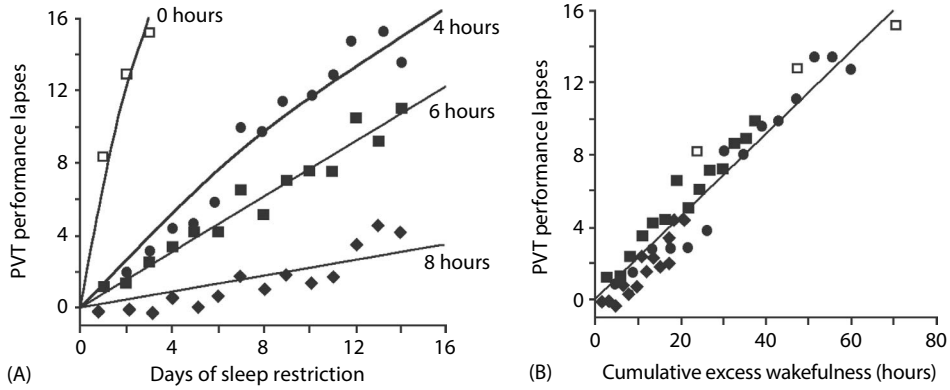


FIGURE 10.32 The effects of chronic sleep restriction. Chronic sleep restriction (i.e., reduced number of hours of sleep on several consecutive nights) leads to an increase in performance lapses in a psychomotor vigilance test. Adult humans generally need slightly more than 8 hours of sleep per night. The graph on the left (A) shows that restriction of sleep to only 6 or 4 hours per night greatly increases the number of performance lapses. After 10 days on 6 hours of sleep per night, people commit as many performance lapses as people who are deprived of sleep for a full night (0 hours of sleep). The graph on the right (B) shows that the important factor in the increase of performance lapses is cumulative excess wakefulness (i.e., how long one has gone without getting enough sleep). Different symbols refer to different conditions of sleep restriction as indicated by the adjacent labels. (Data from van Dongen, H.P.A. et al., *Sleep*, 26, 117, 2003.)

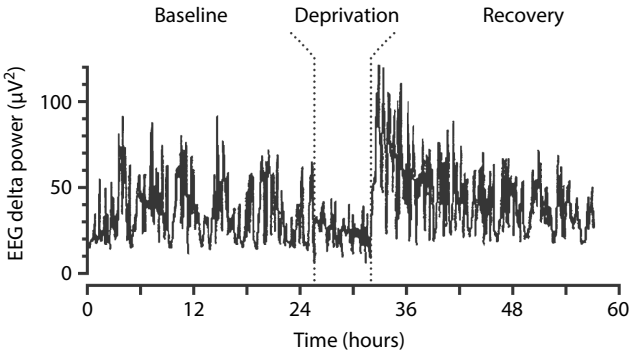


FIGURE 10.33 Sleep is a homeostatic process. The graph shows the variation in EEG delta power (a standard measure of sleepiness) of a laboratory rat (*Rattus norvegicus*) during a baseline interval of 24 hours, a sleep-deprivation interval of 6 hours, and a recovery interval of 24 hours. Daily rhythmicity of sleepiness had been eliminated as a consequence of surgical ablation of the circadian clock in the brain. Notice a clear homeostatic rebound in EEG delta power immediately after the imposed interval of sleep deprivation. (Adapted from Trachsel, L. et al., *Brain Res.*, 589, 253, 1992.)

restorative process. You got sleepier and sleepier as the night progressed, but somehow you found a "second wind" by the time the Sun came up. That is, your sleep propensity decreased in the morning even though you did not get any sleep (and even though you did not go outside to be affected by the rising Sun). Evidently, sleep is under the control of the circadian system. Indeed, we saw in Chapter 5 that daily or circadian rhythmicity of electroencephalographically monitored sleep has been documented in controlled laboratory conditions in a variety of animals, including rodents,[506,527,563,564,571,572,576–588] humans,[589–595] and other vertebrates.[377,562,596–599] Therefore, sleep is not just a restorative process. It is a restorative process *gated by the circadian system*. This concept of a two-process regulation of sleep was first formalized in 1984[600] and has been supported by numerous empirical studies since then.[591,601–612]

Figure 10.34 is a simplified diagram of the two-process regulation of sleep. The circadian drive for sleepiness is represented by a slightly distorted sine wave describing the average human partition of a circadian day into 16 hours of wakefulness and 8 hours of sleep. The homeostatic drive is represented by a linear rise during wake hours and a linear fall during sleep hours. The rise in homeostatic drive for sleepiness corresponds to the accumulation of adenosine and is most likely a decelerated function rather than a linear one as depicted in the figure for simplicity. At time point *a* in the figure, the person has just woken up and has no homeostatic drive. The circadian drive is still falling, which accounts for the usual drowsiness at wake-up time. From time point *b* onward, the two drives rise slowly. At time point *c*, both drives reach their maxima for the day, and the individual is ready to go to bed. Both drives fall through the hours of night sleep until wake-up time at point *d* (which is the same as *a*).

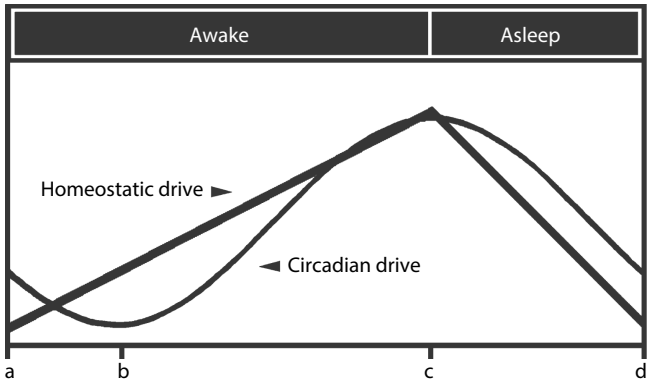


FIGURE 10.34 Sleep is under both homeostatic and circadian control. Under ideal conditions, the homeostatic drive to sleep and the circadian drive to sleep reinforce each other, as shown in this diagram. The homeostatic drive is depicted as a linear function for simplicity. See text for details.

The diagram in Figure 10.35 can help you understand what happened that night when you did not sleep. Time point *a* was your usual bedtime. The homeostatic drive was high because you had been awake for 16 hours, and the circadian drive was also high. However, you felt that the job or the party was more important than sleep, so you fought both drives. At time point *b*, the homeostatic drive was still high (and getting higher), but the circadian drive was well on its way down. So, you got your "second wind" even though you had gotten no sleep. At time point *c* you felt amazingly well, as the circadian drive reached its daily minimum. In contrast, you felt horrible at time point *d*, when the circadian drive reached its peak *and* you had accumulated 40 consecutive hours of wakefulness. You went home and crashed in bed.

If we compute the exact expected waveform of vigilance during a 64-hour interval of continuous wakefulness (by combining the homeostatic drive and the circadian drive), we obtain the curve shown in Panel B of Figure 10.36. For comparison, the curve in Panel A describes the actual average performance of 13 subjects in a reaction time task. Notice that the two curves are very similar, which indicates that the two-process model is quite accurate, considering the sizable noise inherent in biological systems. I should indicate, however, that the authors of the study from which these data were taken felt that the approximation was *not* good enough. In their opinion, a model based on the additive effects of the homeostatic and circadian drives is unsatisfactory.[607]

Finally, Figure 10.37 shows that the homeostatic and circadian components of sleep can be disentangled by means of the

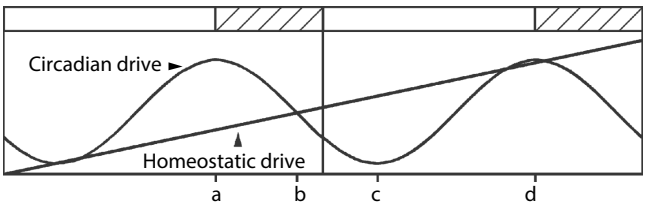


FIGURE 10.35 The homeostatic and circadian components of sleep may oppose each other. Attempts to stay up for two consecutive nights create the conditions for a conflict between the homeostatic and the circadian components of sleep regulation, as shown in this diagram. The hatched rectangles indicate sleep time. See text for details.

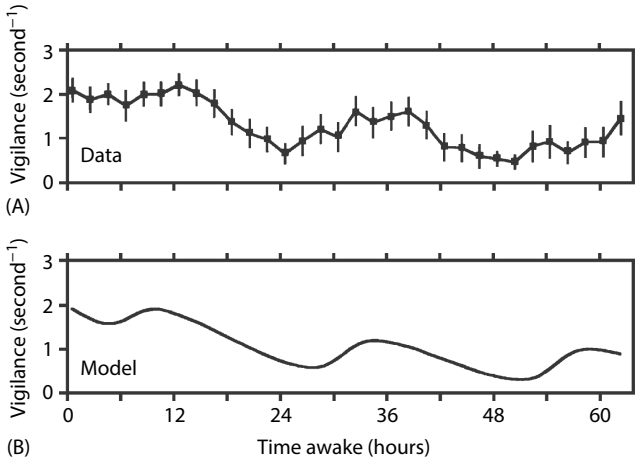


FIGURE 10.36 Measuring vigilance. The graphs show actual (A) and predicted (B) variations in vigilance of human subjects undergoing 64 consecutive hours of wakefulness. The data points correspond to the means (± SE) of 13 subjects. Vigilance is expressed as the reciprocal of reaction time in a standardized test. The predicted values (model) were computed under the assumption of additive effects of the homeostatic and circadian components. The approximation of the model to the actual data is reasonably good and describes the daily oscillation in vigilance as well as the gradual decline in its mean level. (Adapted from van Dongen, H.P.A. and Dinges, D.F., *J. Sleep Res.*, 12, 181, 2003.)

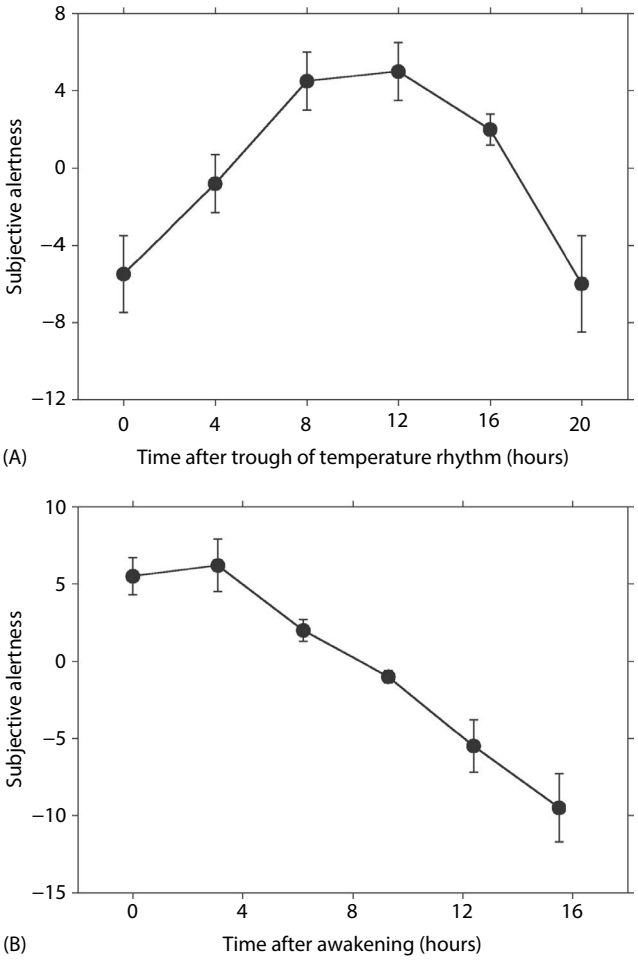


FIGURE 10.37 Isolating the effects of the homeostatic and circadian components. The experimental procedure of forced desynchrony allows the isolation of the effects of the homeostatic and circadian determinants of alertness. Human subjects were forced to live under 28-hour days, so that the endogenous circadian rhythm could not follow along the imposed rhythm of sleep and rest. The data points shown correspond to the means (± SE) of 9 subjects. When the data are plotted in reference to the circadian component (A), a clear circadian oscillation in subjective alertness is seen. When the data are plotted in reference to the homeostatic component (B), a gradual fall in alertness is seen. (Data from Johnson, M.P. et al., *J. Sleep Res.*, 1, 24, 1992.)

forced-desynchrony paradigm. In this paradigm, subjects are required to follow a 28-hour rest–activity cycle, which causes desynchrony between the endogenous 24-hour T_b rhythm and the imposed 28-hour sleep–wake rhythm. Consequently, the subjects' waking hours occur at all different phases of the T_b cycle, so that tests of alertness can be conducted during circadian times when the subjects would normally be asleep (and, therefore, unable to perform any requested tasks).[602] Panel A in Figure 10.37 shows the variation in subjective alertness as a function of time after the trough of the T_b rhythm. There is a clear circadian pattern, which reveals the circadian component of alertness (or its opposite, sleepiness). Panel B shows the variation in subjective alertness as a function of time after awakening. It is clear that alertness decreases progressively during the hours of wakefulness, which reveals the homeostatic component of alertness (or sleepiness).

The sleep homeostat is not a clock (a self-sustained oscillator), but, because sleep is a restorative process, the homeostat constitutes a timer of the hourglass type (as defined in Chapter 6). With basis on the output of a mathematical model of sleep, it has been proposed that this hourglass mechanism can account for ultradian oscillations in rest and activity without the need to postulate the existence of a separate ultradian clock.[613]

10.2.2 Homeostatic and Circadian Control of Feeding

The two preceding subsections showed that homeostatic and circadian mechanisms interact in the control of T_b and sleep. One can expect that similar interactions will characterize the control of all other processes in the body. In this subsection, we will consider the process of *feeding* (Figure 10.38).

Animals, unlike plants, cannot photosynthesize. This means that all energy that can be transformed within the body must be brought in through feeding. The energy contained in the ingested food follows various paths (Figure 10.39). Some energy cannot be extracted from the food and is excreted in the urine or feces. About half of the metabolizable energy is, from a nutritional perspective, wasted as thermic energy—that is, it is used in diet-induced thermogenesis (DIT) and cold-induced thermogenesis. The net energy is used for maintenance of tissues, for performance of muscular work, for growth, and for production of various life-sustaining substances such as hormones, neurotransmitters, and reproductive cells.[614]



FIGURE 10.38 Lunch time! Eating is an essential part of the life of all animals. (Copyright ArtToday, Tucson, AZ.)

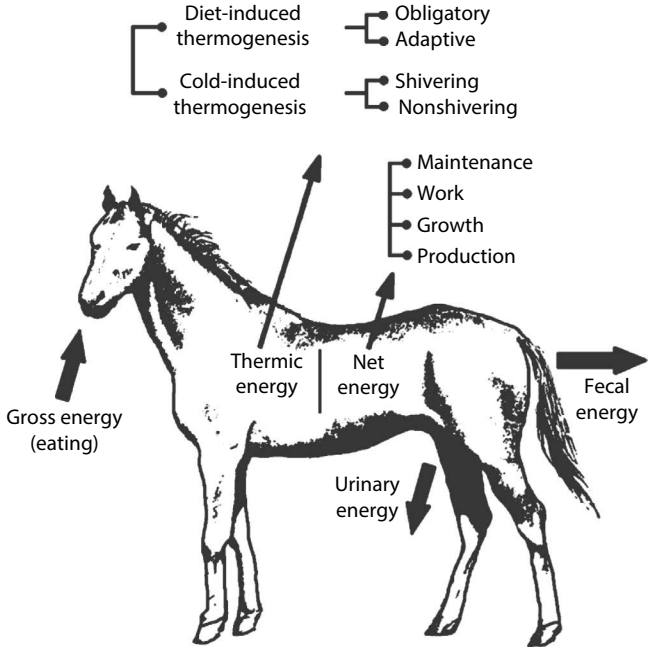


FIGURE 10.39 Where does the food go? In animals, ingested food is the sole source of energy that can be converted for multiple uses. Some of the ingested energy cannot be used and is eventually lost through the feces and urine. Some of the energy is wasted in thermal processes such as cold-induced thermogenesis and DIT. The remaining portion (less than 50% of the ingested energy) is used for the maintenance of body tissues, growth, production of various substances, and work (such as reading this book or riding a bicycle). (Adapted from Girardier, L. and Stock, M.J., *Mammalian Thermogenesis*, Chapman and Hall, London, U.K., 1983.)

Although food intake is determined by a variety of signals that will be discussed in Chapter 11, it is evident that the ingestion of nutrients must match the energetic needs of the organism. Thus, ingestive behavior serves the homeostatic purpose of maintaining a relatively stable supply of nutrients in the body. Figure 10.40 shows the mean meal duration of rats whose diet was temporarily diluted with cellulose so that it would yield only 75% of the energy of the unadulterated diet.

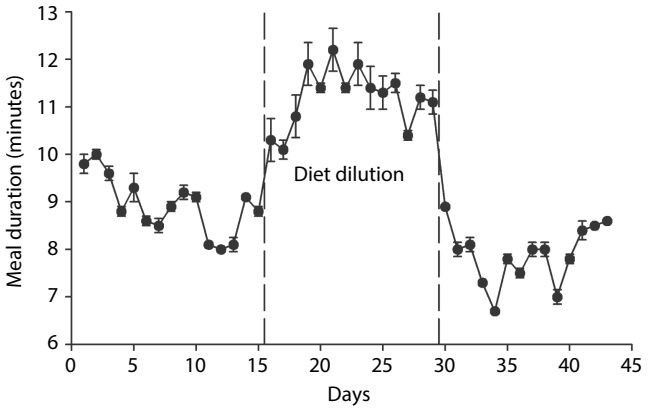


FIGURE 10.40 Ingestive behavior is homeostatically controlled. Depending on how one defines a meal, rats (*Rattus norvegicus*) eat about 10 meals a day, spending about 9 minutes per meal. The graph shows the mean (± SE) meal duration of rats whose diet was temporarily diluted with cellulose (thus yielding 75% of the energy of the unadulterated diet). Meal duration increased during the period of diet dilution as a homeostatic adjustment for the reduction in the energy content of the diet. (Adapted from Strubbe, J.H. and van Dijk, G., *Neurosci. Biobehav. Rev.*, 26, 485, 2002.)

Meal duration clearly increased during the period of diet dilution as a homeostatic adjustment for the reduction in the energy content of the diet.

When you think of being hungry or being satiated, you probably think of these two states as opposites. If you do not eat for many hours, you get hungry; if you eat, you get satiated. However, if you think about it more carefully, you will realize that hunger and satiety must involve distinct processes (Figure 10.41). Hunger can be evoked as a response to the depletion of energy stores in the body, but, because the digestion of a meal and the assimilation of its nutrients into body organs takes many hours, you would continue eating (and potentially explode!) if you did not have a short-term satiety mechanism to terminate the meal. Thus, there must be separate hunger and satiety loops. The hunger loop is based on the monitoring of energy stored in the form of adipose tissue, whereas the satiety loop is based on the monitoring of meal-generated signals such as gastric distention and various peptides secreted by the gastrointestinal system.[615–617]

We saw in Chapter 5 that daily and/or circadian rhythmicity of feeding has been extensively documented in laboratory rats[545,618–640] as well as in other rodents,[578,580,641–645] birds,[376,646–658] and other animals,[659–668] including nonhuman primates[512,669–677] and humans.[678–684] Consequently, the circadian system places temporal constraints on the homeostatic regulation of feeding.[685] It is reasonable to assume that greater constraints are placed on the long-term hunger loop, but I am unaware of any study that has specifically addressed the differential action of the circadian system on the hunger loop and the satiety loop. At least in rats, meal size seems to be associated mostly with the hunger loop, whereas meal frequency is associated mostly with the satiety loop.[615,685] A recent study in human subjects verified that the subjective feelings of hunger and appetite are stronger in the evening than at other times of

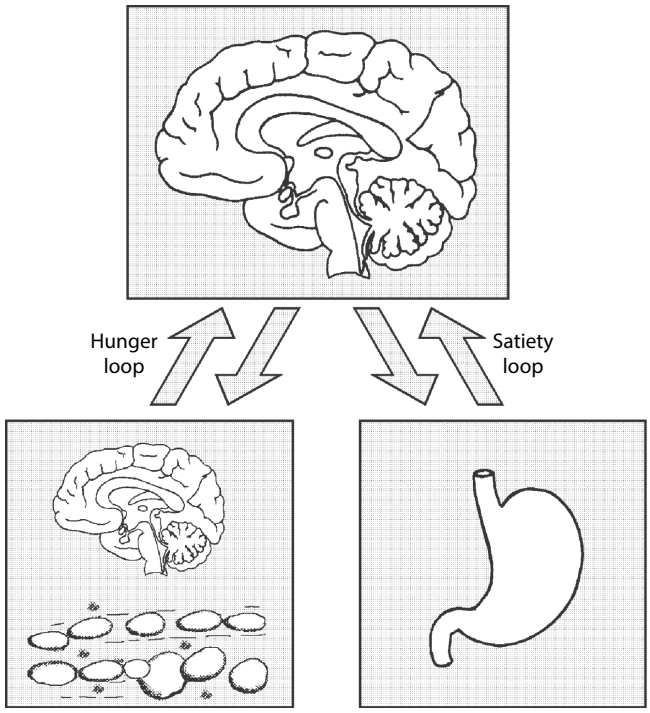


FIGURE 10.41 Two homeostatic loops. Although meals are normally initiated in response to hunger (and feeding eventually leads to a reduction in hunger), meals are terminated much before the digestive system has fully absorbed the ingested nutrients. Meal termination requires a satiety loop to temporarily inhibit the hunger loop.

the day even under a strict constant-routine protocol,[681] but no attempt was made to assess the hunger loop and the satiety loop separately.

So, the control of feeding involves an interaction of homeostatic and circadian processes similar to that previously discussed for the control of T_b and sleep. A peculiar feature of the control of feeding is its interaction with the control of overall metabolism and T_b. We saw earlier in this chapter that fasted animals experience a reduction in MR and a fall in T_b.[67,297,373–380,686,687] To better describe this phenomenon, Figure 10.42 shows T_b records of two speckled mousebirds (*Colius striatus*) with food freely available (Panel A) or after food deprivation for a day (Panel B). Notice the exaggerated fall in T_b when food is not available. What is especially interesting about this phenomenon is its modulation by the circadian system. The hypothermia induced by food deprivation (or chronic food restriction) does not occur indiscriminately. Instead, it is restricted to the inactive phase of the circadian cycle. Some animals have a natural disposition to exhibit daily torpor even when fed regularly,[38,338,532,688–698] but various true homeotherms exhibit circadian-modulated starvation-induced hypothermia. This has been documented in doves,[699] owls,[700] pigeons,[376,377,701–703] quail,[704] mousebirds,[496,705] finches,[706] pygmy mice,[707] deer mice,[708] domestic mice,[709–711] rats,[378,379,507,712–714] lemurs,[715,716] sheep,[717] goats,[718] and camels.[719] Figure 10.43 exemplifies the phenomenon in sheep. The T_b of five sheep were recorded every 2 hours as

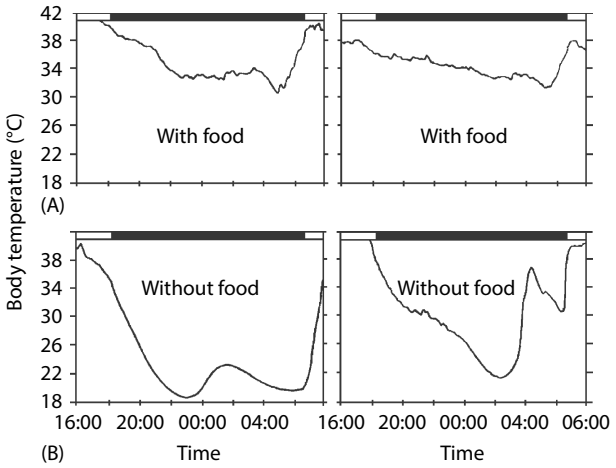


FIGURE 10.42 Starvation-induced hypothermia in a small bird. Food deprivation for even a single day greatly enhances the nocturnal fall in the body temperature (T_b) of the speckled mousebird (*Colius striatus*), a 50 g passerine bird. The graphs show segments of the T_b records of two mousebirds maintained under a 24-hour light–dark cycle (LD 12:12) at 15°C with (A) or without (B) food. (Adapted from McKechnie, A.E. and Lovegrove, B.G., *J. Comp. Physiol. B*, 171, 507, 2001.)

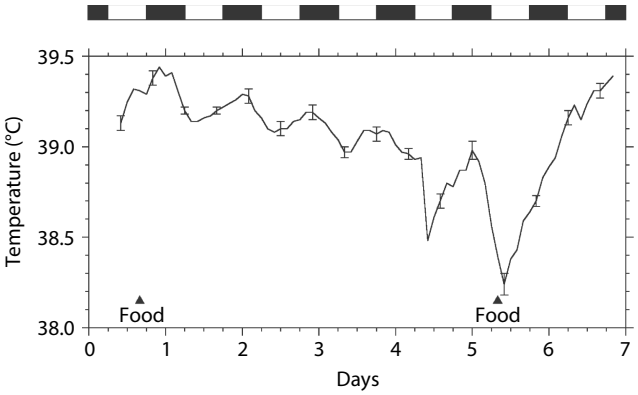


FIGURE 10.43 Starvation-induced hypothermia in a much larger mammal. Food deprivation for several days enhances the amplitude of the daily rhythm of body temperature (T_b) in the sheep (*Ovis aries*), a 40 kg mammal. The graph shows the mean (± SE) rectal temperature of 5 sheep measured at 2-hour intervals for a week. The dark and white horizontal bars indicate the duration of the dark and light phases of the light–dark cycle, respectively. The arrowheads indicate feeding times. Notice that T_b is gradually lowered (without a change in the amplitude of the rhythm) for the first 3 days of food deprivation. On the 4th and 5th days, the daily fall is greatly enhanced. (Data from Piccione, G. et al., *Chronobiol. Int.*, 19, 531, 2002.)

the animals spent 5 days without food. Although mean T_b initially fell gradually both during the day and the night, a partial circadian modulation of starvation-induced hypothermia was evident. At sunrise on day 4, mean T_b of the sheep fell to 38.5°C but subsequently rose to 39.0°C before falling to 38.2°C on the following morning. Feeding was resumed at this point, and T_b rose continuously for the next 24 hours, resuming the normal daily rhythm on the following day.

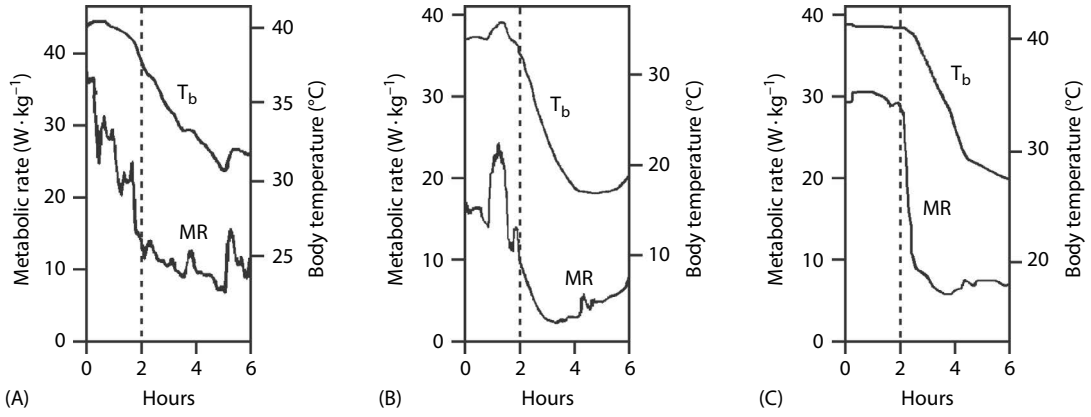


FIGURE 10.44 Fall in metabolic rate (MR) precedes fall in body temperature (T_b). In animals entering daily torpor, the fall in MR generally precedes the fall in T_b, as indicated by these records from representative individuals of three species. (A) Speckled mousebird (*Colius striatus*), a diurnal bird. (B) Siberian hamster (*Phodopus sungorus*), a nocturnal rodent. (C) Blue-naped mousebird (*Urocolius macrourus*), a diurnal bird. The vertical dashed lines indicate the day–night or night–day transition. (Data from McKechnie, A.E. and Lovegrove, B.G., *J. Comp. Physiol. B*, 171, 507, 2001; Heldmaier, G. and Ruf, T., *J. Comp. Physiol. B*, 162, 696, 1992; Prinzinger, R. et al., *J. Ornithol.*, 133, 446, 1992.)

Evidently, starvation-induced hypothermia is an energy-saving process similar to torpor and hibernation. As a matter of fact, Siberian hamsters (*Phodopus sungorus*) exhibit spontaneous torpor (i.e., torpor when food is freely available) only under winter-like short photoperiods,[689,690] but they also exhibit starvation-induced hypothermia when deprived of food under long photoperiods.[720,721] The lowering of MR and T_b allows conservation of energy in response to the immediate (in the case of food deprivation) or expected (in the case of short photoperiod) shortage of food. How the lowering of MR and the lowering of T_b relate to each other has been the topic of some controversy. There are two main possibilities here: either MR is actively downregulated (and T_b follows along because of the reduced heat production) or T_b is downregulated (and MR follows along according to a natural Q_{10} effect). The truth seems to be that the two processes are utilized at different stages of the torpid state. Figure 10.44 shows records of T_b and MR of individuals of three different species (speckled mousebird, Siberian hamster, and blue-naped mousebird) during the initial stage of torpor. In all three cases, although more clearly so in the speckled mousebird (A), MR starts to fall before T_b. Therefore, the fall in T_b cannot possibly be the cause of the lowering in MR.[722] Also, we saw earlier (Figure 10.11) that during deep hibernation MR is raised when T_b approaches dangerously low levels.[215,723] On the other hand, during most of the stable stage of torpor and hibernation, MR does fall along with T_b according to a Q_{10} between 2 and 3.[724–727]

It is probably impossible to distinguish starvation-induced hypothermia from daily torpor. It is likely that starvation-induced hypothermia refers to cases studied under laboratory conditions, where the exact effect of food restriction can be determined, whereas daily torpor refers to cases studied in the field, where the extent of food deprivation cannot be determined. As a matter of fact, even daily torpor and hibernation, which used to be thought of as distinct processes, are now considered to be only different manifestations of the same strategy of energy conservation.[728–730]

Torpor and starvation-induced hypothermia are phenomena associated with limited availability of food. When there is an *excess* of food, *obesity* may ensue. Because of DIT, excessive food intake is usually not sufficient to produce obesity. A reduction in energy expenditure is usually required to produce obesity. An interesting possibility is that obesity may result from reduced energy expenditure achieved by a regulated lowering of T_b. That is, a lowering of T_b may allow the storage of energy that would otherwise have been spent in thermogenic processes necessary for the maintenance of a higher level of T_b. In rodents, genetically obese (*ob/ob*) mice have been consistently reported to have lower T_b than lean mice.[285,731–734] My colleagues and I conducted a study on dogs (taking advantage again of the large variation of body size between breeds) and found that, for the same body size, obese dogs have lower rectal temperatures than lean dogs,[735] consistently with the results from studies in mice. Two studies in humans found no evidence that obese people have lower T_b than lean people,[736,737] but a recent, well-controlled study indicated that obese people do have lower T_b than lean people, although only during the day (i.e., not at night).[738] Because the studies with positive results (as well as the studies with negative results) were all correlational studies that started after obesity had already developed, we do not know whether the positive result of chronically lowered T_b preceded or followed the inception of obesity. Therefore, we cannot distinguish the direction of causality in the association between obesity and reduced T_b. If the lowering of T_b follows the development of obesity, then it cannot possibly cause it. However, if the lowering of T_b precedes or accompanies the development of obesity, then the hypothesis of obesity being caused by lower T_b might be correct.

10.2.3 Homeostatic and Circadian Control of Energy Expenditure

In this last subsection of the chapter, we will examine a very interesting aspect of the interaction of homeostasis and circadian rhythmicity in the control of *energy expenditure* (Figure 10.45). We briefly addressed the issue of energy expenditure when we discussed feeding in the preceding subsection. We shall now address the issue in greater detail. As diagrammed in Figure 10.46, the energy acquired through feeding may be either wasted (if it is not metabolizable), expended, or stored for later use. Energy is expended for the performance of essential body functions (basal MR [BMR]), for exercise, for thermoregulatory shivering, or for NST (which includes both diet- and cold-induced thermogenesis).[614]

BMR is the fraction of energy expenditure used to rebuild tissues, support the operation of the brain, keep the heart pumping, and all other processes required to sustain life at rest in a postabsorptive state (i.e., after food has been digested and absorbed). Larger animals have higher BMRs, as exemplified for rats in Figure 10.47. This is quite intuitive, as larger animals are made up of a larger number of cells. However, notice in the figure that the relationship between body mass and MR is not linear. The function is decelerated, which means that larger animals actually have lower MRs per unit body mass.[739] This is quite evident if we compare a mouse and an elephant. As indicated in Figure 10.48, a mouse weighs about 0.03 kg and has a MR of 0.2 W. An elephant weighs as much as 100,000 mice (3,000 kg) and, therefore, should have a MR of 22,000 W. Yet, the elephant's actual MR is only 1500 W.

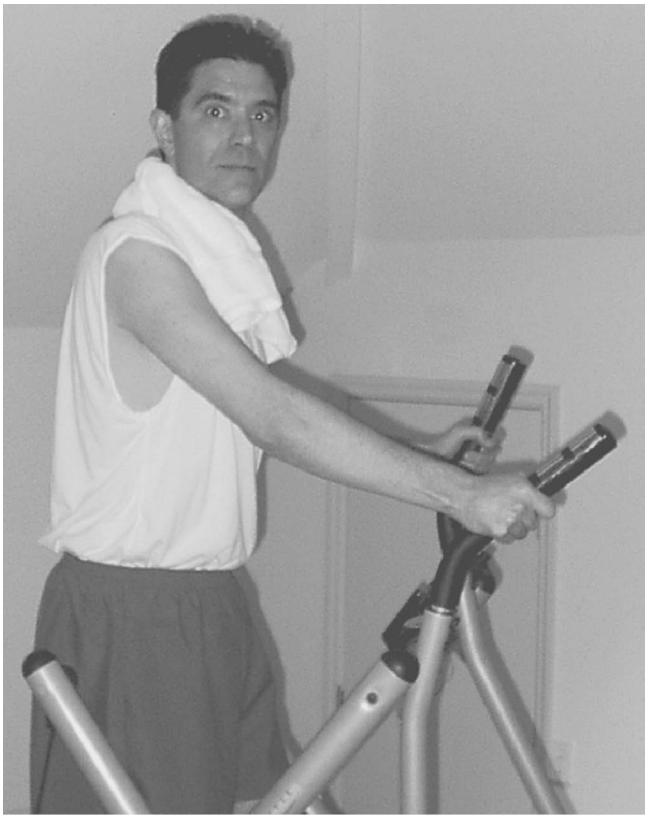


FIGURE 10.45 Working out a sweat. Physical exercise requires an elevation in energy expenditure. (Photo by Theresa Tolleson.)

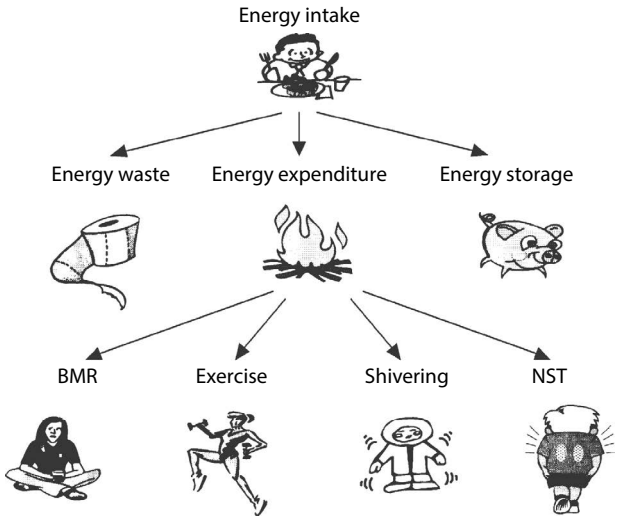


FIGURE 10.46 Energy expenditure. Energy that is ingested and is not wasted or stored is eventually expended. Energy expenditure generally falls into one of four categories: BMR, physical exercise, shivering, and nonshivering thermogenesis.

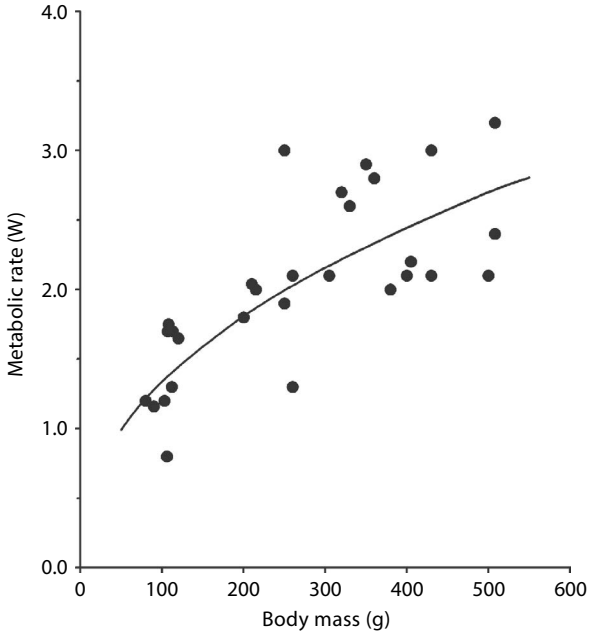


FIGURE 10.47 Energy expenditure and body size. The graph shows the relationship between BMR and body size for 30 laboratory rats (*Rattus norvegicus*). Notice the deceleration of the function as body size increases. (Data from Refinetti, R., *Exp. Biol.*, 48, 291, 1989.)

Why larger animals have proportionally lower MRs has been argued for generations. As early as 1838, before actual data had been collected, Pierre Sarrus and J. F. Rameaux proposed that MR should be proportional to the surface area of the animals—because heat can only leave the body through

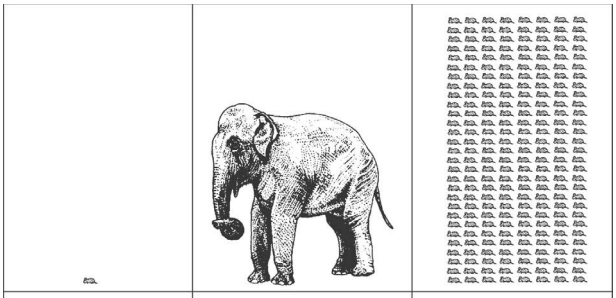


FIGURE 10.48 Big but slow. The elephant is bigger than the mouse and has a greater metabolic rate (MR). However, its MR is much lower than that of a group of mice large enough to match the elephant's body size. (The mouse is drawn out of proportion so that it can be visible in the figure.)

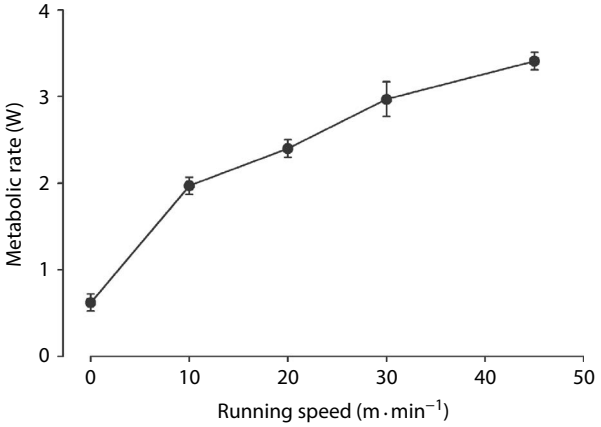


FIGURE 10.49 Rodent gym. The graph shows the average metabolic rates of antelope ground squirrels (*Ammospermophilus leucurus*) running on a motor-driven treadmill at different speeds. The data points correspond to the means (± SE) of 10 squirrels. Notice that more energy is expended at higher speeds. (Data from Yousef, M.K. et al., *Physiol. Zool.*, 46, 139, 1973.)

its surface.[740] Considering animals to be somewhat spherical, they reasoned that increases in body size (or mass, M) should lead to greater increases in volume (which is proportional to M^3) than in surface (which is proportional to M^2). Thus, in order to prevent overheating of large animals, MR would have to be proportional not to M^1 but to $M^{2/3}$. This could certainly account for the decelerated function relating MR to body mass. Actual experimental data indicating a decelerated function were collected by other investigators in the nineteenth century.[741,742] However, when Max Kleiber, in 1947, compiled data from a large number of studies conducted during the previous half century, he found that MR was proportional to $M^{3/4}$, not to $M^{2/3}$.[743] This meant that the surface area explanation was incorrect, but the correct explanation was nowhere to be found. The issue has been debated since then,[744–757] with no clear winner.

Exercise is one of the avenues of energy expenditure above basal metabolism. As physical activity increases, MR increases.[25,336,371,387,390–392,758–761] An example is given in Figure 10.49. Antelope ground squirrels (*Ammospermophilus leucurus*) were forced to run in a motor-driven treadmill at different speeds, and their MR was recorded. The function is decelerated, suggesting lower cost at high speeds. Notice, however, that the main change is from rest (0 m · min^{-1}) to slow running (10 m · min^{-1}). Within a wide range of speeds, the relationship between running speed and MR is generally linear.[8]

Shivering was discussed earlier in this chapter. Cold-induced *NST* was also discussed, but we have not addressed DIT. DIT is the fraction of energy expenditure induced by the ingestion of food. Some authors include BMR in the definition of DIT.[6] There are two types of DIT. One is called "obligatory" because it cannot be avoided. After a meal is ingested, MR is temporarily elevated.[547,762–771] This elevation is believed to be due partially to the energetic cost of digestion and partially to a cephalic component involving mastication as well as arousal ("palatability").[772–778] The other type of DIT is called "adaptive" because its magnitude can be adapted to conditions of shortage or excess of food supply. That is, DIT can be increased after overeating and be reduced during starvation or food restriction.[763,779–788] As was the case for cold-induced thermogenesis (see *thermoregulatory NST* in Section 10.1), DIT in mammals seems to depend strongly on the activation of BAT.[789–795] The drug *ephedrine*, used in several weight loss products, is a potent stimulator of DIT in BAT.[129,796–800]

According to the traditional homeostatic view, animals and humans spend metabolic energy at a constant rate, just like a light bulb spends electric energy, so that energy expenditure can be expressed in units of joules per second (i.e., in watts). Fifty years ago, Aschoff proposed the hypothesis that energy expenditure in animals might be primarily under circadian control rather than homeostatic control.[801] Because the natural alternation of day and night imposes on most animals a daily cycle of food availability, mating opportunities, and so on, Aschoff reasoned that energy expenditure might be regulated on a daily (circadian) basis rather than on a constant-rate (homeostatic) basis. Thus, MR would be properly expressed not in units of joules per second but in units of joules per circadian cycle. If this seems confusing to you, Figure 10.50 may help. Instead of metabolic energy, think of some paint. If we pour paint into three different containers of different sizes (Panel A), we can have two outcomes depending on how we proceed. If we pour the paint at a constant rate, then an amount of paint necessary to fill the 28-hour container will fill more than one 24-hour container (Panel B). This corresponds to the *homeostatic* control of energy expenditure. However, if we adjust the flow of paint according to the size of the containers, then all containers will end up with the same amount of paint (Panel C). This corresponds to the *circadian* control of energy expenditure, as proposed by Aschoff.

Indirect supporting evidence for Aschoff's hypothesis came from his laboratory observation that human subjects living on long days (say, 28-hour long) consume the same amount of food per day (i.e., per circadian cycle) as subjects living on short days (say, 20-hour long).[802] Assuming that body mass was not affected, these results suggest that

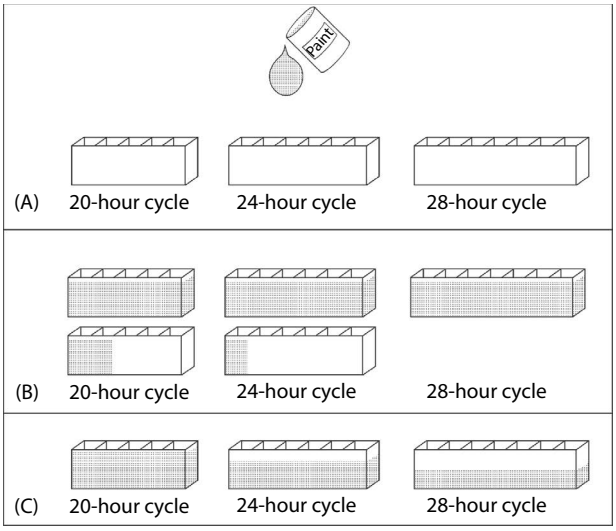


FIGURE 10.50 Circadian conservation of energy expenditure? These diagrams provide an analogy for the issue of the temporal organization of energy expenditure. Assume that some paint must be poured into containers of different capacities (A). Pouring at a constant rate requires more small containers than large containers (B), but an equal number of containers of different capacities can be used if the rate of pouring is adjusted proportionally (C).

energy expenditure is regulated on a circadian basis (i.e., not on a constant-rate basis) and that MR should be expressed not in watts but in *aschoffs* (i.e., joules per circadian cycle). This new conception of MR would instate a true revolution in the study of homeostasis. It might not be of great consequence for normal humans living under the usual conditions where the duration of a day is the same as the duration of the entrained circadian cycle (i.e., 24 hours), but it would have great practical importance for humans living under artificial working conditions. The planning of food supply for special worker groups (such as sailors in submarines and workers in underground facilities living under non-24-hour days) would certainly be affected by the new conception of MR. Furthermore, seasonal changes in photoperiod have physiological effects similar to changes in circadian period, as in both cases, there is a reduction or extension of the daily segment of activity. In the winter, when days are shorter, diurnal animals (including humans and livestock) have fewer hours each day in which to perform their daytime activities. Nocturnal animals have a shorter segment of activity during the summer but a longer one during the winter. A longer daily segment of activity at a time when food supply is reduced constitutes an energetic challenge, and the regulation of energy expenditure on a circadian basis could reduce the magnitude of the challenge.

Aschoff's additional studies showed that the amount of locomotion and the pattern of defecation were also conserved on a circadian basis rather than on a homeostatic basis.[803,804] However, a separate group of investigators found that human subjects freerunning with a period of 25 hours consumed less food per cycle than when entrained to a 24-hour routine.[678] Also, rats entrained to light–dark cycles with periods ranging from 23 to 28 hours ate the same amount of food *per 24 hours*, not *per circadian cycle*.[638]

Two studies published in 1997 took advantage of the *tau* mutation in the golden hamster. You will remember from Chapter 6 that wild-type hamsters have an endogenous circadian period of approximately 24 hours, but homozygous *tau*-mutant hamsters have a period of 20 hours as the result of a single gene mutation. One of the studies found that the MRs of wild-type and homozygous mutant hamsters were different when expressed in the traditional, homeostatic form (i.e., energy expended per unit of time) but indistinguishable when expressed in circadian form (i.e., energy expended per circadian cycle).[805] These findings support Aschoff's hypothesis that energy expenditure is conserved on a circadian basis. However, the other study produced opposite results. In two separate experiments, the authors measured energy expenditure as running-wheel activity and as oxygen consumption. The data for running-wheel activity are shown in Figure 10.51. Notice that the number of wheel revolutions performed per circadian cycle is lower in mutant hamsters than in wild types (because the circadian cycle is shorter in the mutants), but that mutants and wild types do not differ significantly when activity is expressed per 24 hours.[806] Similar results were obtained in a third laboratory.[807] Also, running-wheel activity of mice maintained under 24-hour light–dark cycles with different proportions of light and darkness was compressed under short scotophases (as previously discussed in Chapter 7) but remained constant in terms of daily totals (Figure 10.52).

The data for MR from the study on *tau*-mutant hamsters are shown in Figure 10.53. Notice that energy expenditure per circadian cycle is lower in mutant hamsters than in wild types (because the circadian cycle is shorter in the mutants), but that mutants and wild types do not differ significantly when energy expenditure is expressed per 24 hours. This indicates

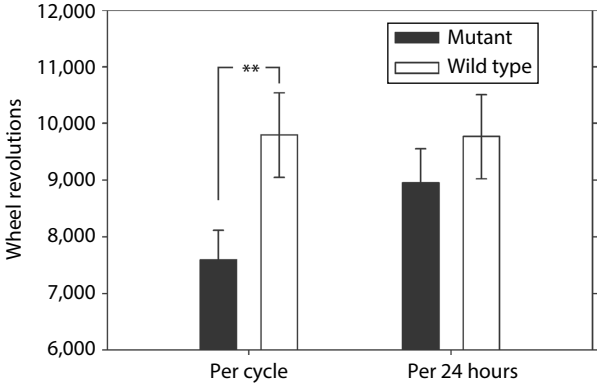


FIGURE 10.51 Running-wheel activity: homeostasis by circadian period. The graph shows the mean number of wheel revolutions of *tau*-mutant and wild-type golden hamsters (*Mesocricetus auratus*) maintained in constant darkness. The bars correspond to the means (± SE) of 32 mutants (τ = 20.4 hours) and 32 wild types (τ = 24.1 hours). The two groups are significantly different when activity is expressed per circadian cycle but statistically indistinguishable when activity is expressed per 24 hours. (Data from Refinetti, R. and Menaker, M., *J. Physiol.*, 501, 449, 1997.)

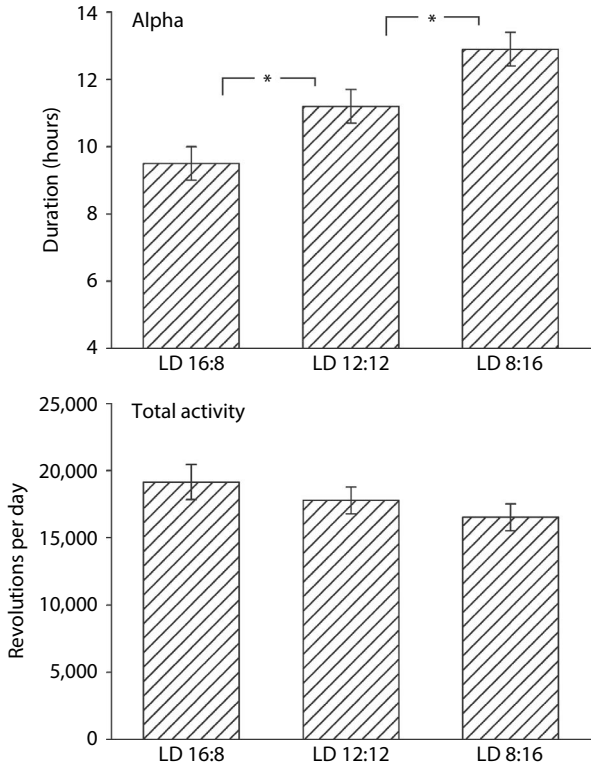


FIGURE 10.52 Running-wheel activity: homeostasis by photoperiod. The graphs show the mean duration of the activity phase (Alpha) and the number of wheel revolutions per day of domestic mice (*Mus musculus*) maintained under light–dark cycles with different proportions of light and darkness. Each bar corresponds to the mean (± SE) of 30 mice. Notice that alpha is longer when the dark phase of the light–dark cycle is longer, but total daily activity is not significantly different between the groups. (Data from Refinetti, R., *Integr. Physiol. Behav. Sci.*, 37, 114, 2002.)

that energy expenditure is conserved on the traditional unit-time basis, not on a circadian basis. Why the results of this study[806] contradict the results of the other study[805] is not clear. In the other study, the wild-type hamsters were significantly heavier (132 g) than the mutants (107 g), and difficulties in the comparison of MR between animals of different body sizes are well recognized in the physiological literature.[747,808–812] If this is the cause of the difference in the results of the two studies, then Aschoff's hypothesis of circadian conservation of energy expenditure cannot be supported. I conducted an additional study with Nile grass rats, golden hamsters, and domestic mice and again found no evidence of circadian (or photoperiodic) conservation of energy expenditure.[813] I am convinced that the traditional homeostatic view of energy expenditure is correct. As much as I admired Aschoff, I cannot name a unit of energy expenditure after him.

SUMMARY

1. Body temperature is under both homeostatic control and circadian control. The two mechanisms act independently on effector organs responsible for the regulation of T_b. The circadian system generates circadian rhythmicity of T_b. The thermoregulatory system restricts this rhythmicity according to its set point and its range of hysteresis error.
2. Sleep is a restorative (homeostatic) process gated by the circadian system. Feeding is also a homeostatic process modulated by the circadian system. Food restriction in endotherms often leads to hypothermia limited to the inactive phase of the circadian cycle. Energy expenditure is modulated by the circadian system but is conserved on a homeostatic basis, not on a circadian basis.

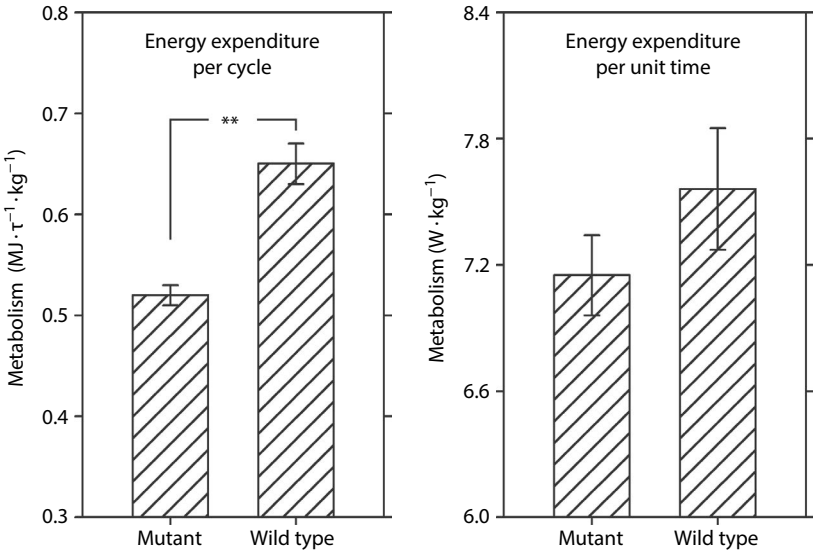


FIGURE 10.53 Metabolism: homeostasis by circadian period. The graph shows the mean energy expenditure of *tau*-mutant and wild-type golden hamsters (*Mesocricetus auratus*) maintained in constant darkness. The bars correspond to the means (± SE) of 8 mutants ($\tau = 20.4$ hours) and 8 wild types ($\tau = 24.1$ hours). The two groups are significantly different when energy expenditure is expressed per circadian cycle but statistically indistinguishable when expenditure is expressed per unit time. (Data from Refinetti, R. and Menaker, M., *J. Physiol.*, 501, 449, 1997.)

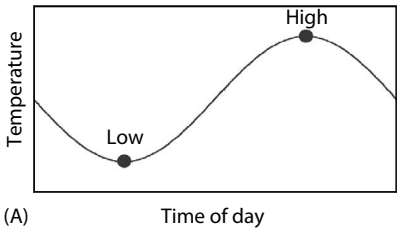
EXERCISES

10.1 Circadian modulation of thermoregulatory behavior. The interaction between homeostasis and circadian rhythmicity in the control of T_b was discussed in Section 10.1. Behavioral adjustment is one of the mechanisms used by the homeostatic (thermoregulatory) system to control T_b. In this exercise, we will examine how a behavioral response (thermal sensation) is affected by the circadian system.

1. You can be your own subject, although it would be easier to work in groups of two.
2. You measured your T_b rhythm in previous exercises and should have a graph that looks like the left panel (A) in Figure 10.54. Basically, you need to know approximately at what time of day your temperature rhythm reaches its lowest value and its highest value. For most people, the lowest temperature occurs around wake-up time, and the highest temperature occurs 10–12 hours later.
3. The equipment that you will need for this exercise is a set of five constant-temperature water containers. If you have the resources, thermostatically controlled water baths would be ideal. Otherwise, a set of five small Styrofoam boxes filled with appropriate mixtures of cold and hot water would be adequate. In this case, you will need an accurate thermometer to measure the water temperature.
4. Although you may have to adjust the values based on preliminary trials, the temperature of the water baths should generally be set 1°C (2°F) apart, starting at 31°C (88°F). Usually, 33°C feels neither cold nor warm, so that you will have two stimuli on the warm side and two on the cold side. If the 31°C–35°C range does not work for you, you should adjust it to your personal range.
5. Your task as the subject will be to subjectively assess the temperature of the water baths with your hand. Here is where a second person will be helpful. If you know which water bath is at which temperature, you will likely allow this knowledge to interfere with your true sensations. As a matter of fact, your partner should have you try the different water temperatures in a different order each time so that you cannot guess which one is supposed to be warm and which one is supposed to be cold. Make sure to dry your hand and wait a few minutes between trials.
6. The right panel (B) in Figure 10.54 is a guide for your data collection protocol. Twice a day for several days, you will judge the temperature of the five water baths. You do not need to estimate the actual temperature. A judgment of *warm* or *cold* is enough. As a matter of fact, concentrating on the *pleasantness* of the sensation, rather than on an objective measurement of temperature, is likely to yield more robust results. Obviously, you will feel very uncertain when you try the neutral temperature, but you should force yourself to decide between warm and cold for every stimulus.
7. After you finish collecting the data, you can start to analyze them. Presumably, you will have called all 35°C stimuli warm and all 31°C stimuli cold. The interesting values are those between these two extremes. Can you notice a consistent difference between the early morning values (low) and the late afternoon values (high)? Did you tend to judge more stimuli as warm in the late afternoon? If you did, it was probably because you were warmer at this time of day and your thermoregulatory system shifted your sense of thermoneutrality so that you would seek cooler temperatures (by perceiving the stimuli as warmer than they are). That is, the homeostatic system tried to reduce the elevation in T_b caused by the circadian system.

10.2 Homeostatic and circadian control of sleep. The interaction between homeostasis and circadian rhythmicity in the control of sleep was discussed in Section 10.2. In this exercise, you will have the opportunity to experience it yourself.

1. You will be your own subject. The central variable of study will be general sleepiness. To measure it, you can use the same subjective scale (0, very awake; 5, very sleepy) that you used in Exercise 9.1. You should also monitor the state of your circadian system by measuring your own T_b. (Look back at Exercise 1.2 if you need instructions on how to monitor your T_b rhythm.)
2. The goal of this exercise is to uncouple the two systems that control sleep by maintaining the circadian



(A)

	Low	High	Low	High	
31°C					
32°C					
33°C					
34°C					
35°C					

(B)

FIGURE 10.54 Testing the daily variation in thermal sensation. This figure will help you conduct Exercise 10.1. Panel A indicates the two daily time points for data collection (in reference to your body temperature rhythm). Panel B illustrates a form for registering the data.

system undisturbed while the homeostatic system is manipulated. This will be achieved by sleep deprivation.

3. Start by recording sleepiness and T_b every waking hour for at least 1 day (2 or 3 days would be better). Then, do an *all-nighter* so that you can keep recording data throughout the night.
4. Even though you stayed up all night, you probably will not be able to sleep in the morning. However, it is important that you try to go to sleep in the afternoon to pay back your sleep debt and be refreshed for your second consecutive all-nighter. Yes, you will do a second all-nighter. In the first all-nighter, you simply skipped a night of sleep. In the second one, you will try to go to bed much earlier than usual so that you can be awake during your usual bedtime later on. That is, you will still be up all night, but you will not be sleep deprived.
5. Because you may have difficulty in sleeping in the afternoon, some advice may be helpful. You should choose a comfortable surface (preferably your own bed) in a dark room with soft monotonous background sound. The room should not be hot, but it should not be cold either. Usually, a good meal at lunchtime helps you initiate sleep in the afternoon.
6. When you are finished collecting data, you should plot the two variables on Cartesian coordinates (time in the X-axis, variable in the Y-axis).
7. The records of T_b will hopefully show that your circadian system was not disturbed by the experimental procedure (i.e., the daily oscillations will be similar during the baseline days and the two experimental days).
8. In contrast to the T_b records, the records of subjective sleepiness should show an interesting pattern. During the baseline days, sleepiness will be minimal after you are fully awake in the morning, may increase a bit in the early afternoon, go down again, and then go up as your usual bedtime approaches. During the first all-nighter, sleepiness will go up throughout the night but will go down in the morning even though you did not get any sleep. Of course, because you did not get any sleep, the reduction in sleepiness in the morning must be due to the circadian system.
9. The most interesting comparison is that between the first and the second sleepless night. Although the influence of the circadian system is the same on both nights, the influence of the homeostatic system should be much greater in the first night than in the second night (because you slept in the afternoon preceding the second sleepless night). Can you notice any difference in your records? If so, can you still see the influence of the circadian system?

SUGGESTIONS FOR FURTHER READING

Van Dongen, H. P. A., Maislin, G., Mullington, J. M., and Dinges, D. F. (2003). The cumulative cost of additional wakefulness: Dose–response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep* 26: 117–126.

By now, you should be familiar with the idea that suggestions for further reading on specific topics can be gathered from the list of source articles in the "References" section and that only general readings are listed in this section. However, I would like to explicitly list this research article here, because it is an exceptionally good example of a well-written article with rigorous research methods and clear-cut results.

Blumberg, M. S. (2002). *Body Heat: Temperature and Life on Earth*. Cambridge, MA: Harvard University Press.

An easy-reading book on the homeostatic aspect of T_b regulation written for general audiences.

Mrosovsky, N. (1990). *Rheostasis: The Physiology of Change*. New York: Oxford University Press.

A 25-year-old introduction to the debate on the interaction of homeostasis and biological rhythmicity. Mrosovsky anticipated much of the current debate and placed it in the broader context of physiological change.

Heller, H. C., Edgar, D. M., Grahan, D. A., and Glotzbach, S. F. (1996). Sleep, thermoregulation, and circadian rhythms. In: Fregly, M. J. and Blatteis, C. M. (Eds.), *Handbook of Physiology. Section 4: Environmental Physiology*, Vol. 2. New York: Oxford University Press, pp. 1361–1374.

A review article, with emphasis on animal studies, that discusses the interactions among sleep, circadian rhythmicity, and hibernation.

Dement, W. C. and Vaughan, C. (1999). *The Promise of Sleep*. New York: Delacorte.

Written for general audiences by a pioneer in sleep research, this book provides basic information on the homeostatic control of sleep and helpful advice on how to manage sleep for health and happiness.

Danguir, J. (1996). The relationship between food and sleep. In: Fregly, M. J. and Blatteis, C. M. (Eds.), *Handbook of Physiology. Section 4: Environmental Physiology*, Vol. 2. New York: Oxford University Press, pp. 1375–1387.

A review article on the interactions between the homeostatic mechanisms controlling food intake and sleep.

WEBSITES TO EXPLORE

American Physiological Society: http://www.the-aps.org/.

Center for Sleep and Circadian Biology at Northwestern University: http://www.northwestern.edu/cscb/.

Lecture on Homeostasis: http://pespmc1.vub.ac.be/HOMEOSTA.html.

National Center on Sleep Disorders Research: http://www.nhlbi.nih.gov/about/org/ncsdr/.

Sleep and Circadian Neuroscience Institute at the University of Oxford: http://www.eye.ox.ac.uk/research/sleep-circadian-neuroscience-institute.

Stanford Center for Sleep Sciences and Medicine: http://sleep.stanford.edu/.

Thermal Biology Institute (Montana State University): http://tbi.montana.edu/.

Thermal Physiology and Pharmacology Home Page (IUPS): http://thermal.uow.edu.au/.

REFERENCES

1. Michael, J., Modell, H., McFarland, J., and Cliff, W. (2009). The "core principles" of physiology: What should students understand? *Advances in Physiology Education* 33: 10–16.
2. Bernard, C. (1872). *De la Physiologie Générale*. Paris, France: Librairie Hachette.
3. Mrosovsky, N. (1990). *Rheostasis: The Physiology of Change*. New York: Oxford University Press.
4. Bonny, O. and Firsov, D. (2009). Circadian clock and the concept of homeostasis. *Cell Cycle* 8: 4015–4016.
5. Hirsch, E. D. (1987). *Cultural Literacy: What Every American Needs to Know*. Boston, MA: Houghton Mifflin.
6. IUPS Thermal Commission. (2001). Glossary of terms for thermal physiology. *Japanese Journal of Physiology* 51: 245–280.
7. Cossins, A. R. and Bowler, K. (1987). *Temperature Biology of Animals*. London, U.K.: Chapman and Hall.
8. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th edn. New York: Cambridge University Press.
9. Montgomery, J. C. and Macdonald, J. A. (1990). Effects of temperature on nervous system: Implications for behavioral performance. *American Journal of Physiology* 259: R191–R196.
10. Girguis, P. R. and Lee, R. W. (2006). Thermal preference and tolerance of alvinellids. *Science* 312: 231.
11. Kashefi, K. and Lovley, D. R. (2003). Extending the upper temperature limit for life. *Science* 301: 934.
12. Costanzo, J. P. and Lee, R. E. (1994). Biophysical and physiological responses promoting freeze tolerance in vertebrates. *News in Physiological Sciences* 9: 252–256.
13. Fletcher, G. L., Hew, C. L., and Davies, P. L. (2001). Antifreeze proteins of teleost fishes. *Annual Review of Physiology* 63: 359–390.
14. Stevens, J. C. and Stevens, S. S. (1960). Warmth and cold: Dynamics of sensory intensity. *Journal of Experimental Psychology* 60: 183–192.
15. Mower, G. D. (1976). Perceived intensity of peripheral thermal stimuli is independent of internal body temperature. *Journal of Comparative and Physiological Psychology* 90: 1152–1155.
16. Stevens, J. C. (1979). Variation of cold sensitivity over the body surface. *Sensory Processes* 3: 317–326.
17. Refinetti, R. (1988). Magnitude estimation of warmth in children. *Journal of Thermal Biology* 13: 85–88.
18. Refinetti, R. (1990). Is there modal opponency in the thermal senses? *Journal of Thermal Biology* 15: 255–258.
19. McArthur, A. J. and Clark, J. A. (1988). Body temperature of homeotherms and the conservation of energy and water. *Journal of Thermal Biology* 13: 9–13.
20. Else, P. L. and Hulbert, A. J. (1981). Comparison of the "mammal machine" and the "reptile machine": Energy production. *American Journal of Physiology* 240: R3–R9.
21. Hulbert, A. J. and Else, P. L. (1989). Evolution of mammalian endothermic metabolism: Mitochondrial activity and cell composition. *American Journal of Physiology* 256: R63–R69.
22. Graham, J. B. and Dickson, K. A. (1981). Physiological thermoregulation in the albacore *Thunnus alalunga*. *Physiological Zoology* 54: 470–486.
23. Holland, K. N., Brill, R. W., Chang, R. K. C., Sibert, J. R., and Fournier, D. A. (1992). Physiological and behavioral thermoregulation in bigeye tuna (*Thunnus obesus*). *Nature* 358: 410–412.
24. Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A. et al. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293: 1310–1314.
25. Blank, J. M., Farwell, C. J., Morrissette, J. M., Schallert, R. J., and Block, B. A. (2007). Influence of swimming speed on metabolic rates of juvenile Pacific bluefin tuna and yellowfin tuna. *Physiological and Biochemical Zoology* 80: 167–177.
26. James, M. C. and Mrosovsky, N. (2004). Body temperatures of leatherback turtles (*Dermochelys coriacea*) in temperate waters off Nova Scotia, Canada. *Canadian Journal of Zoology* 82: 1302–1306.
27. Chown, S. L. and Scholtz, C. H. (1993). Temperature regulation in the nocturnal melolonthine *Sparrmannia flava*. *Journal of Thermal Biology* 18: 25–33.
28. Knutson, R. M. (1974). Heat production and temperature regulation in eastern skunk cabbage. *Science* 186: 746–747.
29. Nagy, K. A., Odell, D. K., and Seymour, R. S. (1972). Temperature regulation by the inflorescence of philodendron. *Science* 178: 1195–1197.
30. Brattstrom, B. H. (1972). Temperature changes in heat producing plants. *Bulletin of the Southern California Academy of Sciences* 71: 54–55.
31. Seymour, R. S. and Schultze-Motel, P. (1996). Thermoregulating lotus flowers. *Nature* 383: 305.
32. Lamprecht, I., Seymour, R. S., and Schultze-Motel, P. (1998). Direct and indirect calorimetry of thermogenic flowers of the sacred lotus, *Nelumbo nucifera*. *Thermochimica Acta* 309: 5–16.
33. Buggeln, R. G. and Meeuse, B. J. D. (1971). Hormonal control of the respiratory climateric in *Sauromatum guttatum* (Araceae). *Canadian Journal of Botany* 49: 1373–1377.
34. Raskin, I., Ehmann, A., Melander, W. R., and Meeuse, B. J. D. (1987). Salicylic acid: A natural inducer of heat production in *Arum* lilies. *Science* 237: 1601–1602.
35. Vane, J. R. and Botting, R. M. (2003). The mechanism of action of aspirin. *Thrombosis Research* 110: 255–258.
36. Wooden, K. M. and Walsberg, G. E. (2002). Effect of environmental temperature on body temperature and metabolic heat production in a heterothermic rodent, *Spermophilus tereticaudus*. *Journal of Experimental Biology* 205: 2099–2105.
37. Wooden, K. M. and Walsberg, G. E. (2004). Body temperature and locomotor capacity in a heterothermic rodent. *Journal of Experimental Biology* 207: 41–46.
38. Refinetti, R. (1996). The body temperature rhythm of the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *Physiological Zoology* 69: 270–275.
39. Bennett, N. C., Jarvis, J. U. M., and Cotterill, F. P. D. (1993). Poikilothermic traits and thermoregulation in the Afrotropical social subterranean Mashona mole-rat (*Cryptomys hottentotus darlingi*) (Rodentia: Bathyergidae). *Journal of Zoology* 231: 179–186.
40. Buffenstein, R. and Yahav, S. (1991). Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *Journal of Thermal Biology* 16: 227–232.

41. Bligh, J., Ingram, D. L., Keynes, R. D., and Robinson, S. G. (1965). The deep body temperature of an unrestrained Welsh mountain sheep recorded by a radiotelemetric technique during a 12-month period. *Journal of Physiology* 176: 136–144.
42. Hardy, J. D. (1965). The "set-point" concept in physiological temperature regulation. In: Yamamoto, W. S. and Brobeck, J. R. (Eds.), *Physiological Controls and Regulations*. Philadelphia, PA: Saunders, pp. 98–116.
43. Hammel, H. T., Jackson, D. C., Stolwijk, J. A. J., Hardy, J. D., and Strømme, S. B. (1963). Temperature regulation by hypothalamic proportional control with an adjustable set point. *Journal of Applied Physiology* 18: 1146–1154.
44. Lipton, J. M. and Ticknor, C. B. (1979). Influence of sex and age on febrile responses to peripheral and central administration of pyrogens in the rabbit. *Journal of Physiology* 295: 263–272.
45. Morimoto, A., Watanabe, T., Ono, T., Sakata, Y., and Murakami, N. (1986). Rat endogenous pyrogen and fever. *American Journal of Physiology* 250: R776–R782.
46. Dascombe, M. J., Rothwell, N. J., Sagay, B. O., and Stock, M. J. (1989). Pyrogenic and thermogenic effects of interleukin 1 β in the rat. *American Journal of Physiology* 256: E7–E11.
47. Horan, M. A., Little, R. A., Rothwell, N. J., and Strijbos, P. J. L. M. (1989). Comparison of the effects of several endotoxin preparations on body temperature and metabolic rate in the rat. *Canadian Journal of Physiology and Pharmacology* 67: 1011–1014.
48. Tanaka, H., Kanosue, K., Yanase, M., and Nakayama, T. (1990). Effects of pyrogen administration on temperature regulation in exercising rats. *American Journal of Physiology* 258: R842–R847.
49. Bruguerolle, B. and Roucoules, X. (1994). Time-dependent changes in body temperature rhythm induced in rats by brewer's yeast injection. *Chronobiology International* 11: 180–186.
50. Cabanac, M., Duclaux, R., and Gillet, A. (1970). Thermorégulation comportementale chez le chien: Effets de la fièvre et de la thyroxine. *Physiology and Behavior* 5: 697–704.
51. Blatteis, C. M. and Smith, K. A. (1980). Behavioral fever induced in guinea-pigs by intrapreoptic pyrogens. *Experientia* 36: 1086–1088.
52. Marques, P. R., Spencer, R. L., Burks, T. F., and McDougal, J. N. (1984). Behavioral thermoregulation, core temperature, and motor activity: Simultaneous quantitative assessment in rats after dopamine and prostaglandin E_1. *Behavioral Neuroscience* 98: 858–867.
53. Sugimoto, N., Shido, O., Sakurada, S., and Nagasaka, T. (1996). Day–night variations of behavioral and autonomic thermoregulatory responses to lipopolysaccharide in rats. *Japanese Journal of Physiology* 46: 451–456.
54. Briese, E. (1997). Selected temperature correlates with intensity of fever in rats. *Physiology and Behavior* 61: 659–660.
55. Reynolds, W. W., Casterlin, M. E., and Covert, J. B. (1976). Behavioural fever in teleost fishes. *Nature* 259: 41–42.
56. Casterlin, M. E. and Reynolds, W. W. (1977). Behavioral fever in crayfish. *Hydrobiologia* 56: 99–101.
57. Myhre, K., Cabanac, M., and Myhre, G. (1977). Fever and behavioural temperature regulation in the frog *Rana esculenta*. *Acta Physiologica Scandinavica* 101: 219–229.
58. Cabanac, M. and Le Guelte, L. (1980). Temperature regulation and prostaglandin E_1 fever in scorpions. *Journal of Physiology* 303: 365–370.
59. Cabanac, M. (1989). Fever in the leech, *Nephelopsis obscura* (Annelida). *Journal of Comparative Physiology B* 159: 281–285.
60. Refinetti, R. (1988). The concept of set point (goal value) in thermal physiology. *Manuscrito* 11: 47–56.
61. Bligh, J. (1979). The central neurology of mammalian thermoregulation. *Neuroscience* 4: 1213–1236.
62. Werner, J. (1980). The concept of regulation for human body temperature. *Journal of Thermal Biology* 5: 75–82.
63. Bligh, J. (1984). Temperature regulation: A theoretical consideration incorporating Sherringtonian principles of central neurology. *Journal of Thermal Biology* 9: 3–6.
64. McAllen, R. M., Tanaka, M., Ootsuka, Y., and McKinley, M. J. (2010). Multiple thermoregulatory effectors with independent central controls. *European Journal of Applied Physiology* 109: 27–33.
65. Hochachka, P. W. and Somero, G. N. (1984). *Biochemical Adaptation*. Princeton, NJ: Princeton University Press.
66. Blatteis, C. M. and Fregly, M. J. (1996). Introduction. In: Fregly, M. J. and Blatteis, C. M. (Eds.), *Handbook of Physiology, Section 4: Environmental Physiology*, Vol. 1. New York: Oxford University Press, pp. xix–xxii.
67. Duchamp, C., Barré, H., Delage, D., Rouanet, J. L., Cohen-Adad, F., and Minaire, Y. (1989). Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. *American Journal of Physiology* 257: R744–R751.
68. West, G. C. (1965). Shivering and heat production in wild birds. *Physiological Zoology* 38: 111–120.
69. Hohtola, E. and Stevens, E. D. (1986). The relationship of muscle electrical activity, tremor and heat production to shivering thermogenesis in Japanese quail. *Journal of Experimental Biology* 125: 119–135.
70. Steen, J. and Enger, P. S. (1957). Muscular heat production in pigeons during exposure to cold. *American Journal of Physiology* 191: 157–158.
71. Saarela, S. and Heldmaier, G. (1987). Effect of photoperiod and melatonin on cold resistance, thermoregulation and shivering/nonshivering thermogenesis in Japanese quail. *Journal of Comparative Physiology B* 157: 625–633.
72. Barré, H., Cohen-Adad, F., Duchamp, C., and Rouanet, J. L. (1986). Multilocular adipocytes from muscovy ducklings differentiated in response to cold acclimation. *Journal of Physiology* 375: 27–38.
73. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. I. Effector mechanisms. *Pflügers Archiv* 386: 173–179.
74. Marjoniemi, K. and Hohtola, E. (2000). Does cold acclimation induce nonshivering thermogenesis in juvenile birds? Experiments with Pekin ducklings and Japanese quail chicks. *Journal of Comparative Physiology B* 170: 537–543.
75. Korhonen, K. (1989). Heat loss of willow grouse (*Lagopus lagopus*) in a snowy environment. *Journal of Thermal Biology* 14: 27–31.
76. Lim, T. P. K. (1960). Central and peripheral control mechanisms of shivering and its effects on respiration. *Journal of Applied Physiology* 15: 567–574.
77. Pohl, H. (1965). Temperature regulation and cold acclimation in the golden hamster. *Journal of Applied Physiology* 20: 405–410.
78. Oufara, S., Barré, H., Rouanet, J. L., and Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *American Journal of Physiology* 253: R39–R45.
79. Davis, T. R. A. and Mayer, J. (1955). Nature of the physiological stimulus for shivering. *American Journal of Physiology* 181: 669–674.

80. Spaan, G. and Klussmann, F. W. (1970). Die Frequenz des Kältezitterns bei Tierarten verschiedener Größe. *Pflügers Archiv* 320: 318–333.
81. Slee, J. (1972). Habituation and acclimatization of sheep to cold following exposures of varying length and severity. *Journal of Physiology* 227: 51–70.
82. Fuller, C. A., Horwitz, B. A., and Horowitz, J. M. (1975). Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming. *American Journal of Physiology* 228: 1519–1524.
83. Sellers, E. A., Scott, J. W., and Thomas, N. (1954). Electrical activity of skeletal muscle of normal and acclimated rats on exposure to cold. *American Journal of Physiology* 177: 372–376.
84. Griggio, M. A. (1982). The participation of shivering and nonshivering thermogenesis in warm and cold-acclimated rats. *Comparative Biochemistry and Physiology A* 73: 481–484.
85. Hart, J. S., Heroux, O., and Depocas, F. (1956). Cold acclimation and the electromyogram of unanesthetized rats. *Journal of Applied Physiology* 9: 404–408.
86. Heroux, O., Hart, J. S., and Depocas, F. (1956). Metabolism and muscle activity of anesthetized warm and cold acclimated rats on exposure to cold. *Journal of Applied Physiology* 9: 399–403.
87. Moriya, K., Arnold, F., and LeBlanc, J. (1988). Shivering and nonshivering thermogenesis in exercised cold-deacclimated rats. *European Journal of Applied Physiology* 57: 467–473.
88. Harri, M., Dannenberg, T., Oksanen-Rossi, R., Hohtola, E., and Sundin, U. (1984). Related and unrelated changes in response to exercise and cold in rats: A reevaluation. *Journal of Applied Physiology* 57: 1489–1497.
89. Pohl, H. and Hart, J. S. (1965). Thermoregulation and cold acclimation in a hibernator, *Citellus tridecemlineatus*. *Journal of Applied Physiology* 20: 398–404.
90. Heath, M. and Ingram, D. L. (1983). Thermoregulatory heat production in cold-reared and warm-reared pigs. *American Journal of Physiology* 244: R273–R278.
91. Berthon, D., Herpin, P., and Le Dividich, J. (1994). Shivering thermogenesis in the neonatal pig. *Journal of Thermal Biology* 19: 413–418.
92. Israel, D. J. and Pozos, R. S. (1989). Synchronized slow-amplitude modulations in the electromyograms of shivering muscles. *Journal of Applied Physiology* 66: 2358–2363.
93. Brück, K., Baum, E., and Schwennicke, H. P. (1976). Cold-adaptive modifications in man induced by repeated short-term cold-exposures and during a 10-day and -night cold-exposure. *Pflügers Archiv* 363: 125–133.
94. Bawa, P., Matthews, P. B. C., and Mekjavic, I. B. (1987). Electromyographic activity during shivering of muscles acting at the human elbow. *Journal of Thermal Biology* 12: 1–4.
95. Refinetti, R. (1990). Peripheral nervous control of cold-induced reduction in the respiratory quotient of the rat. *International Journal of Biometeorology* 34: 24–27.
96. Barré, H., Bailly, L., and Rouanet, J. L. (1987). Increased oxidative capacity in skeletal muscles from cold-acclimated ducklings: A comparison with rats. *Comparative Biochemistry and Physiology B* 88: 519–522.
97. Duchamp, C., Barré, H., Rouanet, J. L., Lanni, A., Cohen-Adad, F., Berne, G., and Brebion, P. (1991). Nonshivering thermogenesis in king penguin chicks. I. Role of skeletal muscle. *American Journal of Physiology* 261: R1438–R1445.
98. Duchamp, C., Cohen-Ada, F., Rouanet, J. L., and Barré, H. (1992). Histochemical arguments for muscular non-shivering thermogenesis in muscovy ducklings. *Journal of Physiology* 457: 27–45.
99. Chaffee, R. R. J., Mayhew, W. W., Drebin, M., and Cassuto, Y. (1963). Studies on thermogenesis in cold-acclimated birds. *Canadian Journal of Biochemistry and Physiology* 41: 2215–2220.
100. Kronfeld-Schor, N., Haim, A., Dayan, T., Zisapel, N., Klingenspor, M., and Heldmaier, G. (2000). Seasonal thermogenic acclimation of diurnally and nocturnally active desert spiny mice. *Physiological and Biochemical Zoology* 73: 37–44.
101. Haim, A. and Martinez, J. J. (1992). Seasonal acclimatization in the migratory hamster *Cricetulus migratorius*: The role of photoperiod. *Journal of Thermal Biology* 17: 347–351.
102. Harlow, H. J. (1997). Winter body fat, food consumption and nonshivering thermogenesis of representative spontaneous and facultative hibernators: The white-tailed prairie dog and black-tailed prairie dog. *Journal of Thermal Biology* 22: 21–30.
103. Haim, A. (1987). Thermoregulation and metabolism of Wagner's gerbil (*Gerbillus dasyurus*, a rock dwelling rodent adapted to arid and mesic environments. *Journal of Thermal Biology* 12: 45–48.
104. Haim, A. and Levi, G. (1990). Role of body temperature in seasonal acclimatization: Photoperiod-induced rhythms and heat production in *Meriones crassus*. *Journal of Experimental Zoology* 256: 237–241.
105. Wünnenberg, W. (1983). Thermosensitivity of the preoptic region and the spinal cord in the golden hamster. *Journal of Thermal Biology* 8: 381–384.
106. Banin, D., Haim, A., and Arad, Z. (1994). Metabolism and thermoregulation in the Levant vole *Microtus guentheri*: The role of photoperiodicity. *Journal of Thermal Biology* 19: 55–62.
107. Saarela, S. and Hissa, R. (1993). Metabolism, thermogenesis and daily rhythm of body temperature in the wood lemming, *Myopus schisticolor*. *Journal of Comparative Physiology B* 163: 546–555.
108. Cottle, W. H. (1963). Calorigenic response of cold-adapted rabbits to adrenaline and to noradrenaline. *Canadian Journal of Biochemistry and Physiology* 41: 1334–1337.
109. Heldmaier, G., Steinlechner, S., and Rafael, J. (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *Journal of Comparative Physiology* 149: 1–9.
110. Heldmaier, G. and Jablonka, B. (1985). Seasonal differences in thermogenic adaptation evoked by daily injections of noradrenaline. *Journal of Thermal Biology* 10: 97–99.
111. Davis, T. R. A., Johnston, D. R., Bell, F. C., and Cremer, B. J. (1960). Regulation of shivering and non-shivering heat production during acclimation of rats. *American Journal of Physiology* 198: 471–475.
112. Hsieh, A. C. L., Carlson, L. D., and Gray, G. (1957). Role of the sympathetic nervous system in the control of chemical regulation of heat production. *American Journal of Physiology* 190: 247–251.
113. Cottle, W. H. and Carlson, L. D. (1956). Regulation of heat production in cold-adapted rats. *Proceedings of the Society for Experimental Biology and Medicine* 92: 845–849.
114. Davis, T. R. A. and Mayer, J. (1955). Demonstration and quantitative determination of the contributions of physical and chemical thermogenesis on acute exposure to cold. *American Journal of Physiology* 181: 675–678.
115. Morimoto, A., Murakami, N., Nakamori, T., and Watanabe, T. (1986). Suppression of non-shivering thermogenesis in the rat by heat-seeking behaviour during cold exposure. *Journal of Physiology* 380: 541–549.

116. Arnold, J., LeBlanc, J., Cote, J., Lalonde, J., and Richard, D. (1986). Exercise suppression of thermoregulatory thermogenesis in warm- and cold-acclimated rats. *Canadian Journal of Physiology and Pharmacology* 64: 922–926.
117. Banet, M. and Hensel, H. (1977). The control of shivering and non-shivering thermogenesis in the rat. *Journal of Physiology* 269: 669–676.
118. Vallerand, A. L., Pérusse, F., and Bukowiecki, L. J. (1990). Stimulatory effects of cold exposure and cold acclimation on glucose uptake in rat peripheral tissues. *American Journal of Physiology* 259: R1043–R1049.
119. McDonald, R. B., Horwitz, B. A., Hamilton, J. S., and Stern, J. S. (1988). Cold- and norepinephrine-induced thermogenesis in younger and older Fischer 344 rats. *American Journal of Physiology* 254: R457–R462.
120. Nagashima, T., Kuroshima, A., and Yoshida, T. (1996). The role of β- and α-adrenoceptors on blood flow and temperature of brown adipose tissue and involvement of nitric oxide in their effects. *Journal of Thermal Biology* 21: 313–318.
121. Haim, A., Racey, P. A., Speakman, J. R., Ellison, G. T. H., and Skinner, J. D. (1991). Seasonal acclimatization and thermoregulation in the pouched mouse *Saccostomus campestris*. *Journal of Thermal Biology* 16: 13–17.
122. Haim, A. (1996). Food and energy intake, non-shivering thermogenesis and daily rhythm of body temperature in the bushy-tailed gerbil *Sekeetamys calurus*: The role of photoperiod manipulations. *Journal of Thermal Biology* 21: 37–42.
123. Tomasi, T. E., Hamilton, J. S., and Horwitz, B. A. (1987). Thermogenic capacity in shrews. *Journal of Thermal Biology* 12: 143–147.
124. Smith, R. E. and Roberts, J. C. (1964). Thermogenesis of brown adipose tissue in cold-acclimated rats. *American Journal of Physiology* 206: 143–148.
125. Holloway, B. R., Stribling, D., Freeman, S., and Jamieson, L. (1985). The thermogenic role of adipose tissue in the dog. *International Journal of Obesity* 9: 423–432.
126. Huttunen, P., Hirvonen, J., and Kinnula, V. (1981). The occurrence of brown adipose tissue in outdoor workers. *European Journal of Applied Physiology* 46: 339–345.
127. Smith, R. E. and Hock, R. J. (1963). Brown fat: Thermogenic effector of arousal in hibernators. *Science* 140: 199–200.
128. Heim, T. and Hull, D. (1966). The blood flow and oxygen consumption of brown adipose tissue in the new-born rabbit. *Journal of Physiology* 186: 42–55.
129. Astrup, A., Bülow, J., Madsen, J., and Christensen, N. J. (1985). Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *American Journal of Physiology* 248: E507–E515.
130. Foster, D. O. and Frydman, M. L. (1979). Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: The dominant role of brown adipose tissue in the replacement of shivering by non-shivering thermogenesis. *Canadian Journal of Physiology and Pharmacology* 57: 257–270.
131. Bukowiecki, L., Collet, A. J., Follea, N., Guay, G., and Jahjah, L. (1982). Brown adipose tissue hyperplasia: A fundamental mechanism of adaptation to cold and hyperphagia. *American Journal of Physiology* 242: E353–E359.
132. Schönbaum, E., Johnson, G. E., Sellers, E. A., and Gill, M. J. (1966). Adrenergic β-receptors and non-shivering thermogenesis. *Nature* 210: 426.
133. Rafael, J. and Vsiansky, P. (1985). Photoperiodic control of the thermogenic capacity in brown adipose tissue of the Djungarian hamster. *Journal of Thermal Biology* 10: 167–170.
134. Bourhim, M., Barré, H., Oufara, S., Minaire, Y., Chatonnet, J., Cohen-Adad, F., and Rouanet, J. L. (1990). Increase in cytochrome oxidase capacity of BAT and other tissues in cold-acclimated gerbils. *American Journal of Physiology* 258: R1291–R1298.
135. Kurosawa, M. (1991). Reflex changes in thermogenesis in the interscapular brown adipose tissue in response to thermal stimulation of the skin via sympathetic efferent nerves in anesthetized rats. *Journal of the Autonomic Nervous System* 33: 15–24.
136. Rothwell, N. J. and Stock, M. J. (1985). Biological distribution and significance of brown adipose tissue. *Comparative Biochemistry and Physiology A* 82: 745–751.
137. Gordon, C. J. (1983). Influence of heating rate on control of heat loss from the tail in mice. *American Journal of Physiology* 244: R778–R784.
138. Brown, R. T. and Baust, J. G. (1980). Time course of peripheral heterothermy in a homeotherm. *American Journal of Physiology* 239: R126–R129.
139. Rand, R. P., Burton, A. C., and Ing, T. (1965). The tail of the rat, in temperature regulation and acclimatization. *Canadian Journal of Physiology and Pharmacology* 43: 257–267.
140. Raman, E. R., Roberts, M. F., and Vanhuyse, V. J. (1983). Body temperature control of rat tail blood flow. *American Journal of Physiology* 245: R426–R432.
141. Young, A. A. and Dawson, N. J. (1982). Evidence for on–off control of heat dissipation from the tail of the rat. *Canadian Journal of Physiology and Pharmacology* 60: 392–398.
142. Romanovsky, A. A., Ivanov, A. I., and Shimansky, Y. P. (2002). Ambient temperature for experiments in rats: A new method for determining the zone of thermal neutrality. *Journal of Applied Physiology* 92: 2667–2679.
143. Gwosdow, A. R. and Besch, E. L. (1985). Effect of thermal history on the rat's response to varying environmental temperature. *Journal of Applied Physiology* 59: 413–419.
144. Shido, O. (1987). Day–night variation of thermoregulatory responses to intraperitoneal electric heating in rats. *Journal of Thermal Biology* 12: 273–279.
145. Caputa, M., Kamari, A., and Wachulec, M. (1991). Selective brain cooling in rats resting in heat and during exercise. *Journal of Thermal Biology* 16: 19–24.
146. Caputa, M., Demicka, A., Dokladny, K., and Kurowicka, B. (1996). Anatomical and physiological evidence for efficacious selective brain cooling in rats. *Journal of Thermal Biology* 21: 21–28.
147. Folkow, B., Fox, R. H., Krog, J., Odelram, H., and Thorén, O. (1963). Studies on the reactions of the cutaneous vessels to cold exposure. *Acta Physiologica Scandinavica* 58: 342–354.
148. Grant, R. T. (1963). Vasodilatation and body warming in the rat. *Journal of Physiology* 167: 311–317.
149. Webb-Peploe, M. M. and Shepherd, J. T. (1968). Response of dogs' cutaneous veins to local and central temperature changes. *Circulation Research* 23: 693–699.
150. Webb-Peploe, M. M. and Shepherd, J. T. (1968). Peripheral mechanism involved in response of dogs' cutaneous veins to local temperature change. *Circulation Research* 23: 701–708.
151. Mohler, F. S. and Heath, J. E. (1988). Comparison of IR thermography and thermocouple measurement of heat loss from rabbit pinna. *American Journal of Physiology* 254: R389–R395.
152. Grayson, J. (1949). Reactions of the peripheral circulation to external heat. *Journal of Physiology* 109: 53–63.
153. Werner, J. (1977). Influences of local and global temperature stimuli on the Lewis reaction. *Pflügers Archiv* 367: 291–294.

154. Taylor, C. R. and Lyman, C. P. (1972). Heat storage in running antelopes: Independence of brain and body temperatures. *American Journal of Physiology* 222: 114–117.
155. Schroter, R. C., Robertshaw, D., and Filali, R. Z. (1989). Brain cooling and respiratory heat exchange in camels during rest and exercise. *Respiration Physiology* 78: 95–105.
156. Scholander, P. F. and Schevill, W. E. (1955). Counter-current vascular heat exchange in the fins of whales. *Journal of Applied Physiology* 8: 279–282.
157. Heyning, J. E. and Mead, J. G. (1997). Thermoregulation in the mouths of feeding gray whales. *Science* 278: 1138–1139.
158. Tattersall, G. J., Andrade, D. V., and Abe, A. S. (2009). Heat exchange from the toucan bill reveals a controllable vascular thermal radiator. *Science* 325: 468–470.
159. Carey, F. G., Teal, J. M., Kanwisher, J. W., Lawson, K. D., and Beckett, J. S. (1971). Warm-bodied fish. *American Zoologist* 11: 137–145.
160. Randall, W. C. (1947). Local sweat gland activity due to direct effects of radiant heat. *American Journal of Physiology* 150: 365–371.
161. Fox, R. H., Goldsmith, R., Hampton, I. F. G., and Lewis, H. E. (1964). The nature of the increase in sweating capacity produced by heat acclimatization. *Journal of Physiology* 171: 368–376.
162. Elizondo, R. S. and Bullard, R. W. (1971). Local determinants of sweating and the assessment of the "set point". *International Journal of Biometeorology* 15: 273–280.
163. Nadel, E. R., Bullard, R. W., and Stolwijk, J. A. J. (1971). Importance of skin temperature in the regulation of sweating. *Journal of Applied Physiology* 31: 80–87.
164. Nadel, E. R., Mitchell, J. W., and Stolwijk, J. A. J. (1973). Differential thermal sensitivity in the human skin. *Pflügers Archiv* 340: 71–76.
165. Sato, K. and Sato, F. (1983). Individual variations in structure and function of human eccrine sweat gland. *American Journal of Physiology* 245: R203–R208.
166. Amoros, C., Sagot, J. C., Libert, J. P., and Candas, V. (1986). Sweat gland response to local heating during sleep in man. *Journal de Physiology* 81: 209–215.
167. Aoki, K., Kondo, N., Shibasaki, M., Takano, S., Tominaga, H., and Katsuura, T. (1997). Circadian variation of sweating responses to passive heat stress. *Acta Physiologica Scandinavica* 161: 397–402.
168. Adolph, E. F. (1947). Tolerance to heat and dehydration in several species of mammals. *American Journal of Physiology* 151: 564–575.
169. Bartholomew, G. A. and Wilke, F. (1956). Body temperature in the northern fur seal, *Callorhinus ursinus*. *Journal of Mammalogy* 37: 327–337.
170. Richards, S. A. (1971). The significance of changes in the temperature of the skin and body core of the chicken in the regulation of heat loss. *Journal of Physiology* 216: 1–10.
171. Blatt, C. M., Taylor, C. R., and Habal, M. B. (1972). Thermal panting in dogs: The lateral nasal gland, a source of water for evaporative cooling. *Science* 177: 804–805.
172. Le Maho, Y., Goffart, M., Rochas, A., Felbabel, H., and Chatonnet, J. (1981). Thermoregulation in the only nocturnal simian: The night monkey *Aotus trivirgatus*. *American Journal of Physiology* 240: R156–R165.
173. Whittow, G. C., Pettit, T. N., Ackerman, R. A., and Paganelli, C. V. (1987). Temperature regulation in a burrow-nesting tropical seabird, the wedge-tailed shearwater (*Puffinus pacificus*). *Journal of Comparative Physiology B* 157: 607–614.
174. van Kampen, M. (1988). Effects of drinking water temperature and leg cooling on heat stress of laying hens (*Gallus domesticus*). *Journal of Thermal Biology* 13: 43–47.
175. Kasa, I. W. and Thwaites, C. J. (1992). The effect of infra-red radiation on rectal temperature and respiration rate of unacclimated female New Zealand white rabbits. *Journal of Thermal Biology* 17: 293–296.
176. Ellis, H. I., Maskrey, M., Pettit, T. N., and Whittow, G. C. (1995). Thermoregulation in the brown noddy (*Anous stolidus*). *Journal of Thermal Biology* 20: 307–313.
177. Hainsworth, F. R. (1967). Saliva spreading, activity, and body temperature regulation in the rat. *American Journal of Physiology* 212: 1288–1292.
178. Malan, A. and Hildwein, G. (1967). La thermolyse latente en fonction de la température centrale chez deux rongeurs, le rat blanc et le hamster d'Europe. *Comptes Rendus de Séances de la Société de Biologie de Strasbourg* 161: 185–189.
179. Hainsworth, F. R. (1968). Evaporative water loss from rats in the heat. *American Journal of Physiology* 214: 979–982.
180. Roberts, W. W., Mooney, R. D., and Martin, J. R. (1974). Thermoregulatory behaviors of laboratory rodents. *Journal of Comparative and Physiological Psychology* 86: 693–699.
181. Refinetti, R. (1983). Conflito entre ingestão de água e termorregulação no rato. *Ciência e Cultura* 35(S): 821.
182. Buffenstein, R. (1984). The importance of microhabitat in thermoregulation and thermal conductance in two Namib rodents. *Journal of Thermal Biology* 9: 235–241.
183. Yanase, M., Kanosue, K., Yasuda, H., and Tanaka, H. (1991). Salivary secretion and grooming behaviour during heat exposure in freely moving rats. *Journal of Physiology* 432: 585–592.
184. Hahn, P. (1956). The development of thermoregulation. III. The significance of fur in the development of thermoregulation in rats. *Physiologia Bohemoslovenica* 5: 428–431.
185. Johansen, K. (1962). Buoyancy and insulation in the muskrat. *Journal of Mammalogy* 43: 64–68.
186. Walsberg, G. E. (1988). Heat flow through avian plumages: The relative importance of conduction, convection, and radiation. *Journal of Thermal Biology* 13: 89–92.
187. Sellers, E. A., You, S. S., and Thomas, N. (1951). Acclimatization and survival of rats in the cold: Effects of clipping, of adrenalectomy and of thyroidectomy. *American Journal of Physiology* 165: 481–485.
188. Bartlett, R. G., Mantel, N., Foster, G. L., and Bernstein, P. (1958). Core to surface thermal gradients in the rat at several environmental temperatures. *American Journal of Physiology* 193: 541–546.
189. Herreid, C. F. and Kessel, B. (1967). Thermal conductance in birds and mammals. *Comparative Biochemistry and Physiology* 21: 405–414.
190. Piccione, G., Caola, G., and Refinetti, R. (2002). Effect of shearing on the core body temperature of three breeds of Mediterranean sheep. *Small Ruminant Research* 46: 211–215.
191. Kauffman, A. S., Paul, M. J., and Zucker, I. (2004). Increased heat loss affects hibernation in golden-mantled ground squirrels. *American Journal of Physiology* 287: R167–R173.
192. Rust, C. C., Shackelford, R. M., and Meyer, R. K. (1965). Hormonal control of pelage cycles in the mink. *Journal of Mammalogy* 46: 549–565.
193. Rust, C. C. (1962). Temperature as a modifying factor in the spring pelage change of sort-tailed weasels. *Journal of Mammalogy* 43: 323–328.

194. Cooper, S. J. (2002). Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. *Physiological and Biochemical Zoology* 75: 386–395.
195. Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M., and Morton, M. L. (2003). Effects of temperature on photoperiodically induced reproductive development, circulating luteinizing hormone and thyroid hormones, body mass, fat deposition and molt in mountain white-crowned sparrows, *Zonotrichia leucophrys oriantha*. *General and Comparative Endocrinology* 131: 143–158.
196. Harris, G. D., Huppi, H. D., and Gessaman, J. A. (1985). The thermal conductance of winter and summer pelage of *Lepus californicus*. *Journal of Thermal Biology* 10: 79–81.
197. Walsberg, G. E. (1991). Thermal effects of seasonal coat change in three subarctic mammals. *Journal of Thermal Biology* 16: 291–296.
198. Al-Hilli, F. and Wright, E. A. (1988). The effects of environmental temperature on the hair coat of the mouse. *Journal of Thermal Biology* 13: 21–24.
199. Duncan, M. J. and Goldman, B. D. (1984). Hormonal regulation of the annual pelage color cycle in the Djungarian hamster, *Phodopus sungorus*. I. Role of the gonads and the pituitary. *Journal of Experimental Zoology* 230: 89–95.
200. Goldman, S. L., Dhandapani, K., and Goldman, B. D. (2000). Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 417–428.
201. Kuhlmann, M. T., Clemen, G., and Schlatt, S. (2003). Molting in the Djungarian hamster (*Phodopus sungorus* Pallas): Seasonal or continuous process? *Journal of Experimental Zoology* 295A: 160–171.
202. Palchykova, S., Deboer, T., and Tobler, I. (2003). Seasonal aspects of sleep in the Djungarian hamster. *BMC Neuroscience* 4: art. 9.
203. Burkhardt, J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *Journal of Agricultural Science* 37: 64–68.
204. Lincoln, G. A., Andersson, H., and Hazlerigg, D. (2003). Clock genes and the long-term regulation of prolactin secretion: Evidence for a photoperiodic/circannual timer in the pars tuberalis. *Journal of Neuroendocrinology* 15: 390–397.
205. Hart, J. S. (1964). Geography and season: Mammals and birds. In: Dill, D. B. (Ed.), *Handbook of Physiology, Section 4*. Washington, DC: American Physiological Society, pp. 295–321.
206. Jansky, L. (1967). Evolutionary adaptations of temperature regulation in mammals. *Zeitschrift für Säugetierkunde* 32: 167–172.
207. Paladino, F. V., O'Connor, M. P., and Spotila, J. R. (1990). Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* 344: 858–860.
208. Bergmann, C. (1847). Über die Verhältnisse der wärmeökonomie der Thiere zu ihrer Größe. *Göttinger Studien (Abtheilung 1)* 3: 595–708.
209. Allen, J. A. (1877). The influence of physical conditions in the genesis of species. *Radical Review* 1: 108–140.
210. Scholander, P. F. (1955). Evolution of climatic adaptation in homeotherms. *Evolution* 9: 15–26.
211. Mayr, E. (1956). Geographical character gradients and climatic adaptation. *Evolution* 10: 105–108.
212. Weaver, M. E. and Ingram, D. L. (1969). Morphological changes in swine associated with environmental temperature. *Ecology* 50: 710–713.
213. Sumner, F. B. (1915). Some studies of environmental influence, heredity, correlation and growth, in the white mouse. *Journal of Experimental Zoology* 18: 325–432.
214. Ogle, C. (1934). Climatic influence on the growth of the male albino mouse. *American Journal of Physiology* 107: 635–640.
215. Buck, C. L. and Barnes, B. M. (2000). Effects of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *American Journal of Physiology* 279: R255–R262.
216. Heldmaier, G., Ortmann, S., and Elvert, R. (2004). Natural hypometabolism during hibernation and daily torpor in mammals. *Respiratory Physiology and Neurobiology* 141: 317–329.
217. Geiser, F. (2004). Metabolic rate and body temperature reduction during hibernation and daily torpor. *Annual Review of Physiology* 66: 239–274.
218. Zhang, P., Watanabe, K., and Eishi, T. (2007). Habitual hot-spring bathing by a group of Japanese macaques (*Macaca fuscata*) in their natural habitat. *American Journal of Primatology* 69: 1425–1430.
219. Briscoe, N. J., Handasyde, K. A., Griffiths, S. R., Porter, W. P., Krockenberger, A., and Kearney, M. R. (2014). Tree-hugging koalas demonstrate a novel thermoregulatory mechanism for arboreal mammals. *Biology Letters* 10: 20140235.
220. Herter, K. (1924). Untersuchungen über den Temperatursinn einiger Insekten. *Zeitschrift für Vergleichende Physiologie* 1: 221–288.
221. Refinetti, R. (1982). Termopreferendum da formiga saúva fora do ninho. *Ciência e Cultura* 34(S): 897.
222. Dreisig, H. (1980). Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia* 44: 376–389.
223. Murphy, B. F. (1986). The effect of acclimation temperature and the removal of peripheral temperature receptors on body temperature preference in the cockroach, *Periplaneta americana*. *Journal of Thermal Biology* 11: 209–212.
224. Takeuchi, K., Nakano, Y., Kato, U., Kaneda, M., Aizu, M., Awano, W., Yonemura, S. et al. (2009). Changes in temperature preferences and energy homeostasis in dystroglycan mutants. *Science* 323: 1740–1743.
225. Krafft, B. (1967). Thermopreferendum de l'araignée sociale *Agelena consociata* Denis. *Insectes Sociaux* 14: 161–182.
226. Barlow, C. A. and Kuenen, D. J. (1957). A new thermopreferendum apparatus for terrestrial isopods. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Ser. C* 60: 240–250.
227. Refinetti, R. (1984). Behavioral temperature regulation in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 47: 29–43.
228. Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemistry and Physiology A* 77: 513–517.
229. Crawshaw, L. I. (1971). Temperature selection and activity in the crayfish, *Orconectes immunis*. *Journal of Comparative Physiology* 95: 315–322.
230. Díaz-Iglesias, E., Díaz-Herrera, F., Re-Araujo, A. D., Báez-Hidalgo, M., López-Zenteno, M., Valdés-Sánchez, G., and López-Murillo, A. K. (2004). Temperatura preferida y consumo de oxígeno circadiano de la langosta roja, *Panulirus interruptus* (Randall, 1842). *Ciencias Marinas* 30: 169–178.
231. Bernardi, N. (1990). Temperature influence upon food ingestion and spontaneous locomotion of the freshwater prawn *Macrobrachium acanthurus*. *Journal of Thermal Biology* 15: 33–36.

232. Kivivuori, L. and Lagerspetz, K. Y. H. (1990). Temperature selection behaviour of the Isopod *Saduria entomon*. *Journal of Thermal Biology* 15: 83–86.
233. Espina, S., Herrera, F. D., and Bückle, L. F. (1993). Preferred and avoided temperatures in the crawfish *Procambarus clarkii* (Decapoda: Cambaridae). *Journal of Thermal Biology* 18: 35–39.
234. Kivivuori, L. A. (1994). Temperature selection behaviour of cold- and warm-acclimated crayfish (*Astacus astacus*). *Journal of Thermal Biology* 19: 291–297.
235. Bückle-Ramírez, L. F., Díaz-Herrera, F., Correa-Sandoval, F., Barón-Sevilla, B., and Hernández-Rodríguez, M. (1994). Diel thermoregulation of the crawfish *Procambarus clarkii* (Crustacea: Cambaridae). *Journal of Thermal Biology* 19: 419–422.
236. Neill, W. H., Magnuson, J. J., and Chipman, G. G. (1972). Behavioral thermoregulation by fishes: A new experimental approach. *Science* 176: 1443–1445.
237. Roy, A. W. and Johansen, P. H. (1970). The temperature selection of small hypophysectomized goldfish (*Carassius auratus* L.). *Canadian Journal of Zoology* 48: 323–326.
238. Ingersoll, C. G. and Claussen, D. L. (1984). Temperature selection and critical thermal maxima of the fantail darter, *Etheostoma flabellare*, and johnny darter, *E. nigrum*, related to habitat and season. *Environmental Biology of Fishes* 11: 131–138.
239. McCauley, R. W. and Thomson, D. A. (1988). Thermoregulatory activity in the Tecopa pupfish, *Cyprinodon nevadensis amargosae*, an inhabitant of thermal spring. *Environmental Biology of Fishes* 23: 135–139.
240. Reynolds, W. W., Thomson, D. A., and Casterlin, M. E. (1977). Responses of young California grunion, *Leuresthes tenuis*, to gradients of temperature and light. *Copeia* 1977: 144–149.
241. Reynolds, W. W., McCauley, R. W., Casterlin, M. E., and Crawshaw, L. I. (1976). Body temperatures of behaviorally thermoregulating largemouth blackbass (*Micropterus salmoides*). *Comparative Biochemistry and Physiology A* 54: 461–463.
242. Sullivan, C. M. and Fisher, K. C. (1954). The effects of light on temperature selection in speckled trout *Salvelinus fontinalis* (Mitchill). *Biological Bulletin* 107: 278–288.
243. Beitinger, T. L. and Magnuson, J. J. (1975). Influence of social rank and size on thermoselection behavior of bluegill (*Lepomis macrochirus*). *Journal of the Fisheries Research Board of Canada* 32: 2133–2136.
244. Carpenter, S. J., Erickson, J. M., and Holland, F. D. (2003). Migration of a Late Cretaceous fish. *Nature* 423: 70–74.
245. Reynolds, W. W., Casterlin, M. E., Matthey, J. K., Millington, S. T., and Ostrowski, A. C. (1978). Diel patterns of preferred temperature and locomotor activity in the goldfish *Carassius auratus*. *Comparative Biochemistry and Physiology A* 59: 225–227.
246. Kavaliers, M. and Ralph, C. L. (1980). Pineal involvement in the control of behavioral thermoregulation of the white sucker, *Catostomus commersoni*. *Journal of Experimental Zoology* 212: 301–303.
247. Crawshaw, L. I. (1975). Attainment of the final thermal preferendum in brown bullheads acclimated to different temperatures. *Comparative Biochemistry and Physiology A* 52: 171–173.
248. Nelson, D. O. and Prosser, C. L. (1979). Effect of preoptic lesions on behavioral thermoregulation of green sunfish, *Lepomis cyanellus*, and of goldfish, *Carassius auratus*. *Journal of Comparative Physiology* 129: 193–197.
249. Schurmann, H. and Christiansen, J. S. (1994). Behavioral thermoregulation and swimming activity of two arctic teleosts (subfamily Gadinae): The Polar cod (*Boreogadus saida*) and the navaga (*Eleginus navaga*). *Journal of Thermal Biology* 19: 207–212.
250. Feder, M. E. and Pough, F. H. (1975). Temperature selection by red-backed salamander, *Plethodon c. cinereus* (Green) (Caudata: Plethodontidae). *Comparative Biochemistry and Physiology A* 50: 91–98.
251. Duclaux, R., Fantino, M., and Cabanac, M. (1973). Comportement thermoregulateur chez *Rana esculenta*. *Pflügers Archiv* 342: 347–358.
252. Sievert, L. M. (1991). Thermoregulatory behaviour in the toads *Bufo marinus* and *Bufo cognatus*. *Journal of Thermal Biology* 16: 309–312.
253. Smith, E. N. (1975). Thermoregulation of the American alligator, *Alligator mississippiensis*. *Physiological Zoology* 48: 177–194.
254. Crawshaw, L. I., Johnston, M. H., and Lemons, D. E. (1980). Acclimation, temperature selection, and heat exchange in the turtle, *Chrysemys scripta*. *American Journal of Physiology* 238: R443–R446.
255. Myhre, K. and Hammel, H. T. (1969). Behavioral regulation of internal temperature in the lizard *Tiliqua scincoides*. *American Journal of Physiology* 217: 1490–1495.
256. Gleeson, T. T. (1981). Preferred body temperature, aerobic scope, and activity capacity in the monitor lizard, *Varanus salvator*. *Physiological Zoology* 54: 423–429.
257. Schuett, G. W. and Gatten, R. E. (1980). Thermal preference in snapping turtles (*Chelydra serpentina*). *Copeia* 1980: 149–152.
258. King, D., Green, B., and Herrera, E. (1994). Thermoregulation in a large teiid lizard, *Tupinambis teguixin*, in Venezuela. *Copeia* 1994: 806–808.
259. Innicenti, A., Minutini, L., and Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
260. Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. *Journal of Comparative Physiology A* 179: 135–142.
261. Sievert, L. M. and Hutchison, V. H. (1988). Light versus heat: Thermoregulatory behavior in a nocturnal lizard (*Gekko gecko*). *Herpetologica* 44: 266–273.
262. Autumn, K. and De Nardo, D. F. (1995). Behavioral thermoregulation increases growth rate in a nocturnal lizard. *Journal of Herpetology* 29: 157–162.
263. Brown, R. P. (1996). Thermal biology of the gecko *Tarentola boettgeri*: Comparisons among populations from different elevations within Gran Canaria. *Herpetologica* 52: 396–405.
264. Keraney, M. and Predavec, M. (2000). Do nocturnal ectotherms thermoregulate? A study of the temperate gecko *Christinus marmoratus*. *Ecology* 81: 2984–2996.
265. Regal, P. J. (1967). Voluntary hypothermia in reptiles. *Science* 155: 1551–1553.
266. Cowgell, J. and Underwood, H. (1979). Behavioral thermoregulation in lizards: A circadian rhythm. *Journal of Experimental Zoology* 210: 189–194.
267. Rismiller, P. D. and Heldmaier, G. (1991). Seasonal changes in daily metabolic patterns of *Lacerta viridis*. *Journal of Comparative Physiology B* 161: 482–488.
268. Refinetti, R. and Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.

269. Wilhoft, D. C. and Anderson, J. D. (1960). Effect of acclimation on the preferred body temperature of the lizard, *Sceloporus occidentalis*. *Science* 131: 610–611.
270. Cannon, J. G. and Kluger, M. J. (1985). Altered thermoregulation in the iguana *Dipsosaurus dorsalis* following exercise. *Journal of Thermal Biology* 10: 41–45.
271. Christian, K. A., Tracy, C. R., and Porter, W. P. (1985). Inter- and intra-individual variation in body temperatures of the Galapagos land iguana (*Conolophus pallidus*). *Journal of Thermal Biology* 10: 47–50.
272. van Damme, R., Bauwens, D., and Verheyen, R. F. (1986). Selected body temperature in the lizard *Lacerta vivipara*: Variation within and between populations. *Journal of Thermal Biology* 11: 219–222.
273. Sievert, L. M. and Hutchison, V. H. (1989). Influences of season, time of day, light and sex on the thermoregulatory behaviour of *Crotaphytus collaris*. *Journal of Thermal Biology* 14: 159–165.
274. Jarling, C., Scarperi, M., and Bleichert, A. (1989). Circadian rhythm in the temperature preference of the turtle, *Chrysemys scripta elegans*, in a thermal gradient. *Journal of Thermal Biology* 14: 173–178.
275. Shine, R. and Lambeck, R. (1990). Seasonal shifts in the thermoregulatory behaviour of Australian blacksnakes, *Pseudechis porphyriacus* (Serpentes: Elapidae). *Journal of Thermal Biology* 15: 301–305.
276. Skinner, D. C. (1991). Effect of intraperitoneal melatonin injections on thermoregulation in the Transvaal girdled lizard, *Cordylus vittifer*. *Journal of Thermal Biology* 16: 179–184.
277. Sievert, L. M. and Paulissen, M. A. (1996). Temperature selection and thermoregulatory precision of bisexual and parthenogenetic *Cnemidophorus* lizards from southern Texas. *Journal of Thermal Biology* 21: 15–20.
278. Christian, K. and Bedford, G. (1996). Thermoregulation by the spotted tree monitor, *Varanus scalaris*, in the seasonal tropics of Australia. *Journal of Thermal Biology* 21: 67–73.
279. Cotton, P. A. (2003). Avian migration phenology and global climate change. *Proceedings of the National Academy of Sciences United States America* 100: 12219–12222.
280. Modrey, P. and Nichelmann, M. (1992). Development of autonomic and behavioural thermoregulation in turkeys (*Meleagris gallopavo*). *Journal of Thermal Biology* 17: 287–292.
281. Hull, J. and Hull, D. (1982). Behavioral thermoregulation in newborn rabbits. *Journal of Comparative and Physiological Psychology* 96: 143–147.
282. Gumma, M. R. and South, F. E. (1970). Hypothermia and behavioural thermoregulation by the hamster (*Mesocricetus auratus*). *Animal Behaviour* 18: 504–511.
283. Herter, K. (1936). Das thermotaktische Optimum bei Nagetieren, ein mendelndes art- und Rassenmerkmal. *Zeitschrift für Vergleichende Physiologie* 23: 605–650.
284. Gordon, C. J. (1983). Behavioral and autonomic thermoregulation in mice exposed to microwave radiation. *Journal of Applied Physiology* 55: 1242–1248.
285. Carlisle, H. J. and Dubuc, P. U. (1984). Temperature preference of genetically obese (ob/ob) mice. *Physiology and Behavior* 33: 899–902.
286. Briese, E. (1985). Rats prefer ambient temperatures out of phase with their body temperature circadian rhythm. *Brain Research* 345: 389–393.
287. Gordon, C. J. (1986). Relationship between behavioral and autonomic thermoregulation in the guinea pig. *Physiology and Behavior* 38: 827–831.
288. Gordon, C. J. (1987). Relationship between preferred ambient temperature and autonomic thermoregulatory function in rat. *American Journal of Physiology* 252: R1130–R1137.
289. Gordon, C. J. (1985). Relationship between autonomic and behavioral thermoregulation in the mouse. *Physiology and Behavior* 34: 687–690.
290. Gordon, C. J., Fehlner, K. S., and Long, M. D. (1986). Relationship between autonomic and behavioral thermoregulation in the golden hamster. *American Journal of Physiology* 251: R320–R324.
291. Ogilvie, D. M. and Stinson, R. H. (1966). Temperature selection in *Peromyscus* and laboratory mice, *Mus musculus*. *Journal of Mammalogy* 47: 655–660.
292. Lynch, G. R., Lynch, C. B., Dube, M., and Allen, C. (1976). Early cold exposure: Effects on behavioral and physiological thermoregulation in the house mouse, *Mus musculus*. *Physiological Zoology* 49: 191–199.
293. Refinetti, R. and Horvath, S. M. (1989). Thermopreferendum of the rat: Inter- and intra-subject variability. *Behavioral and Neural Biology* 52: 87–94.
294. Laughter, J. S. and Blatteis, C. M. (1985). A system for the study of behavioral thermoregulation of small animals. *Physiology and Behavior* 35: 993–997.
295. Gordon, C. J., Lee, K. A., Chen, T. A., Killough, P., and Ali, J. S. (1991). Dynamics of behavioral thermoregulation in the rat. *American Journal of Physiology* 261: R705–R711.
296. Gordon, C. J., Becker, P., and Ali, J. S. (1998). Behavioral thermoregulatory responses of single- and group-housed mice. *Physiology and Behavior* 65: 255–262.
297. Klein, M. S., Conn, C. A., and Kluger, M. J. (1992). Behavioral thermoregulation in mice inoculated with influenza virus. *Physiology and Behavior* 52: 1133–1139.
298. Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology A* 119: 295–300.
299. Gordon, C. J. (1993). Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiology and Behavior* 53: 257–263.
300. Gordon, C. J. (1994). 24-Hour control of body temperature in rats. I. Integration of behavioral and autonomic effectors. *American Journal of Physiology* 267: R71–R77.
301. Refinetti, R. (1996). Rhythms of body temperature and temperature selection are out of phase in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 60: 959–961.
302. Refinetti, R. (1998). Body temperature and behavior of tree shrews and flying squirrels in a thermal gradient. *Physiology and Behavior* 63: 517–520.
303. Sargeant, G. A., Eberhardt, L. E., and Peek, J. M. (1994). Thermoregulation by mule deer (*Odocoileus hemionus*) in arid rangelands of southcentral Washington. *Journal of Mammalogy* 75: 536–544.
304. Gelineo, S. and Gelineo, A. (1951). Sur la thermorégulation du rat nouveau-né et la température du nid. *Comptes Rendus de l'Académie des Sciences* 232: 1031–1032.
305. Leonard, C. M. (1982). Shifting strategies for behavioral thermoregulation in developing golden hamsters. *Journal of Comparative and Physiological Psychology* 96: 234–243.
306. Fewell, J. E., Kang, M., and Eliason, H. L. (1997). Autonomic and behavioral thermoregulation in guinea pigs during postnatal maturation. *Journal of Applied Physiology* 83: 830–836.
307. Gordon, C. J., Long, M. D., and Fehlner, K. S. (1984). Behavioural and autonomic thermoregulation in hamsters during microwave-induced heat exposure. *Journal of Thermal Biology* 9: 271–277.

308. Downs, C. T. and Perrin, M. R. (1990). Thermal parameters of four species of *Gerbillurus*. *Journal of Thermal Biology* 15: 291–300.
309. Lee, H., Iida, T., Mizuno, A., Suzuki, M., and Caterina, M. J. (2005). Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. *Journal of Neuroscience* 25: 1304–1310.
310. Malvin, G. M. and Wood, S. C. (1992). Behavioral hypothermia and survival of hypoxic protozoans *Paramecium caudatum*. *Science* 255: 1423–1425.
311. Mendelssohn, M. (1902). Recherches sur l'interférence de la thermotaxie avec d'autres tactismes et sur le méchanism du mouvement thermotactique. *Journal de Physiologie* 4: 475–488.
312. Jeddi, E., Guerin, J. F., and Czyba, J. C. (1977). Have human spermatozoa a thermopreferendum? *Biology of Reproduction* 16: 561–563.
313. Hernandez, J., Bonnedahl, J., Waldenström, J., Palmgren, H., and Olsen, B. (2003). *Salmonella* in birds migrating through Sweden. *Emerging Infectious Diseases* 9: 753–755.
314. Froy, O., Gotter, A. L., Casselman, A. L., and Reppert, S. M. (2003). Illuminating the circadian clock in monarch butterfly migration. *Science* 300: 1303–1305.
315. Hunter, E., Metcalfe, J. D., and Reynolds, J. D. (2003). Migration route and spawning area fidelity by North Sea plaice. *Proceedings of the Royal Society of London B* 270: 2097–2103.
316. Rattenborg, N. C., Mandt, B. H., Obermeyer, W. H., Winsauer, P. J., Huber, R., Wikelski, M., and Benca, R. M. (2004). Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLoS Biology* 2: 924–936.
317. Altizer, S., Bartel, R., and Han, B. A. (2011). Animal migration and infectious disease risk. *Science* 331: 296–302.
318. Milner-Gulland, E. J., Fryxell, J. M., and Sinclair, A. R. E. (Eds.) (2011). *Animal Migration: A Sythesis*. New York: Oxford University Press.
319. Le Maho, Y., Delclitte, P., and Chatonnet, J. (1976). Thermoregulation in fasting emperor penguins under natural conditions. *American Journal of Physiology* 231: 913–922.
320. Lustick, S., Battersby, B., and Kelty, M. (1978). Behavioral thermoregulation: Orientation toward the sun in herring gulls. *Science* 200: 81–83.
321. Wells, R. T. (1978). Thermoregulation and activity rhythms in the hairy-nosed wombat, *Lasiorhinus latifrons* (Owen), (Vombatidae). *Australian Journal of Zoology* 26: 639–651.
322. Swift, R. W. and Forbes, R. M. (1939). The heat production of the fasting rat in relation to the environmental temperature. *Journal of Nutrition* 18: 307–318.
323. Schmidek, W. R., Hoshino, K., Schmidek, M., and Timo-Iaria, C. (1972). Influence of environmental temperature on the sleep-wakefulness cycle in the rat. *Physiology and Behavior* 8: 363–371.
324. McEwen, G. N. and Heath, J. E. (1973). Resting metabolism and thermoregulation in the unrestrained rabbit. *Journal of Applied Physiology* 35: 884–886.
325. Chevillard-Hugot, M. C., Müller, E. F., and Kulzer, E. (1980). Oxygen consumption, body temperature and heart rate in the coati (*Nasua nasua*). *Comparative Biochemistry and Physiology A* 65: 305–309.
326. Øritsland, N. A. (1970). Temperature regulation of the polar bear (*Thalarctos maritimus*). *Comparative Biochemistry and Physiology* 37: 225–233.
327. Macari, M. (1983). Efeito do cruzamento de suínos sobre o comportamento termoregulador. *Ciência e Cultura* 35: 1145–1150.
328. Moinard, C., Doncaster, C. P., and Barré, H. (1992). Indirect calorimetry measurements of behavioral thermoregulation in a semiaquatic social rodent, *Myocastor coypus*. *Canadian Journal of Zoology* 70: 907–911.
329. Pivnick, K. A. and McNeil, J. N. (1986). Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). *Ecology* 67: 1024–1035.
330. Rutowski, R. L., Demlong, M. J., and Leffingwell, T. (1994). Behavioural thermoregulation at mate encounter sites by male butterflies (*Asterocampa leilia*, Nymphalidae). *Animal Behaviour* 48: 833–841.
331. Lillywhite, H. B. (1970). Behavioral temperature regulation in the bullfrog, *Rana catesbeiana*. *Copeia* 1970: 158–168.
332. Bogert, C. M. (1959). How reptiles regulate their body temperature. *Scientific American* 200(4): 105–120.
333. Fielder, D. P. (2012). Seasonal and diel dive performance and behavioral ecology of the bimodally respiring freshwater turtle *Myuchelys bellii* of eastern Australia. *Journal of Comparative Physiology A* 198: 129–143.
334. Kleiber, M. and Winchester, C. (1933). Temperature regulation in baby chicks. *Proceedings of the Society for Experimental Biology and Medicine* 31: 158–159.
335. Webb, D. R. (1993). Maternal-nestling contact geometry and heat transfer in an altricial bird. *Journal of Thermal Biology* 18: 117–124.
336. Mount, L. E. and Willmott, J. V. (1967). The relation between spontaneous activity, metabolic rate and the 24 hour cycle in mice at different environmental temperatures. *Journal of Physiology* 190: 371–380.
337. Sealander, J. A. (1952). The relationship of nest protection and huddling to survival of *Peromyscus* at low temperature. *Ecology* 33: 63–71.
338. Vogt, F. D. and Lynch, G. R. (1982). Influence of ambient temperature, nest availability, huddling, and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus*. *Physiological Zoology* 55: 56–63.
339. Alberts, J. R. (1978). Huddling by rat pups: Group behavioral mechanisms of temperature regulation and energy conservation. *Journal of Comparative and Physiological Psychology* 92: 231–245.
340. Stanier, M. W. (1975). Effect of body weight, ambient temperature and huddling on oxygen consumption and body temperature of young mice. *Comparative Biochemistry and Physiology A* 51: 79–82.
341. Batchelder, P., Kinney, R. O., Demlow, L., and Lynch, C. B. (1983). Effects of temperature and social interactions on huddling behavior in *Mus musculus*. *Physiology and Behavior* 31: 97–102.
342. Withers, P. C. and Jarvis, J. U. M. (1980). The effect of huddling on thermoregulation and oxygen consumption for the naked mole-rat. *Comparative Biochemistry and Physiology A* 66: 215–219.
343. Contreras, L. C. (1984). Bioenergetics of huddling: Test of a psycho-physiological hypothesis. *Journal of Mammalogy* 65: 256–262.
344. Andrews, R. V. and Belknap, R. W. (1986). Bioenergetic benefits of huddling by deer mice (*Peromyscus maniculatus*). *Comparative Biochemistry and Physiology A* 85: 775–778.
345. Newkirk, K. D., Silverman, D. A., and Wynne-Edwards, K. E. (1995). Ontogeny of thermoregulation in the Djungarian hamster (*Phodopus campbelli*). *Physiology and Behavior* 57: 117–124.
346. Sokoloff, G., Blumberg, M. S., and Adams, M. M. (2000). A comparative analysis of huddling in infant Norway rats and Syrian golden hamsters: Does endothermy modulate behavior? *Behavioral Neuroscience* 114: 585–593.

347. Arnold, W., Heldmaier, G., Ortmann, S., Pohl, H., Ruf, T., and Steinlechner, S. (1991). Ambient temperatures in hibernacula and their energetic consequences for Alpine marmots (*Marmota marmota*). *Journal of Thermal Biology* 16: 223–226.
348. Brown, C. R. (1999). Metabolism and thermoregulation of individual and clustered long-fingered bats, *Miniopterus schreibersii*, and the implications for roosting. *South African Journal of Zoology* 34: 166–172.
349. Schino, G. and Troisi, A. (1990). Behavioral thermoregulation in long-tailed macaques: Effect on social preference. *Physiology and Behavior* 47: 1125–1128.
350. Harrison, J. F., Fewell, J. H., Roberts, S. P., and Hall, H. G. (1996). Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science* 274: 88–90.
351. Heinrich, B. (1981). The regulation of temperature in the honeybee swarm. *Scientific American* 244(6): 120–129.
352. Kronenberg, F. and Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *Journal of Comparative Physiology* 148: 65–76.
353. Southwick, E. E. (1987). Cooperative metabolism in honey bees: An alternative to antifreeze and hibernation. *Journal of Thermal Biology* 12: 155–158.
354. Gasnier, A. and Mayer, A. (1939). Recherches sur la régulation de la nutrition. III. Mécanismes régulateurs de la nutrition et intensité du métabolisme. *Annales de Physiologie Physicochimique et Biologique* 15: 186–194.
355. Brobeck, J. R. (1948). Food intake as a mechanism of temperature regulation. *Yale Journal of Biology and Medicine* 20: 545–549.
356. Fregly, M. J., Marshall, N. B., and Mayer, J. (1957). Effect of changes in ambient temperature on spontaneous activity, food intake and body weight of goldthioglucose-obese and nonobese mice. *American Journal of Physiology* 188: 435–438.
357. Iampietro, P. F., Bass, D. E., and Buskirk, E. R. (1958). Caloric intake during prolonged cold exposure. *Metabolism* 7: 149–153.
358. Witty, R. T. and Long, J. F. (1970). Effect of ambient temperature on gastric secretion and food intake in the rat. *American Journal of Physiology* 219: 1359–1363.
359. Jakubczak, L. F. (1976). Food and water intakes of rats as a function of strain, age, temperature, and body weight. *Physiology and Behavior* 17: 251–258.
360. Cormarèche-Leydier, M. (1981). The effect of ambient temperature on rectal temperature, food intake and short term body weight in the capsaicin desensitized rat. *Pflügers Archiv* 389: 171–174.
361. Yamauchi, C., Fujita, S., Obara, T., and Ueda, T. (1981). Effects of room temperature on reproduction, body and organ weights, food and water intake, and hematology in rats. *Laboratory Animal Science* 31: 251–258.
362. Nakatsuka, H., Shoji, Y., and Tsuda, T. (1983). Effects of cold exposure on gaseous metabolism and body composition in the rat. *Comparative Biochemistry and Physiology A* 75: 21–25.
363. Armitage, G., Harris, R. B. S., Hervey, G. R., and Tobin, G. (1984). The relationship between energy expenditure and environmental temperature in congenitally obese and nonobese Zucker rats. *Journal of Physiology* 350: 197–207.
364. Savory, C. J. (1986). Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowls. *Physiology and Behavior* 38: 353–357.
365. Manning, J. M. and Bronson, F. H. (1990). The effects of low temperature and food intake on ovulation in domestic mice. *Physiological Zoology* 63: 938–948.
366. Hahn, G. L., Chen, Y. R., Nienaber, J. A., Eigenberg, R. A., and Parkhurst, A. M. (1992). Characterizing animal stress through fractal analysis of thermoregulatory responses. *Journal of Thermal Biology* 17: 115–120.
367. Henderson, D., Fort, M. M., Rashotte, M. E., and Henderson, R. P. (1992). Ingestive behavior and body temperature of pigeons during long-term cold exposure. *Physiology and Behavior* 52: 455–469.
368. Pouliquen-Young, O. (1994). Thermoregulatory behaviour of outdoor and commensal populations of mice (*Mus musculus domesticus* and *Mus spretus*) in response to cold. *Journal of Thermal Biology* 19: 213–217.
369. Agetsuma, N. (2000). Influence of temperature on energy intake and food selection by macaques. *International Journal of Primatology* 21: 103–111.
370. Kauffman, A. S., Cabrera, A., and Zucker, I. (2001). Energy intake and fur in summer- and winter-acclimated Siberian hamsters (*Phodopus sungorus*). *American Journal of Physiology* 281: R519–R527.
371. Vaanholt, L. M., Garland, T., Daan, S., and Visser, G. H. (2007). Wheel-running activity and energy metabolism in relation to ambient temperature in mice selected for high wheel-running activity. *Journal of Comparative Physiology B* 177: 109–118.
372. Kaiyala, K. J., Morton, G. J., Thaler, J. P., Meek, T. H., Tylee, T., Ogimoto, K., and Wisse, B. E. (2012). Acutely decreased thermoregulatory energy expenditure or decreased activity energy expenditure both acutely reduce food intake in mice. *PLOS ONE* 7: e41473.
373. Horst, K., Mendel, L. B., and Benedict, F. G. (1930). The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. *Journal of Nutrition* 3: 177–200.
374. Campbell, B. A. and Lynch, G. S. (1968). Influence of hunger and thirst on the relationship between spontaneous activity and body temperature. *Journal of Comparative and Physiological Psychology* 65: 492–498.
375. Bolles, R. C. and Duncan, P. M. (1969). Daily course of activity and subcutaneous body temperature in hungry and thirsty rats. *Physiology and Behavior* 4: 87–89.
376. Rashotte, M. E., Basco, P. S., and Henderson, R. P. (1995). Daily cycles in body temperature, metabolic rate, and substrate utilization in pigeons: Influence of amount and timing of food consumption. *Physiology and Behavior* 57: 731–746.
377. Rashotte, M. E., Pastukhov, I. F., Poliakov, E. L., and Henderson, R. P. (1998). Vigilance states and body temperature during the circadian cycle in fed and fasted pigeons (*Columbia livia*). *American Journal of Physiology* 275: R1690–R1702.
378. Yoda, T., Crawshaw, L. I., Yoshida, K., Su, L., Hosono, T., Shido, O., Sakurada, S., Fukuda, Y., and Kanosue, K. (2000). Effects of food deprivation on daily changes in body temperature and behavioral thermoregulation in rats. *American Journal of Physiology* 278: R133–R139.
379. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T., and Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *Journal of Physiology* 526: 417–424.
380. MacMillen, R. E. (1965). Aestivation in the cactus mouse, *Peromyscus eremicus*. *Comparative Biochemistry and Physiology* 16: 227–248.
381. Ingram, D. L. and Legge, K. F. (1974). Effects of environmental temperature on food intake in growing pigs. *Comparative Biochemistry and Physiology A* 48: 573–581.

382. Kraly, F. S. and Blas, E. M. (1976). Mechanisms for enhanced feeding in the cold in rats. *Journal of Comparative and Physiological Psychology* 90: 714–726.
383. Macari, M., Dauncey, M. J., and Ingram, D. L. (1983). Changes in food intake in response to alterations in the ambient temperature: Modifications by previous thermal and nutritional experience. *Pflügers Archiv* 396: 231–237.
384. Refinetti, R. (1988). Effects of food temperature and ambient temperature during a meal on food intake in the rat. *Physiology and Behavior* 43: 245–247.
385. Caputa, M. and Cabanac, M. (1980). Muscular work as thermal behavior. *Journal of Applied Physiology* 48: 1020–1023.
386. Cabanac, M. and Leblanc, J. (1983). Physiological conflict in humans: Fatigue vs. cold discomfort. *American Journal of Physiology* 244: R621–R628.
387. Paladino, F. V. and King, J. R. (1984). Thermoregulation and oxygen consumption during terrestrial locomotion by white-crowned sparrows *Zonotrichia leucophrys gambelii*. *Physiological Zoology* 57: 226–236.
388. Webster, M. D. and Weathers, W. W. (1990). Heat produced as a by-product of foraging activity contributes to thermoregulation by verdins, *Auriparus flaviceps*. *Physiological Zoology* 63: 777–794.
389. Zerba, E. and Walsberg, G. E. (1992). Exercise-generated heat contributes to thermoregulation by Gambel's quail in the cold. *Journal of Experimental Biology* 171: 409–422.
390. Chappell, M. A., Garland, T., Rezende, E. L., and Gomes, F. R. (2004). Voluntary running in deer mice: Speed, distance, energy costs and temperature effects. *Journal of Experimental Biology* 207: 3839–3854.
391. Wunder, B. A. (1970). Energetics of running activity in Merriam's chipmunk, *Eutamias merriami*. *Comparative Biochemistry and Physiology* 33: 821–836.
392. Yousef, M. K., Robertson, W. D., Dill, D. B., and Johnson, H. D. (1973). Energetic cost of running in the antelope ground squirrel *Ammospermophilus leucurus*. *Physiological Zoology* 46: 139–147.
393. Girardier, L., Clark, M. G., and Seydoux, J. (1995). Thermogenesis associated with spontaneous activity: An important component of thermoregulatory needs in rats. *Journal of Physiology* 488: 779–787.
394. Doss, D. and Ohnesorge, F. K. (1966). Motilität und Körpertemperatur von Mäusen in verschiedenen Umgebungstemperaturen. *Pflügers Archiv* 289: 91–97.
395. Aujard, F. and Vasseur, F. (2001). Effect of ambient temperature on the body temperature rhythm of male gray mouse lemurs (*Microcebus murinus*). *International Journal of Primatology* 22: 43–56.
396. Yang, Y. and Gordon, C. J. (1996). Ambient temperature limits and stability of temperature regulation in telemetered male and female rats. *Journal of Thermal Biology* 21: 353–363.
397. Lyman, C. P. (1954). Activity, food consumption and hoarding in hibernators. *Journal of Mammalogy* 35: 545–552.
398. Abreu-Vieira, G., Xiao, C., Gavrilova, O., and Reitman, M. L. (2015). Integration of body temperature into the analysis of energy expenditure in the mouse. *Molecular Metabolism* 4: 461–470.
399. Wadley, L., Sievers, C., Bamford, M., Goldberg, P., Berna, F., and Miller, C. (2011). Middle Stone Age bedding construction and settlement patterns at Sibudu, South Africa. *Science* 334: 1388–1391.
400. Olesen, B. W. and Seelen, J. (1993). Criteria for a comfortable indoor environment in buildings. *Journal of Thermal Biology* 18: 545–549.
401. Givoni, B. (1974). Architectural and urban planning in relation to weather and climate. *Progress in Biometeorology* 1: 183–193.
402. Leff, L. G. and McArthur, J. V. (1989). Notes on the use of wood refugia by *Physella heterostropha* (Say) in a thermally disturbed swamp. *Journal of Thermal Biology* 14: 179–182.
403. Grassé, P. P. and Noirot, C. (1961). Nouvelles recherches sur la systématique et l'éthologie des termites champignonnistes du genre *Bellicositermes* Emerson. *Insectes Sociaux* 8: 311–357.
404. Scherba, G. (1962). Mound temperatures of the ant *Formica ulkei* Emery. *American Midland Naturalist* 67: 373–385.
405. Lensky, Y. (1964). Comportement d'une colonie d'abeilles a des temperatures extremes. *Journal of Insect Physiology* 10: 1–12.
406. Degrandi-Hoffman, G., Spivak, M., and Martin, J. S. (1993). Role of thermoregulation by nestmates on the development time of honey bee (Hymenoptera: Apidae) queens. *Annals of the Entomological Society of America* 86: 165–172.
407. McGinnis, S. M. and Voigt, W. G. (1971). Thermoregulation in the desert tortoise, *Gopherus agassizii*. *Comparative Biochemistry and Physiology A* 40: 119–126.
408. Autumn, K., Weinstein, R. B., and Full, R. J. (1994). Low cost of locomotion increases performance at low temperature in a nocturnal lizard. *Physiological Zoology* 67: 238–262.
409. Coulianos, C. C. and Johnels, A. G. (1963). Note on the subnivean environment of small mammals. *Arkiv för Zoologi* 15: 363–370.
410. Elvert, R., Kronfeld, N., Dayan, T., Haim, A., Zisapel, N., and Heldmaier, G. (1999). Telemetric field studies of body temperature and activity rhythms of *Acomys russatus* and *A. cahirinus* in the Judean Desert of Israel. *Oecologia* 119: 484–492.
411. Lovegrove, B. G., Heldmaier, G., and Knight, M. (1991). Seasonal and circadian energetic patterns in an arboreal rodent, *Thallomys paedulcus*, and a burrow-dwelling rodent, *Aethomys namaquensis*, from the Kalahari desert. *Journal of Thermal Biology* 16: 199–209.
412. Dufour, B. A. (1978). Le terrier d'*Apodemus sylvaticus* L.: sa construction en terrarium et son adaptation a des facteurs externes et internes. *Behavioural Processes* 3: 57–76.
413. Rode, P. (1929). Contribuition a l'étude du fouissement chez les petits rongeurs. *Bulletin de la Société Zoologique de France* 54: 573–588.
414. Kuhnen, G. (1986). O_2 and CO_2 concentrations in burrows of euthermic and hibernating golden hamsters. *Comparative Biochemistry and Physiology A* 84: 517–522.
415. Stark, H. E. (1963). Nesting habits of the California vole, *Microtus californicus*, and microclimatic factors affecting its nests. *Ecology* 44: 663–669.
416. Lee, C. T. and Wong, P. T. P. (1970). Temperature effect and strain differences in the nest-building behavior of inbred mice. *Psychonomic Science* 20: 9–10.
417. Kenagy, G. J., Vásquez, R. A., Barnes, B. M., and Bozinovic, F. (2004). Microstructure of summer activity bouts of degus in a thermally heterogeneous habitat. *Journal of Mammalogy* 85: 260–267.
418. Lynch, C. B. (1973). Environmental modification of nest-building in the white-footed mouse, *Peromyscus leucopus*. *Animal Behaviour* 22: 405–409.
419. Ivey, R. D. (1949). Life history notes on three mice from the Florida east coast. *Journal of Mammalogy* 30: 157–162.
420. Puchalski, W., Bulova, S. J., Lynch, C. B., and Lynch, G. R. (1988). Photoperiod, temperature and melatonin effects on thermoregulatory behavior in Djungarian hamsters. *Physiology and Behavior* 42: 173–177.

421. Kinder, E. F. (1927). A study of the nest-building activity of the albino rat. *Journal of Experimental Zoology* 47: 117–161.
422. Boice, R. (1977). Burrows of wild and albino rats: Effects of domestication, outdoor raising, age, experience, and maternal state. *Journal of Comparative and Physiological Psychology* 91: 649–661.
423. Pisano, R. G. and Storer, T. I. (1948). Burrows and feeding of the Norway rat. *Journal of Mammalogy* 29: 374–383.
424. Refinetti, R. (1983). Construção de ninho no rato: efeitos da temperatura na fase de coleta. *Ciência e Cultura* 35(S): 820–821.
425. McDevitt, R. M. and Andrews, J. F. (1994). The importance of nest utilization as a behavioural thermoregulatory strategy in *Sorex minutus*, the pygmy shrew. *Journal of Thermal Biology* 19: 97–102.
426. Hut, R. A., Barnes, B. M., and Daan, S. (2002). Body temperature patterns before, during, and after semi-natural hibernation in the European ground squirrel. *Journal of Comparative Physiology B* 172: 47–58.
427. Mayer, W. V. (1955). The protective value of the burrow system to the hibernating arctic squirrel, *Spermophilus undulatus*. *Anatomical Record* 122: 437–438.
428. Michener, G. R. (1983). Spring emergence schedules and vernal behavior of Richardson's ground squirrels: Why do males emerge from hibernation before females? *Behavioral Ecology and Sociobiology* 14: 29–38.
429. Long, R. A., Martin, T. J., and Barnes, B. M. (2005). Body temperature and activity patterns in free-living Arctic ground squirrels. *Journal of Mammalogy* 86: 314–322.
430. Fadem, B. H., Kraus, D. B., and Sheffet, R. H. (1986). Nest-building in gray short-tailed opossums: Temperature effects and sex differences. *Physiology and Behavior* 36: 667–670.
431. Deutsch, J. A. (1957). Nest building behaviour of domestic rabbits under semi-natural conditions. *British Journal of Animal Behaviour* 5: 53–54.
432. Buech, R. R. and Rugg, D. J. (1989). Temperature in beaver lodges and bank dens in a near-boreal environment. *Canadian Journal of Zoology* 67: 1061–1066.
433. Dyck, A. P. and MacArthur, R. A. (1992). Seasonal patterns of body temperature in free-ranging beaver (*Castor canadensis*). *Canadian Journal of Zoology* 70: 1668–1672.
434. Lancia, R. A., Dodge, W. E., and Larson, J. S. (1982). Winter activity patterns of two radio-marked beaver colonies. *Journal of Mammalogy* 63: 598–606.
435. Linduska, J. P. (1947). Winter den studies of the cottontail in southern Michigan. *Ecology* 28: 448–454.
436. Bakko, E. B., Porter, W. P., and Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
437. Downey, P. and Jahan-Parwar, B. (1972). Cooling as reinforcing stimulus in *Aplysia*. *American Zoologist* 12: 507–512.
438. Rozin, P. N. and Mayer, J. (1961). Thermal reinforcement and thermoregulatory behavior in the goldfish, *Carassius auratus*. *Science* 134: 942–943.
439. Laudenslager, M. L. and Hammel, H. T. (1977). Environmental temperature selection by the Chukar partridge, *Alectoris chukar*. *Physiology and Behavior* 19: 543–548.
440. Schmidt, I. and Rautenberg, W. (1975). Instrumental thermoregulatory behavior in pigeons. *Journal of Comparative Physiology* 101: 225–235.
441. Schmidt, I. (1976). Effect of central thermal stimulation on the thermoregulatory behavior of the pigeon. *Pflügers Archiv* 363: 271–272.
442. Horowitz, K. A., Scott, N. R., Hillman, P. E., and Van Tienhoven, A. (1978). Effects of feathers on instrumental thermoregulatory behavior in chickens. *Physiology and Behavior* 21: 233–238.
443. Budgell, P. (1971). Behavioural thermoregulation in the Barbary dove (*Streptopelia risoria*). *Animal Behaviour* 19: 524–531.
444. Richards, S. A. (1976). Behavioural temperature regulation in the fowl. *Journal of Physiology* 258: 122P–123P.
445. Refinetti, R. (1983). Thermoregulatory behaviour of Budgerigars in a cold environment. *Bird Behaviour* 4: 90–92.
446. Revusky, S. H. (1966). Cold acclimatization in hairless mice measured by behavioral thermoregulation. *Psychonomic Science* 6: 209–210.
447. Baldwin, B. A. (1968). Behavioural thermoregulation in mice. *Physiology and Behavior* 3: 401–407.
448. Dauncey, M. J. (1984). Behavioural and autonomic thermoregulation in lean and genetically obese (*ob/ob*) mice. *Journal of Thermal Biology* 9: 247–253.
449. Chen, X. M., Hosono, T., Mizuno, A., Yoda, T., Yoshida, K., Aoyagi, Y., and Kanosue, K. (1998). New apparatus for studying behavioral thermoregulation in rats. *Physiology and Behavior* 64: 419–424.
450. Epstein, A. N. and Milestone, R. (1968). Showering as a coolant for rats exposed to heat. *Science* 160: 895–896.
451. Carlton, P. L. and Marks, R. A. (1958). Cold exposure and heat reinforced operant behavior. *Science* 128: 1344.
452. Weiss, B. (1957). Thermal behavior of the subnourished and pantothenic-acid-deprived rat. *Journal of Comparative and Physiological Psychology* 50: 481–485.
453. Sabourin, M., Deschambault, A., and Ducharme, R. (1973). Effect of light on thermal reinforcement in behavioral thermoregulation. *Perceptual and Motor Skills* 37: 611–614.
454. Wright, J. W. and Meyer, M. E. (1969). A new technique for measuring thermoregulatory behavior in the rat. *Journal of the Experimental Analysis of Behavior* 12: 999–1002.
455. Carlisle, H. J. (1968). Initiation of behavioral responding for heat in a cold environment. *Physiology and Behavior* 3: 827–830.
456. Carlisle, H. J. (1969). Effect of fixed-ratio thermal reinforcement on thermoregulatory behavior. *Physiology and Behavior* 4: 23–28.
457. Matthews, T. J. (1971). Thermal motivation in the rat. *Journal of Comparative and Physiological Psychology* 74: 240–247.
458. Leeming, F. C. (1968). Response rate as a function of magnitude and schedule of heat reinforcement. *Journal of Experimental Psychology* 76: 74–77.
459. Scott, D. M. and Blackwood, V. (1973). Response latencies of rats during behavioral thermoregulation. *Perception and Psychophysics* 14: 531–541.
460. Weiss, B. and Laties, V. G. (1960). Magnitude of reinforcement as a variable in thermoregulatory behavior. *Journal of Comparative and Physiological Psychology* 53: 603–608.
461. Kristt, D. A. and Sechzer, J. A. (1969). Behavioral thermoregulation at high environmental temperatures: Effect of variation in intensity of heat stimulus and magnitude of reinforcement. *Psychonomic Science* 16: 1–2.
462. Refinetti, R. and Carlisle, H. J. (1987). Behavioral heat intake as a function of reward duration in rats. *Animal Learning and Behavior* 15: 228–237.
463. Szymusiak, R., DeMory, A., Kittrell, E. M. W., and Satinoff, E. (1985). Diurnal changes in thermoregulatory behavior in rats with medial preoptic lesions. *American Journal of Physiology* 249: R219–R227.

464. Cabanac, M. and Dib, B. (1983). Behavioural responses to hypothalamic cooling and heating in the rat. *Brain Research* 264: 79–87.
465. Jakubczak, L. F. (1966). Behavioral thermoregulation in young and old rats. *Journal of Applied Physiology* 21: 19–21.
466. Schulze, G. and Bürgel, P. (1977). The influence of age and drugs on the thermoregulatory behaviour of rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 298: 143–147.
467. Jänicke, B. and Schulze, G. (1986). Capacity of thermoregulation in different aged rats: Age as a factor modifying thermoregulation. *Pharmacopsychiatry* 19: 312–313.
468. Baldwin, B. A. and Ingram, D. L. (1968). Factor influencing behavioral thermoregulation in the pig. *Physiology and Behavior* 3: 409–415.
469. Baldwin, B. A. and Ingram, D. L. (1968). The effects of food intake and acclimatization to temperature on behavioral thermoregulation in pigs and mice. *Physiology and Behavior* 3: 395–400.
470. Carlisle, H. J. and Ingram, D. L. (1973). The effects of heating and cooling the spinal cord and hypothalamus on thermoregulatory behaviour in the pig. *Journal of Physiology* 231: 353–364.
471. Carlisle, H. J. (1966). Heat intake and hypothalamic temperature during behavioral temperature regulation. *Journal of Comparative and Physiological Psychology* 61: 388–397.
472. Adair, E. R., Casby, J. U., and Stolwijk, J. A. J. (1970). Behavioral temperature regulation in the squirrel monkey: Changes induced by shifts in hypothalamic temperature. *Journal of Comparative and Physiological Psychology* 72: 17–27.
473. Adair, E. R. and Wright, B. A. (1976). Behavioral thermoregulation in the squirrel monkey when response effort is varied. *Journal of Comparative and Physiological Psychology* 90: 179–184.
474. Refinetti, R. and Carlisle, H. J. (1987). A computational formula for heat influx in animal experiments. *Journal of Thermal Biology* 12: 263–266.
475. Refinetti, R. (1982). Custo da resposta termoregulatória de pressão à barra. *Ciência e Cultura* 34(S): 896–897.
476. Refinetti, R. and Carlisle, H. J. (1986). Complementary nature of heat production and heat intake during behavioral thermoregulation in the rat. *Behavioral and Neural Biology* 46: 64–70.
477. Ingram, D. L. (1975). The efficiency of operant thermoregulatory behaviour in pigs as determined from the rate of oxygen consumption. *Pflügers Archiv* 353: 139–149.
478. Schmidt, I. and Simon, E. (1979). Interaction of behavioral and autonomic thermoregulation in cold exposed pigeons. *Journal of Comparative Physiology* 133: 151–157.
479. Schmidt, I. (1978). Interactions of behavioral and autonomic thermoregulation in heat stressed pigeons. *Pflügers Archiv* 374: 47–55.
480. Waterhouse, J., Drust, B., Weinert, D., Edwards, B., Gregson, W., Atkinson, G., Kao, S., Aizawa, S., and Reilly, T. (2005). The circadian rhythm of core temperature: Origin and some implications for exercise performance. *Chronobiology International* 22: 207–225.
481. Refinetti, R. (2010). The circadian rhythm of body temperature. *Frontiers in Bioscience* 15: 564–594.
482. Aschoff, J. (1982). The circadian rhythm of body temperature as a function of body size. In: Taylor, C. R., Johansen, K., and Bolis, L. (Eds.), *A Companion to Animal Physiology.* Cambridge, U.K.: Cambridge University Press, pp. 173–188.
483. Clarke, A. and Rothery, P. (2008). Scaling of body temperature in mammals and birds. *Functional Ecology* 22: 58–67.
484. Piccione, G., Fazio, F., Giudice, E., and Refinetti, R. (2009). Body size and the daily rhythm of body temperature in dogs. *Journal of Thermal Biology* 34: 171–175.
485. Mortola, J. P. and Lanthier, C. (2004). Scaling the amplitudes of the circadian pattern of resting oxygen consumption, body temperature and heart rate in mammals. *Comparative Biochemistry and Physiology A* 139: 83–95.
486. Morrison, P. R. and Ryser, F. A. (1952). Weight and body temperature in mammals. *Science* 116: 231–232.
487. Poole, S. and Stephenson, J. D. (1977). Core temperature: Some shortcomings of rectal temperature measurements. *Physiology and Behavior* 18: 203–205.
488. Georgiev, J. (1978). Influence of environmental conditions and handling on the temperature rhythm of the rat. *Biotelemetry and Patient Monitoring* 5: 229–234.
489. Berkey, D. L., Meeuwsen, K. W., and Barney, C. C. (1990). Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. *American Journal of Physiology* 258: R743–R749.
490. Cabanac, A. and Briese, E. (1991). Handling elevates the colonic temperature of mice. *Physiology and Behavior* 51: 95–98.
491. Harkin, A., Connor, T. J., O'Donnell, J. M., and Kelly, J. P. (2002). Physiological and behavioral responses to stress: What does a rat find stressful? *Lab Animal* 31: 42–50.
492. Lovegrove, B. G. (2003). The influence of climate on the basal metabolic rate of small mammals: A slow-fast metabolic continuum. *Journal of Comparative Physiology B* 173: 87–112.
493. Refinetti, R. (1997). The effects of ambient temperature on the body temperature rhythm of rats, hamsters, gerbils, and tree shrews. *Journal of Thermal Biology* 22: 281–284.
494. Fuller, C. A. (1984). Circadian brain and body temperature rhythms in the squirrel monkey. *American Journal of Physiology* 246: R242–R246.
495. Piccione, G., Gianesella, M., Morgante, M., and Refinetti, R. (2013). Daily rhythmicity of core and surface temperatures of sheep kept under thermoneutrality or in the cold. *Research in Veterinary Science* 95: 261–265.
496. McKechnie, A. E. and Lovegrove, B. G. (2001). Heterothermic responses in the speckled mousebird (*Colius striatus*). *Journal of Comparative Physiology B* 171: 507–518.
497. Downs, C. T. and Brown, M. (2002). Nocturnal heterothermy and torpor in the Malachite sunbird (*Nectarinia famosa*). *Auk* 119: 251–260.
498. Roussel, B., Chouvet, G., and Debilly, G. (1976). Rythmes circadiens des températures internes et ambiance thermique chez le rat. *Pflügers Archiv* 365: 183–189.
499. Gordon, C. J. (1993). *Temperature Regulation in Laboratory Rodents.* New York: Cambridge University Press.
500. Piccione, G., Caola, G., and Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
501. Kittrell, E. M. W. and Satinoff, E. (1986). Development of the circadian rhythm of body temperature in rats. *Physiology and Behavior* 38: 99–104.
502. Piccione, G. Caola, G., and Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.
503. Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.

504. Osborne, A. R. and Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.
505. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
506. Stephan, F. K. and Nunez, A. A. (1977). Elimination of circadian rhythms in drinking, activity, sleep, and temperature by isolation of the suprachiasmatic nuclei. *Behavioral Biology* 20: 1–16.
507. Liu, S., Chen, X. M., Yoda, T. Nagashima, K., Fukuda, Y., and Kanosue, K. (2002). Involvement of the suprachiasmatic nucleus in body temperature modulation by food deprivation in rats. *Brain Research* 929: 26–36.
508. Wachulec, M., Li, H., Tanaka, H., Peloso, E., and Satinoff, E. (1997). Suprachiasmatic nuclei lesions do not eliminate homeostatic thermoregulatory responses in rats. *Journal of Biological Rhythms* 12: 226–234.
509. Aschoff, J. (1970). Circadian rhythm of activity and of body temperature. In: Hardy, J. D., Gagge, A. P., and Stolwijk, J. A. J. (Eds.), *Physiological and Behavioral Temperature Regulation*. Springfield, IL: Charles C. Thomas, pp. 905–919.
510. Hensel, H. (1981). *Thermoreception and Temperature Regulation*. New York: Academic Press.
511. Kluger, M. J. (1991). Fever: Role of pyrogens and cryogens. *Physiological Reviews* 71: 93–128.
512. Robinson, E. L. and Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
513. Simon, E., Pierau, F. K., and Taylor, D. C. M. (1986). Central and peripheral thermal control of effectors in homeothermic temperature regulation. *Physiological Reviews* 66: 235–300.
514. Satinoff, E. (1978). Neural organization and evolution of thermal regulation in mammals. *Science* 201: 16–22.
515. Van Someren, E. J. W., Raymann, R. J. E. M., Scherder, E. J. A., Daanen, H. A. M., and Swaab, D. F. (2002). Circadian and age-related modulation of thermoreception and temperature regulation: Mechanisms and functional implications. *Ageing Research Reviews* 1: 721–778.
516. Cabanac, M., Cunningham, D. J., and Stolwijk, J. A. J. (1971). Thermoregulatory set point during exercise: A behavioral approach. *Journal of Comparative and Physiological Psychology* 76: 94–102.
517. Nadel, E. R. (1983). Factors affecting the regulation of body temperature during exercise. *Journal of Thermal Biology* 8: 165–169.
518. Watts Jr., R. H. and Refinetti, R. (1996). Circadian modulation of cold-induced thermogenesis in the golden hamster. *Biological Rhythm Research* 27: 87–94.
519. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. II. Spinal thermosensitivity. *Pflügers Archiv* 386: 181–185.
520. Heller, H. C., Graf, R., and Rautenberg, W. (1983). Circadian and arousal state influences on thermoregulation in the pigeon. *American Journal of Physiology* 245: R321–R328.
521. Wenger, C. B., Roberts, M. F., Stolwijk, J. A. J., and Nadel, E. R. (1976). Nocturnal lowering of thresholds for sweating and vasodilation. *Journal of Applied Physiology* 41: 15–19.
522. Stephenson, L. A., Wenger, C. B., O'Donovan, B. H., and Nadel, E. R. (1984). Circadian rhythm in sweating and cutaneous blood flow. *American Journal of Physiology* 246: R321–R324.
523. Aoki, K., Stephens, D. P., Saad, A. R., and Johnson, J. M. (2003). Cutaneous vasoconstrictor response to whole body skin cooling is altered by time of day. *Journal of Applied Physiology* 94: 930–934.
524. Beste, R., Kaiser, K., Issing, K., and Hensel, H. (1978). Perception of local thermal stimuli in the course of day. *Pflügers Archiv* 377(S): R56.
525. Carlisle, H. J., Wilkinson, C. W., Laudenslager, M. L., and Keith, L. D. (1979). Diurnal variation of heat intake in ovariectomized, steroid-treated rats. *Hormones and Behavior* 12: 232–242.
526. Briese, E. (1986). Circadian body temperature rhythm and behavior of rats in thermoclines. *Physiology and Behavior* 37: 839–847.
527. Ray, B., Mallick, H. N., and Kumar, V. M. (2004). Changes in thermal preference, sleep-wakefulness, body temperature and locomotor activity of rats during continuous recording for 24 hours. *Behavioural Brain Research* 154: 519–526.
528. Ray, B., Mallick, H. N., and Kumar, V. M. (2005). Changes in sleep-wakefulness in medial preoptic area lesioned rats: Role of thermal preference. *Behavioural Brain Research* 158: 43–52.
529. Refinetti, R. (1995). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behavioral Neuroscience* 109: 523–527.
530. Jefimow, M., Wojciechowski, M., and Tegowska, E. (2004). Seasonal changes in the thermoregulation of laboratory golden hamsters during acclimation to seminatural outdoor conditions. *Comparative Biochemistry and Physiology A* 139: 379–388.
531. Mojciechowski, M. S. and Jefimow, M. (2006). Is torpor only an advantage? Effect of thermal environment on torpor use in the Siberian hamster (*Phodopus sungorus*). *Journal of Physiology and Pharmacology* 57: 83–92.
532. Song, X., Körtner, G., and Geiser, F. (1998). Temperature selection and use of torpor by the marsupial *Sminthopsis macroura*. *Physiology and Behavior* 64: 675–682.
533. Aujard, F., Séguy, M., Terrien, J., Botalla, R., Blanc, S., and Perret, M. (2006). Behavioral thermoregulation in a non human primate: Effects of age and photoperiod on temperature selection. *Experimental Gerontology* 41: 784–792.
534. Terai, Y., Asayama, M., Ogawa, T., Sugenoya, J., and Miyagawa, T. (1985). Circadian variation of preferred environmental temperature and body temperature. *Journal of Thermal Biology* 10: 151–156.
535. Pöllmann, L. (1994). Circadian and circannual variations in the evaluation of thermal comfort in a constant climate. *Indoor Environment* 3: 145–148.
536. Kim, H. E. and Tokura, H. (1994). Effects of time of day on dressing behavior under the influence of ambient temperature fall from 30°C to 15°C. *Physiology and Behavior* 55: 645–650.
537. Grivel, F. and Candas, V. (1991). Ambient temperatures preferred by young European males and females at rest. *Ergonomics* 34: 365–378.
538. Shoemaker, J. A. and Refinetti, R. (1996). Day–night difference in the preferred ambient temperature of human subjects. *Physiology and Behavior* 59: 1001–1003.
539. Refinetti, R. (1997). Homeostasis and circadian rhythmicity in the control of body temperature. *Annals of the New York Academy of Sciences* 813: 63–70.
540. Briese, E. (1998). Normal body temperature of rats: The set-point controversy. *Neuroscience and Biobehavioral Reviews* 22: 427–436.

541. Satinoff, E., Liran, J., and Clapman, R. (1982). Aberrations of circadian body temperature rhythms in rats with medial preoptic lesions. *American Journal of Physiology* 242: R352–R357.
542. Briese, E. (1989). Do medial preoptic lesions interfere with the set-point of temperature regulation? *Brain Research Bulletin* 23: 137–144.
543. Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429.
544. Aschoff, J. (1983). Circadian control of body temperature. *Journal of Thermal Biology* 8: 143–147.
545. Shido, O., Sugano, Y., and Nagasaka, T. (1986). Circadian change of heat loss in response to change in core temperature in rats. *Journal of Thermal Biology* 11: 199–202.
546. Fuller, C. A., Sulzman, F. M., and Moore-Ede, M. C. (1985). Role of heat loss and heat production in generation of the circadian temperature rhythm of the squirrel monkey. *Physiology and Behavior* 34: 543–546.
547. Kräuchi, K. and Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *American Journal of Physiology* 267: R819–R829.
548. Sarabia, J. A., Rol, M. A., Mendiola, P., and Madrid, J. A. (2008). Circadian rhythm of wrist temperature in normal-living subjects: A candidate for a new index of the circadian system. *Physiology and Behavior* 95: 570–580.
549. Varela, M., Cuesta, D., Madrid, J. A., Churruca, J., Miro, P., Ruiz, P., and Martinez, C. (2009). Holter monitoring of central and peripheral temperature: Possible uses and feasibility study in outpatient settings. *Journal of Clinical Monitoring and Computing* 23: 209–216.
550. Kolodyazhniy, V., Späti, J., Frey, S., Götz, T., Wirz-Justice, A., Kräuchi, K., Cajochen, C., and Wilhelm, F. H. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. *Journal of Biological Rhythms* 26: 55–67.
551. Shechter, A., Boudreau, P., Varin, F., and Boivin, D. B. (2011). Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. *Journal of Biological Rhythms* 26: 260–270.
552. Refinetti, R. and Menaker, M. (1992). The circadian rhythm of body temperature. *Physiology and Behavior* 51: 613–637.
553. Grigg, G. C., Beard, L. A., and Augee, M. L. (2004). The evolution of endothermy and its diversity in mammals and birds. *Physiological and Biochemical Zoology* 77: 982–997.
554. Lovegrove, B. G. (2012). The evolution of endothermy in Cenozoic mammals: A plesiomorphic–apomorphic continuum. *Biological Reviews* 87: 128–162.
555. Liu, Y., Merrow, M., Loros, J. J., and Dunlap, J. C. (1998). How temperature changes reset a circadian oscillator. *Science* 281: 825–829.
556. Brown, S. A., Zumbrunn, G., Fleury-Olela, F., Preitner, N., and Schibler, U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Current Biology* 12: 1574–1583.
557. Ruoff, P. and Rensing, L. (2004). Temperature effects on circadian clocks. *Journal of Thermal Biology* 29: 445–456.
558. Buhr, E. D., Yoo, S. H., and Takahashi, J. S. (2010). Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* 330: 379–385.
559. Morf, J., Ray, G., Schneider, K., Stratmann, M., Fujita, J., Naef, F., and Schibler, U. (2012). Cold-inducible RNA-binding protein modulates circadian gene expression posttranscriptionally. *Science* 338: 379–383.
560. Morf, J and Schibler, U. (2013). Body temperature cycles: Gatekeepers of circadian clocks. *Cell Cycle* 12: 539–540.
561. Van Dongen, H. P. A., Maislin, G., Mullington, J. M., and Dinges, D. F. (2003). The cumulative cost of additional wakefulness: Dose–response effects on neurobehavioral functions and sleep physiology from chronic restriction and total sleep deprivation. *Sleep* 26: 117–126.
562. Lancel, M., van Riezen, H., and Glatt, A. (1991). Effects of circadian phase and duration of sleep deprivation on sleep and EEG power spectra in the cat. *Brain Research* 548: 206–214.
563. Franken, P., Dijk, D. J., Tobler, I., and Borbély, A. A. (1991). Sleep deprivation in rats: Effects on EEG power spectra, vigilance states, and cortical temperature. *American Journal of Physiology* 261: R198–R208.
564. Yoshida, Y., Fujiki, N., Nakajima, T., Ripley, B., Matsumura, H., Yoneda, H., Mignot, E., and Nishino, S. (2001). Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light–dark cycle and sleep–wake activities. *European Journal of Neuroscience* 14: 1075–1081.
565. Dijk, D. J. and Beersma, D. G. M. (1989). Effects of SWS deprivation on subsequent EEG power density and spontaneous sleep duration. *Electroencephalography and Clinical Neurophysiology* 72: 312–320.
566. Almirall, H., Aguirre, A., Rial, R. V., Daurat, A., Foret, J., and Benoit, O. (1993). Temperature drop and sleep: Testing the contribution of SWS in keeping cool. *NeuroReport* 5: 177–180.
567. Coolen, A., Hoffmann, K., Barf, R. P., Fuchs, E., and Meerlo, P. (2012). Telemetric study of sleep architecture and sleep homeostasis in the day-active tree shrew *Tupaia belangeri*. *Sleep* 35: 879–888.
568. Mistlberger, R. E., Bergmann, B. M., Waldenar, W., and Rechtschaffen, A. (1983). Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep* 6: 217–233.
569. Trachsel, L., Edgar, D. M., Seidel, W. F., Heller, H. C., and Dement, W. C. (1992). Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: Effects of sleep deprivation and triazolam administration. *Brain Research* 589: 253–261.
570. Mendelson, W. B., Bergmann, B. M., and Tung, A. (2003). Baseline and post-deprivation recovery sleep in SCN-lesioned rats. *Brain Research* 980: 185–190.
571. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D., and Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
572. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P., and Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
573. Porkka-Heiskanen, T., Strecker, R. E., Thakkar, M., Bjørkum, A. A., Greene, R. W., and McCarley, R. W. (1997). Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. *Science* 276: 1265–1268.
574. Saper, C. B., Scammell, T. E., and Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature* 437: 1257–1263.
575. Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J. et al. (2013). Sleep drives metabolite clearance from the adult brain. *Science* 342: 373–377.
576. Naylor, E., Bergmann, B. M., Krauski, K., Zee, P. C., Takahashi, J. S., Vitaterna, M. H., and Turek, F. W. (2000). The circadian clock mutation alters sleep homeostasis in the mouse. *Journal of Neuroscience* 20: 8138–8143.

577. Wisor, J. P., O'Hara, B. F., Terao, A., Selby, C. P., Kilduff, T. S., Sancar, A., Edgar, D. M., and Franken, P. (2002). A role for *cryptochromes* in sleep regulation. *BMC Neuroscience* 3: art. 20.
578. Edgar, D. M., Martin, C. E., and Dement, W. C. (1991). Activity feedback to the mammalian circadian pacemaker: Influence on observed measures of rhythm period length. *Journal of Biological Rhythms* 6: 185–199.
579. Sei, H., Oishi, K., Morita, Y., and Ishida, N. (2001). Mouse model for morningness/eveningness. *NeuroReport* 12: 1461–1464.
580. Edgar, D. M. and Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
581. Kas, M. J. H. and Edgar, D. M. (1998). Crepuscular rhythms of EEG sleep–wake in a hystricomorph rodent, *Octodon degus*. *Journal of Biological Rhythms* 13: 9–17.
582. Deboer, T., Vyazovskiy, V. V., and Tobler, I. (2000). Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 429–436.
583. Borbély, A. A. and Neuhaus, H. U. (1978). Circadian rhythm of sleep and motor activity in the rat during skeleton photoperiod, continuous darkness and continuous light. *Journal of Comparative Physiology* 128: 37–46.
584. Eastman, C. and Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
585. Ikeda, M., Sagara, M., and Inoué, S. (2000). Continuous exposure to dim illumination uncouples temporal patterns of sleep, body temperature, locomotion and drinking behavior in the rat. *Neuroscience Letters* 279: 185–189.
586. Adrien, J., Dugovic, C., and Martin, P. (1991). Sleep-wakefulness patterns in the helpless rat. *Physiology and Behavior* 49: 257–262.
587. Deboer, T., Vansteensel, M. J., Détári, L., and Meijer, J. H. (2003). Sleep states alter activity of suprachiasmatic nucleus neurons. *Nature Neuroscience* 6: 1086–1090.
588. Li, H. and Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
589. Tzischinsky, O. and Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.
590. Hayashi, M., Morikawa, T., and Hori, T. (2002). Circasemidian 12 h cycle of slow wave sleep under constant darkness. *Clinical Neurophysiology* 113: 1505–1516.
591. Tan, X., Uchiyama, M., Shibui, K., Tagaya, H., Suzuki, H., Kamei, Y., Kim, K., Aritaka, S., Ozaki, A., and Takahashi, K. (2003). Circadian rhythms in human's delta sleep electroencephalogram. *Neuroscience Letters* 344: 205–208.
592. Gradisar, M. and Lack, L. (2004). Relationships between the circadian rhythms of finger temperature, core temperature, sleep latency, and subjective sleepiness. *Journal of Biological Rhythms* 19: 157–163.
593. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C., and Knauer, R. S. (1980). Human sleep: Its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
594. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J., and Van Cauter, E. (1997). Sleepiness, performance, and neuroendocrine function during sleep deprivation: Effects of exposure to bright light or exercise. *Journal of Biological Rhythms* 12: 245–258.
595. Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep–wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
596. Berger, R. J. and Phillips, N. H. (1994). Constant light suppresses sleep and circadian rhythms in pigeons without consequent sleep rebound in darkness. *American Journal of Physiology* 267: R945–R952.
597. Szymczak, J. T. (1987). Distribution of sleep and wakefulness in 24-h light–dark cycles in the juvenile and adult magpie, *Pica pica*. *Chronobiologia* 14: 277–287.
598. Kuwabara, N., Seki, K., and Aoki, K. (1986). Circadian, sleep and brain temperature rhythms in cats under sustained daily light–dark cycles and constant darkness. *Physiology and Behavior* 38: 283–289.
599. Marks, G. A. and Shaffery, J. P. (1996). A preliminary study of sleep in the ferret, *Mustela putorius furo*: A carnivore with an extremely high proportion of REM sleep. *Sleep* 19: 83–93.
600. Daan, S., Beersma, D. G. M., and Borbély, A. A. (1984). Timing of human sleep: Recovery process gated by a circadian pacemaker. *American Journal of Physiology* 246: R161–R178.
601. Dijk, D. J., Beersma, G. M., Daan, S., and Lewy, A. J. (1989). Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *American Journal of Physiology* 256: R106–R111.
602. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M., and Czeisler, C. A. (1992). Short-term memory, alertness and performance: A reappraisal of their relationship to body temperature. *Journal of Sleep Research* 1: 24–29.
603. Dijk, D. J. and Czeisler, C. A. (1994). Paradoxical timing of the circadian rhythm of sleep propensity serves to consolidate sleep and wakefulness in humans. *Neuroscience Letters* 166: 63–68.
604. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A., and Dijk, D. J. (1999). Circadian temperature and melatonin rhythms, sleep, and neurobehavioral function in humans living on a 20-h day. *American Journal of Physiology* 277: R1152–R1163.
605. Klerman, E. B., Boulos, Z., Edgar, D. M., Mistlberger, R. E., and Moore-Ede, M. C. (1999). Circadian and homeostatic influences on sleep in the squirrel monkey: Sleep after sleep deprivation. *Sleep* 22: 45–59.
606. Zeitzer, J. M., Buckmaster, C. L., Parker, K. J., Hauck, C. M., Lyons, D. M., and Mignot, E. (2003). Circadian and homeostatic regulation of hypocretin in a primate model: Implications for the consolidation of wakefulness. *Journal of Neuroscience* 23: 3555–3560.
607. Van Dongen, H. P. A. and Dinges, D. F. (2003). Investigating the interaction between the homeostatic and circadian processes of sleep–wake regulation for the prediction of waking neurobehavioural performance. *Journal of Sleep Research* 12: 181–187.
608. Hull, J. T., Wright, K. P., and Czeisler, C. A. (2003). The influence of subjective alertness and motivation on human performance independent of circadian and homeostatic regulation. *Journal of Biological Rhythms* 18: 329–338.
609. Varkevisser, M. and Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: Validating a self-constructed test battery. *Chronobiology International* 20: 109–121.
610. Horowitz, T. S., Cade, B. E., Wolfe, J. M., and Czeisler, C. A. (2003). Searching night and day: A dissociation of effects of circadian phase and time awake on visual selective attention and vigilance. *Psychological Science* 14: 549–557.

611. Miró, E., Cano, M. C., Espinosa-Fernández, L., and Buela-Casal, G. (2003). Time estimation during prolonged sleep deprivation and its relation to activation measures. *Human Factors* 45: 148–159.
612. Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A., and Cajochen, C. (2004). Circadian and wake-dependent modulation of fastest and slowest reaction times during psychomotor vigilance task. *Physiology and Behavior* 80: 695–701.
613. Phillips, A. J. K., Fulcher, B. D., Robinson, P. A., and Klerman, E. B. (2013). Mammalian test/activity patterns explained by physiologically based modeling. *PLoS Computational Biology* 9: e1003213.
614. Girardier, L. and Stock, M. J. (1983). Mammalian thermogenesis: An introduction. In: Girardier, L. and Stock, M. J. (Eds.), *Mammalian Thermogenesis*. London, U.K.: Chapman and Hall, pp. 1–7.
615. Woods, S. C., Seeley, R. J., Porte, D., and Schwartz, M. W. (1998). Signals that regulate food intake and energy homeostasis. *Science* 280: 1378–1383.
616. Funahashi, H., Takenoys, F., Guan, J. L., Kageyama, H., Yada, T., and Shioda, S. (2003). Hypothalamic neuronal networks and feeding-related peptides involved in the regulation of feeding. *Anatomical Science International* 78: 123–138.
617. Badman, M. K. and Flier, J. S. (2005). The gut and energy balance: Visceral allies in the obesity wars. *Science* 307: 1909–1914.
618. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K., and Shiraishi, T. (1988). Disruption of light–dark cycle of feeding and drinking behavior, and ambulatory activity induced by development of obesity in the Zucker rat. *International Journal of Obesity* 12: 481–490.
619. Drewnowski, A., Grinker, J. A., Gruen, R., and Sullivan, A. C. (1985). Effects of inhibitors of carbohydrate absorption or lipid metabolism on meal patterns of Zucker rats. *Pharmacology, Biochemistry and Behavior* 23: 811–821.
620. Mistlberger, R. E., Lukman, H., and Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: Assessment and intervention. *Appetite* 30: 255–267.
621. Shido, O., Sakurada, S., Kohda, W., and Nagasaka, T. (1994). Day–night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
622. Kittrell, E. M. W. and Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.
623. Ikeda, M. and Inoué, S. (1998). Simultaneous recording of circadian rhythms of brain and intraperitoneal temperatures and locomotor and drinking activities in the rat. *Biological Rhythm Research* 29: 142–150.
624. Francis, A. J. P. and Coleman, G. J. (1988). The effect of ambient temperature cycles upon circadian running and drinking activity in male and female laboratory rats. *Physiology and Behavior* 43: 471–477.
625. Balagura, S. and Coscina, D. V. (1968). Periodicity of food intake in the rat as measured by an operant response. *Physiology and Behavior* 3: 641–643.
626. Alingh Prins, A., de Jong-Nagelsmit, A., Keijser, J., and Strubbe, J. H. (1986). Daily rhythms of feeding in the genetically obese and lean Zucker rats. *Physiology and Behavior* 38: 423–426.
627. Cunningham, J. T., Beltz, T., Johnson, R. F., and Johnson, A. K. (1992). The effects of ibotenate lesions of the median preoptic nucleus on experimentally-induced and circadian drinking behavior in rats. *Brain Research* 580: 325–330.
628. Velasco-Plaza, A., Granda, T. G., and Cachero, M. T. G. (1993). Circadian rhythms of food and water intake and urine excretion in diabetic rats. *Physiology and Behavior* 54: 665–670.
629. Zucker, I. (1971). Light–dark rhythms in rat eating and drinking behavior. *Physiology and Behavior* 6: 115–126.
630. Barney, C. C., Vergoth, C., Renkema, L. A., and Meeuwsen, K. W. (1995). Nycthemeral variation in thermal dehydration-induced thirst. *Physiology and Behavior* 58: 329–335.
631. Williams, T. D., Chambers, J. B., May, O. L., Henderson, R. P., Rashotte, M. E., and Overton, J. M. (2000). Concurrent reductions in blood pressure and metabolic rate during fasting in the unrestrained SHR. *American Journal of Physiology* 278: R255–R262.
632. Strubbe, J. H., Spiteri, N. J., and Alingh Prins, A. J. (1986). Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats. *Physiology and Behavior* 36: 647–651.
633. Witte, K. and Lemmer, B. (1999). Development of inverse circadian blood pressure pattern in transgenic hypertensive TGR(mREN2)27 rats. *Chronobiology International* 16: 293–303.
634. Galbicka, G., Smurthwaite, S., Riggs, R., and Tang, L. W. (1997). Daily rhythms in a complex operant: Targeted percentile shaping of run lengths in rats. *Physiology and Behavior* 62: 1165–1169.
635. De Castro, J. M. (1978). Diurnal rhythms of behavioral effects on core temperature. *Physiology and Behavior* 21: 883–886.
636. Rosenwasser, A. M., Boulos, Z., and Terman, M. (1983). Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiology and Behavior* 30: 353–359.
637. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: Effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
638. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M., and Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
639. Fischette, C. T., Edinger, H. M., and Siegel, A. (1981). Temporary desynchronization among circadian rhythms with lateral fornix ablation. *Brain Research* 229: 85–101.
640. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K., Uchimura, K., and Asano, C. (1992). Advance shift of feeding circadian rhythm induced by obesity progression in Zucker rats. *American Journal of Physiology* 263: R1169–R1175.
641. Jilge, B. (1985). The rhythm of food and water ingestion, faeces excretion and locomotor activity in the guinea pig. *Zeitschrift für Versuchstierkunde* 27: 215–225.
642. Büttner, D. (1992). Social influences on the circadian rhythm of locomotor activity and food intake of guinea pigs. *Journal of Interdisciplinary Cycle Research* 23: 100–112.
643. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A., and Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
644. Gerkema, M. P., Groos, G. A., and Daan, S. (1990). Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. *Journal of Biological Rhythms* 5: 81–95.
645. Edgar, D. M., Kilduff, T. S., Martin, C. E., and Dement, W. C. (1991). Influence of running wheel activity on free-running sleep/wake and drinking circadian rhythms in mice. *Physiology and Behavior* 50: 373–378.

646. Shapiro, C. J. and Weathers, W. W. (1981). Metabolic and behavioral responses of American kestrels to food deprivation. *Comparative Biochemistry and Physiology A* 68: 11–114.
647. Kadono, H., Besch, E. L., and Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
648. Aschoff, J. and von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
649. Houdelier, C., Guyomarc'h, C., Lumineau, S., and Richard, J. P. (2002). Circadian rhythms of oviposition and feeding activity in Japanese quail: Effects of cyclic administration of melatonin. *Chronobiology International* 19: 1107–1119.
650. Lumineau, S. and Guyomarc'h, C. (2000). Circadian rhythm of activity during the annual phases in the European quail, *Coturnix coturnix*. *Comptes Rendus de l'Académie des Sciences* 323: 793–799.
651. Guyomarc'h, C., Lumineau, S., and Richard, J. P. (1998). Circadian rhythm of activity in Japanese quail in constant darkness: Variability of clarity and possibility of selection. *Chronobiology International* 15: 219–230.
652. Lumineau, S. and Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
653. Ebihara, S. and Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
654. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
655. Hau, M. and Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
656. Heigl, S. and Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
657. Reierth, E. and Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
658. Kumar, V. and Gwinner, E. (2005). Pinealectomy shortens resynchronisation times of house sparrow (*Passer domesticus*) circadian rhythms. *Naturwissenschaften* 92: 419–422.
659. Casey, T. M., Joos, B., Fitzgerald, T. D., Yurlina, M. E., and Young, P. A. (1988). Synchronized group foraging, thermoregulation, and growth of Eastern tent caterpillars in relation to microclimate. *Physiological Zoology* 61: 372–377.
660. Ryer, C. H. and Boehlert, G. W. (1983). Feeding chronology, daily ration, and the effects of temperature upon gastric evacuation in the pipefish, *Syngnathus fuscus*. *Environmental Biology of Fishes* 9: 301–306.
661. Chen, W. M., Naruse, M., and Tabata, M. (2002). Circadian rhythms and individual variability of self-feeding activity in groups of rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquaculture Research* 33: 491–500.
662. Sánchez-Vázquez, F. J., Iigo, M., Madrid, J. A., and Tabata, M. (2000). Pinealectomy does not affect the entrainment to light nor the generation of the circadian demand-feeding rhythms of rainbow trout. *Physiology and Behavior* 69: 455–461.
663. Bovet, J. (1994). Missed opportunities in the study of activity and body temperature of beaver (*Castor canadensis*). *Canadian Journal of Zoology* 72: 567–569.
664. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
665. Jilge, B. (1991). Restricted feeding: A nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
666. Bobbert, A. C. and Riethoven, J. J. (1991). Feedback in the rabbit's central circadian system, revealed by the changes in its free-running food intake pattern induced by blinding, cervical sympathectomy, pinealectomy, and melatonin administration. *Journal of Biological Rhythms* 6: 263–278.
667. Araki, C. T., Nakamura, R. M., and Kam, L. W. G. (1987). Diurnal temperature sensitivity of dairy cattle in a naturally cycling environment. *Journal of Thermal Biology* 12: 23–26.
668. Xia, C., Liu, W., Xu, W., Yang, W., Xu, F., and Blank, D. (2013). Diurnal time budgets and activity rhythms of the Asiatic wild ass *Equus hemionus* in Xinjiang, Western China. *Pakistan Journal of Zoology* 45: 1241–1248.
669. Rauth-Widmann, B., Fuchs, E., and Erkert, H. G. (1996). Infradian alteration of circadian rhythms in owl monkeys (*Aotus lemurinus griseimembra*): An effect of estrous? *Physiology and Behavior* 59: 11–18.
670. Hoban, T. M., Levine, A. H., Shane, R. B., and Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
671. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
672. Moore-Ede, M. C., Kass, D. A., and Herd, J. A. (1977). Transient circadian internal desynchronization after light–dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
673. Fuller, C. A. and Edgar, D. M. (1986). Effects of light intensity on the circadian temperature and feeding rhythms in the squirrel monkey. *Physiology and Behavior* 36: 687–691.
674. Boulos, Z., Frim, D. M., Dewey, L. K., and Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
675. Sulzman, F. M., Fuller, C. A., and Moore-Ede, M. C. (1977). Feeding time synchronizes primate circadian rhythms. *Physiology and Behavior* 18: 775–779.
676. Barger, L. K., Hoban-Higgins, T. M., and Fuller, C. A. (2008). Assessment of circadian rhythms throughout the menstrual cycle of female rhesus monkeys. *American Journal of Primatology* 70: 19–25.
677. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
678. Green, J., Pollak, C. P., and Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
679. De Castro, J. M. (2001). Heritability of diurnal changes in food intake in free-living humans. *Nutrition* 17: 713–720.
680. De Castro, J. M. (1987). Circadian rhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology and Behavior* 40: 437–446.
681. Scheer, F. A. J., Morris, C. J., and Shea, S. A. (2013). The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. *Obesity* 21: 421–423.
682. Setas, C. D., Pinhão, S. C., Carvalho, D. M., Correia, F. C., and Medina, J. L. (2004). Avaliação da ingestão energética circadiana num grupo de trabalhadores da segurança social do Porto. *Acta Médica Portuguesa* 17: 417–426.

683. De Graaf, C., Jas, P., van der Kooy, K., and Leenen, R. (1993). Circadian rhythms of appetite at different stages of a weight loss programme. *International Journal of Obesity* 17: 521–526.
684. Waterhouse, J., Kao, S., Edwards, B., Weinert, D., Atkinson, G., and Reilly, T. (2005). Transient changes in the pattern of food intake following a simulated time-zone transition to the East across eight time zones. *Chronobiology International* 22: 299–319.
685. Strubbe, J. H. and van Dijk, G. (2002). The temporal organization of ingestive behaviour and its interaction with regulation of energy balance. *Neuroscience and Biobehavioral Reviews* 26: 485–498.
686. Westman, W. and Geiser, F. (2004). The effect of metabolic fuel availability on thermoregulation and torpor in a marsupial hibernator. *Journal of Comparative Physiology B* 174: 49–57.
687. Geiser, F., Holloway, J. C., and Körtner, G. (2007). Thermal biology, torpor and behaviour in sugar gliders: A laboratory-field comparison. *Journal of Comparative Physiology B* 177: 495–501.
688. Tannenbaum, M. G. and Pivorun, E. B. (1987). Differential effect of food restriction on the induction of daily torpor in *Peromyscus maniculatus* and *P. leucopus*. *Journal of Thermal Biology* 12: 159–162.
689. Heldmaier, G., Steinlechner, S., Ruf, T., Wiesinger, H., and Klingenpor, M. (1989). Photoperiod and thermoregulation in vertebrates: Body temperature rhythms and thermogenic acclimation. *Journal of Biological Rhythms* 4: 251–265.
690. Ruby, N. F., Ibuka, N., Barnes, B. M., and Zucker, I. (1989). Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *American Journal of Physiology* 257: R210–R215.
691. Ellison, G. T. H. and Skinner, J. D. (1992). The influence of ambient temperature on spontaneous daily torpor in pouched mice (*Saccostomus campestris*: Rodentia: Cricetidae) from southern Africa. *Journal of Thermal Biology* 17: 25–31.
692. Prinzinger, R., Schäfer, T., and Schuchmann, K. L. (1992). Energy metabolism, respiratory quotient and breathing parameters in two convergent small bird species: The fork-tailed sunbird and the Chilean hummingbird. *Journal of Thermal Biology* 17: 71–79.
693. Geiser, F. and Masters, P. (1994). Torpor in relation to reproduction in the mulgara, *Dasycercus cristicauda* (Dasyuridae: Marsupialia). *Journal of Thermal Biology* 19: 33–40.
694. Holloway, J. C. and Geiser, F. (1996). Reproductive status and torpor of the marsupial *Sminthopsis crassicaudata*: Effect of photoperiod. *Journal of Thermal Biology* 21: 373–380.
695. Ortmann, S. and Heldmaier, G. (1997). Spontaneous daily torpor in Malagasy mouse lemurs. *Naturwissenschaften* 84: 28–32.
696. Turbill, C., Law, B. S., and Geiser, F. (2003). Summer torpor in a free-ranging bat from subtropical Australia. *Journal of Thermal Biology* 28: 223–226.
697. Turbill, C., Körtner, G., and Geiser, F. (2003). Natural use of heterothermy by a small, tree-roosting bat during summer. *Physiological and Biochemical Zoology* 76: 868–876.
698. Cooper, C. E. and Withers, P. C. (2004). Patterns of body temperature variation and torpor in the numbat, *Myrmecobius fasciatus* (Marsupialia: Myrmecobiidae). *Journal of Thermal Biology* 29: 277–284.
699. MacMillen, R. E. and Trost, C. H. (1967). Nocturnal hypothermia in the Inca dove, *Scardafella inca*. *Comparative Biochemistry and Physiology* 23: 243–253.
700. Thouzeau, C., Duchamp, C., and Handrich, Y. (1999). Energy metabolism and body temperature of barn owls fasting in the cold. *Physiological and Biochemical Zoology* 72: 170–178.
701. Rashotte, M. E. and Henderson, D. (1988). Coping with rising food costs in a closed economy: Feeding behavior and nocturnal hypothermia in pigeons. *Journal of the Experimental Analysis of Behavior* 50: 441–456.
702. Phillips, N. H. and Berger, R. J. (1991). Regulation of body temperature, metabolic rate, and sleep in fasting pigeons diurnally infused with glucose or saline. *Journal of Comparative Physiology B* 161: 311–318.
703. Ostheim, J. (1992). Coping with food-limited conditions: Feeding behavior, temperature preference, and nocturnal hypothermia in pigeons. *Physiology and Behavior* 51: 353–361.
704. Hohtola, E., Hissa, R., Pyörnilä, A., Rintamäki, H., and Saarela, S. (1991). Nocturnal hypothermia in fasting Japanese quail: The effect of ambient temperature. *Physiology and Behavior* 49: 563–567.
705. Prinzinger, R., Schleucher, E., and Preßmar, A. (1992). Langzeittelemetrie der Körpertemperatur mit synchroner Bestimmung des Energiestoffwechsels beim Blaunackenmausvogel (*Urocolius macrourus*) unter Normal- und Lethargiebedingungen (Torpor). *Journal für Ornithologie* 133: 446–450.
706. McKechnie, A. E. and Lovegrove, B. G. (2003). Facultative hypothermic responses in an Afrotropical arid-zone passerine, the red-headed finch (*Amadina erythrocephala*). *Journal of Comparative Physiology B* 173: 339–346.
707. Hudson, J. W. (1965). Temperature regulation and torpidity in the pigmy mouse, *Baiomys taylori*. *Physiological Zoology* 38: 243–254.
708. Nestler, J. R. (1990). Relationships between respiratory quotient and metabolic rate during entry to and arousal from daily torpor in deer mice (*Peromyscus maniculatus*). *Physiological Zoology* 63: 504–515.
709. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes and Development* 14: 2950–2961.
710. Satoh, Y., Kawai, H., Kudo, N., Kawashima, Y., and Mitsumoto, A. (2006). Time-restricted feeding entrains daily rhythms of energy metabolism in mice. *American Journal of Physiology* 290: R1276–R1283.
711. Zhang, J., Kaasik, K., Blackburn, M. R., and Lee, C. C. (2006). Constant darkness is a circadian metabolic signal in mammals. *Nature* 439: 340–343.
712. Pecoraro, N., Gomez, F., Laugero, K., and Dallman, M. F. (2002). Brief access to sucrose engages food-entrainable rhythms in food-deprived rats. *Behavioral Neuroscience* 116: 757–776.
713. Nagashima, K., Nakai, S., Matsue, K., Konishi, M., Tanaka, M., and Kanosue, K. (2003). Effects of fasting on thermoregulatory processes and the daily oscillations in rats. *American Journal of Physiology* 284: R1486–R1493.
714. Smith, S. E., Ramos, F. A., Refinetti, R., Farthing, J. P., and Paterson, P. G. (2013). Protein-energy malnutrition induces an aberrant acute-phase response and modifies the circadian rhythm of core temperature. *Applied Physiology, Nutrition and Metabolism* 38: 844–853.
715. Perret, M. and Aujard, F. (2001). Daily hypothermia and torpor in a tropical primate: Synchronization by 24-h light–dark cycle. *American Journal of Physiology* 281: R1925–R1933.

716. Génin, F. and Perret, M. (2003). Daily hypothermia in captive grey mouse lemurs (*Microcebus murinus*): Effects of photoperiod and food restriction. *Comparative Biochemistry and Physiology B* 136: 71–81.
717. Piccione, G., Caola, G., and Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
718. Piccione, G., Caola, G., and Refinetti, R. (2003). Circadian rhythms of body temperature and liver function in fed and food-deprived goats. *Comparative Biochemistry and Physiology A* 134: 563–572.
719. Bouâouda, H., Achâaban, M. R., Ouassat, M., Oukassou, M., Piro, M., Challet, E., El Allali, K., and Pévet, P. (2014). Daily regulation of body temperature rhythm in camel (*Camelus dromedarius*) exposed to experimental desert conditions. *Physiological Reports* 2: e12151.
720. Ruby, N. F. and Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *American Journal of Physiology* 263: R353–R362.
721. Paul, M. J., Kauffman, A. S., and Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
722. Heldmaier, G. and Ruf, T. (1992). Body temperature and metabolic rate during natural hypothermia in endotherms. *Journal of Comparative Physiology B* 162: 696–706.
723. Pivorun, E. B. (1976). A biotelemetry study of the thermoregulatory patterns of *Tamias striatus* and *Eutamias minimus* during hibernation. *Comparative Biochemistry and Physiology A* 53: 265–271.
724. Geiser, F. (1988). Reduction of metabolism during hibernation and daily torpor in mammals and birds: Temperature effect or physiological inhibition? *Journal of Comparative Physiology B* 158: 25–37.
725. Song, X., Körtner, G., and Geiser, F. (1995). Reduction of metabolic rate and thermoregulation during daily torpor. *Journal of Comparative Physiology B* 165: 291–297.
726. Zimmer, M. B. and Milsom, W. K. (2001). Effects of changing ambient temperature on metabolic, heart, and ventilation rates during steady state hibernation in golden-mantled ground squirrels (*Spermophilus lateralis*). *Physiological and Biochemical Zoology* 74: 714–723.
727. Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M., and Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* 293: 2248–2251.
728. Wilz, M. and Heldmaier, G. (2000). Comparison of hibernation, estivation and daily torpor in the edible dormouse, *Glis glis*. *Journal of Comparative Physiology B* 170: 511–521.
729. Cooper, C. E. and Geiser, F. (2008). The "minimal boundary curve for endothermy" as a predictor of heterothermy in mammals and birds: A review. *Journal of Comparative Physiology B* 178: 1–8.
730. Heldmaier, G. (2011). Life on low flame in hibernation. *Science* 331: 866–867.
731. Ohtake, M., Bray, G. A., and Azukizawa, M. (1977). Studies on hypothermia and thyroid function in the obese (ob/ob) mouse. *American Journal of Physiology* 233: R110–R115.
732. Trayhurn, P. and James, W. P. (1978). Thermoregulation and non-shivering thermogenesis in the genetically obese (ob/ob) mouse. *Pflügers Archiv* 373: 189–193.
733. Batt, R. A. and Hambi, M. (1982). Development of hypothermia in obese mice (genotype ob/ob). *International Journal of Obesity* 6: 391–397.
734. Wilson, L. M., Currie, P. J., and Gilson, T. L. (1991). Thermal preference behavior in preweaning genetically-obese (ob/ob) and lean (+/?, +/+) mice. *Physiology and Behavior* 50: 155–160.
735. Piccione, G., Giudice, E., Fazio, F., and Refinetti, R. (2011). Association between obesity and reduced body temperature in dogs. *International Journal of Obesity* 35: 1011–1018.
736. Heikens, M. J., Gorbach, A. M., Eden, H. S., Savastano, D. M., Chen, K. Y., Skarulis, M. C., and Yanovski, J. A. (2011). Core body temperature in obesity. *American Journal of Clinical Nutrition* 93: 963–967.
737. Hoffman, M. E., Rodriguez, S. M., Zeiss, D. M., Wachsberg, K. N., Kushner, R. F., Landsberg, L., and Linsenmeier, R. A. (2012). 24-hour core temperature in obese and lean men and women. *Obesity* 20: 1585–1590.
738. Grimaldi, D., Provini, F., Pierangeli, G., Mazzella, N., Zamboni, G., Marchesini, G., and Cortelli, P. (2015). Evidence of a diurnal thermogenic handicap in obesity. *Chronobiology International* 32: 299–302.
739. Refinetti, R. (1989). Body size and metabolic rate in the laboratory rat. *Experimental Biology* 48: 291–294.
740. Sarrus, P. and Rameaux, J. F. (1838). Rapport sur un mémoire adressé à l'Académie royale de médecine. *Bulletin de L'Académie Royale de Médecine* 3: 1094–1100.
741. Regnault, V. and Reiset, J. (1849). Recherches chimiques sur la respiration des animaux des diverses classes. *Annales de Chimie et de Physique, Series 3* 26: 299–519.
742. Richet, C. (1884). Le calorimètre a siphon et la production de chaleur. *Comptes Rendus des Séances de la Société de Biologie de Paris* 36: 707–715.
743. Kleiber, M. (1947). Body size and metabolic rate. *Physiological Reviews* 27: 511–541.
744. Kayser, C. and Heusner, A. (1964). Étude comparative de métabolisme énergétique dans la série animale. *Journal de Physiologie* 56: 489–524.
745. Lasiewski, R. C. and Dawson, W. R. (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. *Condor* 69: 13–23.
746. Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *Journal of Applied Physiology* 22: 453–460.
747. Thonney, M. L., Touchberry, R. W., Goodrich, R. D., and Meiske, J. C. (1976). Intraspecies relationship between fasting heat production and body weight. *Journal of Animal Science* 43: 692–704.
748. Poczopko, P. (1979). Metabolic rate and body size relationships in adult and growing homeotherms. *Acta Theriologica* 24: 125–136.
749. Bartels, H. (1982). Metabolic rate of mammals equals the 0.75 power of their body weight. In: Gärtner, K., Hackbarth, H., and Stolte, H. (Eds.), *Research Animals and Concepts of Applicability to Clinical Medicine*. Basel, Switzerland: S. Karger, pp. 1–11.
750. Feldman, H. A. and McMahon, T. A. (1983). The 3/4 mass exponent for energy metabolism is not a statistical artifact. *Respiration Physiology* 52: 149–163.
751. Wieser, W. (1984). A distinction must be made between the ontogeny and the phylogeny of metabolism in order to understand the mass exponent of energy metabolism. *Respiration Phsyiology* 55: 1–9.
752. Heusner, A. A. (1984). Biological similitude: Statistical and functional relationships in comparative physiology. *American Journal of Physiology* 246: R839–R845.

753. Calder, W. A. (1987). Scaling energetics of homeothermic vertebrates: An operational allometry. *Annual Review of Physiology* 49: 107–120.
754. Spatz, H. C. (1991). Circulation, metabolic rate, and body size in mammals. *Journal of Comparative Physiology B* 161: 231–236.
755. West, G. B., Brown, J. H., and Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* 276: 122–126.
756. West, G. B., Brown, J. H., and Enquist, B. J. (1999). The fourth dimension of life: Fractal geometry and allometric scaling of organisms. *Science* 284: 1677–1679.
757. White, C. R. and Seymour, R. S. (2003). Mammalian basal metabolic rate is proportional to body mass$^{2/3}$. *Proceedings of the National Academy of Sciences United States America* 100: 4046–4049.
758. Lefèvre, J. and Auguet, A. (1931). Le problème des relations entre les chaleurs du travail et du repos. *Comptes Rendus de l'Académie des Sciences* 192: 819–821.
759. Margaria, R., Cerretelli, P., Aghemo, P., and Sassi, G. (1963). Energy cost of running. *Journal of Applied Physiology* 18: 367–370.
760. Taylor, C. R., Schmidt-Nielsen, K., and Raab, J. L. (1970). Scaling of energetic cost of running to body size in mammals. *American Journal of Physiology* 219: 1104–1107.
761. Saltin, B., Gagge, A. P., and Stolwijk, J. A. J. (1970). Body temperature and sweat during thermal transients caused by exercise. *Journal of Applied Physiology* 28: 318–327.
762. Wang, L. C. H. (1980). Modulation of maximum thermogenesis by feeding in the white rat. *Journal of Applied Physiology* 49: 975–978.
763. Forsum, E., Hillman, P. E., and Nesheim, M. C. (1981). Effect of energy restriction on total heat production, basal metabolic rate, and specific dynamic action of food in rats. *Journal of Nutrition* 111: 1691–1697.
764. Welle, S., Lilavivat, U., and Campbell, R. G. (1981). Thermic effect of feeding in man: Increased plasma norepinephrine levels following glucose but not protein or fat consumption. *Metabolism* 30: 953–958.
765. Rothwell, N. J. and Stock, M. J. (1983). Acute effects of fat and carbohydrate on metabolic rate in normal, cold-acclimated and lean and obese (fa/fa) Zucker rats. *Metabolism* 32: 371–376.
766. Glick, Z., Wickler, S. J., Stern, J. S., and Horwitz, B. A. (1984). Regional blood flow in rats after a single low-protein, high-carbohydrate test meal. *American Journal of Physiology* 247: R160–R166.
767. Maehlum, S., Grandmontage, M., Newsholme, E. A., and Sejersted, O. M. (1986). Magnitude and duration of excess postexercise oxygen consumption in healthy young subjects. *Metabolism* 35: 425–429.
768. Poehlman, E. T., Melby, C. L., and Badylak, S. F. (1988). Resting metabolic rate and postprandial thermogenesis in highly trained and untrained males. *American Journal of Clinical Nutrition* 47: 793–798.
769. Hill, J. O., Anderson, J. C., Lin, D., and Yakubu, F. (1988). Effects of meal frequency on energy utilization in rats. *American Journal of Physiology* 255: R616–R621.
770. Romom, M., Edme, J. J., Boulenguez, C., Lescroart, J. L., and Frimat, P. (1993). Circadian variation of diet-induced thermogenesis. *American Journal of Clinical Nutrition* 57: 476–480.
771. Maffeis, C., Schutz, Y., Grezzani, A., Provera, S., Piacentini, G., and Tatò, L. (2001). Meal-induced thermogenesis and obesity: Is a fat meal a risk factor for fat gain in children? *Journal of Clinical Endocrinology and Metabolism* 86: 214–219.
772. Diamond, P., Brondel, L., and LeBlanc, J. (1985). Palatability and postprandial thermogenesis in dogs. *American Journal of Physiology* 248: E75–E79.
773. LeBlanc, J. and Diamond, P. (1986). Effect of meal size and frequency on postprandial thermogenesis in dogs. *American Journal of Physiology* 250: E144–E147.
774. Heymsfield, S. B., Hill, J. O., Evert, M., Casper, K., and DiGirolamo, M. (1987). Energy expenditure during continuous intragastric infusion of fuel. *American Journal of Clinical Nutrition* 45: 526–533.
775. Diamond, P. and LeBlanc, J. (1987). Hormonal control of postprandial thermogenesis in dogs. *American Journal of Physiology* 253: E521–E529.
776. Allard, M. and LeBlanc, J. (1988). Effects of cold acclimation, cold exposure, and palatability on postprandial thermogenesis in rats. *International Journal of Obesity* 12: 169–178.
777. Arnold, J., Shipley, K. A., Scott, N. A., Little, R. A., and Irving, M. H. (1989). Thermic effect of parenteral nutrition in septic and nonseptic individuals. *American Journal of Clinical Nutrition* 50: 853–860.
778. LeBlanc, J., Mercier, I., and Nadeau, A. (1993). Components of postprandial thermogenesis in relation to meal frequency in humans. *Canadian Journal of Physiology and Pharmacology* 71: 879–883.
779. Grande, F., Anderson, J. T., and Keys, A. (1958). Changes of basal metabolic rate in man in semistarvation and refeeding. *Journal of Applied Physiology* 12: 230–238.
780. Bray, G. A. (1969). Effect of caloric restriction on energy expenditure in obese patients. *Lancet* 7617: 397–398.
781. Boyle, P. C., Storlien, L. H., and Keesey, R. E. (1978). Increased efficiency of food utilization following weight loss. *Physiology and Behavior* 21: 261–264.
782. Dauncey, M. J. (1980). Metabolic effects of altering the 24-h energy intake in man using direct and indirect calorimetry. *British Journal of Nutrition* 43: 257–269.
783. Boyle, P. C., Storlien, L. H., Harper, A. E., and Keesey, R. E. (1981). Oxygen consumption and locomotor activity during restricted feeding and realimentation. *American Journal of Physiology* 241: R392–R397.
784. Gleeson, M., Brown, J. F., and Waring, J. J. (1982). The effects of physical exercise on metabolic rate and dietary-induced thermogenesis. *British Journal of Nutrition* 47: 173–181.
785. Stock, M. J. and Rothwell, N. J. (1982). Evidence for diet-induced thermogenesis in hyperphagic cafeteria-fed rats. *Proceedings of the Nutrition Society* 41: 133–135.
786. Verboeket-van-de-Venne, W. P. H. G., Westerterp, K. R., Hermans-Limpens, T. J. F. M. B., de Graaf, C., van het Hof, K. H., and Weststrate, J. A. (1996). Long-term effects of consumption of full-fat or reduced-fat products in healthy non-obese volunteers: Assessment of energy expenditure and substrate oxidation. *Metabolism* 45: 1004–1010.
787. Levine, J. A., Eberhardt, N. L., and Jensen, M. D. (1999). Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 283: 212–214.
788. Bachman, E. S., Dhillon, H., Zhang, C. Y., Cinti, S., Bianco, A. C., Kobilka, B. K., and Lowell, B. B. (2002). Beta-AR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297: 843–845.
789. Rothwell, N. J. and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281: 31–35.
790. Himms-Hagen, J., Triandafillou, J., and Gwilliam, C. (1981). Brown adipose tissue of cafeteria-fed rats. *American Journal of Physiology* 241: E116–E120.

791. Trayhurn, P., Jones, P. M., McGuckin, M. M., and Goodbody, A. E. (1982). Effects of overfeeding on energy balance and brown fat thermogenesis in obese (*ob/ob*) mice. *Nature* 295: 323–325.
792. Triandafillou, J. and Himms-Hagen, J. (1983). Brown adipose tissue in genetically obese (*fa/fa*) rats: Response to cold and diet. *American Journal of Physiology* 244: E145–E150.
793. Glick, Z., Wickler, S. J., Stern, J. S., and Horwitz, B. A. (1984). Blood flow into brown fat of rats is greater after a high carbohydrate than after a high fat test meal. *Journal of Nutrition* 114: 1934–1939.
794. Glick, Z., Wu, S. Y., Lupien, J., Reggio, R., Bray, G. A., and Fisher, D. A. (1985). Meal-induced brown fat thermogenesis and thyroid hormone metabolism in rats. *American Journal of Physiology* 249: E519–E524.
795. Himms-Hagen, J. (1990). Brown adipose tissue thermogenesis: Interdisciplinary studies. *FASEB Journal* 4: 2890–2898.
796. Tulp, O. L. and Buck, C. L. (1986). Caffeine and ephedrine stimulated thermogenesis in LA-corpulent rats. *Comparative Biochemistry and Physiology C* 85: 17–19.
797. Bailey, C. J., Thornburn, C. C., and Flatt, P. R. (1986). Effects of ephedrine and atenolol on the development of obesity and diabetes in *ob/ob* mice. *General Pharmacology* 17: 243–246.
798. Dulloo, A. G. and Miller, D. S. (1987). Aspirin as a promoter of ephedrine-induced thermogenesis: Potential use in the treatment of obesity. *American Journal of Clinical Nutrition* 45: 564–569.
799. Dulloo, A. G. and Miller, D. S. (1987). Prevention of genetic *fa/fa* obesity with an ephedrine-methylxanthines thermogenic mixture. *American Journal of Physiology* 252: R507–R513.
800. Dulloo, A. G. and Miller, D. S. (1987). Reversal of obesity in the genetically obese fa/fa Zucker rat with an ephedrine/methylxanthines thermogenic mixture. *Journal of Nutrition* 117: 383–389.
801. Aschoff, J. (1964). Survival value of diurnal rhythms. *Symposia of the Zoological Society of London* 13: 79–98.
802. Aschoff, J., von Goetz, C., Wildbruber, C., and Wever, R. (1986). Meal timing in humans during isolation without time cues. *Journal of Biological Rhythms* 1: 151–162.
803. Aschoff, J. (1993). On the relationship between motor activity and the sleep-wake cycle in humans during temporal isolation. *Journal of Biological Rhythms* 8: 33–46.
804. Aschoff, J. (1994). The timing of defecation within sleep–wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
805. Oklejewicz, M., Hut, R. A., Daan, S., Loudon, A. S. I., and Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.
806. Refinetti, R. and Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
807. Osiel, S., Golombek, D. A., and Ralph, M. R. (1998). Conservation of locomotor behavior in the golden hamster: Effects of light cycle and a circadian period mutation. *Physiology and Behavior* 65: 123–131.
808. Conrad, M. C. and Miller, A. T. (1956). Age changes in body size, body composition and basal metabolism. *American Journal of Physiology* 186: 207–210.
809. Chiu, C. C. and Hsieh, A. C. L. (1960). A comparative study of four means of expressing the metabolic rate of rats. *Journal of Physiology* 150: 694–706.
810. Heusner, A. and Harmelin, M. L. (1963). Relation entre le poids et la consommation d'oxygène. I. Etude chez le rat. *Comptes Rendus de Séances de la Société de Biologie de Strasbourg* 157: 376–380.
811. Refinetti, R. (1989). Computation of the effective body mass for metabolic studies of lean and obese rats. *Metabolism* (Erratum: vol. 39, p. 215) 38: 763–766.
812. Even, P. C., Rolland, V., Roseau, S., Bouthegourd, J. C., and Tomé, D. (2001). Prediction of basal metabolism from organ size in the rat: Relationship to strain, feeding, age, and obesity. *American Journal of Physiology* 280: R1887–R1896.
813. Refinetti, R. (2007). Absence of circadian and photoperiodic conservation of energy expenditure in three rodent species. *Journal of Comparative Physiology B* 177: 309–318.
814. Rubal, A., Choshniak, I., and Haim, A. (1992). Daily rhythms of metabolic rate and body temperature of two murids from extremely different habitats. *Chronobiology International* 9: 341–349.
815. Lovegrove, B. G. and Heldmaier, G. (1994). The amplitude of circadian body temperature rhythms in three rodents (*Aethomys namaquensis*, *Thallomys paedulcus* and *Cryptomys damarensis*) along the arboreal-subterranean gradient. *Australian Journal of Zoology* 42: 65–78.
816. Körtner, G. and Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiology and Behavior* 58: 31–36.
817. Gebczynski, A. K. and Taylor, J. R. E. (2004). Daily variation of body temperature, locomotor activity and maximum nonshivering thermogenesis in two species of small rodents. *Journal of Thermal Biology* 29: 123–131.
818. McElhinny, T. L., Smale, L., and Holekamp, K. E. (1997). Patterns of body temperature, activity, and reproductive behavior in a tropical murid rodent, *Arvicanthis niloticus*. *Physiology and Behavior* 62: 91–96.
819. Blanchong, J. A., McElhinny, T. L., Mahoney, M. M., and Smale, L. (1999). Nocturnal and diurnal rhythms in the unstriped Nile rate, *Arvicanthis niloticus*. *Journal of Biological Rhythms* 14: 364–377.
820. Rose, R. W., Swain, R., and Bryant, S. L. (1990). Body temperature: Rhythm and regulation in the Tasmanian bettong (*Bettongia gaimardi*) (Marsupialia: Potoroidae). *Comparative Biochemistry and Physiology A* 97: 573–576.
821. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A., and Littledike, E. T. (1990). Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. *Journal of Animal Science* 68: 2658–2665.
822. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L., and Bitman, J. (1999). Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. *Domestic Animal Endocrinology* 16: 41–55.
823. Refinetti, R. and Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.
824. Jessen, C. and Kuhnen, G. (1996). Seasonal variations of body temperature in goats living in an outdoor environment. *Journal of Thermal Biology* 21: 197–204.
825. Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D., and Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflügers Archiv* 436: 659–666.
826. Winget, C. M., Card, D. H., and Hetherington, N. W. (1968). Circadian oscillations of deep-body temperature and heart rate in a primate (*Cebus albafrons*). *Aerospace Medicine* 39: 350–353.
827. Oshima, I. and Ebihara, S. (1988). The measurement and analysis of circadian locomotor activity and body temperature rhythms by a computer-based system. *Physiology and Behavior* 43: 115–119.

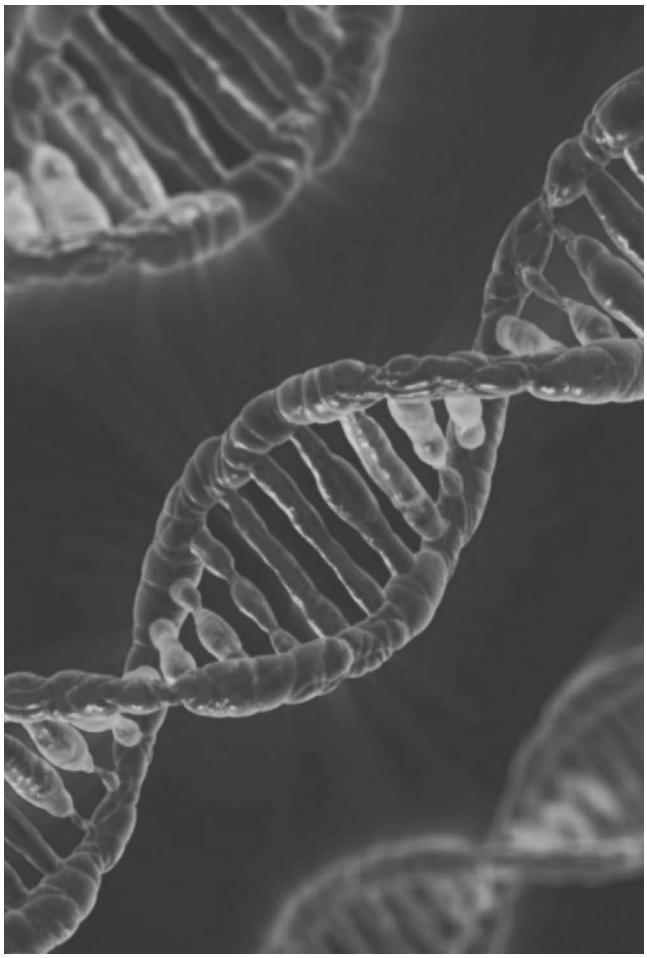
828. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
829. Harlow, H. J., Phillips, J. A., and Ralph, C. L. (1982). Circadian rhythms and the effects of exogenous melatonin in the nine-banded armadillo, *Dasypus novemcinctus*: A mammal lacking a distinct pineal gland. *Physiology and Behavior* 29: 307–313.
830. Gemmell, R. T., Turner, S. J., and Krause, W. J. (1997). The circadian rhythm of body temperature of four marsupials. *Journal of Thermal Biology* 22: 301–307.
831. Piccione, G., Caola, G., and Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
832. Fowler, P. A. and Racey, P. A. (1990). Daily and seasonal cycles of body temperature and aspects of heterothermy in the hedgehog *Erinaceus europaeus*. *Journal of Comparative Physiology B* 160: 299–307.
833. Johnson, R. F. and Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
834. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., and Johnson, R. F. (1987). A two-peak circadian system in body temperature and activity in the domestic cat, *Felis catus*. *Journal of Thermal Biology* 12: 27–37.
835. Michels, H., Herremans, M., and Decuypere, E. (1985). Light–dark variations of oxygen consumption and subcutaneous temperature in young *Gallus domesticus*: Influence of ambient temperature and depilation. *Journal of Thermal Biology* 10: 13–20.
836. Winget, C. M. and Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
837. Riccio, A. P. and Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
838. Wever, R. and Zink, R. A. (1971). Fortlaufende Registrierung der Rectaltemperatur des Menschen unter extremen Bedingungen. *Pflügers Archiv* 327: 186–190.
839. Souetre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B., and Darcourt, G. (1988). Twenty-four-hour profiles of body temperature and plasma TSH in bipolar patients during depression and during remission and in normal control subjects. *American Journal of Psychiatry* 145: 1133–1137.
840. Kleitman, N. and Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
841. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N., and Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.
842. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J. et al. (2001). Sleep, performance, circadian rhythms, and light–dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
843. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
844. Barrett, J., Lack, L., and Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
845. Pollak, C. P. and Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
846. Cagnacci, A., Arangino, S., Tuveri, F., Paoletti, A. M., and Volpe, A. (2002). Regulation of the 24-h body temperature rhythm of women in luteal phase: Role of gonadal steroids and prostaglandins. *Chronobiology International* 19: 721–730.
847. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K., and Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
848. Kattapong, K. R., Fogg, L. F., and Eastman, C. I. (1995). Effect of sex, menstrual phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International* 12: 257–266.
849. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
850. Scales, W. E., Vander, A. J., Brown, M. B., and Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
851. Lee, Y. H. and Tokura, H. (1993). Circadian rhythm of human rectal and skin temperatures under the influences of three different kinds of clothing. *Journal of Interdisciplinary Cycle Research* 24: 33–42.
852. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T., and Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
853. Takasu, N., Nigi, H., and Tokura, H. (2002). Effects of diurnal bright/dim light intensity on circadian core temperature and activity rhythms in the Japanese macaque. *Japanese Journal of Physiology* 52: 573–578.
854. Tapp, W. N. and Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
855. Honnebier, M. B. O. M., Jenkins, S. L., and Nathanielsz, P. W. (1992). Circadian timekeeping during pregnancy: Endogenous phase relationships between maternal plasma hormones and the maternal body temperature rhythm in pregnant rhesus monkeys. *Endocrinology* 131: 2051–2058.
856. Simpson, S. and Galbraith, J. J. (1906). Observations on the normal temperature of the monkey and its diurnal variation, and on the effect of changes in the daily routine on this variation. *Transactions of the Royal Society of Edinburgh* 45: 65–104.
857. McCarron, H. C. K., Buffenstein, R., Fanning, F. D., and Dawson, T. J. (2001). Free-ranging heart rate, body temperature and energy metabolism in eastern grey kangaroos (*Macropus giganteus*) and red kangaroos (*Macropus rufus*) in the arid regions of South East Australia. *Journal of Comparative Physiology B* 171: 401–411.
858. Hayes, S. R. (1976). Daily activity and body temperature of the southern woodchuck, *Marmota monax monax*, in northwestern Arkansas. *Journal of Mammalogy* 57: 291–299.
859. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W., and Bacon, W. L. (2000). Circadian rhythm of the preovulatory surge of luteinizing hormone and its relationships to rhythms of body temperature and locomotor activity in turkey hens. *Biology of Reproduction* 62: 1452–1458.
860. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
861. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
862. Conn, C. A., Borer, K. T., and Kluger, M. J. (1990). Body temperature rhythm and response to pyrogen in exercising and sedentary hamsters. *Medicine and Science in Sports and Exercise* 22: 636–642.

863. Perret, M., Aujard, F., Séguy, M., and Schilling, A. (2003). Olfactory bulbectomy modifies photic entrainment and circadian rhythms of body temperature and locomotor activity in a nocturnal primate. *Journal of Biological Rhythms* 18: 392–401.
864. Kramer, K., Voss, H. P., Grimbergen, J., and Bast, A. (1998). Circadian rhythms of heart rate, body temperature, and locomotor activity in freely moving mice measured with radio telemetry. *Lab Animal* 27(8): 23–26.
865. Leon, L. R., Walker, L. D., DuBose, D. A., and Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: Impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
866. Irizarry, R. A., Tankersley, C., Frank, R., and Flanders, S. (2001). Assessing homeostasis through circadian patterns. *Biometrics* 57: 1228–1237.
867. Keeney, A. J., Hogg, S., and Marsden, C. A. (2001). Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. *Physiology and Behavior* 74: 177–184.
868. Weinert, D. and Waterhouse, J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. *Physiology and Behavior* 63: 837–843.
869. Conn, C. A., Franklin, B., Freter, R., and Kluger, M. J. (1991). Role of gram-negative and gram-positive gastrointestinal flora in temperature regulation of mice. *American Journal of Physiology* 261: R1358–R1363.
870. Kluger, M. J., Conn, C. A., Franklin, B., Freter, R., and Abrams, G. D. (1990). Effect of gastrointestinal flora on body temperature of rats and mice. *American Journal of Physiology* 258: R552–R557.
871. Goel, N. and Lee, T. M. (1995). Social cues accelerate reentrainment of circadian rhythms in diurnal female *Octodon degus* (Rodentia: Octodontidae). *Chronobiology International* 12: 311–323.
872. Labyak, S. E. and Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
873. Varosi, S. M., Brigmon, R. L., and Besch, E. L. (1990). A simplified telemetry system for monitoring body temperature in small animals. *Laboratory Animal Science* 40: 299–302.
874. Recabarren, S. E., Vergara, M., Llanos, A. J., and Serón-Ferré, M. (1987). Circadian variation of rectal temperature in newborn sheep. *Journal of Developmental Physiology* 9: 399–408.
875. Miles, G. H. (1962). Telemetering techniques for periodicity studies. *Annals of the New York Academy of Sciences* 98: 858–865.
876. Refinetti, R. (1990). Experimentally induced disruption of the diurnal rhythm of body temperature of the rat. *Biotemas* 3(2): 47–58.
877. Honma, K. and Hiroshige, T. (1978). Internal synchronization among several circadian rhythms in rats under constant light. *American Journal of Physiology* 235: R243–R249.
878. Satinoff, E., Kent, S., and Hurd, M. (1991). Elevated body temperature in female rats after exercise. *Medicine and Science in Sports and Exercise* 23: 1250–1253.
879. Refinetti, R., Ma, H., and Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
880. Meinrath, M. and D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
881. Deprés-Brummer, P., Metzger, G., and Lévi, F. (1996). Analyses des rythmes de la température corporelle du rat en libre cours. *Pathologie Biologie* 44: 150–156.
882. Benstaali, C., Bogdan, A., and Touitou, Y. (2002). Effect of a short photoperiod on circadian rhythms of body temperature and motor activity in old rats. *Pflügers Archiv* 444: 73–79.
883. Severinsen, T. and Øritsland, N. A. (1991). Endotoxin induced prolonged fever in rats. *Journal of Thermal Biology* 16: 167–171.
884. Peloso, E., Wachulec, M., and Satinoff, E. (2002). Stress-induced hyperthermia depends on both time of day and light condition. *Journal of Biological Rhythms* 17: 164–170.
885. Heusner, A. (1959). Variation nycthémérale de la température centrale chez le rat adapté à la neutralité thermique. *Comptes Rendus de Séances de la Société de Biologie de Strasbourg* 153: 1258–1261.
886. Morley, R. M., Conn, C. A., Kluger, M. J., and Vander, A. J. (1990). Temperature regulation in biotelemetered spontaneously hypertensive rats. *American Journal of Physiology* 258: R1064–R1069.
887. Goldman, B. D., Goldman, S. L., Riccio, A. P., and Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
888. Muchlinski, A. E., Baldwin, B. C., Padick, D. A., Lee, B. Y., Salguero, H. S., and Gramajo, R. (1998). California ground squirrel body temperature regulation patterns measured in the laboratory and in the natural environment. *Comparative Biochemistry and Physiology A* 120: 365–372.
889. Lee, T. M., Holmes, W. G., and Zucker, I. (1990). Temperature dependence of circadian rhythms in golden-mantled ground squirrels. *Journal of Biological Rhythms* 5: 25–34.
890. Ishii, K., Uchino, M., Kuwahara, M., Tsubone, H., and Ebukuro, S. (2002). Diurnal fluctuations of heart rate, body temperature and locomotor activity in the house musk shrew (*Suncus murinus*). *Experimental Animals* 51: 57–62.
891. Ingram, D. L. and Mount, L. E. (1973). The effects of food intake and fasting on 24-hourly variations in body temperature in the young pig. *Pflügers Archiv* 339: 299–304.
892. Haim, A., Downs, C. T., and Raman, J. (2001). Effects of adrenergic blockade on the daily rhythms of body temperature and oxygen consumption of the black-tailed tree rat (*Thallomys nigricauda*) maintained under different photoperiods. *Journal of Thermal Biology* 26: 171–177.
893. Refinetti, R. and Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
894. Peters, D. G. and Rose, *R*. W. (1979). The oestrous cycle and basal body temperature in the common wombat (*Vombatus ursinus*). *Journal of Reproduction and Fertility* 57: 453–460.

This page intentionally left blank

Section IV

Physical Substrates



A DNA molecule symbolizing the molecular bases of circadian rhythms. (Pixabay public domain image.)

This page intentionally left blank

11 Receptors

11.1 SENSORY INPUT

So far, our journey through the study of circadian physiology has covered the phenomenology of circadian rhythms in Section II and the physiological mechanisms responsible for rhythmicity in Section III. Here in Section IV, we will discuss the *physical substrates* of circadian rhythms—that is, the cellular and molecular phenomena that underlie physiological processes. We will not go as far down as the level of basic interactions between the chemical elements (Figure 11.1), but we will descend into the labyrinth of that class of organic compounds (DNA and proteins) responsible for the generation of circadian rhythmicity and for life itself.

In cybernetic terms, the operation of an organism can be conceptualized as an input–processor–output triad of information processing (Figure 11.2, Panel A). That is, the organism receives information from the environment (*input*), processes it (*processor*), and acts on it (*output*).[1,2] In terms of the circadian system, operation may be thought of as a sensors–pacemaker–effectors triad (Panel B). That is, the organism senses stimuli in the environment (*sensors*), the information from the stimuli affects the pacemaker's operation (*pacemaker*), and the pacemaker controls effector mechanisms such as locomotor activity and body temperature (*effectors*). A more detailed diagram for the circadian triad is shown in Panel C. As discussed in Section III, we know that sensors in the circadian system include both photic and nonphotic receptors. Afferent pathways take information to the pacemaker, and efferent pathways take information from the pacemaker to the effector organs. Effector organs are either muscles or glands but, in complex organisms, the brain serves as an additional effector organ for the generation of thoughts and emotions. In this chapter, we will cover the *receptors.* The *pacemaker* will be discussed in Chapter 12 and the *afference* and *efference* in Chapter 13. However, before you get the impression that things are simpler than they really are, have a look at Figure 11.3. Be forewarned that, although the main flow of information in the circadian system may be conceptualized as the receptors–pacemaker–effectors triad, the actual system involves many physical components of each member of the triad as well as multiple feedback loops among them.[3] Slave pacemakers will be discussed in Section 12.4.

You probably learned in kindergarten that humans have five senses: vision, hearing, smell, taste, and touch. If you share Robert Fulghum's opinion that *All I Really Need to Know I Learned in Kindergarten*,[4] then you are about to have an epiphany. As shown in Table 11.1, we have at least nine somatic (conscious) senses as well as numerous autonomic senses.

For each sensory modality, Table 11.1 indicates the nature of the stimulus being sensed (the "adequate stimulus") and the structure that senses the stimulus (the "sense organ"). Different senses make use of different sensory receptors. *Sensory receptors* are specialized neural structures that perform the task of transducing specific forms of energy (such as light, sound, or pressure) into changes in the polarization of afferent neurons. This use of the term "receptor" should be distinguished from that of synaptic or humoral (hormonal) receptors, which are specific cell membrane structures to which neurotransmitters or hormones can bind (see Chapter 2). Some sensory receptors are as simple as a synaptic or humoral receptor, but many are much more complex.

As reviewed in Chapter 2, cells of the nervous system communicate by electric currents—or, more precisely, by movement of ions along the cytoplasm and across semipermeable membranes. Thus, any information about physical stimuli (such as electromagnetic radiation, which is responsible for vision, or gastric distention, which is partially responsible for the feeling of satiety) must be transduced into ionic flow if it is to reach the brain in usable form (Figure 11.4). In order for the circadian pacemaker to be affected by photic and nonphotic stimuli, these stimuli must be sensed and transduced. We will discuss the sensing and transduction of photic stimuli in Section 11.2 and of nonphotic stimuli in Section 11.3.

11.2 PHOTIC RECEPTORS

Photic receptors transduce light (electromagnetic radiation). Of course, transduction of light is the domain of vision, and the eyes are the organs specialized in vision. Therefore, it is natural to start the discussion of circadian photic receptors by discussing the role of the eyes.

11.2.1 Eyes

Plants, fungi, archaea, and bacteria do not have eyes and must perceive light by other means. However, eyes are very common in animals.[5] We usually think of the eyes as the organs of vision, but they serve other purposes as well. As indicated in Figure 11.5, the vertebrate eye is involved in vision, oculomotor reflex, pupillary reflex, circadian resetting, and masking (which includes the photic suppression of melatonin secretion). To avoid potential confusion, many authors refer to vision as "image-forming photoreception" and to the other functions of the eye as "irradiance detection." Given the nonvisual functions of the eye, it is not surprising that the circadian system of visually blind humans[6] and nonhuman primates[7] can be affected by photic stimuli. The circadian system of blind subterranean mole rats, which have atrophied eyes underneath the skin, also can be affected by photic stimuli.[8] However, the eye is the only photosensitive organ in mammals, whether it contributes to vision or not. Removal of the eyes eliminates all photic input to the circadian system,[9–18] as exemplified

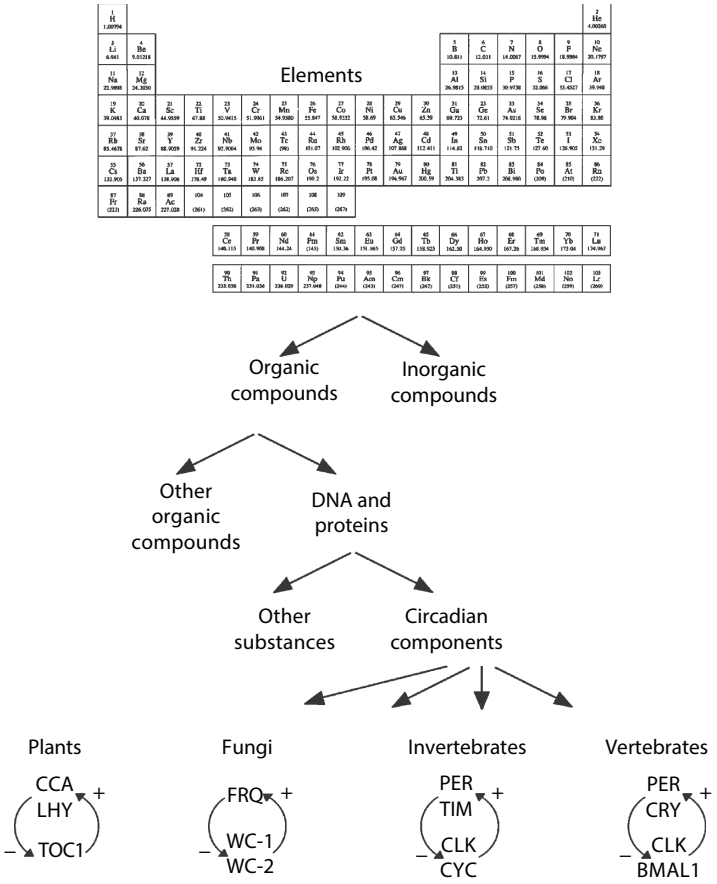


FIGURE 11.1 Physical substrate of life. Life is the result of a unique organization of basic elements, particularly carbon, oxygen, hydrogen, and nitrogen. Substances responsible for circadian rhythmicity are DNA and proteins involved in translation/transcription loops that will be discussed in Chapters 12 and 13.

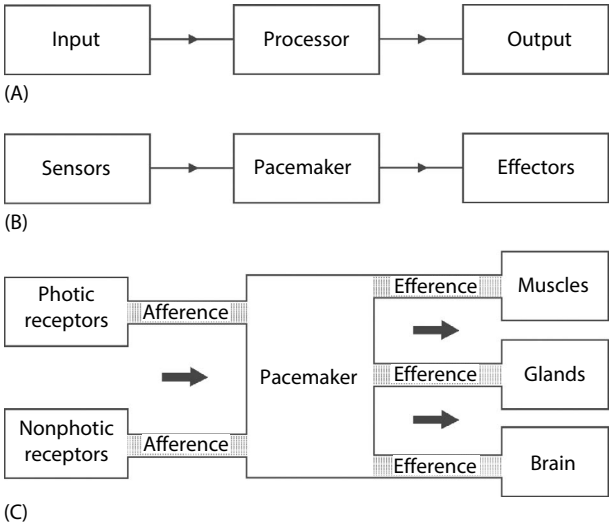


FIGURE 11.2 Sensory input. Sensory input in organisms can be conceptualized in cybernetic terms as the first member of the input–processor–output triad of information processing (A), which translates into the chronobiological triad of sensors–pacemaker–effectors (B). A more detailed diagram for the chronobiological triad is shown in Panel C. Photic and nonphotic receptors are discussed in this chapter. The pacemaker will be discussed in Chapter 12. Afference and efference will be discussed in Chapter 13.

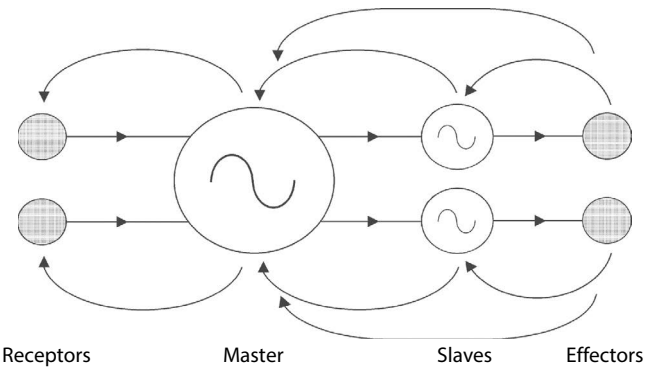


FIGURE 11.3 Recognizing the complexity of biological systems. This diagram emphasizes the complexity of the circadian system. Although the main flow of information may be conceptualized as the receptors–pacemaker–effectors triad, there seem to be many physical components of each member of the triad as well as multiple feedback loops among them.

TABLE 11.1
Mammalian Sensory Systems

	Sensory Modality	Adequate Stimulus	Sense Organ
Somatic Senses			
1	Vision	Light (electromagnetic radiation)	Eye (retina)
2	Hearing	Sound (air vibration)	Ear (cochlea)
3	Taste	Chemicals in fluids	Tongue (taste buds)
4	Smell	Chemicals in air	Nose (olfactory mucosa)
5	Touch	Mechanical pressure	Skin (mechanoreceptors)
6	Warmth/cold	Skin temperature	Skin (thermoreceptors)
7	Pain	Tissue damage	Skin/organs (nociceptors)
8	Equilibrium	Body orientation	Ear (vestibular organs)
9	Kinesthesia	Position of limbs	Muscles, tendons, and joints
Autonomic Senses			
10	Circadian photoreception	Light (electromagnetic radiation)	Eye (retina)
11	Baroreception	Blood pressure	Arterial/venous stretch receptors
12	Osmoreception	Plasma osmotic pressure	Hypothalamic osmoreceptors
13	Central thermoreception	Brain blood temperature	Hypothalamic thermoreceptors
14	Glucoreception	Blood glucose concentration	Hypothalamic glucoreceptors
15		Inflation of lungs	Pulmonary stretch receptors
16		Arterial oxygen concentration	Carotid and aortic bodies
17		pH of cerebrospinal fluid	Receptors in ventral medulla

Sources: Ganong, W.F., *Review of Medical Physiology*, 20th edn., Lange, New York, 2001; Schiffman, H.R., *Sensation and Perception*, 4th edn., Wiley, New York, 1996.

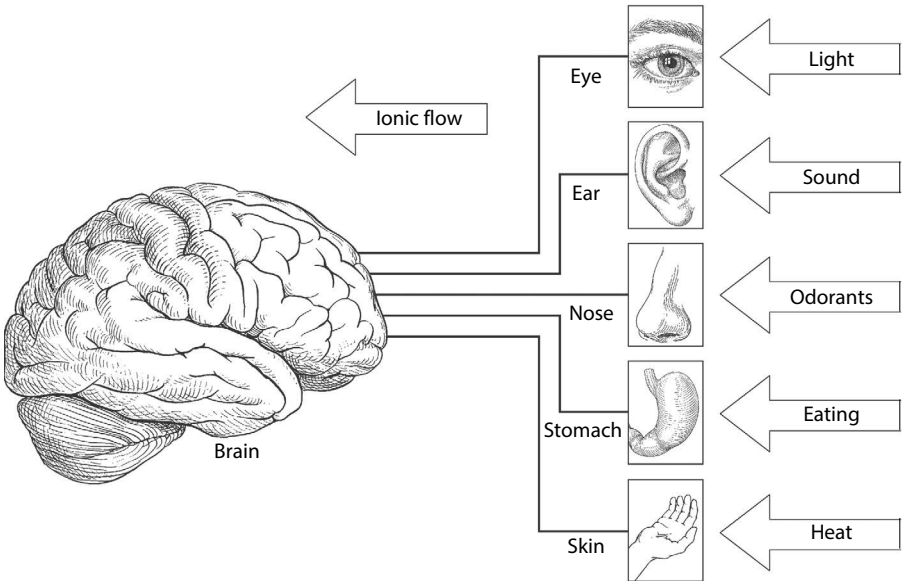


FIGURE 11.4 Sensory systems. Sensory systems convert different forms of energy into ionic flow in nerve cells. This diagram illustrates five of the many sensory systems of mammals: vision, hearing, olfaction, stomach fullness, and skin temperature.

in Figure 11.6. Two golden hamsters (*Mesocricetus auratus*) were housed in a single cage with two running wheels under a 23.3-hour T cycle. One of the hamsters had its eyes surgically removed, whereas the other was left unharmed. The activity records clearly indicate that the T cycle entrained the rhythm of the sighted animal but not that of the blind one.

The mammalian eye is a complex organ that contains millions of nerve cells as well as nonneural tissues (Figure 11.7). Decades of research on the visual system have indicated that photic transduction takes place in the *retina* in the back of the eye. For many years, only two classes of retinal photoreceptors were recognized: rods and cones[19,20] (Figure 11.8).

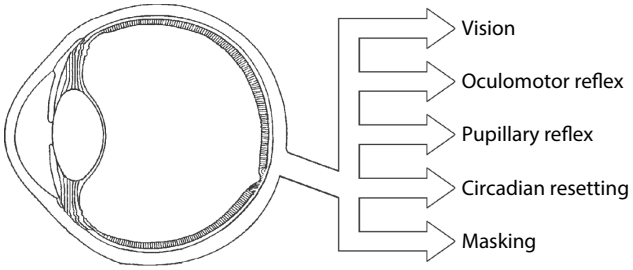


FIGURE 11.5 Sensory systems that use the eye as the receptor organ. The eye is the organ specialized in the transduction of electromagnetic radiation (light). It serves as the receptor organ not only for vision but also for many other functions, including circadian resetting.

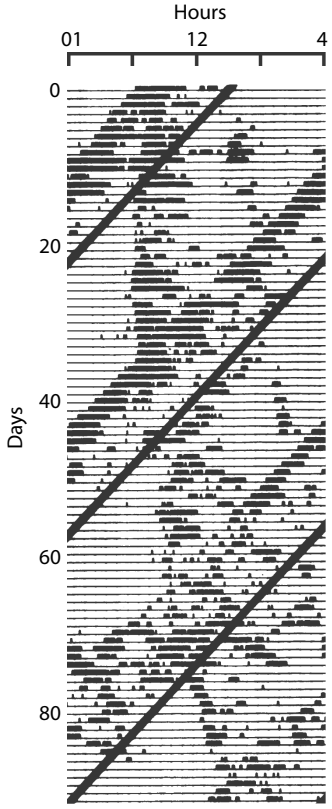


FIGURE 11.6 Missing the eyes. This actogram shows the combined rhythms of running-wheel activity of two male golden hamsters (*Mesocricetus auratus*) maintained under a skeleton light–dark cycle with a period of 23.3 hours (LD 1:22.3), as indicated by the oblique heavy lines. One of the hamsters had intact eyes and was entrained by the light–dark cycle, exhibiting an activity period of 23.3 hours. The other hamster had both eyes surgically removed prior to the beginning of the study and freeran with a period slightly longer than 24.0 hours. (Adapted from Refinetti, R. et al., *J. Comp. Physiol. A*, 170, 181, 1992.)

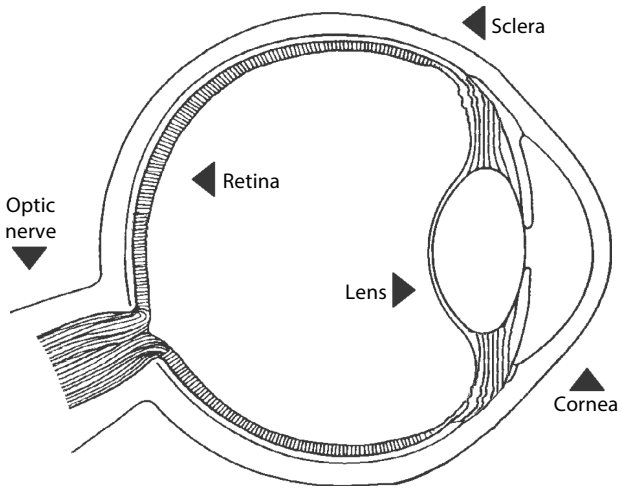


FIGURE 11.7 The human eye. This diagram of the human eye shows its major structures, including the retina (which contains photoreceptor cells) and the optic nerve (which takes information from the eye to the brain). (Adapted from Goldstein, E.B., *Sensation and Perception*, 4th edn., Brooks/Cole, Pacific Grove, CA, 1996.)

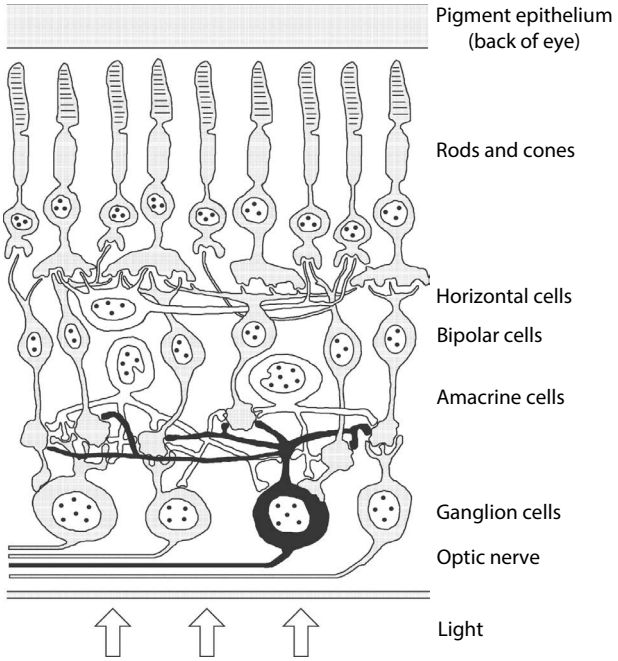


FIGURE 11.8 The retina. This diagram shows the cellular organization of the primate retina. Light traverses the various layers to stimulate the visual photoreceptors (rods and cones) next to the pigment epithelium. Some ganglion cells (such as the cell drawn in black in this diagram) are also photosensitive and provide additional photic input to nonvisual systems such as the circadian system and the system responsible for the pupillary reflex. (Adapted from Dowling, J.E. and Boycott, B.B., *Proc. R. Soc. Lond. B*, 166, 80, 1966.)

Rods are associated with nighttime (black-and-white) vision, whereas *cones* are associated with daytime (color) vision. The mechanism of color vision requires two or more distinct types of cones, and humans have three types of cones that were identified in the early 1960s.[21,22] Rods and cones make complex connections with *bipolar cells*, which on their turn connect with *ganglion cells*. The axons of ganglion cells form the optic nerve, which takes photic information to the brain.

Research conducted in the last 25 years on mice with genetic degeneration of rods and cones revealed that, while rods and cones are indeed needed for vision, they are not needed for

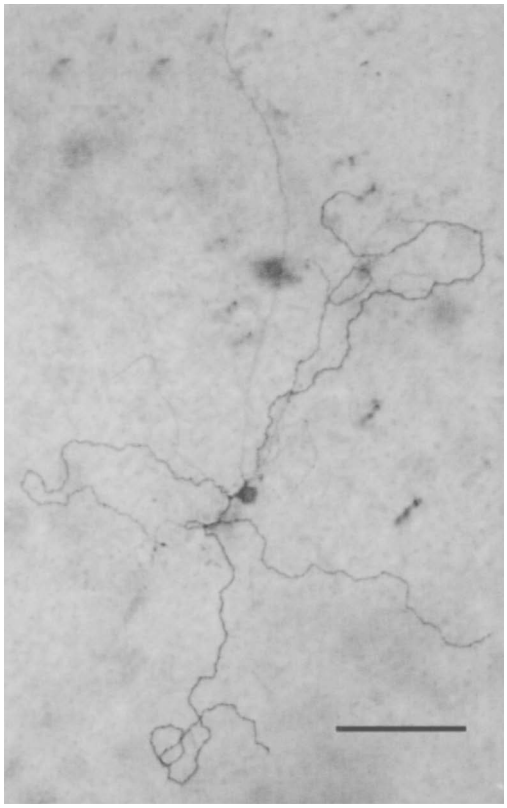


FIGURE 11.9 A close view of a photoreceptive ganglion cell. This microphotograph of the rat retina shows the typical morphology of a photoreceptive ganglion cell that projects to the circadian system. Scale bar: 100 µm. (Reproduced from Warren, E.J. et al., *Eur. J. Neurosci.*, 17, 1727. Copyright 2003 Blackwell Publishing. With permission.)

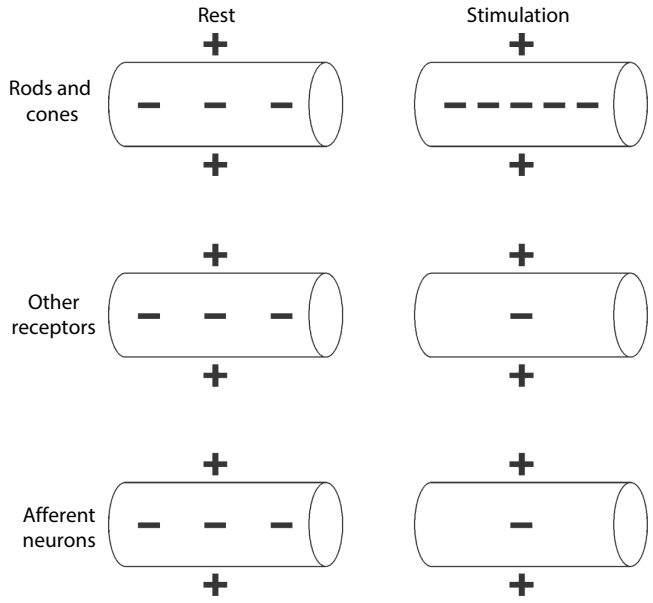


FIGURE 11.10 Changes in the polarization of nerve cells during stimulation. Unlike other sensory receptors and all afferent neurons, rods and cones in the vertebrate retina hyperpolarize in response to stimulation.

photic stimulation of the circadian system.[15,17,23–25] Evidently, some other retinal (or extraretinal) structure must be able to carry out circadian photoreception. More recently, it was found out that some *ganglion cells* can function as photoreceptors.[26] One such cell is depicted in black in the diagram in Figure 11.8. A microphotograph of an actual photosensitive ganglion cell in the rat retina is shown in Figure 11.9. These cells have sparse but wide dendritic fields (0.1 mm^2), and they *depolarize* in response to local photic stimulation.[26,27] Depolarization of a ganglion cell in response to stimulation is what one would expect, except that this is not how retinal photoreceptors normally work. In most sensory modalities, receptors respond to stimulation like normal neurons do—that is, they undergo membrane depolarization (which means that the inside of the cell becomes temporarily less negative than it is at rest). However, as diagrammed in Figure 11.10, rods and cones in the vertebrate retina do exactly the opposite: they hyperpolarize when stimulated. Evidently, photoreceptive retinal ganglion cells behave like afferent neurons, not like retinal photoreceptors in this respect.

At least in rats, intrinsically photosensitive retinal ganglion cells (often abbreviated as ipRGCs) exhibit rapid adaptation to light and darkness like rods and cones do.[28] Morphological and physiological analyses of ipRGCs in a diurnal rodent (*Arvicanthis ansorgei*) revealed few differences from rat ipRGCs.[29]

How the photoreceptive ganglion cells sense light is a more complicated issue. The process of photic transduction in rods and cones has been extensively studied,[19,20] and it is sensible to look at it first. Rods and cones use *photopigments* to absorb incident light. *Rhodopsin*, the photopigment in the rods, is made up of two substances: *retinal* and *opsin*. In darkness, retinal has an isomeric configuration called 11-*cis* because the molecule has a bent tail at the 11th carbon atom (Figure 11.11). When retinal absorbs light, its isomeric configuration changes to all-*trans*, and this is the essence of phototransduction. All-*trans* retinal cannot bind to opsin, so that rhodopsin is depleted in the presence of light. The breakdown of rhodopsin leads to a change in the permeability of the cell membrane and, consequently, to a change in the polarization of the photoreceptor cell.

The opsin in rhodopsin (encoded by the *rho* gene) is distinct from the opsins in cones (encoded by the *opn1* genes), and at first there was uncertainty about which opsin is present in the photoreceptive ganglion cells. As a matter of fact, the substance did not even have to be an opsin. Plants have a blue-light photopigment (*cryptochrome*) that is based on vitamin B_2 rather than on vitamin A (which is used in opsin),[30] and ganglion cells in the mouse retina have been shown to contain cryptochrome.[31] This means that cryptochrome could be the photopigment of photoreceptive ganglion cells. This possibility was reinforced by the observations that transgenic mice with deletion of the cryptochrome genes (*cry1* and *cry2*) exhibit reduced phase shifts in response to light pulses[32] and that mice missing both cryptochrome and rods/cones exhibit reduced pupillary response to photic stimulation.[33] Also, mice missing both cryptochrome and rods/cones show reduced activation of the master pacemaker in the brain when they are photically stimulated.[34]

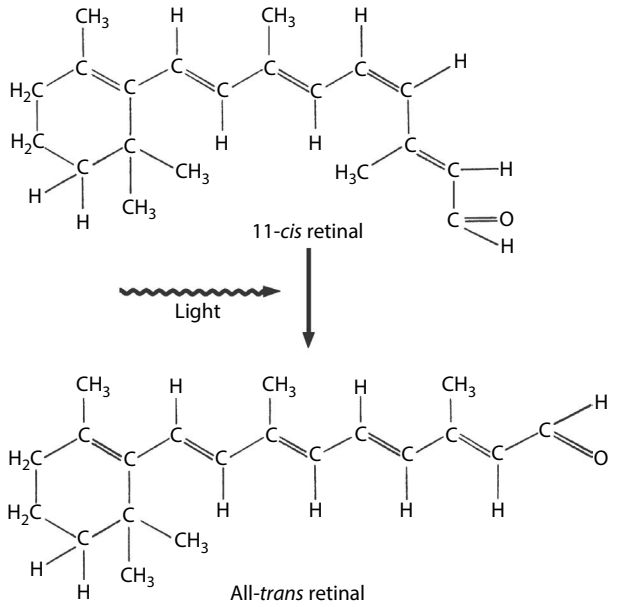


FIGURE 11.11 The chemistry of phototransduction. The photoisomerization of the chemical *retinal* is the key element in the transduction of photic energy in the retina. (Adapted from Bailey, C.H., Visual system I: The retina, in: Kandel, E.R. and Schwartz, J.H., eds., *Principles of Neural Science*, Elsevier, New York, 1981, pp. 213–225.)

The evidence in support of the hypothesis that cryptochrome is the photopigment present in photoreceptive ganglion cells seemed to be strong, but it was misleading. First of all, as we will discuss in greater detail in Chapter 12, cryptochrome turns out to be a major component of the cellular mechanism of the circadian clock.[35] Thus, the impairment of circadian function in mice with cryptochrome deletion was likely the consequence of impairment in the clock itself, not in its photic input. As a matter of fact, mice with deletion of the cryptochrome genes are arrhythmic in constant darkness but retain photic sensitivity.[36–39] Even more importantly, a novel opsin (*opn4*, or *melanopsin*—not to be confused with the hormone *melatonin* or the skin pigment *melanin*) was identified in the retina of mice, monkeys, and humans[40] and was found to be present in photoreceptive ganglion cells but not in nonphotoreceptive cells of the rat retina.[41–43] Furthermore, melanopsin knockout mice (mice with deletion of *opn4*) were found to exhibit reduced responsiveness to photic stimulation.[44–47] As if this were not evidence enough in support of melanopsin's function as a photopigment, researchers expressed mouse melanopsin in frog eggs,[48] or human melanopsin in mouse nerve cells,[49] or mouse melanopsin in human kidney cells,[50] and in all three cases, the previously nonphotoreceptive cells became photoreceptive. Melanopsin mRNA expression in rat retina exhibits the slow adaptation (over many days)[51] that is specific to the circadian system, as discussed in Chapter 7.

A microphotograph of a retinal ganglion cell containing melanopsin is shown in Figure 11.12. Approximately 1% of ganglion cells in the retina of mice,[52] rats,[53] gerbils,[54] and humans[55] contain melanopsin. Melanopsin-containing ganglion cells, just like regular ganglion cells, are innervated by bipolar cells.[56]

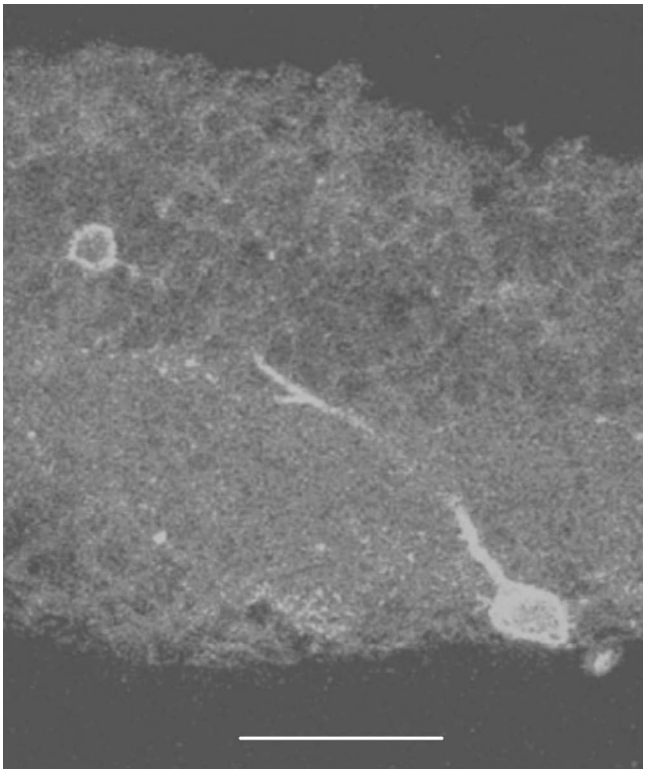


FIGURE 11.12 Melanopsin as a photopigment. This microphotograph of the mouse retina shows a melanopsin-immunopositive cell in the retinal ganglion cell layer with processes extending toward another melanopsin-immunopositive cell deeper in the retina. Scale bar: 50 μm. (Image courtesy of Russell G. Foster, Department of Ophthalmology, University of Oxford, Oxford, U.K.)

The fact that the circadian system of mice deprived of rods and cones can respond to photic stimulation[15,17,23–25,57,58] indicates that rhodopsin and the cone opsins are not necessary for circadian photoreception. This would leave melanopsin as the photopigment used by the circadian system. Indeed, mice lacking rods, cones, *and* melanopsin are not entrained or masked by light–dark cycles and show no pupillary constriction in response to photic stimulation.[59,60] However, the fact that rods and cones are not necessary does not imply that they are not used. The reduction—but not elimination—of photic responsiveness of the circadian system in mice with deletion of melanopsin only[44,45] indicates that rods and cones are also used by the circadian system. Thus, all three of these opsins (*rho*, *opn1*, and *opn4*) are involved in photoreception for the circadian system.[59–62] A novel opsin (*opn5*, or neuropsin) has been recently identified in the eye (and other structures) of mice and humans,[63,64] and its potential role as a circadian photopigment is yet to be investigated.

A potential problem regarding the role of melanopsin as a circadian photopigment derives from the finding that melanopsin is expressed not only in photoreceptive ganglion cells but also in the pigment epithelium of the eye.[65,66] The pigment epithelium (see Figure 11.8) lies behind the rods and cones and has no known photosensory role. Yet, melanopsin is expressed more strongly there than in the retina (Figure 11.13). This may

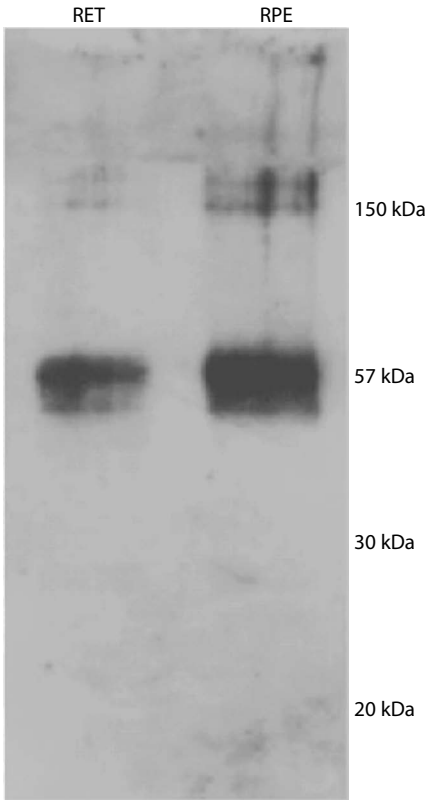


FIGURE 11.13 Melanopsin beyond the retina. This Western blot of protein extracts from the mouse eye shows greater translation of melanopsin (57 kDa band) in the retinal pigment epithelium (RPE) than in the retina itself (RET). For the location of the pigment epithelium, see Figure 11.8. (Adapted from Peirson, S.N. et al., *Mol. Brain Res.*, 123, 132, 2004.)

be inconsequential, but it may also mean that, similarly to cryptochrome, melanopsin is the central component of a different process that only secondarily affects photoreception.

An additional way to investigate the role of the various photoreceptors in the circadian system is to compare the *action spectra* of the photoreceptors with the action spectrum of the full circadian system. An action spectrum is a function that relates the effect of electromagnetic radiation (light) to the wavelength of the radiation. Figure 11.14 shows the action spectra for human scotopic (black-and-white) and photopic (color) vision (Panel A), as well as the action spectra for rhodopsin and the three cone opsins (Panel B). Most mammals have two cone opsins, some have only one, and several primates (including humans) have three.[67] The action spectrum of human scotopic vision follows closely the absorption response of rhodopsin, with peak sensitivity at 505 nm. Photopic vision is a composite of the absorption responses of three distinct types of cones with peak sensitivities at 445 nm (blue), 535 nm (green), and 570 nm (red). The peak sensitivity of human photopic vision is at 555 nm.

Figure 11.15 shows action spectra for various photoreceptive processes (Panels A–D) and for phase shifts of circadian rhythms evoked by photic stimuli of different wavelengths (Panels E–H) in rats and mice. Only two of the spectra (A and B) covered the ultraviolet range ($\lambda < 400$ nm). The peak sensitivity of the rodent retina in the ultraviolet range is approximately at 360 nm.[68,69] No study of circadian phase shifts has mapped sensitivity in the ultraviolet range, but numerous studies have demonstrated that ultraviolet light can entrain circadian rhythms or evoke phase shifts.[70–77] Presumably, circadian sensitivity in the ultraviolet range is due to the cones with peak sensitivity at 360 nm. However, the

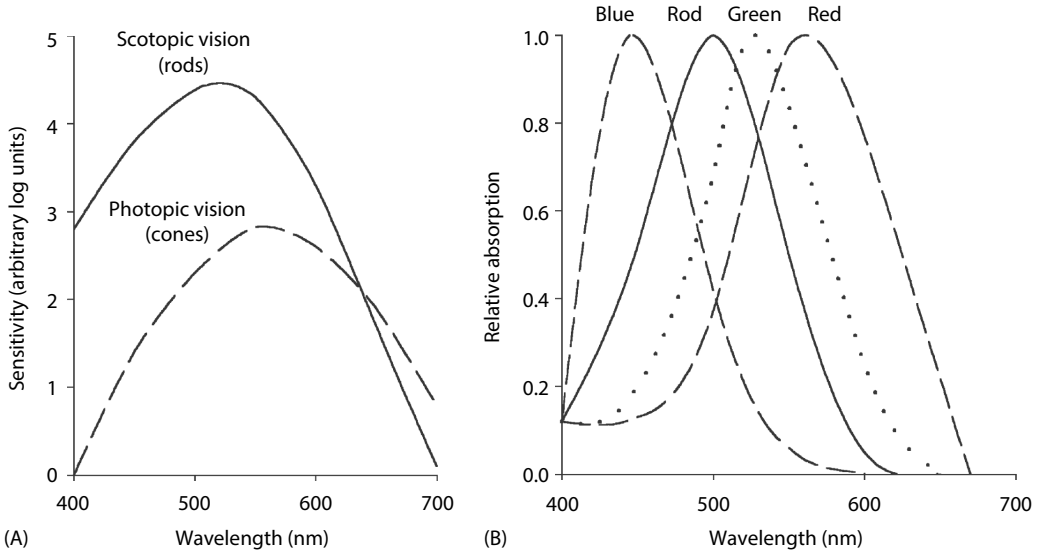


FIGURE 11.14 Spectral sensitivity of human visual receptors. Two processes can be distinguished in human vision (as well as in the vision of numerous other species): scotopic (or black-and-white) vision, which depends on retinal rods, and photopic (or color) vision, which depends on retinal cones (A). The spectral sensitivity of scotopic vision approximates that of rods, whereas the spectral sensitivity of photopic vision is a composite of the spectral sensitivities of the three types of cones (B). Notice that in Panel B, all functions are normalized to facilitate comparison of the spreads of spectral sensitivity. (Adapted from Schiffman, H.R., *Sensation and Perception*, 4th edn., Wiley, New York, 1996.)

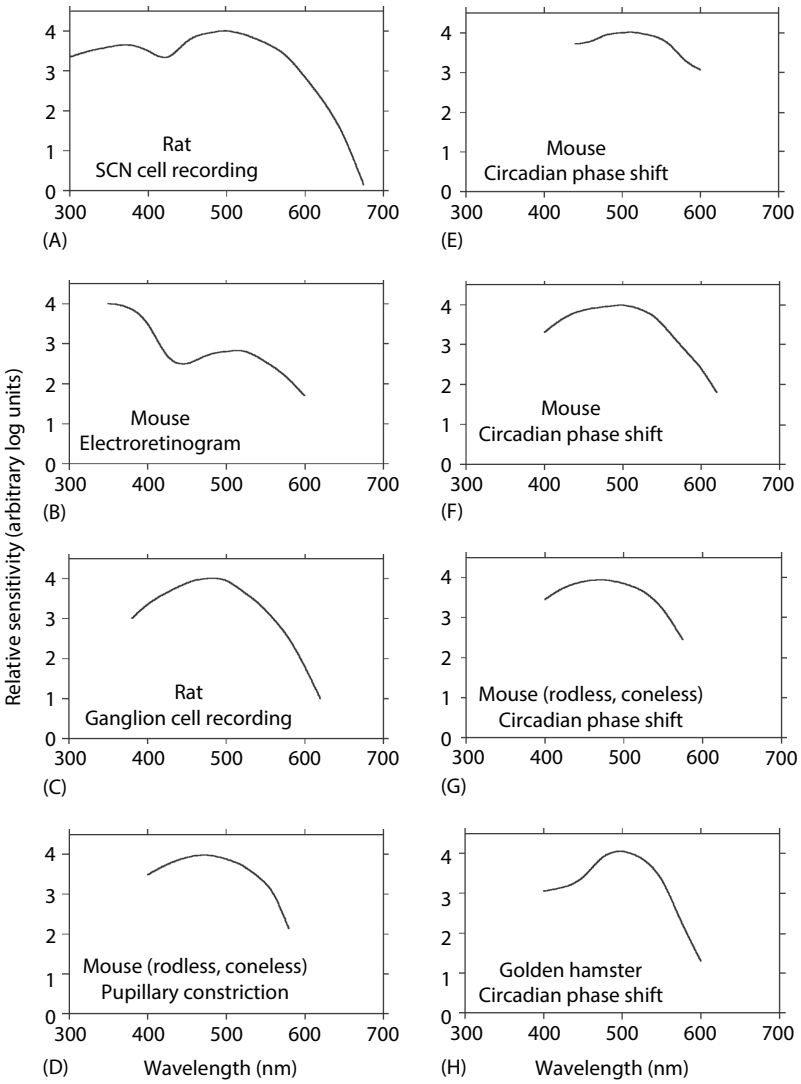


FIGURE 11.15 Comparing various action spectra. These 8 action spectra, including 4 spectra for eye responses (A through D) and 4 spectra for circadian resetting (E through H), provide useful information about the spectral sensitivity of the circadian system in rodents. A peak between approximately 480 and 510 nm (green) is consistently found in all spectra. An additional peak in the ultraviolet range (between 360 and 375 nm) is present in spectra that scanned the ultraviolet range. Informal studies of circadian resetting by ultraviolet light (not shown) have confirmed that the circadian system is responsive to ultraviolet stimulation. (A: From Aggelopoulos, N.C. and Meissl, H., *J. Physiol.*, 523, 211, 2000; B: Jacobs, G.H. et al., *Nature*, 353, 655, 1991; C: Berson, D.M. et al., *Science*, 295, 1070, 2002; D: Lucas, R.J. et al., *Nat. Neurosci.*, 4, 621, 2001; E: Provencio, I. and Foster, R.G., *Brain Res.*, 694, 183, 1995; F: Yoshimura, T. and Ebihara, S., *J. Comp. Physiol. A*, 178, 797, 1996; G: Hattar, S. et al., *Nature*, 424, 76, 2003; H: Takahashi, J.S. et al., *Nature*, 308, 186, 1984.)

only action spectrum available for photoreceptive ganglion cells (Panel C) is incomplete and could very well extend into the ultraviolet range—which means that melanopsin could be the photopigment that transduces ultraviolet light for the circadian system.

As for the visual range, Panels A and B indicate peak sensitivity at 510 nm (green cones) and Panels C and D indicate peak sensitivity at approximately 480 nm (presumably melanopsin, as rats and mice have only green cones in the visual range).[68,69] The spectrum for photoreceptive ganglion cells (Panel C) peaks at approximately 480 nm,[26] which is also the peak wavelength for phase shifts and pupillary reflex in mice with degenerate retina[59,78,79] as well as for long-term retinal adaptation in the human visual system.[80] Furthermore, phase shifts of the human circadian system and melatonin suppression seem to be more easily evoked by light pulses of short wavelength (around 480 nm) than by pulses in the green or red regions.[81–86] Panels E through H (rodent phase-shift data) show that peak sensitivity is at about 480 nm for retinally degenerate mice[59,78] (Panel G) and at 500–515 nm in animals with intact rods and cones.[71,78,87] Thus, it seems that traditional photoreceptors and melanopsin-containing ganglion cells are used by the circadian system under normal conditions, but melanopsin-containing ganglion cells are capable of providing adequate photic input in the absence of rods and cones. Among the traditional photoreceptors, cones are probably more important than rods for circadian photoreception because the photic sensitivity of the circadian system

is much lower than that of the visual system,[87,88] which suggests that the more sensitive rods are not used. Nonetheless, at least in rats, it seems that the presence of rods is necessary for the maintenance of adequate levels of melanopsin in ganglion cells.[89]

The involvement of cones in photic signaling is a necessity given the recent demonstration that the circadian system can use the color of incident light (whose variation is most extreme at dawn and dusk) to fine-tune behavioral entrainment.[90] As mentioned earlier, at least two types of cones are needed for true color discrimination.[91]

11.2.2 Other Photic Receptors

Look at Figure 11.16. Its shows a double-plotted actogram of the perching activity of a house sparrow (*Passer domesticus*) maintained under a light–dark cycle and in constant darkness. There seems to be nothing remarkable about these records: the bird shows entrainment to the light–dark cycle, it shifts its activity when the light–dark cycle is shifted, and it freeruns in constant darkness. What is remarkable is that the bird whose records are shown in the figure had had *both eyes removed* prior to the beginning of the study! Evidently, the circadian system of birds—unlike that of mammals—can obtain photic information from organs other than the eyes.[92–95]

Fruit flies have numerous distinct photoreceptive organs that provide input to the circadian system: the eyes, the circadian pacemaker itself (through cryptochrome), and three other extraocular photoreceptors that have not been clearly characterized.[96,97] Nonmammalian vertebrates also have many photoreceptive structures functionally connected to the circadian system: the eyes, the pineal gland, the parietal eye in lizards (or the parapineal organ in teleost fishes), and additional deep brain photoreceptors.[98–100] Figure 11.17 depicts in diagrammatic form the various vertebrate photoreceptors. Cells with an outer segment resembling retinal cones are found in the parietal eye and the pineal gland. Cells with a disorganized outer segment, cells with limited outer segment, and cells with no outer segment are found in the pineal gland in different taxonomic groups. Rods, cones, and photoreceptive ganglion cells are found in the retina of the eye. Deep brain photoreceptors seem to be cells with limited outer segment, but they have not been well characterized. A study in lizards has identified

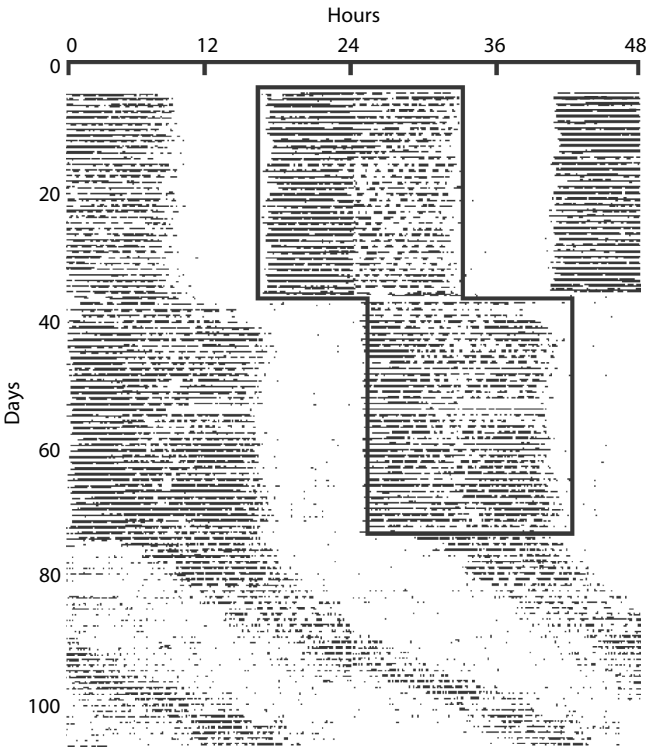


FIGURE 11.16 Blinded but not blind. This double-plotted actogram shows the rhythm of perching activity of a house sparrow (*Passer domesticus*) maintained under a light–dark cycle (as indicated by the single-plotted rectangles). The sparrow had both eyes removed prior to the beginning of the records. Entrainment by the light–dark cycle is evident despite the absence of both eyes, which indicates that the circadian system of the sparrow must receive photic information from alternate photosensitive organs. (Adapted from Menaker, M., *Proc. Natl. Acad. Sci. USA*, 59, 414, 1968.)

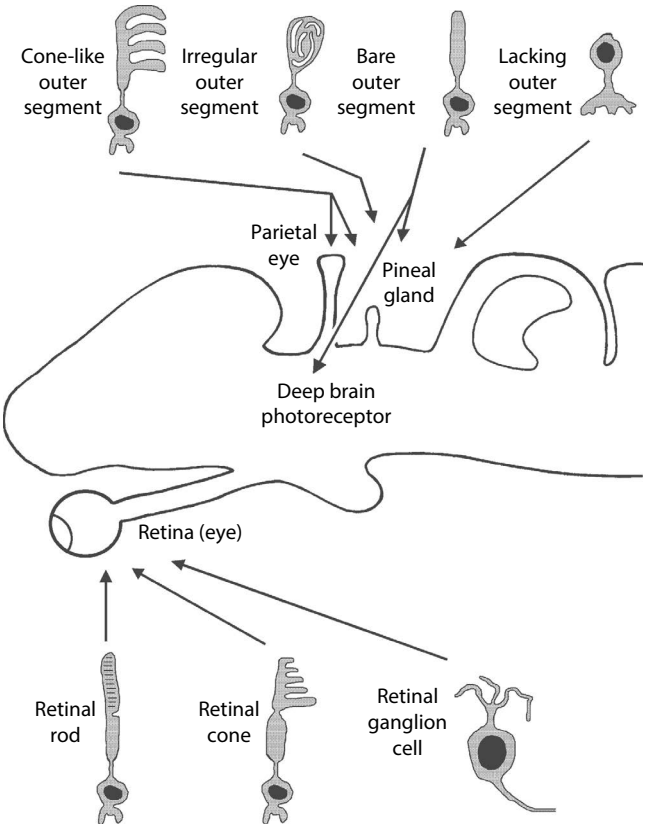


FIGURE 11.17 Vertebrate photoreceptive structures. This diagram of the vertebrate brain shows the approximate location of 7 types of opsin-containing cells. Opsin-containing cells can be found in the retina, in the pineal gland, in the parietal eye, and deep in the brain. Although all vertebrates have eyes (retina), only some groups of vertebrates have the other photoreceptive structures. The retina is the only photoreceptive structure in adult mammals. (Adapted from Foster, R.G. and Menaker, M., Circadian photoreception in mammals and other vertebrates, in: Wettenberg, L., ed., *Light and Biological Rhythms in Man*, Pergamon, New York, 1993, pp. 73–91.)

a novel opsin that is expressed deep in the brain and that is required for photic entrainment of the activity rhythm in animals lacking the other photoreceptive structures (i.e., lateral eyes, parietal eye, and pineal gland).[101] A study in chickens isolated an opsin that is expressed in the hypothalamus and that could operate as a deep brain photoreceptor.[102]

Most vertebrates, including humans, have a pineal gland (Figure 11.18). We have previously discussed that the pineal gland secretes the hormone melatonin and that melatonin secretion is inhibited by photic stimulation in humans,[61,81,82,85,103–116] rodents,[16,117–121] and other vertebrates.[122–125] Suppression of melatonin synthesis in mammals is achieved through photic information received through the eyes, although the pineal gland of newborn rats (up to 4 days of age) seems to be intrinsically photosensitive.[126] In all other vertebrates, the adult pineal gland is intrinsically photosensitive.[99,100,127] An example is shown in Figure 11.19. Pineal cells from newly hatched chicks (*Gallus domesticus*) were cultured *in vitro*. After 10 days under a light–dark cycle, the cultures were placed in constant darkness and were given discrete 4-hour light pulses (as indicated by the vertical gray lines). Notice that not only did the rhythm of melatonin secretion phase shift in response to the light pulse but also that pulses at different circadian times evoked different types of shifts (phase delay *versus* phase advance).[128]

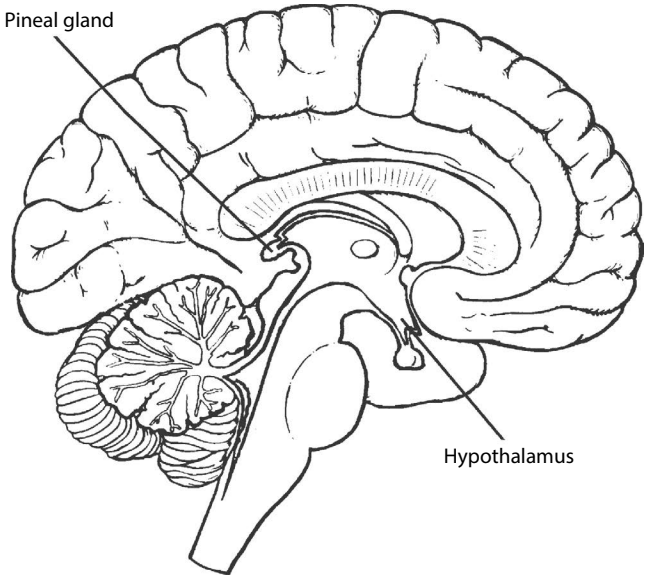


FIGURE 11.18 The pineal gland. This diagram of a midsagittal section of the human brain shows the location of the pineal gland and hypothalamus. Although the pineal gland is not photosensitive in humans, it is an important photosensitive structure in the circadian system of other vertebrates. (Adapted from *Medical Illustration Library*, Williams & Wilkins, Baltimore, MD, 1994.)

The opsin used by photoreceptors in the pineal gland was identified in chicken[129–131] and in pigeon.[132] *Pinopsin*, as it has been called, is expressed in pineal cells but not in the retina and has peak absorption at 470 nm (Figure 11.20). The role of pinopsin in pineal photoreception is not fully elucidated. *Melanopsin*, which—as we saw earlier—is expressed in the retina, is also expressed in the chicken pineal[66,131] and may contribute to pineal photoreception.

Finally, mention should be made of a misfire in the search for extraocular photoreceptors in mammals. In 1998, researchers from Cornell University Medical College (in New York) reported that phase shifts of the human circadian system could be evoked by photic stimulation of the popliteal region (i.e., the back of the knee).[133] These phase shifts were said to be comparable to those obtained by ocular stimulation and to conform to a consistent phase–response curve. Three years later, the same researchers reported that photic stimulation of the popliteal region could also enhance REM sleep in human subjects.[134] Should this be true, it could greatly facilitate the treatment of medical conditions by phototherapy (see Chapters 15 and 16).

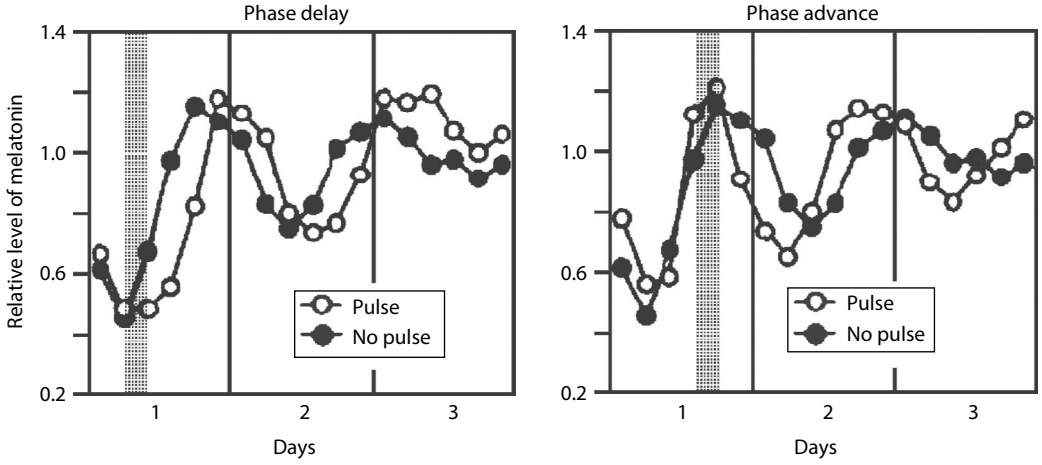


FIGURE 11.19 Intrinsic photosensitivity of the pineal gland in chicken. The graphs show 3-day segments of the records of melatonin secretion of cultures of dispersed pineal cells of young chicken chicks (*Gallus domesticus*). After 10 days under a light–dark cycle, the cultures were placed in constant darkness and received a 4-hour light pulse (300 lux) at the time indicated by the vertical gray lines. Each set of data points corresponds to the means of 3 cell cultures. Notice that photic stimulation during early subjective night caused a phase delay of the rhythm, whereas stimulation during late subjective night caused a phase advance. (Adapted from Kasahara, T. et al., *J. Neurosci.*, 22, 7321, 2002.)

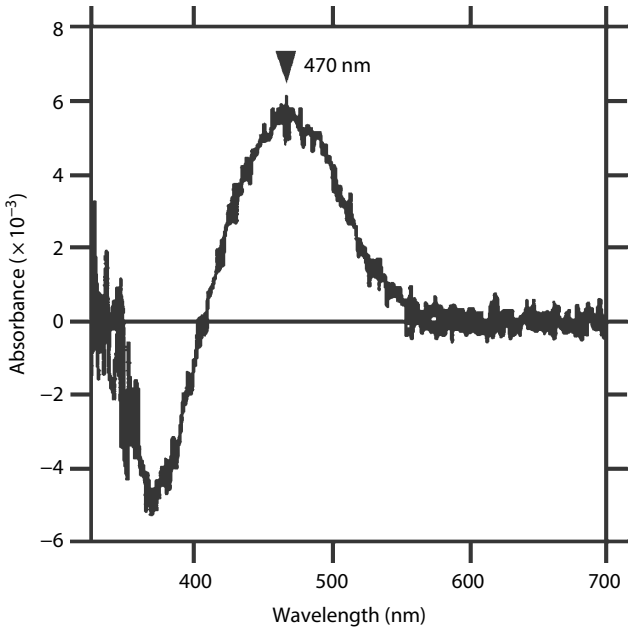


FIGURE 11.20 Pinopsin: the pineal photopigment. The graph shows the difference absorption spectrum (difference between the regenerated photopigment and its photoproducts) of pinopsin, a photopigment found only in the pineal gland. The peak absorption (λ_{max}) is at 470 nm. Pinopsin was extracted from pineal glands of young chicken chicks (*Gallus domesticus*). (Adapted from Okano, T. et al., *Nature*, 372, 94, 1994.)

Yet, seven studies on human subjects[81,108,135–139] and two studies on golden hamsters[16,140] conducted afterward by other research teams failed to detect circadian phase shifts or melatonin suppression in response to photic stimulation of the skin. The idea of photosensitivity in the skin is not absurd, as dermal photosensitivity has been documented in snails,[141] frogs,[142] and lizards.[143] However, the experimental evidence seems conclusive that, if there are photoreceptors in the mammalian skin, they do not provide input to the circadian system.

11.3 NONPHOTIC RECEPTORS

In Chapter 8, we discussed several nonphotic stimuli that are capable of phase-shifting the circadian pacemaker: ambient temperature, food availability, physical activity (exercise), and social contact. Although no one has studied how the circadian system obtains information about these stimuli, much is known about how these stimuli are sensed and processed by other regulatory systems. Therefore, we shall now briefly review the vast body of knowledge on the sensory systems responsible for the skin temperature sense, for the monitoring of body core temperature, and for the monitoring of nutritional state. The sensory processes involved in exercise are likely to involve the monitoring of muscle contraction, tendon distension, vasomotion, partial pressure of oxygen, pH, and too many other variables to be realistically understood at this time. Likewise, I will not even attempt to dissect the sensory processes involved in the sensing of social contact as an environmental stimulus.

11.3.1 Skin Temperature

It was mentioned in Chapter 10 that animals treat the temperature continuum as two separate continua: the cold continuum and the warmth continuum. This is true at the psychophysical (cognitive) level as well as at the level of cellular processes in temperature receptors. At the psychophysical level, cold and warm sensations have different thresholds[144,145] and different sensitivities at suprathreshold intensities.[146–149] Although warm and cold sensations can oppose each other (as if the stimuli cancelled each other off),[150,151] the opposition is only a cognitive combination of distinct sensations because subjects can tell the sensations apart if asked to do so.[152]

Electrophysiological studies of temperature receptors have been conducted since the dawn of sensory neuroscience.[153,154] The first electrophysiological recording of neural activity in single afferent nerve fibers (from frog muscle spindles) was conducted by Edgar Adrian and Yngve Zotterman at the University of Cambridge (in England) in 1926.[155] A few years later, Zotterman (then at the Karolinska Institute, in Sweden) reported the first recording of afferent activity evoked by skin warming in nerves innervating the tongue of the cat.[156] The electrophysiological properties of temperature receptors have been extensively studied since then in rats,[157–159] cats,[157,160–169] rabbits,[170,171] dogs,[157,158] and primates,[172–180] including humans.[181–183] Three basic types of receptors have been identified: cold receptors, warm receptors, and polymodal nociceptors. (*Note*: Although the English language differentiates the adjective *warm* from the noun *warmth*, thermal physiologists routinely use *warm* and *cold* as both adjectives and nouns.)

Polymodal nociceptors are, as the name suggests, pain receptors that respond to various stimuli including heat. They probably do not contribute to the pure sensations of nonnoxious cold and warmth. These are evoked by cold and warm receptors.[184] Cold receptors generally yield a tonic response to thermal stimuli below normal skin temperature (peaking at 25°C), whereas warm receptors yield a tonic response to thermal stimuli above normal skin temperature (peaking at 42°C), but the two types of receptors are more reliably distinguished by their acute responses to changes in temperature. Regardless of the absolute temperature of the stimulus, cold receptors respond to cooling steps with a dynamic overshoot in firing rate and to warming steps with a transient decrease in firing rate, whereas warm receptors show an overshoot on warming steps and an undershoot on cooling steps.[184,185]

The clear electrophysiological distinction between warm and cold receptors is not matched by a clear anatomical distinction. Unlike rods and cones in the retina (responsible for vision) or Pacinian corpuscles in the skin (responsible for the sense of touch), all of which have clear anatomical specializations, warm and cold receptors are simply free nerve endings. As shown in Figure 11.21, warm receptors are generally located in the dermis and are innervated by thin, nonmyelinated fibers, whereas cold receptors are located in the epidermis and are innervated by myelinated fibers. However, they have no clear anatomical specializations. Recent research in mice and rats suggests that the specialization of thermoreceptors

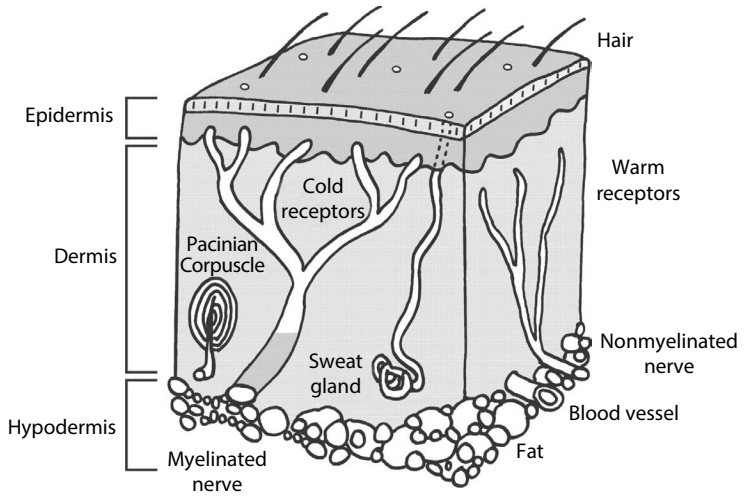


FIGURE 11.21 Skin receptors. This diagram of the human skin shows several specialized structures, including cold receptors in the epidermis and warm receptors in the dermis. Pacinian corpuscles are touch receptors. (From Andrews, G.C., *Diseases of the Skin*, 3rd edn., Saunders, Philadelphia, PA, 1946; Hensel, H., *Thermoreception and Temperature Regulation*, Academic Press, New York, 1981.)

takes place at the level of particular ion channels in the membrane of the afferent fibers.[186–188] To verify that the proteins (ion channels) are indeed responsible for changes in membrane permeability, a research team at the University of California at San Francisco cloned the respective genes and inserted them into frog oocytes, which can be more easily studied *in vitro*. They then recorded changes in inward current evoked not only by cold and heat but also by chemicals that in humans elicit sensations of cold and heat (menthol and capsaicin, respectively).[188] As shown in Figure 11.22, cells

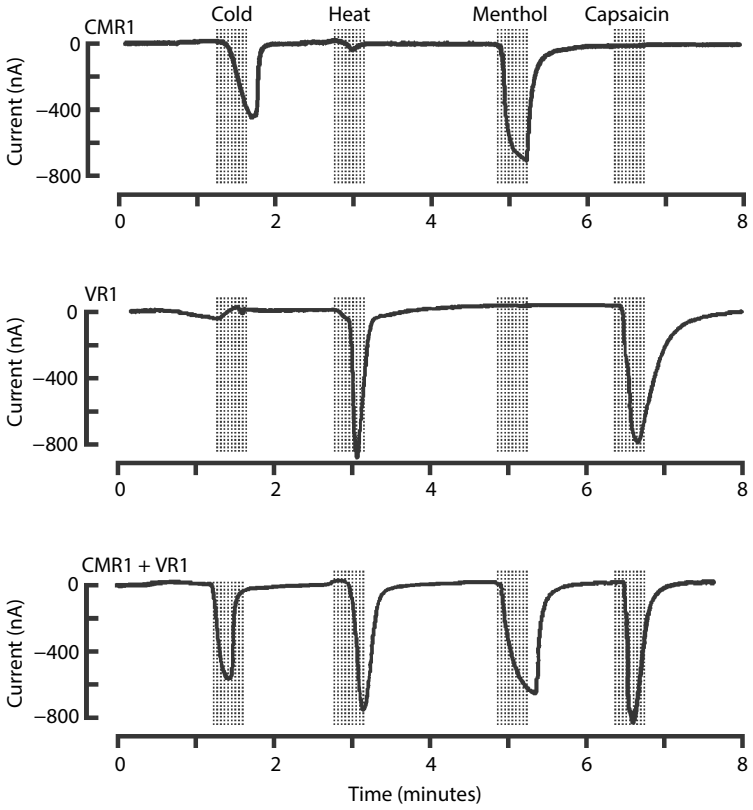


FIGURE 11.22 Transduction of skin temperature. The graphs show electrophysiological records of transmembrane current changes evoked by various stimuli in frog cells expressing rat genes for skin thermoreception. CMR1 stands for cold- and menthol-sensitive receptor. VR1 stands for vanilloid receptor (heat- and capsaicin-sensitive receptor). Stimulation is indicated by the gray vertical bars (cold, 8°C; heat, 50°C; menthol, 100 μM; capsaicin, 1 μM). (Adapted from McKemy, D.D. et al., *Nature*, 416, 52, 2002.)

containing cold- and menthol-sensitive receptors (CMR1) respond to cold and menthol but not to heat and capsaicin. Conversely, cells containing the vanilloid receptor (VR1) respond to heat and capsaicin but not to cold and menthol. Cells containing both receptors respond to all four stimuli.

Whether CMR1 is truly the mammalian cold receptor, and VR1 is the warm receptor, is far from clear. First, the response characteristics of the frog model do not fully resemble those of actual temperature receptors (i.e., they do not exhibit distinct tonic and dynamic components). Second, few real cold receptors respond to temperatures as low as that used in the frog model (8°C), and warm receptors stop responding at temperatures lower than that used in the model (50°C). Third, the CMR1 and VR1 receptors have been identified at the cell bodies of afferent fibers in the trigeminal nucleus or dorsal root ganglia, not at the skin terminals where temperature must be transduced in actual animals. Recent research has added other "transient receptor potential" (TRP) channels to the list of candidate thermoreceptors.[189–192] Although some of the channels are claimed to function in the innocuous range of skin temperature, their properties are still not consistent with those revealed by electrophysiological recordings. A new class of potential cold receptors, guanylyl cyclase G, has been recently described, but this class seems to be restricted to cells innervating the nose.[193] Further research is needed for the full characterization of the cellular mechanisms of cold and warm receptors.

Information about skin temperature ascends through the lateral spinothalamic tract (or trigeminal nucleus) to the ventrobasal complex of the thalamus and then to the somatosensory cortex to generate cold and warm sensations. Afference destined to the hypothalamus to contribute to body temperature regulation seems to diverge at the cervical level and to synapse at the midbrain raphe nuclei.[194] How the information reaches the circadian pacemaker to effect entrainment is not known.

11.3.2 Core Temperature

Studies of thermoregulatory responses evoked by local cooling or warming of various internal organs have shown that thermosensitivity is present in the brain,[195–222] spinal cord,[223–233] and visceral organs.[234–237] Brain thermosensitivity seems to be more important than spinal thermosensitivity in mammals, the opposite being true for birds.[238] In the brain, three areas are particularly thermosensitive: the preoptic area, the anterior hypothalamic area, and the posterior hypothalamic area (Figure 11.23). The posterior hypothalamic area seems to be important only for the activation of behavioral thermoregulatory responses, whereas the preoptic area and anterior hypothalamic area (POAH) are important for both behavioral and autonomic responses.[239–241] Neurons in the POAH play a double role as thermoreceptors and as integrators of thermal information received from other parts of the body,[222,242–259] although only the thermoreceptor aspect will be discussed here.

The electrophysiological properties of preoptic and anterior hypothalamic thermoreceptors have been studied in birds,[260,261] rodents,[262–273] larger mammals,[204,274–280] and lower vertebrates.[281–283] The identification of thermoreceptors in the brain is not, however, as simple as the identification of skin thermoreceptors. First of all, one cannot impose large variations in local temperature without damaging the tissue. More importantly, most body tissues are technically temperature sensitive, as they have a Q_{10} of about 2. This is not a problem for cold-sensitive cells ($Q_{10} < 1$), but some arbitrary criterion is needed to distinguish warm-sensitive cells from non-temperature-sensitive ones. One useful criterion is that firing rates with thermal coefficients greater than 0.8 impulses $\cdot s^{-1} \cdot °C^{-1}$ characterize warm-sensitive cells, whereas firing rates with thermal coefficients smaller than –0.5 impulses $\cdot s^{-1} \cdot °C^{-1}$ characterize cold-sensitive cells.[272]

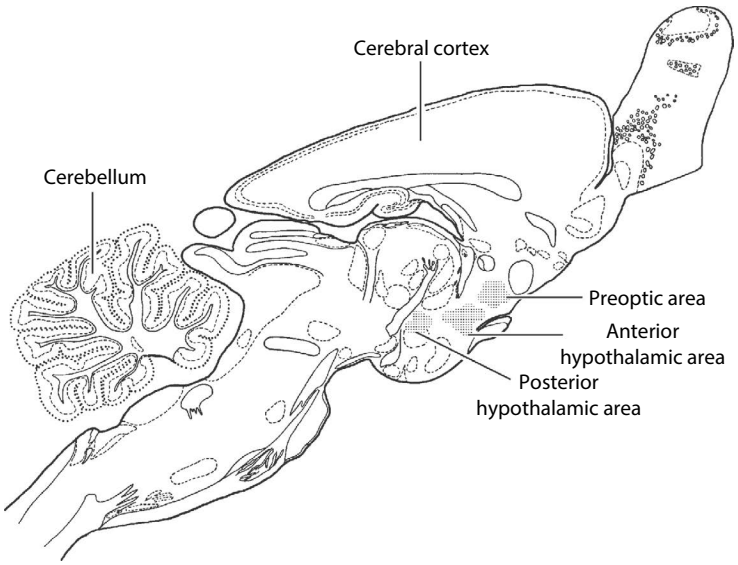


FIGURE 11.23 Intrinsic thermosensitivity in the brain. This diagram of the rodent brain (sagittal section) shows the location of thermosensitive structures: preoptic area, anterior hypothalamic area, and posterior hypothalamic area. (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979.)

A further complication is the distinction between true thermoreceptors (i.e., cells that are intrinsically temperature sensitive) and interneurons that receive excitatory or inhibitory input from thermoreceptors. *In vitro* studies of rat hypothalamic slices showed that approximately 30% of preoptic cells are warm sensitive, 10% are cold sensitive, and 60% are temperature insensitive.[267,272] However, if synaptic communication between cells is chemically blocked, the cold-sensitive cells lose thermosensitivity.[271] This implies that warm-sensitive cells may be genuine warm receptors but cold-sensitive cells may actually be interneurons synaptically modulated by warm receptors. Although several diencephalic areas contain warm- and cold-sensitive cells,[269] most genuine temperature receptors are located in the preoptic area.[270]

Very few studies have addressed the issue of intrinsic thermosensitivity of the circadian pacemaker (in the suprachiasmatic nucleus). We saw in Chapter 6 that the pacemaker is temperature compensated, meaning that its period is not significantly affected by variations in local temperature. However, this does not mean that variations in temperature cannot phase-shift the pacemaker. Approximately 10% of suprachiasmatic cells are warm sensitive and 2% are cold sensitive.[284] As shown in Figure 11.24, temperature pulses delivered to small hypothalamic slices containing the suprachiasmatic nucleus evoke phase shifts of the firing-rate rhythm of the cells in the slice.[285] The fact that the magnitude and direction of the phase shifts depend on the circadian time of stimulus presentation excludes the possibility of a mere Q_{10} effect. The magnitude of the temperature pulses (3°C elevation for 2 hours) in this study was larger than the range of oscillation of the body temperature of the rat (1.6°C) but was comparable to the ranges of oscillation of the body temperature rhythms of many small mammals. This means that, by generating the body temperature rhythm, the circadian pacemaker may subject itself to a phase-shifting stimulus. Of course, the same could be said about the locomotor activity rhythm: by generating a rhythm of locomotor activity, the circadian pacemaker sets the stage for its own photic stimulation as the animal ventures into sunlight. We discussed the issue of photic self-stimulation in Chapter 9, but the implications of thermal self-stimulation to the process of entrainment remain to be determined.

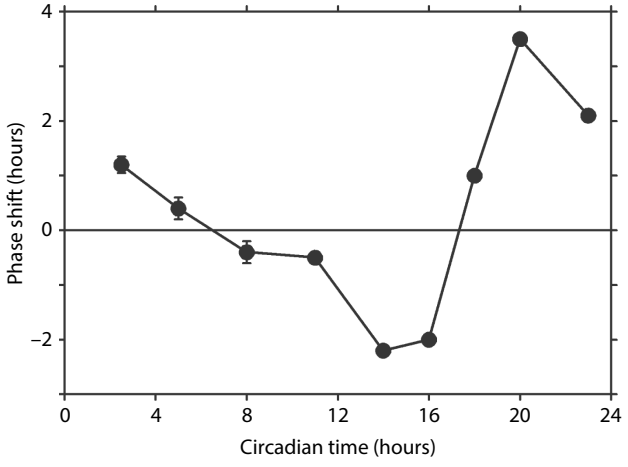


FIGURE 11.24 Intrinsic thermosensitivity of the circadian clock. The graph shows a phase–response curve for temperature pulses. Small slices of rat brain (*Rattus norvegicus*) containing the master circadian clock were kept *in vitro* for several days at 34°C. Electrophysiological recording of extracellular single-cell activity was used to monitor phase shifts. Temperature pulses consisted of elevations of medium temperature to 37°C for 2 hours. A phase-response curve resembling the phase–response curve of whole animals exposed to light pulses can be seen. Each data point corresponds to the mean (± SE) of 2–6 cells. (Data from Ruby, N.F. et al., *J. Neurosci.*, 19, 8630, 1999.)

11.3.3 Nutritional State

Food availability acts as a zeitgeber both for the master circadian pacemaker and for the food-entrainable oscillator (Chapter 8). Very little research has been conducted on the sensory mechanisms that provide the necessary information to the pacemakers, but much is known about how the nutritional state of an organism is sensed as part of the control of ingestive behavior. Therefore, we shall now briefly review the vast body of knowledge on the sensory mechanisms responsible for the monitoring of nutritional state.

The nutrients that animals ingest as food belong to three main categories: carbohydrates, lipids, and protein (Figure 11.25). Through the digestive process, carbohydrates are eventually transformed into glucose, which is the main source of energy for the brain and other organs in the body. Glucose that is not immediately used is stored as glycogen in the liver (short-term storage) or as fat in adipose tissue (long-term storage). Ingested lipids are broken down into fatty acids and glycerol and any excess is also stored in adipose tissue. Ingested protein is broken down into amino acids, which are used to build the proteins needed for growth and maintenance. Any excess of amino acids is converted to fat and stored in adipose tissue. Although not all animals store excess energy as fat, long-term storage in mammals is always in the form of fat, irrespective of the composition of the food.[286]

The procurement and ingestion of food depend on motor processes that need not be addressed here. However, as mentioned in Chapter 10, hunger and satiety must drive the behavioral process of food intake. In the 1940s and 1950s, researchers identified discrete brain centers in the hypothalamus that seemed to be responsible for hunger (the *lateral hypothalamic area*)[287] and satiety (the *ventromedial hypothalamus*).[288] The locations of these and two other sites later identified as being important in the control of food intake (the *paraventricular nucleus* and the *arcuate nucleus*) are shown in Figure 11.26. Today's researchers no longer believe in discrete functional centers, but these four areas are still considered to be essential components of the neural circuit responsible for the control of hunger and satiety.[289–293] In order to appropriately generate hunger and satiety, these hypothalamic areas must be able to sense the nutritional state of the body. Pertinent information

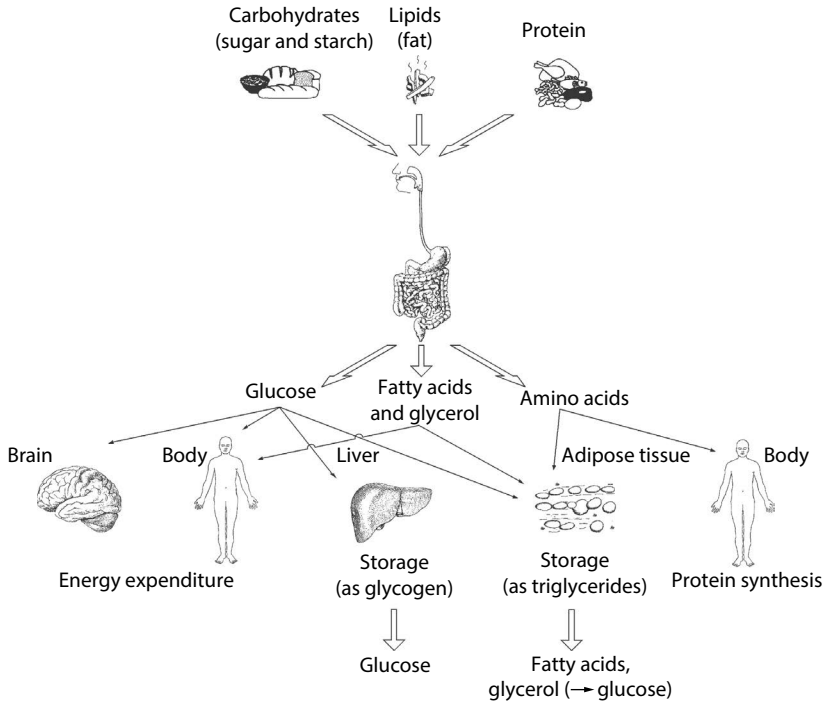


FIGURE 11.25 Origin and destination of nutrients. This diagram shows the three basic types of nutrients (carbohydrates, lipids, and protein), their breakdown by the digestive system, and their three main uses (in energy expenditure, storage, and protein synthesis). For long-term storage, excess energy is stored as fat regardless of the nutrients ingested.

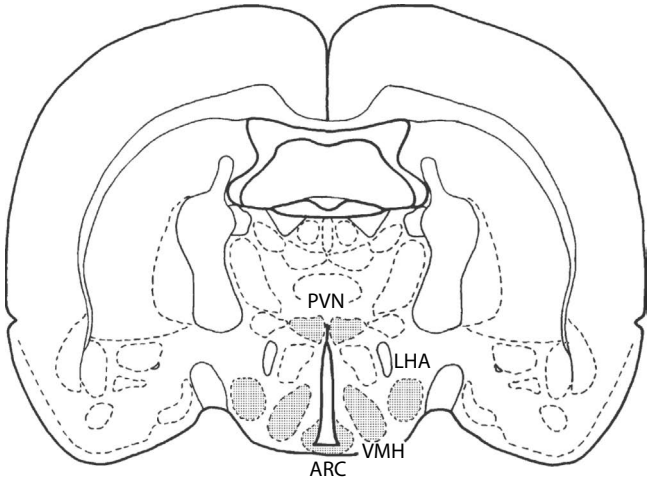


FIGURE 11.26 Brain regions important in the control of food intake. This diagram of the rodent brain (coronal section) shows four regions of the hypothalamus that are involved in the control of food intake: arcuate nucleus of the hypothalamus (ARC), ventromedial nucleus of the hypothalamus (VMH), lateral hypothalamic area (LHA), and paraventricular nucleus of the hypothalamus (PVN). (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979.)

includes blood concentration of nutrients, taste and smell of the food being ingested, gastric distension, gastric contents, and blood levels of hormones secreted by various organs throughout the body.[294] Long-term (hunger) signals must be related to the depletion of energy stores, whereas short-term (satiety) signals may depend on meal-associated factors. Because, as we saw earlier, long-term storage of energy is always in the form of fat, monitoring of fat deposits is a natural candidate for the hunger signal. *Leptin*—the protein product of the *ob* gene[295,296]—seems to fit the bill. Leptin is synthesized by adipocytes (fat cells) and released into the general circulation. Curiously, leptin is actually an anorexic hormone, in the sense that its action on the *arcuate nucleus* is to reduce food intake.[290,291] Presumably, the *default* state of organisms is hunger, not lack thereof, and satiety is attained by long-term and short-term inhibition of hunger.

Depletion of fat stores reduces leptin secretion, and reduced leptin secretion reduces long-term satiety—that is, it increases hunger. Thus, depletion of fat stores increases hunger. This can provide the long-term signal for the control of food intake. Other long-term signals may be used as well. Because the liver provides medium-term storage of energy (as glycogen), depletion of glycogen stores might be used as a hunger signal. This does not seem to happen, however.[297] On the other hand, half-a-century ago, it was suggested that the brain may directly monitor glucose content (or, more precisely, glucose utilization) in the general circulation.[298] This is a more likely possibility, as infusion of glucose into cerebral ventricles inhibits food intake,[299] and neurons sensitive to local concentration of glucose can be found in the hypothalamus.[300–302] An example of a glucose-sensitive neuron is shown in Figure 11.27. A neuron in the lateral hypothalamic area of a rat was stimulated by microinjection (microiontophoresis) of 2-DG (a glucose analog) and orexin-A (a neurotransmitter that is known to stimulate feeding), and its electrical activity

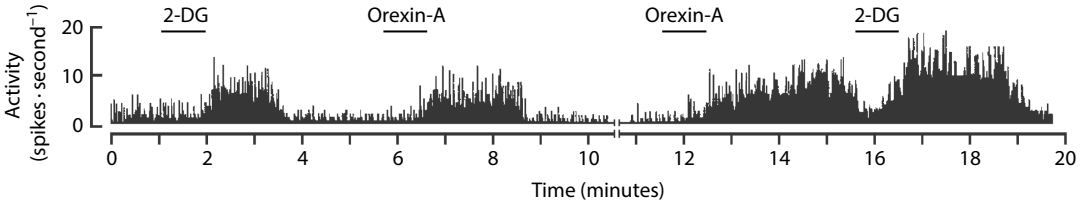


FIGURE 11.27 A glucose-sensitive hypothalamic cell. The graph shows a 20-minute segment of the electrophysiological records of extracellular activity of a glucose-sensitive neuron in the lateral hypothalamus of a rat (*Rattus norvegicus*). The cell was stimulated by microiontophoretic application of either 2-deoxy-glucose (2-DG), a nonmetabolizable analog of glucose, or orexin-A, a hypothalamic peptide previously known to stimulate feeding. (Adapted from Shiraishi, T. et al., *Physiol. Behav.*, 71, 251, 2000.)

was monitored. Notice that the firing rate of the cell was temporarily elevated after delivery of either stimulus.

Various short-term satiety signals, which are necessary for meal termination before the food can be digested and assimilated, have also been identified. Information about gastric distension (stomach fullness) and intestinal assimilation of nutrients is sent to the brain through the vagus nerve and, therefore, provides a short-term satiety signal.[303] The hormone *cholecystokinin* is secreted by the intestine and acts both on vagal terminals and on the brain to inhibit feeding.[304,305] The hormone *insulin* is secreted by the pancreas and acts on insulin receptors in the brain, providing a negative feedback loop for postprandial (after-a-meal) inhibition of food intake.[306,307] The hormone *ghrelin*, which participates also in the regulation of pituitary growth hormone release, is secreted by the stomach and acts on the arcuate nucleus to modulate feeding.[308,309] Curiously, ghrelin is an orexigenic agent (i.e., it increases hunger), which means that the satiety signal must be generated by inhibition rather than by stimulation of ghrelin secretion.

If and how the circadian system utilizes these various food-related signals is not known. A few studies have addressed the issue of nutrient content for synchronization of the food-entrainable oscillator, but the results have not been consistent. In goldfish, the amount of ingested food seems to be more important than the caloric content,[310] whereas the opposite seems to be true for the rat.[311] In mice, a balanced diet seems to be more effective than a pure sugar diet, or a pure protein diet, or a pure oil diet.[312]

In terms of nervous afference from feeding centers to the master circadian pacemaker, anatomical connections have been demonstrated between the arcuate nucleus (one of the aforementioned major feeding centers) and the suprachiasmatic nucleus (the seat of the master circadian pacemaker) in rats.[313]

The limited knowledge about how the circadian system obtains information on nonphotic stimuli may be somewhat frustrating. However, it is very likely that knowledge in this area will increase considerably in the near future. Having discussed sensory receptors in this chapter, we will discuss the cellular and molecular characteristics of the master pacemaker in Chapter 12 and will examine how the information acquired by the receptors is transmitted to the pacemaker in Chapter 13.

SUMMARY

1. The operation of the circadian system may be thought of as a sensors–pacemaker–effectors triad. The organism senses stimuli in the environment, the information from the stimuli affects the pacemaker's operation, and the pacemaker controls effector mechanisms such as locomotor activity and body temperature. Each member of the triad may have many physical components, and the flow of information may include multiple feedback loops.
2. The eye is the only photosensitive organ in mammals. In other vertebrates, photic information can also be acquired by the pineal gland, by the parietal eye (or the parapineal organ), and by additional deep brain photoreceptors. Three types of photoreceptors in the eye provide redundant input to the circadian system: rods, cones, and photosensitive ganglion cells. *Melanopsin* is the photopigment used by photosensitive ganglion cells.
3. Very little is known about how the circadian pacemaker acquires the information about temperature and nutritional state that is needed for nonphotic entrainment. Temperature signals are available from cold- and warm-sensitive cells on the skin and in the body core. Hunger and satiety signals are available from the blood concentration of nutrients, taste and smell of the food being ingested, gastric distension, gastric contents, and blood levels of various hormones secreted by the stomach, by the intestines, and by fat cells.

EXERCISES

11.1 Perception of color. Although the circadian system seems to utilize more photoreceptors than those used for vision, it is very instructive to explore how light of different wavelengths evokes the visual perception of color. Of course, this exercise assumes that you are not *color blind* (that is, that you do not have a deficiency of color vision due to the lack of one or more of the normal visual pigments).

1. The CD that comes with the book contains a short program designed to demonstrate properties of color vision. Although the program does not appear

in the Circadian banner, it should have been copied to your hard drive when the software package was installed. Look for it (**Color.exe**) in the folder where the other programs are (\Program Files\Circadian) and open it.

2. **Color** has two sections. The section on color mixture is quite intuitive and easy to use. You can see for yourself how any color can be produced by the combination of different proportions of the three basic colors (blue, green, and red). This is essentially a consequence of the fact that our visual system uses only three types of color receptors with maximal absorption in the blue, green, and red sections of the spectrum. Notice that, while it may be obvious that the absence of any color is perceived as black, the presence of all three basic colors is perceived as white!
3. The section on color opponency is also simple. Because of the peculiar way in which our photoreceptors connect with bipolar and ganglion cells in the retina, our perception of color is organized in two pairs of opponent colors (red-green and blue-yellow). If you saturate one of the members of a pair, you can experience an afterimage of the other color when you are exposed to a colorless background. Try it out! You may need to blink your eyes briefly after the stimulus disappears in order to see the afterimage. Also, because computer monitors do not produce perfectly monochromatic stimuli, the afterimages may be slightly off-color. Nonetheless, you should be able to see an afterimage in every case.

11.2 Are cold and warmth real? Although light is the major synchronizer of circadian rhythms, other environmental factors, such as ambient temperature and food availability, can affect the phase and period of circadian rhythms. In this brief exercise, we will examine a particular aspect of the sensitivity to ambient temperature.

1. In mammals, as in many other animals, ambient temperature is sensed by specialized skin receptors. Although there is a physical continuum between low and high temperatures, our thermal sensations are processed separately for stimuli below neutrality (cold) and above neutrality (warm). Because "neutrality" is a relative term, "cold" and "warm" must also be relative terms.
2. The equipment needed for the exercise consists simply of three water containers, one filled with cold water (approximately 21°C or 70°F), one filled with "neutral" water (approximately 33°C or 91°F), and one filled with warm water (approximately 38°C or 100°F). The containers should be large enough for you to place your hand inside them.
3. Start by placing your right hand briefly in each of the containers. You should be able to easily distinguish among the three containers even if blindfolded.
4. After a short break, place your right hand in the warm container and your left hand in the cold container. You should again be able to easily feel the difference.
5. After leaving your hands in the two containers for a couple of minutes, move them simultaneously to the neutral container. What happened? Not only is the neutral water no longer neutral, but it actually feels cold to one hand and warm to the other! The same water temperature evokes a sensation of cold in one hand and of warmth in the other hand!
6. What you have just experienced is what the British philosopher John Locke called the "paradox of the basins" in his book *An Essay Concerning Human Understanding*, first published in 1690. Locke's objective was to exemplify the distinction between "primary qualities" and "secondary qualities," which were important concepts in his theory of philosophical empiricism. Today we explain the paradox simply by recognizing that sensory systems were not designed to measure absolute values of environmental variables. Or, to say it more precisely, sensory systems are subject to adaptation. Water at 10°C (50°F) will always feel cold, but, within a wide range of temperatures, the thermal senses will adapt to the current temperature and will label it as "neutral." From that point, a higher temperature will be perceived as warm, even if it is still well below a "normal" neutral temperature. The very opposite is true for high temperatures below 40°C (104°F).

11.3 Electroretinogram (ERG). The electrical activity of cells in the retina can be recorded by electrodes placed on the surface of the eye. *Electroretinogram* is a noninvasive method of neural recording analogous to the *electroencephalogram* (EEG) used to record activity of the brain. Although the ERG is a standard procedure nowadays, it does require sophisticated equipment that is available only in research laboratories and medical establishments. Therefore, you can conduct this exercise only if you have access to a suitable facility. The main investigator (or a technician) at such facility can show you that retinal cells respond reliably to photic stimulation. Based on previous knowledge of intraretinal processes, one can distinguish the responses of rods and cones and of bipolar and ganglion cells that are evoked by discrete pulses of light. The effects of light adaptation can also be easily demonstrated.

SUGGESTIONS FOR FURTHER READING

Warren, E. J., Allen, C. N., Brown, R. L., and Robinson, D. W. (2003). Intrinsic light responses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735.

By now, you should be familiar with the idea that suggestions for further reading on specific topics can be gathered from the list of source articles in the "References" section and that only general readings are listed in this section. However, I would like to explicitly list this research article here because it is an exceptionally good example of a well-written article with rigorous research methods and clear-cut results.

Goldstein, E. B. (2014). *Sensation and Perception*, 9th edn. Belmont, CA: Wadsworth.

A classic undergraduate textbook on sensation and perception. As most textbooks on sensation and perception, it gives prominence to vision and hearing, but the last two chapters cover cutaneous senses and chemical senses.

Fein, A. G. and Szuts, E. Z. (1982). *Photoreceptors: Their Role in Vision*. New York: Cambridge University Press.

An intermediary-level sourcebook on the cellular morphology, pigment chemistry, and receptor physiology of photoreceptors. Although much has been learned about photoreceptors since the publication of this book, the material presented provides a solid background for the understanding of recent developments.

Palmer, S. E. (1999). *Vision Science: Photons to Phenomenology.* Cambridge, MA: MIT Press.

A heavy book (810 pages) on visual perception. Although much of the book deals with higher-level processing, a good deal of material on the anatomy and physiology of photoreceptors is presented.

Hensel, H. (1981). *Thermoreception and Temperature Regulation*. New York: Academic Press.

A classic in thermal physiology. Although this book is over 30 years old, it remains an exceptional summary of scientific research on the temperature senses and their role in body temperature regulation.

WEBSITES TO EXPLORE

Center for Perceptual Systems at the University of Texas: http://www.cps.utexas.edu/.

Physiology of the Senses (University of Western Ontario): http://www.tutis.ca/Senses/.

Vision Science Research (UC Berkeley): http://vision.berkeley.edu/.

Vision Science Resources: http://visionscience.com/.

Vision Sciences Society: http://www.visionsciences.org/.

Webvision: Organization of the Retina and Visual System: http://webvision.med.utah.edu/.

REFERENCES

1. Wiener, N. (1950). *The Human Use of Human Beings: Cybernetics and Society.* Boston, MA: Houghton Mifflin.
2. Pfeifer, R., Lungarella, M., and Iida, F. (2007). Self-organization, embodiment, and biologically inspired robotics. *Science* 318: 1088–1093.
3. Turek, F. W. (1994). Circadian rhythms. *Recent Progress in Hormone Research* 49: 43–90.
4. Fulghum, R. (2003). *All I Really Need to Know I Learned in Kindergarten, 15th Anniversary Edition*. New York: Ballantine.
5. Fernald, R. D. (2006). Casting a genetic light on the evolution of eyes. *Science* 313: 1914–1918.
6. Klerman, E. B., Shanahan, T. L., Brotman, D. J., Rimmer, D. W., Emens, J. S., Rizzo, J. F., and Czeisler, C. A. (2002). Photic resetting of the human circadian pacemaker in the absence of conscious vision. *Journal of Biological Rhythms* 17: 548–555.
7. Silva, M. A. A., Albuquerque, A. M., and Araujo, J. F. (2005). Light–dark cycle synchronization of circadian rhythm in blind primates. *Journal of Circadian Rhythms* 3: art. 10.
8. Goldman, B. D., Goldman, S. L., Riccio, A. P., and Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
9. Halberg, F. (1953). Beobachtungen über 24 Stunden-Periodik in standardisierter Versuchsanordnung vor und nach Epinephrektomie und bilateraler optischer Enucleation. *Berichte über die Gesamte Physiologie und Experimentelle Pharmakologie* 162: 354–355.
10. Halberg, F. and Visscher, M. B. (1954). Some physiologic effects of lighting. In: Tenobergen, J. E. (Ed.), *Proceedings of the First International Photobiological Congress*, Amsterdam, the Netherlands. Wageningen, the Netherlands: Veenman and Zonen, pp. 396–398.
11. Richter, C. P. (1978). "Dark-active" rat transformed into "light-active" rat by destruction of 24-hr clock: Function of 24-hr clock and synchronizers. *Proceedings of the National Academy of Sciences United States of America* 75: 6276–6280.
12. Sato, T. and Kawamura, H. (1984). Effects of bilateral suprachiasmatic nucleus lesions on the circadian rhythms in a diurnal rodent, the Siberian chipmunk (*Eutamias sibiricus*). *Journal of Comparative Physiology A* 155: 745–752.
13. Ruis, J. F., Rietveld, W. J., and Buys, P. J. (1987). Effects of suprachiasmatic nuclei lesions on circadian and ultradian rhythms in body temperature in ocular enucleated rats. *Journal of Interdisciplinary Cycle Research* 18: 259–273.
14. Bobbert, A. C. and Riethoven, J. J. (1991). Feedback in the rabbit's central circadian system, revealed by the changes in its free-running food intake pattern induced by blinding, cervical sympathectomy, pinealectomy, and melatonin administration. *Journal of Biological Rhythms* 6: 263–278.
15. Foster, R. G., Provencio, I., Hudson, D., Fiske, S., De Grip, W., and Menaker, M. (1991). Circadian photoreception in the retinally degenerate mouse (*rd/rd*). *Journal of Comparative Physiology A* 169: 39–50.
16. Yamazaki, S., Goto, M., and Menaker, M. (1999). No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *Journal of Biological Rhythms* 14: 197–201.
17. Freedman, M. S., Lucas, R. J., Soni, B., von Schantz, M., Muñoz, M., David-Gray, Z., and Foster, R. (1999). Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 284: 502–504.
18. Muscat, L. and Morin, L. P. (2005). Binocular contributions to the responsiveness and integrative capacity of the circadian rhythm system to light. *Journal of Biological Rhythms* 20: 513–525.
19. Kandel, E. R., Schwartz, J. H., and Jessell, T. M. (2000). *Principles of Neural Science*, 4th edn. New York: McGraw-Hill.
20. Goldstein, E. B. (2002). *Sensation and Perception*, 6th edn. Pacific Grove, CA: Wadsworth.

21. Marks, W. B., Dobelle, W. H., and MacNichol, E. F. (1964). Visual pigments of single primate cones. *Science* 143: 1181–1182.
22. Brown, P. K. and Wald, G. (1964). Visual pigments in single rods and cones of the human retina. *Science* 144: 45–51.
23. Yoshimura, T., Nishio, M., Goto, M., and Ebihara, S. (1994). Differences in circadian photosensitivity between retinally degenerate CBA/J mice (*rd/rd*) and normal CBA/M mice (+/+). *Journal of Biological Rhythms* 9: 51–60.
24. Yoshimura, T., Yokota, Y., Ishikawa, A., Yasuo, S., Hayashi, N., Suzuki, T., Okabayashi, N., Namikawa, T., and Ebihara, S. (2002). Mapping quantitative trait loci affecting circadian photosensitivity in retinally degenerate mice. *Journal of Biological Rhythms* 17: 512–519.
25. Mrosovsky, N. (2003). Aschoff's rule in retinally degenerate mice. *Journal of Comparative Physiology A* 189: 75–78.
26. Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073.
27. Warren, E. J., Allen, C. N., Brown, R. L., and Robinson, D. W. (2003). Intrinsic light responses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735.
28. Wong, K. Y., Dunn, F. A., and Berson, D. M. (2005). Photoreceptor adaptation in intrinsically photosensitive retinal ganglion cells. *Neuron* 48: 1001–1010.
29. Karnas, D., Hicks, D., Mordel, J., Pévet, P., and Meissl, H. (2013). Intrinsic photosensitive retinal ganglion cells in the diurnal rodent *Arvicanthis ansorgei*. *PLOS ONE* 8: e73343.
30. Cashmore, A. R., Jarillo, J. A., Wu, Y. J., and Liu, D. (1999). Cryptochromes: Blue light receptors for plants and animals. *Science* 284: 760–765.
31. Miyamoto, Y. and Sancar, A. (1998). Vitamin B_2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proceedings of the National Academy of Sciences United States of America* 95: 6097–6102.
32. Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P. et al. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 282: 1490–1494.
33. Van Gelder, R. N., Wee, R., Lee, J. A., and Tu, D. C. (2003). Reduced pupillary light responses in mice lacking cryptochromes. *Science* 299: 222.
34. Thompson, C. L., Selby, C. P., Partch, C. L., Plante, D. T., Thresher, R. J., Araujo, F., and Sancar, A. (2004). Further evidence for the role of cryptochromes in retinohypothalamic photoreception/phototransduction. *Molecular Brain Research* 122: 158–166.
35. Griffin, E. A., Staknis, D., and Weitz, C. J. (1999). Light-independent role of CRY1 and CRY2 in the mammalian clock. *Science* 286: 768–771.
36. Van der Horst, G. T. J., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J. et al. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* 398: 627–630.
37. Okamura, H., Miyake, S., Sumi, Y., Yamaguchi, S., Yasui, A., Muijtjens, M., Hoeijmakers, J. H. J., and van der Horst, G. T. J. (1999). Photic induction of *mPer1* and *mPer2* in *Cry*-deficient mice lacking a biological clock. *Science* 286: 2531–2534.
38. Nagashima, K., Matsue, K., Konishi, M., Iidaka, C., Miyazaki, K., Ishida, N., and Kanosue, K. (2005). The involvement of Cry1 and Cry2 genes in the regulation of the circadian body temperature rhythm in mice. *American Journal of Physiology* 288: R329–R335.
39. Cheng, M. Y., Bittman, E. L., Hattar, S., and Zhou, Q. Y. (2005). Regulation of prokineticin 2 expression by light and the circadian clock. *BMC Neuroscience* 6: art. 17.
40. Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F., and Rollag, M. D. (2000). A novel human opsin in the inner retina. *Journal of Neuroscience* 20: 600–605.
41. Hattar, S., Liao, H. W., Takao, M., Berson, D. M., and Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science* 295: 1065–1069.
42. Gooley, J. J., Lu, J., Fischer, D., and Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience* 23: 7093–7106.
43. Hannibal, J. and Fahrenkrug, J. (2004). Melanopsin-containing retinal ganglion cells are light responsive from birth. *NeuroReport* 15: 2317–2320.
44. Ruby, N. F., Brennan, T. J., Xie, X., Cao, V., Franken, P., Heller, H. C., and O'Hara, B. F. (2002). Role of melanopsin in circadian responses to light. *Science* 298: 2211–2213.
45. Panda, S., Sato, T. K., Castrucci, A. M., Rollag, M. D., DeGrip, W. J., Hogenesch, J. B., Provencio, I., and Kay, S. A. (2002). Melanopsin (*Opn4*) requirement for normal light-induced circadian phase shifting. *Science* 298: 2213–2216.
46. Lucas, R. J., Hattar, S., Takao, M., Berson, D. M., Foster, R. G., and Yau, K. W. (2003). Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* 299: 245–247.
47. Mrosovsky, N. and Hattar, S. (2003). Impaired masking responses to light in melanopsin-knockout mice. *Chronobiology International* 20: 989–999.
48. Panda, S., Nayak, S. K., Campo, B., Walker, J. R., Hogenesch, J. B., and Jegla, T. (2005). Illumination of the melanopsin signaling pathway. *Science* 307: 600–604.
49. Melyan, Z., Tarttelin, E. E., Bellingham, J., Lucas, R. J., and Hankins, M. W. (2005). Addition of human melanopsin renders mammalian cells photoresponsive. *Nature* 433: 741–745.
50. Qiu, X., Kumbalasiri, T., Carlson, S. M., Wong, K. Y., Krishna, V., Provencio, I., and Berson, D. M. (2005). Induction of photosensitivity by heterologous expression of melanopsin. *Nature* 433: 745–749.
51. Hannibal, J., Georg, B., Hindersson, P., and Fahrenkrug, J. (2005). Light and darkness regulate melanopsin in the retinal ganglion cells of the albino Wistar rat. *Journal of Molecular Neuroscience* 27: 147–155.
52. Sekaran, S., Foster, R. G., Lucas, R. J., and Hankins, M. W. (2003). Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Current Biology* 13: 1290–1298.
53. Galindo-Romero, C., Jiménez-López, M., García-Ayuso, D., Salinas-Navarro, M., Nadal-Nicolás, F. M., Aguo-Barriuso, M., Villegas-Pérez, M. P., Avilées-Trigueros, M., and Vidal-Sanz, M. (2013). Number and spatial distribution of intrinsically photosensitive retinal ganglion cells in the adult albino rat. *Experimental Eye Research* 108: 84–93.
54. Jeong, M. J. and Jeon, C. J. (2015). Localization of melanopsin-immunoreactive cells in the Mongolian gerbil retina. *Neuroscience Research* 100: 6–16.
55. Hannibal, J., Hindersson, P., Østergaard, J., Georg, B., Heegaard, S., Larsen, P. J., and Fahrenkrug, J. (2004). Melanopsin is expressed in PACAP-containing retinal ganglion cells of the human retinohypothalamic tract. *Investigative Ophthalmology and Visual Science* 45: 4202–4209.
56. Belenky, M. A., Smeraski, C. A., Provencio, I., Sollars, P. J., and Pickard, G. E. (2003). Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *Journal of Comparative Neurology* 460: 380–393.

57. Semo, M., Peirson, S., Lupi, D., Lucas, R. J., Jeffery, G., and Foster, R. G. (2003). Melanopsin retinal ganglion cells and the maintenance of circadian and pupillary responses to light in aged rodless/coneless (*rd/rd cl*) mice. *European Journal of Neuroscience* 17: 1793–1801.
58. Semo, M., Lupi, D., Peirson, S. N., Butler, J. N., and Foster, R. G. (2003). Light-induced *c-fos* in melanopsin retinal ganglion cells of young and aged rodless/coneless (*rd/rd cl*) mice. *European Journal of Neuroscience* 18: 3007–3017.
59. Hattar, S., Lucas, R. J., Mrosovsky, N., Thompson, S., Douglas, R. H., Hankins, M. W., Lem, J. et al. (2003). Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424: 76–81.
60. Panda, S., Provencio, I., Tu, D. C., Pires, S. S., Rollag, M. D., Castrucci, A. M., Pletcher, M. T. et al. (2003). Melanopsin is required for non-image-forming photic responses in blind mice. *Science* 301: 525–527.
61. Gooley, J. J., Rajaratnam, S. M. W., Brainard, G. C., Kronauer, R. E., Czeisler, C. A., and Lockley, S. W. (2010). Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Science Translational Medicine* 2: 31ra33.
62. Van Diepen, H. C., Ramkisoensing, A., Peirson, S. N., Foster, R. G., and Meijer, J. H. (2013). Irradiance encoding in the suprachiasmatic nuclei by rod and cone photoreceptors. *FASEB Journal* 27: 4204–4212.
63. Tarttelin, E. E., Bellingham, J., Hankins, M. W., Foster, R. G., and Lucas, R. J. (2003). Neuropsin (Opn5): A novel opsin identified in mammalian neural tissue. *FEBS Letters* 554: 410–416.
64. Kojima, D., Mori, S., Torii, M., Wada, A., Morishita, R., and Fukada, Y. (2011). UV-sensitive photoreceptor protein OPN5 in humans and mice. *PLOS ONE* 6: e26388.
65. Peirson, S. N., Bovee-Geurts, P. H. M., Lupi, D., Jeffery, G., DeGrip, W. J., and Foster, R. G. (2004). Expression of the candidate circadian photopigment melanopsin (*Opn4*) in the mouse retinal pigment epithelium. *Molecular Brain Research* 123: 132–135.
66. Chaurasia, S. S., Rollag, M. D., Jiang, G., Hayes, W. P., Haque, R., Natesan, A., Zatz, M. et al. (2005). Molecular cloning, localization and circadian expression of chicken melanopsin (Opn4): Differential regulation of expression in pineal and retinal cell types. *Journal of Neurochemistry* 92: 158–170.
67. Rowe, M. H. (2002). Trichromatic color vision in primates. *News in Physiological Sciences* 17: 93–98.
68. Jacobs, G. H., Neitz, J., and Deegan, J. F. (1991). Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature* 353: 655–656.
69. Lyubarsky, A. L., Falsini, B., Pennesi, M. E., Valentini, P., and Pugh, E. N. (1999). UV- and midwave-sensitive cone-driven retinal responses of the mouse: A possible phenotype for coexpression of cone photopigments. *Journal of Neuroscience* 19: 442–455.
70. McGuire, R. A., Rand, W. M., and Wurtman, R. J. (1973). Entrainment of the body temperature rhythm in rats: Effect of color and intensity of environmental light. *Science* 181: 956–957.
71. Provencio, I. and Foster, R. G. (1995). Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. *Brain Research* 694: 183–190.
72. Von Schantz, M., Argamaso-Hernan, S. M., Szél, A., and Foster, R. G. (1997). Photopigments and photoentrainment in the Syrian golden hamster. *Brain Research* 770: 131–138.
73. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1998). Ultraviolet-light-evoked phase shifts in the locomotor activity rhythm of the field mouse *Mus booduga*. *Journal of Photochemistry and Photobiology B* 45: 83–86.
74. Sharma, V. K. (1999). Ultraviolet light-induced phase response curve for the locomotor activity rhythm of the field mouse *Mus booduga*. *Naturwissenschaften* 86: 96–97.
75. Hut, R. A., Scheper, A., and Daan, S. (2000). Can the circadian system of a diurnal and a nocturnal rodent entrain to ultraviolet light? *Journal of Comparative Physiology A* 186: 707–715.
76. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
77. Van Oosterhout, F., Fisher, S. P., van Diepen, H. C., Watson, T. S., Houben, T., van der Leest, H. T., Thompson, S., Peirson, S. N., Foster, R. G., and Meijer, J. H. (2012). Ultraviolet light provides a major input to non-image-forming light detection in mice. *Current Biology* 22: 1397–1402.
78. Yoshimura, T. and Ebihara, S. (1996). Spectral sensitivity of photoreceptors mediating phase-shifts of circadian rhythms in retinally degenerate CBA/J (*rd/rd*) and normal CBA/N (+/+) mice. *Journal of Comparative Physiology A* 178: 797–802.
79. Lucas, R. J., Douglas, R. H., and Foster, R. G. (2001). Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nature Neuroscience* 4: 621–626.
80. Hankins, M. W. and Lucas, R. J. (2002). The primary visual pathway in humans is regulated according to long-term light exposure through the action of a nonclassical photopigment. *Current Biology* 12: 191–198.
81. Skene, D. J., Lockley, S. W., Thapan, K., and Arendt, J. (1999). Effects of light on human circadian rhythms. *Reproduction Nutrition Development* 39: 295–304.
82. Brainard, G. C., Hanifin, J. P., Greeson, J. M., Byrne, B., Glickman, G., Gerner, E., and Rollag, M. D. (2001). Action spectrum for melatonin regulation in humans: Evidence for a novel circadian photoreceptor. *Journal of Neuroscience* 21: 6405–6412.
83. Lack, L. and Wright, H. (2003). Shorter wavelength light is more effective for changing melatonin phase. *Chronobiology International* 20: 1179–1181.
84. Warman, V. L., Dijk, D. J., Warman, G. R., Arendt, J., and Skene, D. J. (2003). Phase advancing human circadian rhythms with short wavelength light. *Neuroscience Letters* 342: 37–40.
85. Lockley, S. W., Brainard, G. C., and Czeisler, C. A. (2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *Journal of Clinical Endocrinology and Metabolism* 88: 4502–4505.
86. Wright, H. R., Lack, L. C., and Kennaway, D. J. (2004). Differential effects of light wavelength in phase advancing the melatonin rhythm. *Journal of Pineal Research* 36: 140–144.
87. Takahashi, J. S., DeCoursey, P. J., Mauman, L., and Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.
88. Nelson, D. E. and Takahashi, J. S. (1991). Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *Journal of Physiology* 439: 115–145.
89. Sakamoto, K., Liu, C., and Tosini, G. (2004). Classical photoreceptors regulate *melanopsin* mRNA levels in the rat retina. *Journal of Neuroscience* 24: 9693–9697.

90. Walmsley, L., Hanna, L., Mouland, J., Martial, F., West, A., Smedley, A. R., Bechtold, D. A., Webb, A. R., Lucas, R. J., and Brown, T. M. (2015). Colour as a signal for entraining the mammalian circadian clock. *PLoS Biology* 13: e1002127.
91. Szél, A., Ákos, L., Fekete, T., Szepessy, Z., and Röhlich, P. (2000). Photoreceptor distribution in the retinas of subprimate mammals. *Journal of the Optical Society of America A* 17: 568–579.
92. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences United States of America* 59: 414–421.
93. McMillan, J. P., Keatts, H. C., and Menaker, M. (1975). On the role of eyes and brain photoreceptors in the sparrow: Entrainment to light cycles. *Journal of Comparative Physiology* 102: 251–256.
94. Oshima, I., Yamada, H., Goto, M., Sato, K., and Ebihara, S. (1989). Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. *Journal of Comparative Physiology A* 166: 217–226.
95. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
96. Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J. C., and Stanewsky, R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30: 249–261.
97. Rieger, D., Stanewsky, R., and Helfrich-Förster, C. (2003). Cryptochrome, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. *Journal of Biological Rhythms* 18: 377–391.
98. Foster, R. G. and Menaker, M. (1993). Circadian photoreception in mammals and other vertebrates. In: Wetterberg, L. (Ed.), *Light and Biological Rhythms in Man*. Oxford, U.K.: Pergamon, pp. 73–91.
99. Underwood, H. (2001). Circadian organization in nonmammalian vertebrates. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 111–140.
100. Bertolucci, C. and Foà, A. (2004). Extraocular photoreception and circadian entrainment in nonmammalian vertebrates. *Chronobiology International* 21: 501–519.
101. Pasqualetti, M., Bertolucci, C., Ori, M., Innocenti, A., Magnone, M. C., De Grip, W. J., Nardi, I., and Foà, A. (2003). Identification of circadian brain photoreceptors mediating photic entrainment of behavioural rhythms in lizards. *European Journal of Neuroscience* 18: 364–372.
102. Halford, S., Pires, S. S., Turton, M., Zheng, L., González-Menéndez, I., Davies, W. L., Peirson, S. N., García-Fernáandez, J. M., Hankins, M. W., and Foster, R. G. (2009). VA opsin-based photoreceptors in the hypothalamus of birds. *Current Biology* 19: 1396–1402.
103. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J., and Van Cauter, E. (1997). Sleepiness, performance, and neuroendocrine function during sleep deprivation: Effects of exposure to bright light or exercise. *Journal of Biological Rhythms* 12: 245–258.
104. Shanahan, T. L., Kronauer, R. E., Duffy, J. F., Williams, G. H., and Czeisler, C. A. (1999). Melatonin rhythm observed throughout a three-cycle bright-light stimulus designed to reset the human circadian pacemaker. *Journal of Biological Rhythms* 14: 237–253.
105. Zeitzer, J. M., Dijk, D. J., Kronauer, R. E., Brown, E. N., and Czeisler, C. A. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: Melatonin phase resetting and suppression. *Journal of Physiology* 526: 695–702.
106. Hébert, M., Martin, S. K., Lee, C., and Eastman, C. I. (2002). The effects of prior light on the suppression of melatonin by light in humans. *Journal of Pineal Research* 33: 198–203.
107. Glickman, G., Hanifin, J. P., Rollag, M. D., Wang, J., Cooper, H., and Brainard, G. C. (2003). Inferior retinal light exposure is more effective than superior retinal exposure in suppressing melatonin in humans. *Journal of Biological Rhythms* 18: 71–79.
108. Rüger, M., Gordijn, M. C. M., Beersma, D. G. M., de Vries, B., and Daan, S. (2003). Acute and phase-shifting effects of ocular and extraocular light in human circadian physiology. *Journal of Biological Rhythms* 18: 409–419.
109. Smith, K. A., Schoen, M. W., and Czeisler, C. A. (2004). Adaptation of human pineal melatonin suppression by recent photic history. *Journal of Clinical Endocrinology and Metabolism* 89: 3610–3614.
110. Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A., and Markey, S. P. (1980). Light suppresses melatonin secretion in humans. *Science* 210: 1267–1269.
111. Cagnacci, A., Soldani, R., and Yen, S. S. C. (1993). The effect of light on core body temperature is mediated by melatonin in women. *Journal of Clinical Endocrinology and Metabolism* 76: 1036–1038.
112. Lavoie, S., Paquet, J., Selmaoui, B., Rufiange, M., and Dumont, M. (2003). Vigilance levels during and after bright light exposure in the first half of the night. *Chronobiology International* 20: 1019–1038.
113. Rüger, M., Gordijn, M. C. M., Beersma, D. G. M., de Vries, B., and Daan, S. (2005). Nasal versus temporal illumination of the human retina: Effects on core body temperature, melatonin, and circadian phase. *Journal of Biological Rhythms* 20: 60–70.
114. Jasser, S. A., Hanifin, J. P., Rollag, M. D., and Brainard, G. C. (2006). Dim light adaptation attenuates acute melatonin suppression in humans. *Journal of Biological Rhythms* 21: 394–404.
115. Smith, J. S., Kripke, D. F., Elliott, J. A., and Youngstedt, S. D. (2002). Illumination of upper and middle visual fields produces equivalent suppression of melatonin in older volunteers. *Chronobiology International* 19: 883–891.
116. Mien, I. H., Chua, E. C., Lau, P., Tan, L. C., Lee, I. T., Yeo, S. C., Tan, S. S., and Gooley, J. J. (2014). Effects of exposure to intermittent versus continuous red light on human circadian rhythms, melatonin suppression, and pupillary constriction. *PLOS ONE* 9: e96532.
117. Maywood, E. S., Hastings, M. H., Max, M., Ampleford, E., Menaker, M., and Loudon, A. S. I. (1993). Circadian and daily rhythms of melatonin in the blood and pineal gland of free-running and entrained Syrian hamsters. *Journal of Endocrinology* 136: 65–73.
118. Lucas, R. J., Freedman, M. S., Muñoz, M., Garcia-Fernández, J. M., and Foster, R. G. (1999). Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* 284: 505–507.
119. Nakahara, D., Nakamura, M., Iigo, M., and Okamura, H. (2003). Bimodal circadian secretion of melatonin from the pineal gland in a living CBA mouse. *Proceedings of the National Academy of Sciences United States of America* 100: 9584–9589.

120. Kennaway, D. J., Voultsios, A., Varcoe, T. J., and Moyer, R. W. (2002). Melatonin in mice: Rhythms, response to light, adrenergic stimulation, and metabolism. *American Journal of Physiology* 282: R358–R365.
121. Bronstein, D. M., Jacobs, G. H., Haak, K. A., Neitz, J., and Lytle, L. D. (1987). Action spectrum of the retinal mechanism mediating nocturnal light-induced suppression of rat pineal gland *N*-acetyltransferase. *Brain Research* 406: 352–356.
122. Bayarri, M. J., Muñoz-Cueto, J. A., López-Olmeda, J. F., Vera, L. M., Rol-de-Lama, M. A., Madrid, J. A., and Sánchez-Vázquez, F. J. (2004). Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). *Physiology and Behavior* 81: 577–583.
123. Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K., and Tabata, M. (2003). Variations in plasma melatonin levels of the rainbow trout (*Oncorhynchus mykiss*) under various light and temperature conditions. *Zoological Science* 20: 1011–1016.
124. Hasegawa, M. and Ebihara, S. (1992). Circadian rhythms of pineal melatonin release in the pigeon measured by in vivo microdialysis. *Neuroscience Letters* 148: 89–92.
125. Zawilska, J. B., Lorenc, A., Berezinska, M., Vivien-Roels, B., Pévet, P., and Skene, D. J. (2006). Diurnal and circadian rhythms in melatonin synthesis in the turkey pineal gland and retina. *General and Comparative Endocrinology* 145: 162–168.
126. Torres, G. and Lytle, L. D. (1989). Extraretinal mechanisms mediate light-induced changes in neonatal rat pineal gland N-acetyltransferase activity. *Journal of Pineal Research* 7: 211–220.
127. Menaker, M., Moreira, L. F., and Tosini, G. (1997). Evolution of circadian organization in vertebrates. *Brazilian Journal of Medical and Biological Research* 30: 305–313.
128. Kasahara, T., Okano, T., Haga, T., and Fukada, Y. (2002). Opsin-G_{11}-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *Journal of Neuroscience* 22: 7321–7325.
129. Okano, T., Yoshizawa, T., and Fukada, Y. (1994). Pinopsin is a chicken pineal photoreceptive molecule. *Nature* 372: 94–97.
130. Max, M., McKinnon, P. J., Seidenman, K. J., Barrett, R. K., Applebury, M. L., Takahashi, J. S., and Margolskee, R. F. (1995). Pineal opsin: A nonvisual opsin expressed in chick pineal. *Science* 267: 1502–1506.
131. Holthues, H., Engel, L., Spessert, R., and Vollrath, L. (2005). Circadian gene expression patterns of melanopsin and pinopsin in the chick pineal gland. *Biochemical and Biophysical Research Communications* 326: 160–165.
132. Okano, T., Takanaka, Y., Nakamura, A., Hirunagi, K., Adachi, A., Ebihara, S., and Fukada, Y. (1997). Immunocytochemical identification of pinopsin in pineal glands of chicken and pigeon. *Molecular Brain Research* 50: 190–196.
133. Campbell, S. S. and Murphy, P. J. (1998). Extraocular circadian phototransduction in humans. *Science* 279: 396–399.
134. Murphy, P. J. and Campbell, S. S. (2001). Enhancement of REM sleep during extraocular light exposure in humans. *American Journal of Physiology* 280: R1606-R1612.
135. Hébert, M., Martin, S. K., and Eastman, C. I. (1999). Nocturnal melatonin secretion is not suppressed by light exposure behind the knee in humans. *Neuroscience Letters* 274: 127–130.
136. Lindblom, N., Heiskala, H., Hätönen, T., Mustanoja, S., Alfthan, H., Alila-Johansson, A., and Laakso, M. L. (2000). No evidence for extraocular light induced phase shifting of human melatonin, cortisol and thyrotropin rhythms. *NeuroReport* 11: 713–717.
137. Jean-Louis, G., Kripke, D. F., Cole, R. J., and Elliott, J. A. (2000). No melatonin suppression by illumination of popliteal fossae or eyelids. *Journal of Biological Rhythms* 15: 265–269.
138. Lushington, K., Galka, R., Sassi, L. N., Kennaway, D. J., and Dawson, D. (2002). Extraocular light exposure does not phase shift saliva melatonin rhythms in sleeping subjects. *Journal of Biological Rhythms* 17: 377–386.
139. Wright, K. P. and Czeisler, C. A. (2002). Absence of circadian phase resetting in response to bright light behind the knees. *Science* 297: 571.
140. Meijer, J. H., Thio, B., Albus, H., Schaap, J., and Ruijs, A. C. J. (1999). Functional absence of extraocular photoreception in hamster circadian rhythm entrainment. *Brain Research* 831: 337–339.
141. Chono, K., Fujito, Y., and Ito, E. (2002). Non-ocular dermal photoreception in the pond snail *Lymnaea stagnalis*. *Brain Research* 951: 107–112.
142. Moriya, T., Miyashita, Y., Arai, J., Kusunoki, S., Abe, M., and Asami, K. (1996). Light-sensitive response in melanophores of *Xenopus laevis*. I. Spectral characteristics of melanophore response in isolated tail fin of *Xenopus* tadpole. *Journal of Experimental Zoology* 276: 11–18.
143. Tosini, G. and Avery, R. A. (1996). Dermal photoreceptors regulate basking behavior in the lizard *Podarcis muralis*. *Physiology and Behavior* 59: 195–198.
144. Ebaugh, F. G. and Thauer, R. (1950). Influence of various environmental temperatures on the cold and warmth thresholds. *Journal of Applied Physiology* 3: 173–182.
145. Gray, L., Stevens, J. C., and Marks, L. E. (1982). Thermal stimulus thresholds: Sources of variability. *Physiology and Behavior* 29: 355–360.
146. Stevens, J. C. and Stevens, S. S. (1960). Warmth and cold: Dynamics of sensory intensity. *Journal of Experimental Psychology* 60: 183–192.
147. Stevens, S. S. (1965). Matching functions between loudness and ten other continua. *Perception and Psychophysics* 1: 5–8.
148. Banks, W. P. (1969). Temperature sensitivity: One subjective continuum or two? *Perception and Psychophysics* 6: 189–192.
149. Green, B. G. (1984). Thermal perception on lingual and labial skin. *Perception and Psychophysics* 36: 209–220.
150. Green, B. G. (1977). Localization of thermal sensation: An illusion and synthetic heat. *Perception and Psychophysics* 22: 331–337.
151. Young, A. A. (1987). Thermal sensations during simultaneous warming and cooling at the forearm: A human psychophysical study. *Journal of Thermal Biology* 12: 243–247.
152. Refinetti, R. (1990). Is there modal opponency in the thermal senses? *Journal of Thermal Biology* 15: 255–258.
153. Hensel, H. (1974). Thermoreceptors. *Annual Review of Physiology* 36: 233–249.
154. Schepers, R. J. and Ringkamp, M. (2009). Thermoreceptors and thermosensitive afferents. *Neuroscience and Biobehavioral Reviews* 33: 205–212.
155. Adrian, E. D. and Zotterman, Y. (1926). The impulses produced by sensory nerve-endings. Part 2. The response of a single end-organ. *Journal of Physiology* 61: 151–171.
156. Zotterman, Y. (1935). Action potentials in the glossopharyngeal nerve and in the chorda tympani. *Skandinavishes Archiv für Physiologie* 72: 73–77.
157. Iriuchijima, J. and Zotterman, Y. (1960). The specificity of afferent cutaneous C fibres in mammals. *Acta Physiologica Scandinavica* 49: 267–278.
158. Iggo, A. (1969). Cutaneous thermoreceptors in primates and sub-primates. *Journal of Physiology* 200: 403–430.

159. Hellon, R. F., Hensel, H., and Schäfer, K. (1975). Thermal receptors in the scrotum of the rat. *Journal of Physiology* 248: 349–357.
160. Hensel, H. (1953). Das Verhalten der Thermoreceptoren bei Temperatursprüngen. *Pflügers Archiv* 256: 470–487.
161. Hensel, H. and Witt, I. (1959). Spatial temperature gradient and thermoreceptor stimulation. *Journal of Physiology* 148: 180–187.
162. Hensel, H., Iggo, A., and Witt, I. (1960). A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. *Journal of Physiology* 153: 113–126.
163. Martin, H. F. and Manning, J. W. (1969). Rapid thermal cutaneous stimulation: Peripheral nerve responses. *Brain Research* 16: 524–526.
164. Hensel, H. (1968). Spezifische Wärmeimpulse aus der Nasenregion der Katze. *Pflügers Archiv* 302: 374–376.
165. Hensel, H. and Wurster, R. D. (1969). Static behaviour of cold receptors in the trigeminal area. *Pflügers Archiv* 313: 153–154.
166. Hensel, H. and Kenshalo, D. R. (1969). Warm receptors in the nasal region of cats. *Journal of Physiology* 204: 99–112.
167. Kenshalo, D. R. and Brearley, E. A. (1970). Electrophysiological measurements of the sensitivity of cat's upper lip to warm and cool stimuli. *Journal of Comparative and Physiological Psychology* 70: 5–14.
168. Hensel, H., Andres, K. H., and von Düring, M. (1974). Structure and function of cold receptors. *Pflügers Archiv* 352: 1–10.
169. Duclaux, R., Schäfer, K., and Hensel, H. (1980). Response of cold receptors to low skin temperatures in nose of cat. *Journal of Neurophysiology* 43: 1571–1577.
170. Ivanov, K., Konstantinov, V., and Danilova, N. (1982). Thermoreceptor localization in the deep and surface skin layers. *Journal of Thermal Biology* 7: 75–78.
171. Ivanov, K., Konstantinov, V., Danilova, N., Sleptchuck, N., and Rumiantsev, G. (1986). Thermoreceptor distribution in different skin layers and its significance for thermoregulation. *Journal of Thermal Biology* 11: 25–29.
172. Iggo, A. (1964). Temperature discrimination in the skin. *Nature* 204: 481–483.
173. Kenshalo, D. R. and Gallegos, E. S. (1967). Multiple temperature-sensitive spots innervated by single nerve fibers. *Science* 158: 1064–1065.
174. Hensel, H. (1969). Cutane Wärmereceptoren bei Primaten. *Pflügers Archiv* 313: 150–152.
175. Poulos, D. A. and Lende, R. A. (1970). Response of trigeminal ganglion neurons to thermal stimulation of oral-facial regions. *Journal of Neurophysiology* 33: 508–517.
176. Hensel, H. and Iggo, A. (1971). Analysis of cutaneous warm and cold fibres in primates. *Pflügers Archiv* 329: 1–8.
177. Kenshalo, D. R. and Duclaux, R. (1977). Response characteristics of cutaneous cold receptors in the monkey. *Journal of Neurophysiology* 40: 319–332.
178. Darian-Smith, I., Johnson, K. O., LaMotte, C., Shigenaga, Y., Kenins, P., and Champness, P. (1979). Warm fibers innervating palmar and digital skin of the monkey: Responses to thermal stimuli. *Journal of Neurophysiology* 42: 1297–1315.
179. Duclaux, R. and Senshalo, D. R. (1980). Response characteristics of cutaneous warm receptors in the monkey. *Journal of Neurophysiology* 43: 1–15.
180. LaMotte, R. H. and Thalhammer, J. G. (1982). Response properties of high-threshold cutaneous cold receptors in the primate. *Brain Research* 244: 279–287.
181. Hensel, H. and Boman, K. K. A. (1960). Afferent impulses in cutaneous sensory nerves in human subjects. *Journal of Neurophysiology* 23: 564–578.
182. Konietzny, F. and Hensel, H. (1975). Warm fiber activity in human skin nerves. *Pflügers Archiv* 359: 265–267.
183. Konietzny, F. and Hensel, H. (1977). The dynamic response of warm units in human skin nerves. *Pflügers Archiv* 370: 111–114.
184. Hensel, H. (1983). Recent advances in thermoreceptor physiology. *Journal of Thermal Biology* 8: 3–6.
185. Pierau, F. K. (1996). Peripheral thermosensors. In: Fregly, M. J. and Blatteis, C. M. (Eds.), *Handbook of Physiology, Section 4: Environmental Physiology*, Vol. 1. New York: Oxford University Press, pp. 85–104.
186. Caterina, M. J., Leffler, A., Malmberg, A. B., Martin, W. J., Trafton, J., Petersen-Zeitz, K. R., Koltzenburg, M., Basbaum, A. I., and Julius, D. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288: 306–313.
187. Peier, A. M., Reeve, A. J., Andersson, D. A., Moqrich, A., Earley, T. J., Hergarden, A. C., Story, G. M. et al. (2002). A heat-sensitive TRP channel expressed in keratinocytes. *Science* 296: 2046–2049.
188. McKemy, D. D., Neuhausser, W. M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416: 52–58.
189. Lee, H., Iida, T., Mizuno, A., Suzuki, M., and Caterina, M. J. (2005). Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. *Journal of Neuroscience* 25: 1304–1310.
190. Moqrich, A., Hwang, S. W., Earley, T. J., Petrus, M. J., Murray, A. N., Spencer, K. S. R., Andahazy, M., Story, G. M., and Patapoutian, A. (2005). Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. *Science* 307: 1468–1472.
191. Zimmermann, K., Lennerz, J. K., Hein, A., Link, A. S., Kaczmarek, J. S., Delling, M., Uysal, S., Pfeifer, J. D., Riccio, A., and Clapham, D. E. (2011). Transient receptor potential cation channel, subfamily C, member 5 (TRPC5) is a cold-transducer in the peripheral nervous system. *Proceedings of the National Academy of Sciences United States of America* 108: 18114–18119.
192. Pogorzala, L. A., Mishra, S. K., and Hoon, M. A. (2013). The cellular code for mammalian thermosensation. *Journal of Neuroscience* 33: 5533–5541.
193. Chao, Y. C., Chen, C. C., Lin, Y. C., Breer, H., Fleischer, J., and Yang, R. B. (2015). Receptor guanylyl cyclase-G is a novel thermosensory protein activated by cool temperatures. *EMBO Journal* 34: 294–306.
194. Brück, K. and Hinckel, P. (1990). Thermoafferent networks and their adaptive modifications. In: Schönbaum, E. and Lomax, P. (Eds.), *Thermoregulation: Physiology and Biochemistry*. New York: Pergamon, pp. 129–152.
195. Magoun, H. W., Harrison, F., Brobeck, J. R., and Ranson, S. W. (1938). Activation of heat loss mechanisms by local heating of the brain. *Journal of Neurophysiology* 1: 101–114.
196. Folkow, B., Ström, G., and Uvnäs, B. (1949). Cutaneous vasodilatation elicited by local heating of the anterior hypothalamus in cats and dogs. *Acta Physiologica Scandinavica* 17: 317–326.
197. Forster, R. E. and Ferguson, T. B. (1952). Relationship between hypothalamic temperature and thermoregulatory effectors in unanesthetized cat. *American Journal of Physiology* 169: 255–269.
198. Chatonnet, J., Tanche, M., and Cabanac, J. L. (1960). Influence d'un abaissement de température localisé de la région diencéphalique sur l'activité musculaire thermorégulatrice chez le chien éveillé. *Journal de Physiologie* 52: 48–49.

199. Hammel, H. T., Hardy, J. D., and Fusco, M. M. (1960). Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. *American Journal of Physiology* 198: 481–486.
200. Fusco, M. M., Hardy, J. D., and Hammel, H. T. (1961). Interaction of central and peripheral factors in physiological temperature regulation. *American Journal of Physiology* 200: 572–580.
201. Satinoff, E. (1964). Behavioral thermoregulation in response to local cooling of the rat brain. *American Journal of Physiology* 206: 1389–1394.
202. Carlisle, H. J. (1966). Behavioural significance of hypothalamic temperature-sensitive cells. *Nature* 209: 1324–1325.
203. Malan, A. (1969). Controle hypothalamique de la thermorégulation et de l'hibernation chez le hamster d'Europe *Cricetus cricetus*. *Archives des Sciences Physiologiques* 23: 47–87.
204. Cabanac, M. and Hardy, J. D. (1969). Réponses unitaires et thermorégulatrices lors de réchauffements et refroidissements localisés de la région préoptique et du mésencéphale chez le lapin. *Journal de Physiologie* 61: 331–347.
205. Roberts, W. W., Bergquist, E. H., and Robinson, T. C. L. (1969). Thermoregulatory grooming and sleep-like relaxation induced by local warming of preoptic area and anterior hypothalamus in opossum. *Journal of Comparative and Physiological Psychology* 67: 182–188.
206. Adair, E. R. (1970). Control of thermoregulatory behavior by brief displacements of hypothalamic temperature. *Psychonomic Science* 20: 11–13.
207. Adair, E. R., Casby, J. U., and Stolwijk, J. A. J. (1970). Behavioral temperature regulation in the squirrel monkey: Changes induced by shifts in hypothalamic temperature. *Journal of Comparative and Physiological Psychology* 72: 17–27.
208. Gale, C. C., Mathews, M., and Young, J. (1970). Behavioral thermoregulatory responses to hypothalamic cooling and warming in baboons. *Physiology and Behavior* 5: 1–6.
209. Mills, S. H. and Heath, J. E. (1972). Responses to thermal stimulation of the preoptic area in the house sparrow, *Passer domesticus*. *American Journal of Physiology* 222: 914–919.
210. Adair, E. R. and Rawson, R. O. (1974). Autonomic and behavioral temperature regulation: Unilateral vs. bilateral preoptic thermal stimulation. *Pflügers Archiv* 352: 91–103.
211. Fuller, C. A., Horwitz, B. A., and Horowitz, J. M. (1975). Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming. *American Journal of Physiology* 228: 1519–1524.
212. McEwen, G. N. and Heath, J. E. (1975). Thermal responses to preoptic heating and ambient temperature in unrestrained rabbits. *Journal of Thermal Biology* 1: 23–27.
213. Simon, E., Simon-Oppermann, C., Hammel, H. T., Kaul, R., and Maggert, J. (1976). Effects of altering rostral brain stem temperature on temperature regulation in the Adelie penguin, *Pygoscelis adeliae*. *Pflügers Archiv* 362: 7–13.
214. Banet, M. and Hensel, H. (1976). Nonshivering thermogenesis induced by repetitive hypothalamic cooling in the rat. *American Journal of Physiology* 230: 522–526.
215. Schmidt, I. (1976). Effect of central thermal stimulation on the thermoregulatory behavior of the pigeon. *Pflügers Archiv* 363: 271–272.
216. Simon-Oppermann, C., Simon, E., Jessen, C., and Hammel, H. T. (1978). Hypothalamic thermosensitivity in conscious Pekin ducks. *American Journal of Physiology* 235: R130–R140.
217. Carlisle, H. J. and Laudenslager, M. L. (1979). Observations on the thermoregulatory effects of preoptic warming in rats. *Physiology and Behavior* 23: 723–732.
218. Schmidt, I. and Simon, E. (1982). Negative and positive feedback of central nervous system temperature in thermoregulation of pigeons. *American Journal of Physiology* 243: R363–R372.
219. Cabanac, M. and Dib, B. (1983). Behavioural responses to hypothalamic cooling and heating in the rat. *Brain Research* 264: 79–87.
220. Kanosue, K., Nakayama, T., Tanaka, H., Yanase, M., and Yasuda, H. (1990). Modes of action of local hypothalamic and skin thermal stimulation on salivary secretion in rats. *Journal of Physiology* 424: 459–471.
221. Klir, J. J. and Heath, J. E. (1994). Thermoregulatory responses to thermal stimulation of the preoptic anterior hypothalamus in the red fox (*Vulpes vulpes*). *Comparative Biochemistry and Physiology A* 109: 557–566.
222. Kanosue, K., Chen, X. M., Hosono, T., Yoda, T., and Liu, S. (1999). Inhibitory control of shivering and nonshivering thermogenesis by preoptic warm-sensitive neurons. *Journal of Thermal Biology* 24: 351–354.
223. Wünnenberg, W. and Brück, K. (1968). Zur Funktionsweise thermoreceptiver Strukturen im Cervicalmark des Meerschweinchens. *Pflügers Archiv* 299: 1–10.
224. Jessen, C. and Mayer, E. T. (1971). Spinal cord and hypothalamus as core sensors of temperature in the conscious dog. I. Equivalence of responses. *Pflügers Archiv* 324: 189–204.
225. Rautenberg, W., Necker, R., and May, B. (1972). Thermoregulatory responses of the pigeon to changes of the brain and spinal cord temperatures. *Pflügers Archiv* 338: 31–42.
226. Chai, C. Y. and Lin, M. T. (1972). Effects of heating and cooling the spinal cord and medulla oblongata on thermoregulation in monkeys. *Journal of Physiology* 225: 297–308.
227. Carlisle, H. J. and Ingram, D. L. (1973). The effects of heating and cooling the spinal cord and hypothalamus on thermoregulatory behaviour in the pig. *Journal of Physiology* 231: 353–364.
228. Banet, M. and Hensel, H. (1976). The interaction between cutaneous and spinal thermal inputs in the control of oxygen consumption in the rat. *Journal of Physiology* 260: 461–473.
229. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. II. Spinal thermosensitivity. *Pflügers Archiv* 386: 181–185.
230. Avery, P. and Richards, S. A. (1983). Thermosensitivity of the hypothalamus and spinal cord in the domestic fowl. *Journal of Thermal Biology* 8: 237–239.
231. Wünnenberg, W. (1983). Thermosensitivity of the preoptic region and the spinal cord in the golden hamster. *Journal of Thermal Biology* 8: 381–384.
232. Barnas, G. M., Gleeson, M., Nomoto, S., and Rautenberg, W. (1985). Comparison between pigeon and chicken with regard to respiratory and cardiovascular responses to spinal cord cooling at different ambient temperatures. *Journal of Thermal Biology* 10: 51–54.
233. Graf, R., Heller, H. C., Sakaguchi, S., and Krishna, S. (1987). Influence of spinal and hypothalamic warming on metabolism and sleep in pigeons. *American Journal of Physiology* 252: R661–R667.
234. Rawson, R. O. and Quick, K. P. (1970). Evidence of deep-body thermoreceptor response to intra-abdominal heating of the ewe. *Journal of Applied Physiology* 28: 813–820.

235. Riedel, W., Siaplauras, G., and Simon, E. (1973). Intra-abdominal thermosensitivity in the rabbit as compared with spinal thermosensitivity. *Pflügers Archiv* 340: 59–70.
236. Jessen, C. and Feistkorn, G. (1984). Some characteristics of core temperature signals in the conscious goat. *American Journal of Physiology* 247: R456–R464.
237. Merver, J. B. and Simon, E. (1984). A comparison between total body thermosensitivity and local thermosensitivity in mammals and birds. *Pflügers Archiv* 400: 228–234.
238. Simon, E., Pierau, F. K., and Taylor, D. C. M. (1986). Central and peripheral thermal control of effectors in homeothermic temperature regulation. *Physiological Reviews* 66: 235–300.
239. Adair, E. R. (1974). Hypothalamic control of thermoregulatory behavior: Preoptic-posterior hypothalamic interaction. In: Lederis, K. and Cooper, K. E. (Eds.), *Recent Studies of Hypothalamic Function*. Basel, Switzerland: Karger, pp. 341–358.
240. Refinetti, R. and Carlisle, H. J. (1986). Effects of anterior and posterior hypothalamic temperature changes on thermoregulation in the rat. *Physiology and Behavior* 36: 1099–1103.
241. Tanaka, H., Kanosue, K., Nakayama, T., and Shen, Z. (1986). Grooming, body extension, and vasomotor responses induced by hypothalamic warming at different ambient temperatures in rats. *Physiology and Behavior* 38: 145–151.
242. Carlisle, H. J. (1969). Effect of preoptic and anterior hypothalamic lesions on behavioral thermoregulation in the cold. *Journal of Comparative and Physiological Psychology* 69: 391–402.
243. Satinoff, E., Valentino, D., and Teitelbaum, P. (1976). Thermoregulatory cold-defense deficits in rats with preoptic/anterior hypothalamic lesions. *Brain Research Bulletin* 1: 553–565.
244. Toth, D. M. (1973). Temperature regulation and salivation following preoptic lesions in the rat. *Journal of Comparative and Physiological Psychology* 82: 480–488.
245. Morimoto, A. and Murakami, N. (1985). [^{14}C]deoxyglucose incorporation into rat brain regions during hypothalamic or peripheral thermal stimulation. *American Journal of Physiology* 248: R84–R92.
246. Gilbert, T. M. and Blatteis, C. M. (1977). Hypothalamic thermoregulatory pathways in the rat. *Journal of Applied Physiology* 43: 770–777.
247. Han, P. W. and Brobeck, J. R. (1961). Deficits of temperature regulation in rats with hypothalamic lesions. *American Journal of Physiology* 200: 707–710.
248. Briese, E. (1989). Do medial preoptic lesions interfere with the set-point of temperature regulation? *Brain Research Bulletin* 23: 137–144.
249. Lipton, J. M. (1968). Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiology and Behavior* 3: 165–169.
250. Joyce, M. P. and Barr, G. A. (1992). The appearance of Fos protein-like immunoreactivity in the hypothalamus of developing rats in response to cold ambient temperatures. *Neuroscience* 49: 163–173.
251. Ishiwata, T., Hasegawa, H., Yasumatsu, M., Akano, F., Yazawa, T., Otokawa, M., and Aihara, Y. (2001). The role of preoptic area and anterior hypothalamus and median raphe nucleus on thermoregulatory system in freely moving rats. *Neuroscience Letters* 306: 126–128.
252. Bratincsák, A. and Palkovits, M. (2004). Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience* 127: 385–397.
253. Satinoff, E. (1983). Disturbances in circadian body temperature rhythms in rats with hypothalamic damage. *Journal of Thermal Biology* 8: 149–157.
254. Ott, I. (1887). The heat-center in the brain. *Journal of Nervous and Mental Disease* 14: 152–162.
255. Keller, A. D. and Hare, W. K. (1932). The hypothalamus and heat regulation. *Proceedings of the Society for Experimental Biology and Medicine* 29: 1069–1070.
256. White, W. H. (1890). The effect upon the bodily temperature of lesions of the corpus striatum and optic thalamus. *Journal of Physiology* 11: 1–24.
257. Hori, T., Kiyohara, T., Oomura, Y., Nishino, H., Aou, S., and Fujita, I. (1987). Activity of thermosensitive neurons of monkey preoptic hypothalamus during thermoregulatory operant behavior. *Brain Research Bulletin* 18: 649–655.
258. Hagan, A. A. and Heath, J. E. (1980). Effects of preoptic lesions on thermoregulation in ducks. *Journal of Thermal Biology* 5: 141–150.
259. Lepkovsky, S., Snapir, N., and Furuta, F. (1968). Temperature regulation and appetitive behavior in chickens with hypothalamic lesions. *Physiology and Behavior* 3: 911–915.
260. Eissel, K. and Simon, E. (1980). How are neuronal thermosensitivity and lack of thermoreception related in the duck's hypothalamus? A tentative answer. *Journal of Thermal Biology* 5: 219–223.
261. Nakashima, T., Pierau, F. K., Simon, E., and Hori, T. (1987). Comparison between hypothalamic thermoresponsive neurons from duck and rat slices. *Pflügers Archiv* 409: 236–243.
262. Wünnenberg, W., Merker, G., and Speulda, E. (1976). Thermosensitivity of preoptic neurones in a hibernator (golden hamster) in a non-hibernator (guinea pig). *Pflügers Archiv* 363: 119–123.
263. Boulant, J. A. and Bignall, K. E. (1973). Determinants of hypothalamic neuronal thermosensitivity in ground squirrels and rats. *American Journal of Physiology* 225: 306–310.
264. Vasilenko, V. Y. and Gourine, V. N. (1992). Threshold temperature responses of thermosensitive neurons in guinea-pig hypothalamic slices. *Journal of Thermal Biology* 17: 367–374.
265. Glotzbach, S. F. and Heller, H. C. (1984). Changes in the thermal characteristics of hypothalamic neurons during sleep and wakefulness. *Brain Research* 309: 17–26.
266. Jahns, R. and Werner, J. (1974). Special aspects of firing rates and thermosensitivity of preoptic neurons. *Experimental Brain Research* 21: 107–112.
267. Silva, N. L. and Boulant, J. A. (1984). Effects of osmotic pressure, glucose, and temperature on neurons in preoptic tissue slices. *American Journal of Physiology* 247: R335-R345.
268. Baldino, F. and Geller, H. M. (1982). Electrophysiological analysis of neuronal thermosensitivity in rat preoptic and hypothalamic tissue cultures. *Journal of Physiology* 327: 173–184.
269. Dean, J. B. and Boulant, J. A. (1989). In vitro localization of thermosensitive neurons in the rat diencephalon. *American Journal of Physiology* 257: R57-R64.
270. Dean, J. B. and Boulant, J. A. (1989). Effects of synaptic blockade on thermosensitive neurons in rat diencephalon in vitro. *American Journal of Physiology* 257: R65-R73.
271. Kelso, S. R. and Boulant, J. A. (1982). Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. *American Journal of Physiology* 243: R480–R490.
272. Kelso, S. R., Perlmutter, M. N., and Boulant, J. A. (1982). Thermosensitive single-unit activity of in vitro hypothalamic slices. *American Journal of Physiology* 242: R77–R84.

273. Sato, H. (1984). Midbrain neurons of rats responsive to hypothalamic temperature change and their local thermosensitivity. *Journal of Thermal Biology* 9: 39–45.
274. Parmeggiani, P. L., Azzaroni, A., Cevolani, D., and Ferrari, G. (1983). Responses of anterior hypothalamic-preoptic neurons to direct thermal stimulation during wakefulness and sleep. *Brain Research* 269: 382–385.
275. Nakayama, T., Eisenman, J. S., and Hardy, J. D. (1961). Single unit activity of anterior hypothalamus during local heating. *Science* 134: 560–561.
276. Eisenman, J. S. and Jackson, D. C. (1967). Thermal response patterns of septal and preoptic neurons in cats. *Experimental Neurology* 19: 33–45.
277. Hardy, J. D., Hellon, R. F., and Sutherland, K. (1964). Temperature-sensitive neurones in the dog's hypothalamus. *Journal of Physiology* 175: 242–253.
278. Cabanac, M., Stolwijl, J. A. J., and Hardy, J. D. (1968). Effect of temperature and pyrogens on single-unit activity in the rabbit's brain stem. *Journal of Applied Physiology* 24: 645–652.
279. Nakayama, T. and Hardy, J. D. (1969). Unit responses in the rabbit's brain stem to changes in brain and cutaneous temperature. *Journal of Applied Physiology* 27: 848–857.
280. Hellon, R. F. (1972). Temperature-sensitive neurons in the brain stem: Their responses to brain temperature at different ambient temperatures. *Pflügers Archiv* 335: 323–334.
281. Greer, G. L. and Gardner, D. R. (1970). Temperature-sensitive neurons in the brain of brook trout. *Science* 169: 1220–1222.
282. Nelson, D. O. and Prosser, C. L. (1981). Intracellular recordings from thermosensitive preoptic neurons. *Science* 213: 787–789.
283. Cabanac, M., Hammel, T., and Hardy, J. D. (1967). Tiliqua scincoides: Temperature-sensitive units in lizard brain. *Science* 158: 1050–1051.
284. Derambure, P. S. and Boulant, J. A. (1994). Circadian thermosensitive characteristics of suprachiasmatic neurons in vitro. *American Journal of Physiology* 266: R1876–R1884.
285. Ruby, N. F., Burns, D. E., and Heller, H. C. (1999). Circadian rhythms in the suprachiasmatic nucleus are temperature-compensated and phase-shifted by heat pulses *in vitro*. *Journal of Neuroscience* 19: 8630–8636.
286. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th edn. New York: Cambridge University Press.
287. Anand, B. K. and Brobeck, J. R. (1951). Localization of a "feeding center" in the hypothalamus of the rat. *Proceedings of the Society for Experimental Biology and Medicine* 77: 323–324.
288. Hetherington, A. W. and Ranson, S. W. (1940). Hypothalamic lesions and adiposity in the rat. *Anatomical Record* 78: 149–172.
289. Strubbe, J. H. (1994). Regulation of food intake. In: Westerterp-Plantenga, M. S., Fredrix, E. W. H. M., and Steffens, A. B. (Eds.), *Food Intake and Energy Expenditure*. Boca Raton, FL: CRC Press, pp. 141–154.
290. Woods, S. C., Seeley, R. J., Porte, D., and Schwartz, M. W. (1998). Signals that regulate food intake and energy homeostasis. *Science* 280: 1378–1383.
291. Funahashi, H., Takenoys, F., Guan, J. L., Kageyama, H., Yada, T., and Shioda, S. (2003). Hypothalamic neuronal networks and feeding-related peptides involved in the regulation of feeding. *Anatomical Science International* 78: 123–138.
292. Gao, Q. and Horvath, T. L. (2007). Neurobiology of feeding and energy expenditure. *Annual Review of Neuroscience* 30: 367–398.
293. Zheng, H. and Berthoud, H. R. (2008). Neural systems controlling the drive to eat: Mind versus metabolism. *Physiology* 23: 75–83.
294. Kreier, F., Kalsbeek, A., Ruiter, M., Yilmaz, A., Romijn, J. A., Sauerwein, H. P., Fliers, E., and Buijs, R. M. (2003). Central nervous determination of food storage: A daily switch from conservation to expenditure: Implications for the metabolic syndrome. *European Journal of Pharmacology* 480: 51–65.
295. Zhang, Y., Proença, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425–432.
296. Weigle, D. S., Bukowski, T. R., Foster, D. C., Holderman, S., Kramer, J. M., Lasser, G., Lofton-Day, C. E., Prunkard, D. E., Raymond, C., and Kuijper, J. L. (1995). Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. *Journal of Clinical Investigation* 96: 2065–2070.
297. Inoue, S., Satoh, S., Saito, M., Naitoh, M., Suzuki, H., and Egawa, M. (1995). Effects of selective vagotomy on circadian rhythms of plasma glucose, insulin and food intake in control and ventromedial hypothalamic (VMH) lesioned rats. *Obesity Research* 3(S): 747S–752S.
298. Mayer, J. (1955). Regulation of energy intake and body weight: The glucostatic theory and the lipostatic hypothesis. *Annals of the New York Academy of Sciences* 63: 15–43.
299. Davis, J. D., Wirtshafter, D., Asin, K. E., and Brief, D. (1981). Sustained intracerebroventricular infusion of brain fuels reduces body weight and food intake in rats. *Science* 212: 81–82.
300. Boulant, J. A. and Silva, N. L. (1988). Neuronal sensitivities in preoptic tissue slices: Interactions among homeostatic systems. *Brain Research Bulletin* 20: 871–878.
301. Ritter, S., Dinh, T. T., and Zhang, Y. (2000). Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Research* 856: 37–47.
302. Lam, T. K. T., Gutierrez-Juarez, R., Pocai, A., and Rossetti, L. (2005). Regulation of blood glucose by hypothalamic pyruvate metabolism. *Science* 309: 943–947.
303. Phillips, R. J. and Powley, T. L. (1996). Gastric volume rather than nutrient content inhibits food intake. *American Journal of Physiology* 271: R766–R779.
304. Weller, A., Smith, G. P., and Gibbs, J. (1990). Endogenous cholecystokinin reduces feeding in young rats. *Science* 247: 1589–1591.
305. Della-Fera, M. A., Coleman, B. D., and Baile, C. A. (1990). CNS injection of CCK in rats: Effects on real and sham feeding and gastric emptying. *American Journal of Physiology* 258: R1165–R1169.
306. Brüning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., Klein, R., Krone, W., Müller-Wieland, D., and Kahn, C. R. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science* 289: 2122–2125.
307. Schwartz, M. W. and Porte, D. (2005). Diabetes, obesity, and the brain. *Science* 307: 375–379.
308. Tschöp, M., Smiley, D. L., and Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407: 908–913.
309. St.-Pierre, D. H., Wang, L., and Taché, Y. (2003). Ghrelin: A novel player in the gut-brain regulation of growth hormone and energy expenditure. *News in Physiological Sciences* 18: 242–246.

310. Sánchez-Vásquez, F., Aranda, A., and Madrid, J. A. (2001). Differential effects of meal size and food energy density on feeding entrainment in goldfish. *Journal of Biological Rhythms* 16: 58–65.
311. Stephan, F. K. (1997). Calories affect zeitgeber properties of the feeding entrained circadian oscillator. *Physiology and Behavior* 62: 995–1002.
312. Hirao, A., Tahara, Y., Kimura, I., and Shibata, S. (2009). A balanced diet is necessary for proper entrainment signals of the mouse liver clock. *PLOS ONE* 4: e6909.
313. Yi, C. X., van der Vliet, J., Dai, J., Yin, G., Ru, L., and Buijs, R. M. (2006). Ventromedial arcuate nucleus communicates peripheral metabolic information to the suprachiasmatic nucleus. *Endocrinology* 147: 283–294.

This page intentionally left blank

12 Pacemakers

12.1 SUPRACHIASMATIC NUCLEUS

If you have been reading this book from the beginning, you know that organisms have a circadian clock. However, once we know that there is a clock somewhere in the body, how do we go about locating this clock? There is no easy answer to this question, especially when one is dealing with complex organisms such as mammals. As the brain controls most bodily functions, one would be disposed to look at the brain first. But where in the brain? The brain contains about 100 billion nerve cells, which renders the trial-and-error approach useless. Maybe luck? As a matter of fact, the discovery of a "pleasure center" in the brain by James Olds over half a century ago[1] happened by sheer luck. When Olds was a graduate student, he was assigned the job of implanting electrodes (very thin pieces of conducting wire) in the brains of experimental rats for his professor's research. Of course, the electrodes were implanted under anesthesia and were so small that they did not bother the animals afterward. The goal was to pass minute electrical currents to simulate the natural electrical activity of nerve cells. As a beginner, Olds was so inaccurate in the placement of the electrodes that he ended up placing them almost on the opposite side of the brain than that intended by his professor. It ruined the professor's study. However, it resulted in a pleasant surprise: it turned out that direct electrical stimulation of that part of the brain (the *lateral hypothalamus*) resulted in the closest thing to "ultimate pleasure" that a rat could experience. Rats would spend hours performing a dull task (depressing a lever on the cage wall) if this behavior provided them with stimulation of the lateral hypothalamus (Figure 12.1). They would cross a highly charged electrical grid in order to reach the lever and be able to stimulate their own brains. They would forego food or a female in heat in order to self-stimulate. Thus, Olds seemed to have found the *pleasure center* in the brain. By pure luck, he made one of the first discoveries about neural mechanisms involved in motivation.

The discovery of the anatomical site of the master circadian pacemaker in mammals also depended on unplanned actions and the ability to integrate fragmented pieces of evidence. Although much less was known about circadian rhythms in the early 1970s than is known now, researchers were confident that a circadian clock would be found somewhere in the body. As we saw in previous chapters, the study of circadian rhythms had indicated the necessity of the existence of a circadian pacemaker. Researchers also knew that the pacemaker was sensitive to light, although they had no idea whether the pacemaking property of the clock and its sensitivity to light had a single physical substrate or two separate substrates.

12.1.1 Lesion Studies

In the early 1970s, Friedrich Stephan—whose more recent work we discussed in Chapter 8—was a graduate student in Irving Zucker's laboratory at the University of California, Berkeley. Stephan's original interest was in reproductive physiology and behavior. Zucker's main interest, however, was in circadian rhythms, and one need not guess what kind of research Stephan ended up doing. His interest in reproductive physiology came out handy, though. It happened that reproductive physiologists at the time had already found out that the estrous cycle in rats (the equivalent of the menstrual cycle in humans) is dependent on a small part of the brain, namely, the rostral (frontal) part of the hypothalamus (Figure 12.2). When a dependence of the estrous cycle on circadian rhythms was established,[2] Stephan put A and B together and wondered whether the circadian pacemaker might be located in the rostral hypothalamus. Yet, the rostral hypothalamus is not that small. Where in the rostral hypothalamus could the circadian pacemaker be?

The next hint came from findings by neuroanatomist Robert Moore, who at that time was at the University of Chicago. Moore had been studying the nervous pathways that take information about light from the eyes to the brain. One pathway was already well known: the one related to vision. What was not clear was whether there would be a separate pathway responsible for the entrainment of circadian rhythms. What Moore found out—and Stephan heard about it even before publication—was that there was indeed a second path from the retina of the eyes to the rostral hypothalamus, which was accordingly named the *retino-hypothalamic tract* (see Chapter 13). Where did the retino-hypothalamic tract end? At a small bilateral nucleus located at the base of the brain called the *suprachiasmatic nucleus* (SCN). As shown in Figure 12.3, the suprachiasmatic nucleus (or nuclei, since there are two of them) is so called because it sits right above the optic chiasm (the crossing of the optic nerves). To avoid a common mistake made by undergraduate students, notice that the SCN is *suprachiasmatic*, not *supercharismatic* (although circadian physiologists would not object to the characterization of the SCN as charismatic!). So, Stephan now had a specific target to aim at. The SCN had to be the physical substrate of the circadian pacemaker.

Now, once you have a suspicion, how do you proceed? Well, if a brain structure houses the biological clock, then destruction of this structure should eliminate biological timing. Right, but how can you destroy a minute part of the brain of a living animal without damaging everything around it? This is a question that researchers had faced in the late 1800s when they started to systematically study the effects of

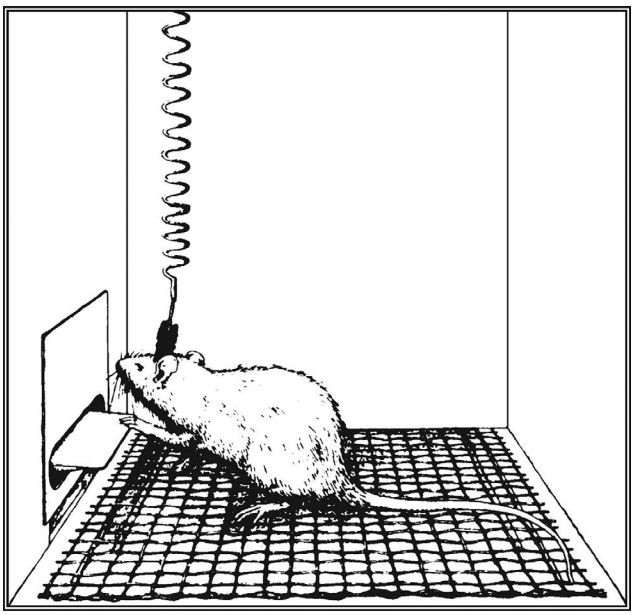


FIGURE 12.1 Having fun. The diagram shows the apparatus used for intracranial self-stimulation. When the rat presses the lever, it triggers a highly pleasurable electric stimulus to the brain. (Adapted from Olds, J., *Sci. Am.*, 195, 105, 1956.)

the destruction of different brain areas on a variety of physiological processes (such as hunger, thirst, and temperature regulation). By the 1950s, procedures for selectively lesioning small areas of the brain had been well established. Although this type of brain surgery, like any type of brain surgery, is

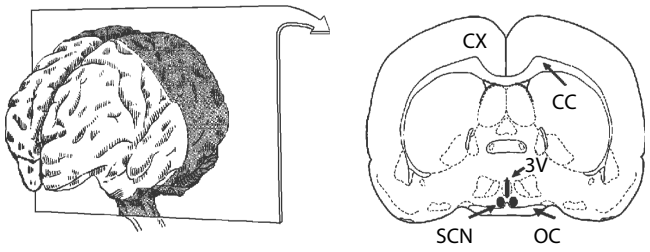


FIGURE 12.3 Locating the suprachiasmatic nucleus (SCN). This diagram shows the location of the SCN in a coronal section of the mammalian brain. *Abbreviations:* 3V, third ventricle; CX, cerebral cortex; CC, corpus callosum; OC, optic chiasm; SCN, suprachiasmatic nucleus.

certainly not something simple, it requires only three basic elements: a stereotaxic atlas, a stereotaxic instrument, and a lesioning electrode. A *stereotaxic atlas* is simply a map of the brain that shows where each structure is in relation to the skull (Figure 12.4). A *stereotaxic instrument* is a precision instrument that allows the investigator to reach structures in the brain with millimetric resolution through a tiny hole drilled in the skull (Figure 12.5). A *lesioning electrode* is an electrode (thin wire) used to lesion the brain tissue, usually by passage of moderate electric current. Thus, the atlas tells you where the particular brain structure is, the stereotaxic instrument allows you to reach the structure without damaging the surrounding tissue, and the electrode provides the means to lesion the structure.

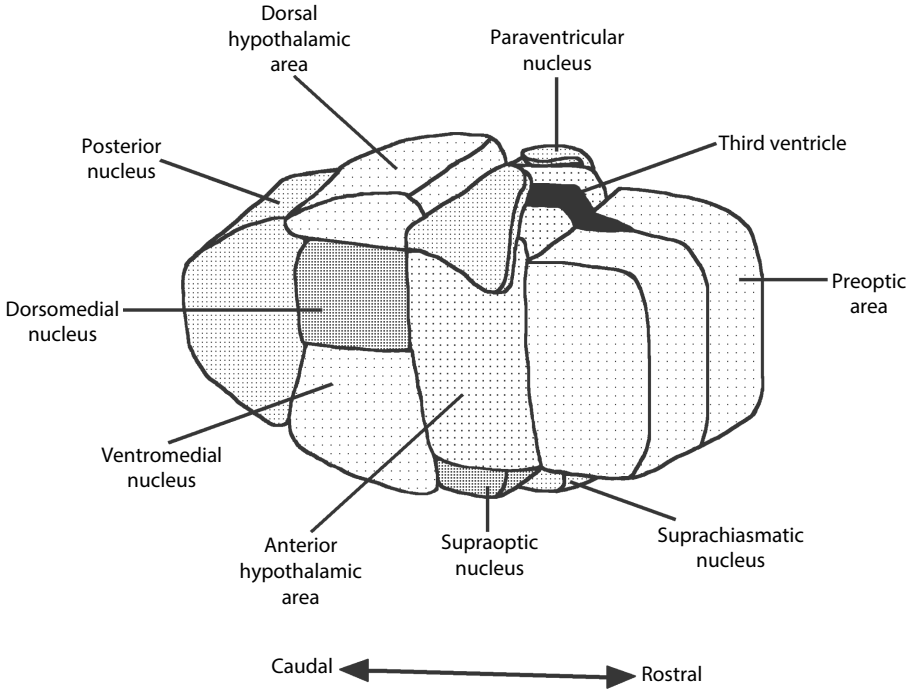


FIGURE 12.2 The hypothalamus. This diagram identifies the various subdivisions of the hypothalamus. The hypothalamus is a small structure at the base of the brain that controls most vital functions in vertebrates. One major subdivision, the lateral hypothalamic area, is not shown. (Adapted from Kalat, J.W., *Biological Psychology*, 7th edn., Wadsworth, Belmont, CA, 2001.)

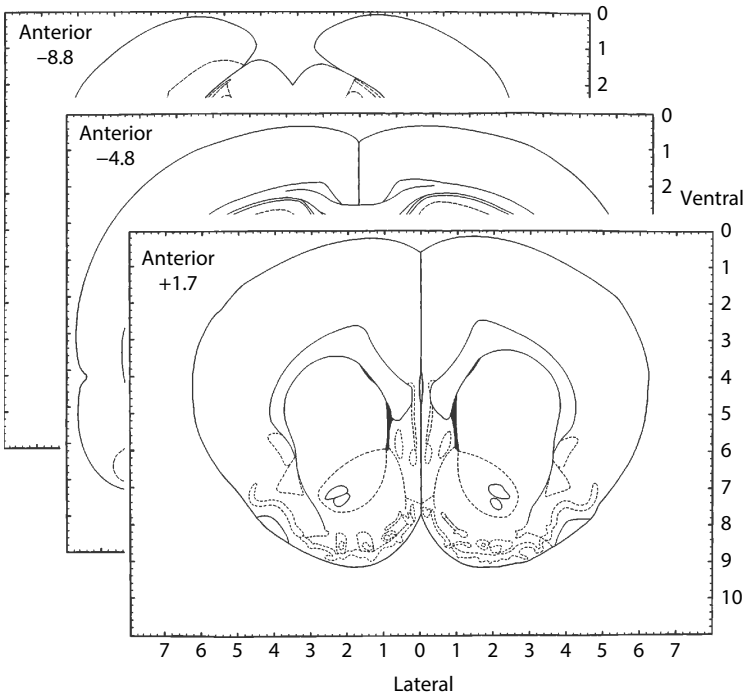


FIGURE 12.4 A stereotaxic atlas. These sample pages from a stereotaxic atlas of the rat brain indicate how brain structures can be located in a coordinate system with three axes: anterior, lateral, and ventral. Measurements in millimeters are given in reference to a skull landmark (bregma). (Adapted from Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, 2nd edn., Academic Press, San Diego, CA, 1986.)

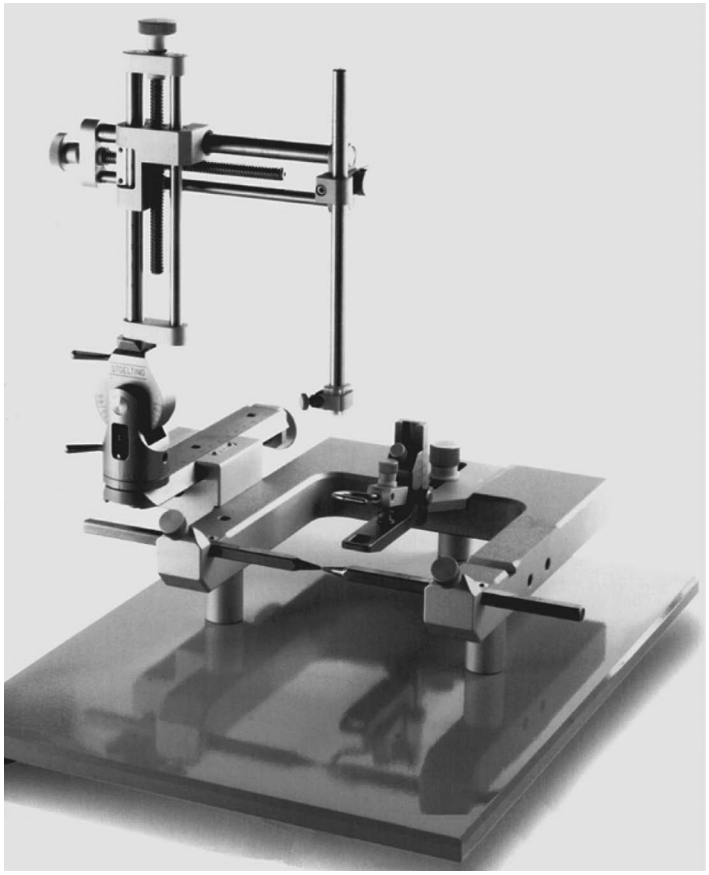


FIGURE 12.5 A stereotaxic instrument. A stereotaxic instrument is a device that allows precise access to brain structures through a small hole in the skull. (Image courtesy of Stoelting Co., Wood Dale, IL.)

So, armed with the suspicion that the circadian pacemaker might be located in the SCN, and the knowledge of how to destroy the SCN without damaging other parts of the brain, Stephan went to work on his project. After many of the usual setbacks and partial accomplishments, he eventually found that complete lesions of the SCN (but not partial ones) eliminated the circadian rhythms of behavioral activity and drinking in rats. Hooray! He had found the physical substrate of the biological clock! A great achievement that, he thought, deserved to be publicized in the top science journal, *Science*. As it turned out, the editors of *Science* were not that impressed with his findings and declined to publish his article. The article was published in the *Proceedings of the National Academy of Sciences* (a very reputable publication) in 1972.[3] Not surprisingly, Moore's team also inferred the role of the SCN in circadian rhythmicity and published results similar to Stephan's in the same year of 1972.[4]

Figure 12.6 shows the ventral parts of coronal slices of golden hamster brains without any lesion (Panel A) and with a large lesion that destroyed the SCN entirely (Panel B). Lesions of this magnitude have been shown to eliminate the circadian rhythm of activity in lizards,[5–7] birds,[8,9] Syrian hamsters,[10–18] Siberian hamsters,[19–21] rats,[22–37] mice,[38–43] and other rodents.[44–47] Destruction of the SCN also eliminated daily rhythmicity of activity in rhesus monkeys[48] and was associated with loss of rhythmicity in a woman with a gunshot wound in the brain.[49]

The effects of complete lesions of the SCN are permanent, as exemplified by the records of running-wheel activity of a Siberian chipmunk (*Eutamias sibiricus*) shown in Figure 12.7. Because large SCN lesions extend into the optic chiasm (thus interrupting the afference of photic information from the eyes), animals with SCN lesions are arrhythmic under light–dark cycles as well as under constant darkness and constant light.

The rhythm of body temperature is also eliminated by SCN lesions, as exemplified in Figure 12.8. This has been documented in golden hamsters,[16,50,51] Siberian hamsters,[19,20,52] rats,[23,24,26,29,31,36,53–55] and ground squirrels.[56] I should point out that several researchers reported persistence of the temperature rhythm in animals whose activity rhythm had been eliminated by SCN lesions.[22,39,40,57–60] Incomplete lesions might have been responsible for the discrepancy, and this became a very likely explanation when it was demonstrated that differences in the robustness of the rhythms of activity and body temperature could account for the apparent elimination of the activity rhythm in animals that still displayed temperature rhythmicity.[16] In other studies, large SCN lesions have been shown to eliminate a variety of other rhythms, including those of feeding and drinking,[22–24,31,42,46,48,61–63] gnawing,[64,65] heart rate,[29,32,65] blood pressure,[30] dentin increment,[33] intraocular pressure,[66] sleep,[45,53,67,68] and secretion of various hormones.[65,69–74] In contrast, circadian rhythmicity was found *not* to be eliminated by lesions of other hypothalamic and extrahypothalamic sites.[75–82]

12.1.2 Monitoring of SCN Activity

Anyone who heard the anecdote about the legless flea knows that lesion studies are not sufficient to settle the issue of the location of the circadian pacemaker. The anecdote goes somewhat like this:

> A flea trainer conducted an experiment with one of his jumping fleas. He approached the flea and said "Jump, flea"—and the flea jumped. So, he wrote down: "A healthy, 6-legged flea will jump on command." Then, he removed one of the flea's legs and said "Jump, flea"—and the flea obliged. The amateur experimenter proceeded meticulously, removing a leg at a time and observing the results. Finally, when the flea had no legs left, it did not jump on command. The flea trainer wrote down: "A flea without legs is deaf."

Obviously, removal of the legs had nothing to do with the flea's ability to hear the commands. The flea could hear perfectly well. It just did not have the legs to jump. Likewise, the SCN might be simply part of the nervous pathway that connects the clock to the effector organs! If the SCN really contains a clock,

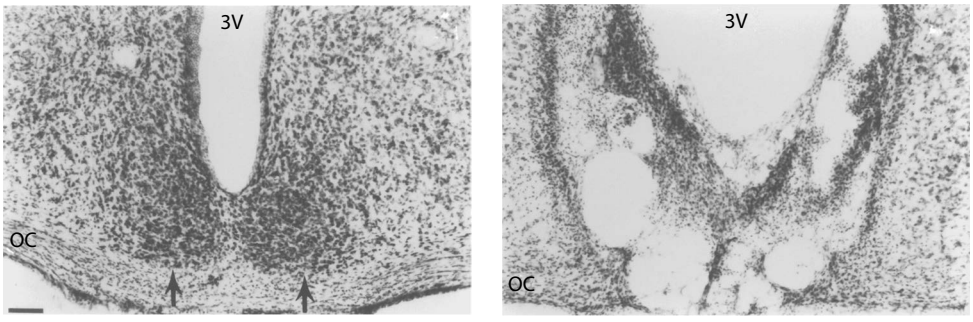


FIGURE 12.6 Lesioning the suprachiasmatic nucleus (SCN). These microphotographs of Nissl-stained coronal sections of the brains of two golden hamsters (*Mesocricetus auratus*) show the region that normally contains the suprachiasmatic nuclei (dorsally to the optic chiasm and ventrally to the third ventricle). The brain on the left (A) has both nuclei intact (arrows). The brain on the right (B) sustained a complete bilateral lesion of the SCN. *Abbreviations:* 3V, third ventricle; OC, optic chiasm. (With kind permission from Springer Science+Business Media: *J. Comp. Physiol. A*, Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters, 175, 1994, 223, Refinetti, R., Kaufman, C.M., and Menaker, M., Copyright 1994.)

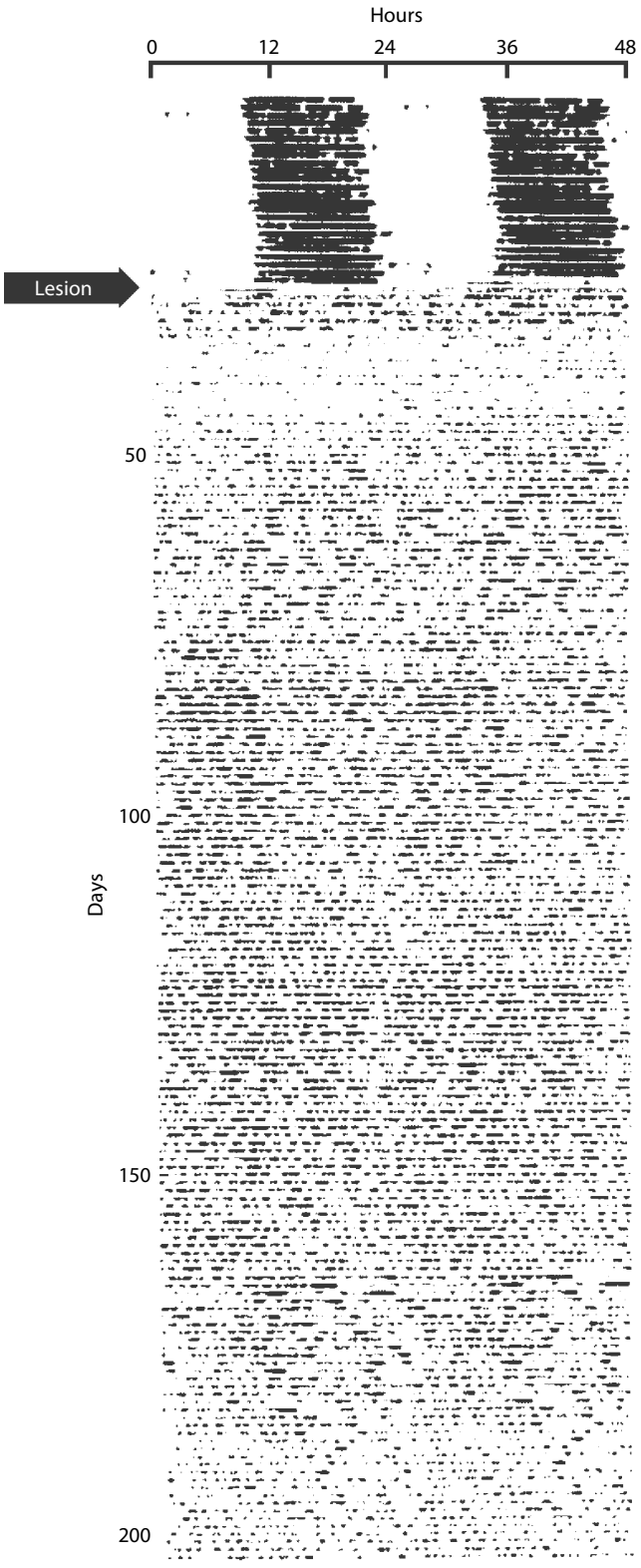


FIGURE 12.7 Suprachiasmatic nucleus (SCN) lesion eliminates rhythm of locomotor activity. This double-plotted actogram shows the rhythm of running-wheel activity of a Siberian chipmunk (*Eutamias sibiricus*) maintained in constant light before and after a complete lesion of the SCN (arrow). Notice the absence of any sign of recovery of circadian rhythmicity for 5 months after the lesion. (Adapted from Sato, T. and Kawamura, H., *J. Comp. Physiol. A*, 155, 745, 1984.)

it should display rhythmicity in isolation. Can we remove the SCN from the brain and study it in a dish? Actually, we can. Electrophysiologists had been studying hypothalamic tissue *in vitro* for years when circadian biologists decided to record the electrical activity of SCN cells from the rat brain. Brain slices cannot be studied for several months, as whole animals can, but clear circadian rhythmicity in the electrical activity of rat SCN cells was recorded continuously for several days by various research groups.[83–92] Although rats are active at night, SCN cells were found to be more active during what would have been subjective day if the animals had not been killed. Similar results were obtained in studies using electrophysiological recording as well as other methods in rats and other species.[93–116]

So, destruction of the SCN in whole animals abolishes circadian rhythmicity, and SCN slices *in vitro* exhibit rhythmicity. Is this proof enough that the SCN is the neural substrate of the circadian pacemaker? Not if you are a compulsive skeptic, as most scientists are. Brain-slice and cell-culture preparations are ostensibly unnatural; would the SCN display rhythmicity *in vivo* (that is, in the intact animal)? This has been investigated in two ways. Some researchers replicated the *in vitro* studies *in vivo*. By using chronically implanted recording electrodes, they recorded the electrical activity of SCN tissue in freely moving animals and found robust circadian rhythmicity.[87,117–126] An example is shown in Figure 12.9. Multiple-unit neural activity was recorded from various brain sites of freely moving golden hamsters. Notice that the firing rate of SCN cells exhibits robust daily rhythmicity. Notice also that SCN cells are more active during the daytime. Rhythmicity in other areas of the brain is not as robust and peaks during the nighttime in this nocturnal species.

Other researchers measured *in vivo* SCN activity using the 2-deoxy-glucose and Fos methodologies. You will remember from Chapter 2 that the 2-deoxy-glucose methodology (2-DG, for short) can determine the parts of the brain that are more active by taking advantage of the fact that structures that are more active accumulate more radioactive 2-DG molecules. The use of this methodology allowed investigators to determine that the SCN of freely moving animals exhibits daily or circadian rhythmicity, being more active during the daytime.[127–132] Figure 12.10 shows autoradiographs of rat brains when the SCN was very active (Panel A) and when it was rather inactive (Panel B). Studies using the Fos methodology also documented *in vivo* rhythmicity of the SCN.[133–138] Regardless of methodology, the SCN was found to be more active during the day (or subjective day) in both nocturnal and diurnal species. The rest of the brain is more active during subjective day in diurnal animals and during subjective night in nocturnal animals. Thus, diurnality or nocturnality is established downstream from the pacemaker.

12.1.3 Transplantation Studies

As if all of the research described so far were not enough to establish beyond doubt that the SCN is the site of the circadian pacemaker, researchers sought even further evidence. If a brain

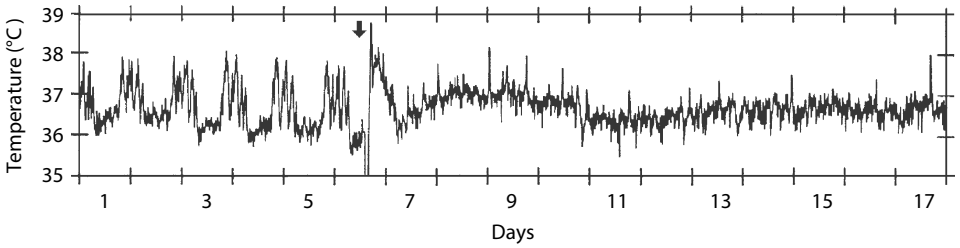


FIGURE 12.8 Suprachiasmatic nucleus (SCN) lesion eliminates rhythm of body temperature. The graph shows a 17-day segment of the records of body temperature of a golden hamster (*Mesocricetus auratus*) obtained by telemetry in 6-minute intervals. On the sixth day, the animal received a complete SCN lesion (arrow) that eliminated the body temperature rhythm. (Data from Osborne, A.R. and Refinetti, R., *NeuroReport*, 6, 2187, 1995.)

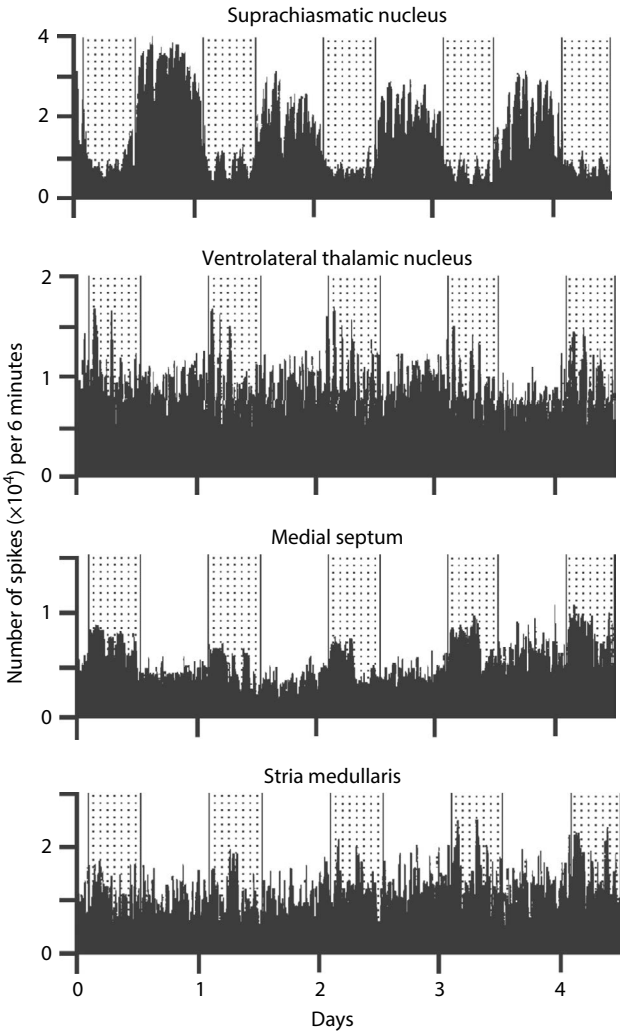


FIGURE 12.9 Suprachiasmatic nucleus (SCN) cells exhibit circadian rhythmicity of electrical activity. The graphs show 4-day segments of the records of multiunit neural activity of four brain regions obtained from freely moving golden hamsters (*Mesocricetus auratus*). Shown is the number of spikes (action potentials) per 6 minutes for representative hamsters. The stippled vertical bars indicate the timing of the dark phase of the prevailing light–dark cycle (LD 14:10). Notice that the SCN exhibits clear daily rhythmicity with greater activity during the light phase. The other brain areas show daily rhythmicity that is not as clear and that peaks during the dark phase of the light–dark cycle in this nocturnal species. (Adapted from Yamazaki, S. et al., *J. Neurosci.*, 18, 10709, 1998.)

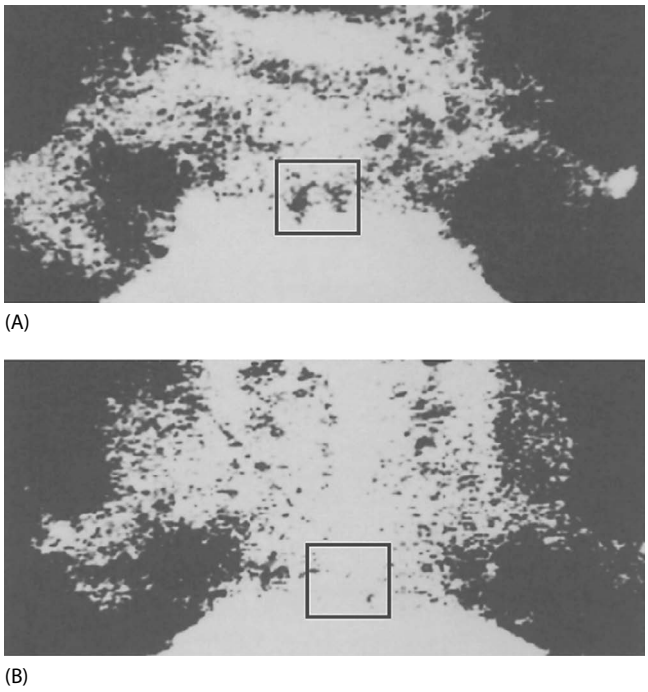


FIGURE 12.10 Suprachiasmatic nucleus (SCN) cells exhibit circadian rhythmicity of metabolic activity. These microphotographs show the ventral sections of coronal slices of the rat brain (*Rattus norvegicus*) in the region of the SCN (indicated by the squares). The images are autoradiographs based on the assimilation of radioactive 2-deoxy-glucose (2-DG) prior to euthanasia. High metabolic activity is associated with high assimilation of 2-DG (A), whereas low metabolic activity is associated with low assimilation (B). (Adapted from Cassone, V.M. et al., *Am. J. Physiol.*, 255, R332, 1988.)

structure houses the biological clock, not only should destruction of this structure eliminate rhythmicity, but replacement of the structure should restore it. Mature brain tissue cannot be successfully transplanted, but fetal brain tissue can be grafted onto an adult brain. Thus, when adult hamsters, rats, or mice rendered arrhythmic by SCN lesions received implants of fetal hypothalamic tissue or immortalized SCN cell lines, circadian rhythmicity was restored.[17,64,97,139–144] Of course, since the circadian periods of the donor and the host are very similar, one cannot be sure that the implants actually replace the clock (rather than provide an essential trophic factor required

by some remaining portion of the host's clock). To eliminate the doubt, other researchers transplanted fetal tissue from one species to SCN-lesioned adults of a different species: from rats to chipmunks[145] and from rats and mice to hamsters.[146,147] Again, rhythmicity was restored, and the period of the rhythm matched, at least closely, that of the donor—not that of the host.

Even more dramatic were the results of transplants utilizing the *tau*-mutant hamster. As described in Chapter 6, these hamsters are genetically set to circadian periods of 20, 22, and 24 hours. What would happen if an adult hamster with a period of 24 hours were rendered arrhythmic by SCN lesion and were implanted with the fetal SCN of a hamster with a period of 20 hours? Yes, you guessed it correctly: experimental results showed that the adult hamster that previously had a 24-hour period started to exhibit 20-hour rhythmicity after a few days of recovery from surgery. The converse was also true: lesioned hamsters with original period of 20 hours started to exhibit 24-hour rhythmicity after receiving a hypothalamic implant from fetuses of the 24-hour period genotype.[12,18,148,149] Furthermore, when animals received implants after having experienced only partial lesion of the SCN, they showed alternating 20- and 24-hour rhythmicity![150,151] An example is shown in Figure 12.11. During the first few days, the hamster exhibited 24-hour rhythmicity. Rhythmicity was impaired but not eliminated by a partial SCN lesion (L). Immediately after the 20-hour brain transplant (T), rhythmicity was even poorer, but, by day 45, 24-hour rhythmicity was clearly seen again. Notice that, starting on day 60, 20-hour rhythmicity predominates for a couple of weeks, being followed by a few days of 24-rhythmicity, then back to 20-hour rhythmicity, and so on. This sort of bizarre behavioral output was later demonstrated even more dramatically in studies using *clock*-mutant mice.[152]

Based on all the experimental evidence described earlier, it seems justified to conclude that the SCN is the physical substrate of the circadian pacemaker. As we look into the biological substrates of the clock in more detail in the other sections of this chapter, however, we will have to place some restrictions in our conclusion. For instance, a few other parts of the brain—particularly in birds and other nonmammalian vertebrates—seem to operate as slave pacemakers that interact with the master pacemaker. Also, the SCN cannot possibly be the anatomical site of the circadian clock in invertebrates, as these do not possess an SCN.

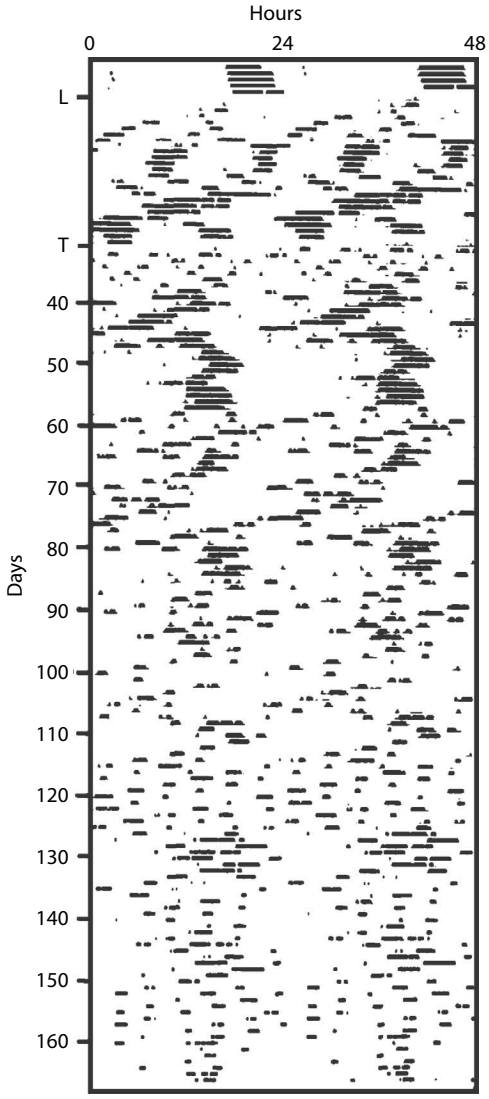


FIGURE 12.11 Suprachiasmatic nucleus (SCN) transplant determines circadian period. This double-plotted actogram shows the rhythm of running-wheel activity of a golden hamster (*Mesocricetus auratus*) that received a partial lesion of the suprachiasmatic nucleus (indicated by L on the left margin) followed by a transplant of fetal hypothalamic tissue from a homozygous *tau*-mutant hamster (indicated by T). After the transplant, the period of the activity rhythm alternated between 24 hours (the host's period) and 20 hours (the donor's period) every few weeks. (Reproduced from Vogelbaum, M.A. and Menaker, M., *J. Neurosci.*, 12, 3619, Copyright 1992, Society for Neuroscience. With permission.)

12.2 CELLULAR PROCESSES

12.2.1 Anatomy of the SCN

The SCN contains small, densely packed cells that are easily distinguishable from the surrounding anterior hypothalamic area (Figure 12.6, Panel A). Each SCN (i.e., the nucleus in each side of the brain) has approximately 8000 neurons with simple dendritic arbors and fine caliber axons.[153] There are few glial cells in the center of the nucleus but many more in the periphery.[154] In the small striped grass mouse (body mass of 40 g), each SCN measures 500 μm rostrocaudally, 400 μm ventrodorsally, and 300 μm mediolaterally.[155]

Anatomically and functionally, the SCN has two main subdivisions: the dorsomedial (or shell) region and the ventrolateral (or core) region (Figure 12.12). The dorsomedial region is indeed dorsal and medial at the center of the nucleus but not quite medial at the anterior end and not quite dorsal at the posterior end. Likewise, the ventrolateral region is not quite lateral at the anterior end and not quite ventral at the posterior end. Thus, the shell–core designation is more consistent, even

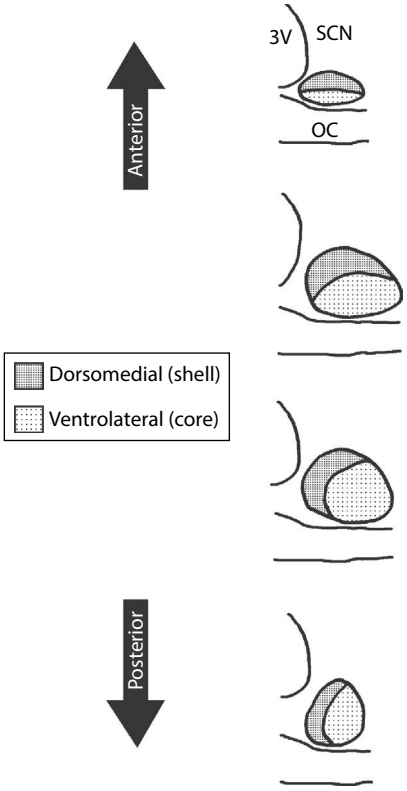


FIGURE 12.12 Major subdivisions of the suprachiasmatic nucleus (SCN). Anatomically and functionally, the SCN can be subdivided into two main regions: the dorsomedial region (or shell) and the ventrolateral region (or core). The dorsomedial–ventrolateral designation has the weight of tradition but is rather inaccurate at the anterior and posterior ends of the SCN. *Abbreviations:* 3V, third ventricle; OC, optic chiasm; SCN, suprachiasmatic nucleus. (Diagram based on Leak, R.K. and Moore, R.Y., *J. Comp. Neurol.*, 433, 312, 2001.)

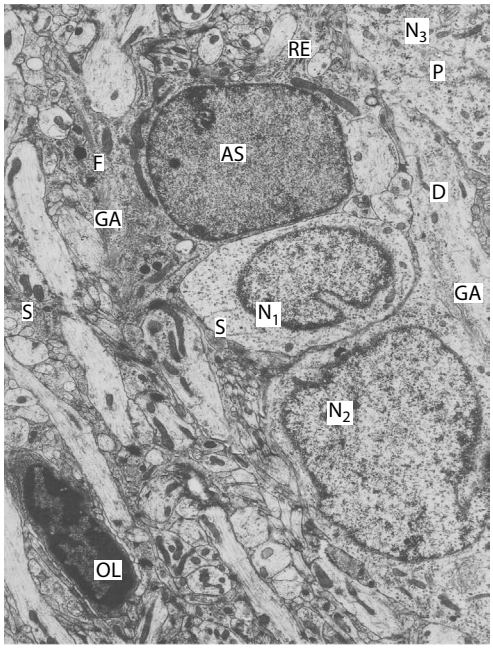


FIGURE 12.13 Cellular organization of the suprachiasmatic nucleus (SCN). This electron microphotograph shows several cellular features of the SCN in the rat (*Rattus norvegicus*). *Abbreviations:* AS, astrocyte; D, dendrite; F, glial filaments; GA, Golgi apparatus; N_1–N_3, neuron; OL, oligodendrocyte; P, perikaryon; RE, rough endoplasmic reticulum; S, synapse. The width of the microphotograph is 15 μm. (Van den Pol, A.N.: The hypothalamic suprachiasmatic nucleus of rat and its intrinsic anatomy. *J. Comp. Neurol.* 1980. 191. 661–702. Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.)

if less descriptive, at least for some species. In golden hamsters, neither the dorsomedial–ventrolateral nor the shell–core classification is ideal.[156]

Cells in the core send many axons to the shell, but there is little projection in the opposite direction.[157] Projections to homologous contralateral areas (on the opposite side of the brain) are common.[153,157]

Figure 12.13 provides a close look at cells in the SCN of the rat. In this electron microphotograph, one can see a neuron (N2) that receives an axo-somatic synapse (S) and that contains fewer organelles than a neighboring neuron (N1).[153] Although not evident in the figure, axo-dendritic synapses (i.e., synapses between the axon of a neuron and dendrites of another neuron) are more common than axo-somatic ones (i.e., synapses between the axon of a neuron and the cell body of another neuron).[154] Two glial cells—an astrocyte (AS) and an oligodendrocyte (OL), both of which have greater nuclear density than the neurons—can also be seen in the figure. We will discuss the neurotransmitters that mediate synaptic communication within the SCN shortly, but it should be mentioned at this point that there is functional and anatomical evidence that SCN neurons can also communicate by electrical coupling.[158–160] Figure 12.14 documents a *gap junction* between two neurons in the SCN of a golden hamster. The neuron marked with the asterisk (*) was microinjected with neurobiotin, a neural tracer that crosses gap junctions. Neurobiotin crossed to the neighboring neuron (indicated by the arrow), presumably through the dendrites that are seen to make contact.[159]

12.2.2 Functional Properties of SCN Cells

As mentioned in Section 12.1, circadian rhythmicity in the activity of SCN cells has been documented by electrophysiological recording as well as other methods in a variety of species.[83–116] Figure 12.15 shows average 24-hour activity profiles of SCN cells recorded from horizontal brain slices of golden hamsters, mice, and rats. In all three species, firing rate is higher during subjective day (i.e., during the light phase of the light–dark cycle to which the animals had been exposed prior to being euthanized). In horizontal slices (but not in coronal slices), the firing-rate rhythm of the golden hamster has two distinct peaks rather than the single peak observed in mice and rats.[107]

Electrophysiological analysis of individual SCN cells has generally revealed a greater proportion of rhythmic cells in the dorsomedial (shell) region than in the ventrolateral

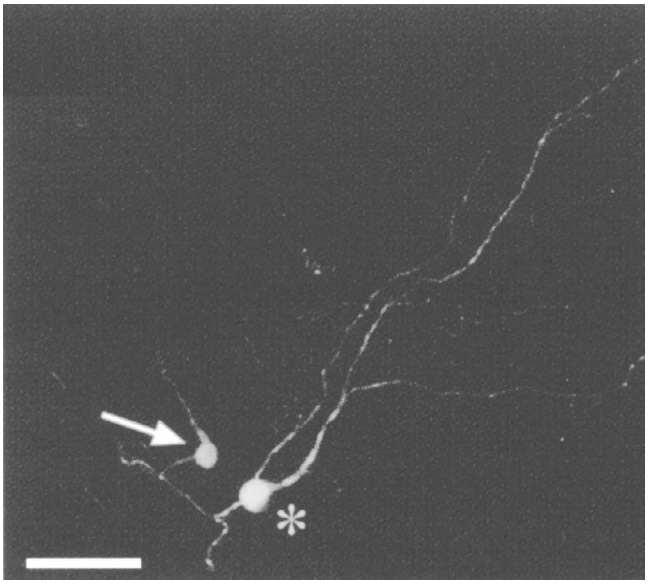


FIGURE 12.14 Gap junction between suprachiasmatic nucleus (SCN) neurons. Some neurons in the SCN communicate through gap junctions rather than through chemical synapses. For this confocal laser scanning microscope image, an individual SCN neuron (*) in a coronal slice of golden hamster brain was microinjected with neurobiotin (a neuronal tracer that crosses gap junctions), and the slice was later processed for immunohistochemistry. Neurobiotin crossed to a neighboring neuron (arrow), presumably through the dendrites that are seen to make contact. The scale bar is 50 μm. (Reproduced from *Neuroscience*, 123, Jobst, E.E., Robinson, D.W., and Allen, C.N., Potential pathways for intercellular communication within the calbindin subnucleus of the hamster suprachiasmatic nucleus, 87–99, Copyright 2004, with permission from Elsevier.)

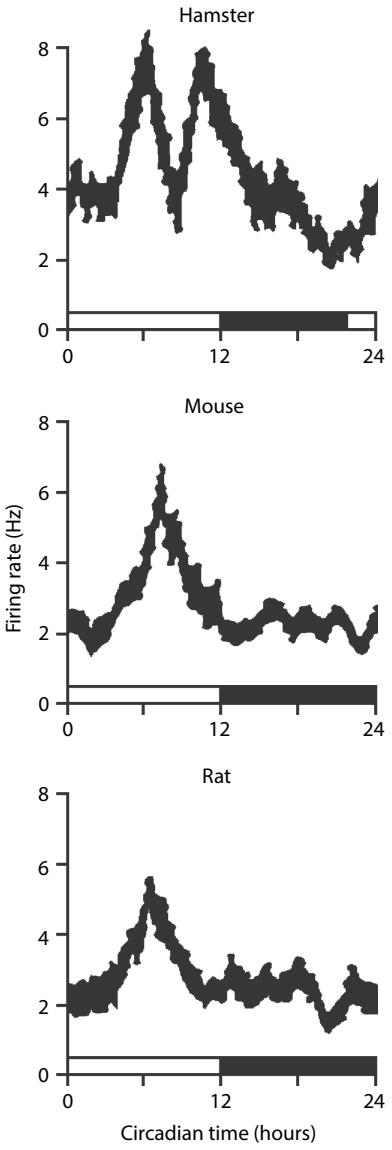


FIGURE 12.15 Circadian variation in the firing rate of suprachiasmatic nucleus (SCN) neurons. The graphs show the circadian variation in mean firing rate of 3–4 single units recorded *in vitro* in horizontal brain slices from golden hamsters (*Mesocricetus auratus*), domestic mice (*Mus musculus*), and laboratory rats (*Rattus norvegicus*). The timing of the light–dark cycle under which the animals were maintained prior to euthanasia is indicated by the horizontal light and dark bars above the abscissa. Neuronal activity is higher during subjective day than during subjective night in all three of these nocturnal species. Notice that in horizontal slices, the hamster SCN exhibits two peaks instead of one. (Adapted from Burgoon, P.W. et al., *J. Comp. Physiol. A*, 190, 167, 2004.)

(core) region,[86,91,115] which is in agreement with *in vivo* studies using the Fos methodology.[133,136] The resting membrane potential of SCN neurons is approximately –50 mV, and the spontaneous firing rate is approximately 2 Hz during subjective night and 8 Hz during subjective day.[115,161–165] Most cells fire regularly, but some fire irregularly.[86,166] As exemplified in Figure 12.16, cells that fire irregularly tend to have lower average firing rates.[166]

In principle, the SCN could generate circadian rhythmicity either by relying on rhythmic cells or by structuring the activity of cells that do not exhibit circadian rhythmicity themselves (a process of *emergent* rhythmicity). Although it was suggested early on that circadian rhythmicity could be derived by the coordination of the activity of multiple ultradian oscillators,[167–169] studies conducted during the past three decades have clearly shown that individual SCN cells display circadian rhythmicity. *In vitro* studies of the electrical activity of single cells in SCN slices or dissociated cell cultures from rodent brain have clearly established that individual cells exhibit circadian rhythmicity, that different cells have different circadian periods, and that the overall period of the circadian oscillation generated by the SCN is the average of the period of the various cells.[170–173] Figure 12.17 shows the activity profiles of four individual cells from a culture of dissociated neurons from neonatal rat SCN. Clearly, individual cells are autonomous circadian oscillators. Synapses formed *in vitro* between the dissociated cells are not necessary for rhythmicity.[170]

The range of periods of individual cells is much wider than that of the whole SCN. For instance, in the rat, whose circadian period is approximately 24.4 hours, the standard deviation of

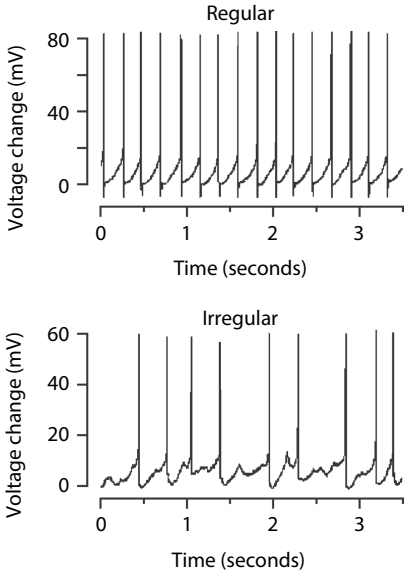


FIGURE 12.16 Firing patterns of suprachiasmatic nucleus (SCN) neurons. These electrophysiological records of single cell activity in brain slices of rats (*Rattus norvegicus*) exemplify the regular and irregular firing patterns of SCN neurons. At rest, cells of the regular type generate action potentials at a frequency of approximately 4 Hz, whereas cells of the irregular type fire irregularly at a frequency of approximately of 2 Hz. (Adapted from Kononenko, N.I. and Dudek, F.E., *J. Neurophysiol.*, 91, 267, 2004.)

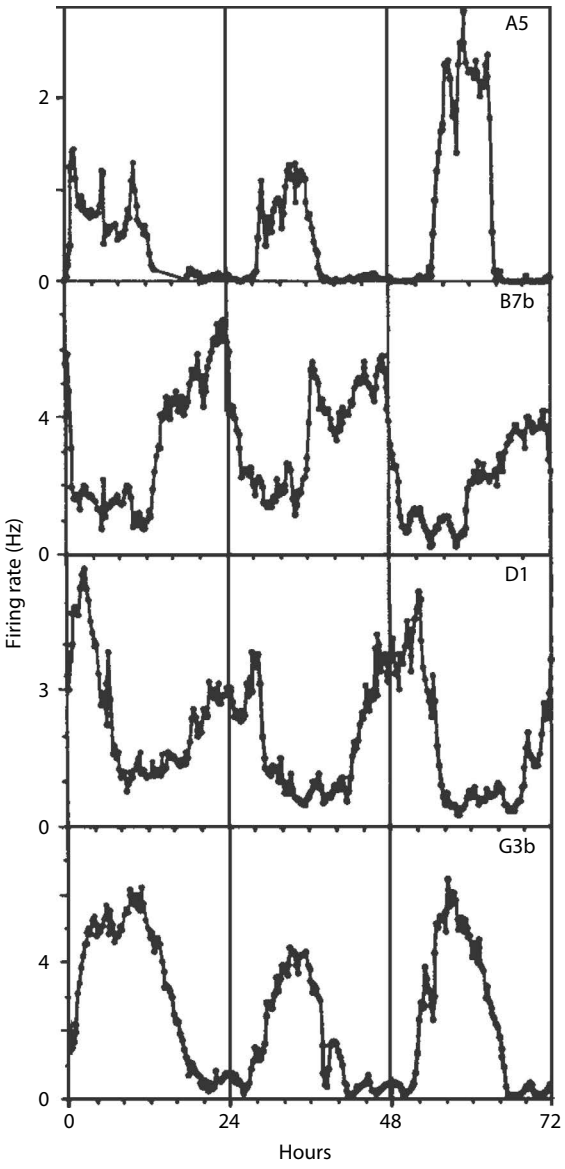


FIGURE 12.17 Activity of isolated suprachiasmatic nucleus (SCN) neurons. The graphs show the circadian variation in firing rate of four individual neurons in a dissociated culture system of SCN cells from the laboratory rat (*Rattus norvegicus*). Different neurons exhibit different circadian periods. (Reproduced from *Neuron*, 14, Welsh, D.K., Logothetis, D.E., Meister, M., and Reppert, S.M., Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms, 697–706, 1995, with permission from Elsevier.)

the mean of individual cells was found to be 1.4 hours (i.e., the periods of individual cells ranged from 20.0 to 28.3 hours), whereas the standard deviation of the mean of the locomotor activity rhythm of the animals was 0.2 hour (i.e., the periods ranged from 24.0 to 24.8 hours).[172] This difference is easily explained by basic statistical properties of the arithmetic mean. Because the value of a mean is, by necessity, never as extreme as the most extreme values of its elements, the variability of sample means is always smaller than the variability of the original population of elements (Figure 12.18). The standard deviation of the distribution of means equals the standard deviation of the distribution of the population divided by the square root of the group size: $S_M = \sigma/\sqrt{n}$. Thus, it can be predicted that the mean period of a group of as few as 50 cells will have a standard deviation of 0.2 hour. As a matter of fact, based on the standard deviation of the individual cells (1.4 hours), one would expect the group of 16,000 SCN cells to have a mean period with a standard deviation slightly larger than 0.01 hour. Evidently, some source of biological noise must be responsible for the increase in the standard deviation from 0.01 to 0.2 hour. A study on mice showed not only that SCN cells have a wider distribution of periods but also that each cell is less precise in the maintenance of its circadian period.[174] An example is shown in Figure 12.19. As indicated by the phase markers (open circles) in the actograms, the circadian periods of the whole organism and of SCN explants are much more precise than that of a single SCN neuron.

The program **Free-run**, which is part of the software package that accompanies this book (see Exercise 12.1), allows you to simulate two particular phenomena: the pooling of SCN cells with varying circadian periods to produce a single *organismal* period and the production of *temporal chimeras* by placing two distinct circadian pacemakers in the same animal (as in hamsters with partial SCN lesions receiving a hypothalamic graft from a genotypically distinct hamster). However, average period is only part of the problem. If the cells were not mutually synchronized, the overall output of the SCN would be temporally uniform, not sinusoidal. Thus, the individual cells must

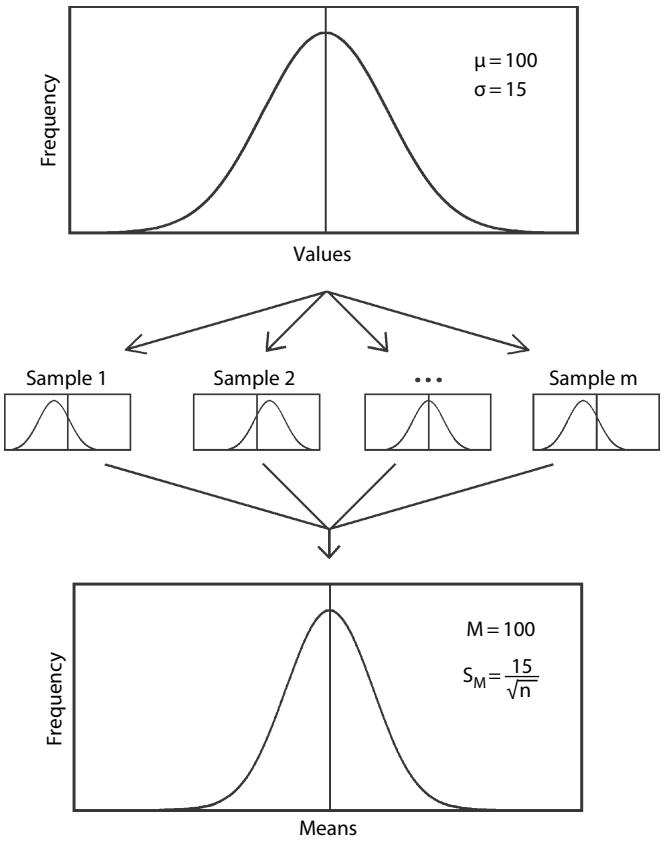


FIGURE 12.18 Variability of the means is smaller than variability of the population. Because the value of a mean is, by necessity, never as extreme as the most extreme values of its elements, the variability of sample means is always smaller than the variability of the original population of elements. As shown in this figure, if we randomly draw many samples of size *n* from a population that has a mean value (μ) of 100 and a standard deviation (σ) of 15, the distribution of the means of the samples will have the same mean as that of the population (M = 100) but a smaller standard deviation (S_M = 15 ÷ a, where a is the square root of the sample size). If n = 2, S_M = 10.6; if n = 10, S_M = 4.7; if n = 100, S_M = 1.5; and so on.

be coupled somehow. Functional models indicate that intercommunication in a population of autonomous oscillators can yield a stable ensemble period.[175–178] Efforts to identify the coupling mechanism have pointed toward synaptic communication between neurons,[179–181] although neuronal communication via gap junctions[160] and influence by glial cells may also be involved.[182,183] Some of the evidence for synaptic communication is shown in Figure 12.20. The study used the technique of genetically engineered bioluminescence described in Chapter 2 (luciferase reporter gene) to investigate the activity of individual SCN cells in mouse brain slices. The figure shows the oscillation in luminescence of six representative cells. During the first 24 hours, clear rhythmicity is seen. The luminescence profiles of the six cells are not identical, but they are all clearly synchronized. Tetrodotoxin (TTX), a substance that blocks action potentials dependent on Na^+ channels, was then introduced into the medium for 7 days (although only the 6th and 7th days are shown). The blockade of action potentials clearly disrupted the synchronization of the cells. Two days later, after TTX had been washed out, synchronization resumed.[181] Thus, synchronization (and ensemble rhythmicity) is dependent on synaptic communication.

12.2.3 Neurotransmitters in the SCN

Much has been learned about the neurotransmitters used for communication between nerve cells in the SCN. Three neurotransmitters are particularly abundant in the SCN: γ-aminobutyric acid (GABA, a ubiquitous inhibitory amino-acid transmitter found throughout the central nervous system), arginine vasopressin (AVP, a neurotransmitter that, as indicated by its name, was first identified by its role in the control of dilation and constriction of blood vessels), and vasoactive intestinal polypeptide (VIP, a peptide first identified by its action on the intestinal wall). The structural formulas of the three neurotransmitters are shown in Figure 12.21.

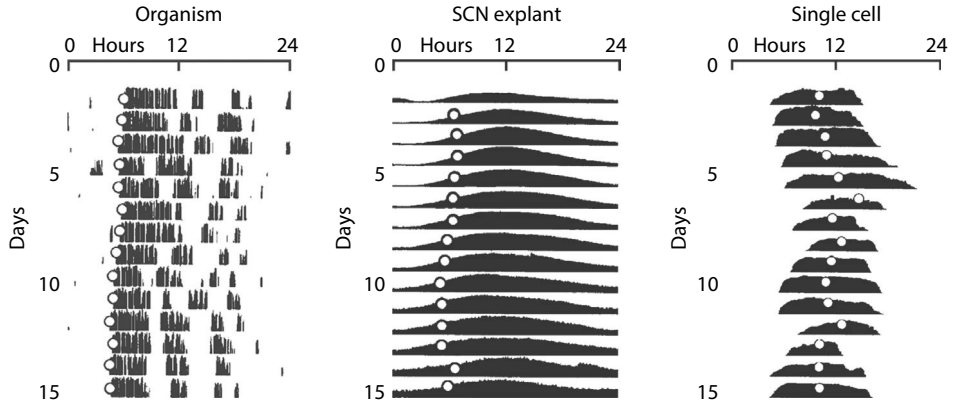


FIGURE 12.19 Precision of circadian period. Not only is there great variability in the circadian period of different suprachiasmatic nucleus (SCN) cells but there is also great variability in the circadian period of each individual cell. As indicated by the phase markers (open circles) in these three actograms, the circadian periods of the whole organism and of SCN explants are much more precise than that of a single SCN neuron. All three variables were recorded from domestic mice (*Mus musculus*). (Adapted from Herzog, E.D. et al., *J. Biol. Rhythms*, 19, 35, 2004.)

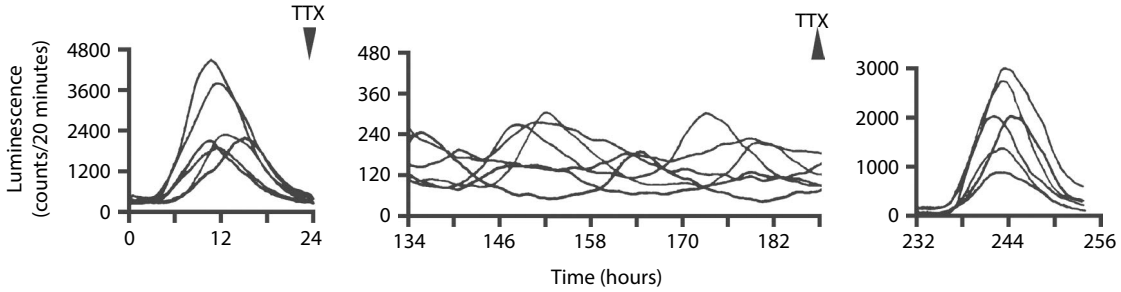


FIGURE 12.20 Synchronization of individual suprachiasmatic nucleus (SCN) neurons. Circadian rhythmicity of six individual SCN neurons in a slice of mouse brain (*Mus musculus*) was measured by variations in luminescence (glow) induced by a luminescence gene attached to a clock gene (*mPer1*-promoter-driven luciferase reporter gene). Luminescence was recorded for over 10 consecutive days. Initially (first 24-hour segment), the six cells were synchronized. After administration of tetrodotoxin (TTX, a substance that inhibits the generation of action potentials and, therefore, interrupts communication between neurons), the cells lost synchronization. After TTX was washed out, synchronization resumed. (Adapted from Yamaguchi, S. et al., *Science*, 302, 1408, 2003.)

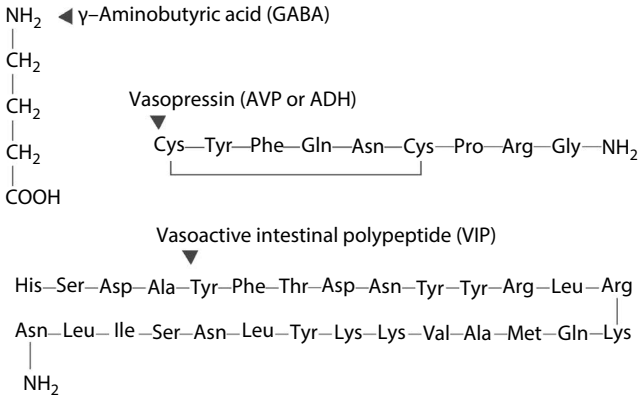


FIGURE 12.21 The main neurotransmitters in the suprachiasmatic nucleus (SCN). Synapses within the SCN involve three main neurotransmitters: γ-aminobutyric acid, arginine vasopressin, and vasoactive intestinal polypeptide. (Formulas from Feldman, R.S. and Quenzer, L.F., *Fundamentals of Neuropsychopharmacology*, Sinauer, Sunderland, MA, 1984; Cooper, J.R. et al., *The Biochemical Basis of Neuropharmacology*, 6th edn., Oxford University Press, New York, 1991.)

Anatomical studies using immunocytochemical methods (that is, methods that utilize specially prepared antibodies that contain dyes or radioactive substances and that attach to the neurotransmitter in an antigen–antibody reaction) have demonstrated the presence of GABA neurons throughout the SCN.[179,184–187] As a matter of fact, GABA seems to be the most common neurotransmitter in the SCN.[185] GABA administration in hypothalamic slices or dissociated cell cultures was shown to inhibit the activity of SCN neurons.[188–190] Of the two main GABA receptors ($GABA_A$ and $GABA_B$), $GABA_A$ seems to be the most common one in SCN cells,[191] although there is functional evidence that $GABA_B$ may also be involved in the operation of the circadian clock.[192] Discrete administration of the $GABA_A$ agonist *muscimol* causes phase shifts in the activity rhythm of SCN cells *in vitro*[190,193] as well as in the activity rhythm of the whole organism.[194,195] Administration of the $GABA_A$ antagonist *bicuculline* abolishes inhibitory postsynaptic currents[196,197] and increases the spontaneous firing rate of SCN cells.[166,179]

AVP is also abundant in the SCN, predominantly in the dorsomedial (shell) section of the nucleus, and is not found in the adjacent hypothalamus (Figure 12.22, Panel A).[155,184,187,198–204] Immunocytochemical and microdialysis studies in brain slices or cultures of SCN cells clearly showed that AVP is released from synaptic terminals at a higher rate during the day than during the night in rodents and humans maintained under a light–dark cycle and that the release remains rhythmic in animals maintained in constant darkness.[91,94,198,199,205–211] (As mentioned earlier, the electrical activity of the SCN is higher during the day than during the night in both diurnal and nocturnal animals, even though the activity of the rest of the brain, and of the whole animal, is higher during the night in nocturnal animals.) Interestingly, a study in voles indicated that animals with a smaller number of AVP immunoreactive cells have more robust rhythms of running-wheel activity than those with a greater number of such cells.[212]

In contrast to AVP, VIP is present mostly in the ventrolateral (core) section of the SCN and in its dorsal projections (Figure 12.22, Panel B).[155,187,198–200,202–204,213,214] (As we will see in Chapter 13, most of the afferent pathways to the SCN end in the ventrolateral section.) VIP is released from synaptic terminals at a higher rate during the night than during the day in rodents and humans maintained under a light–dark cycle, and the release remains rhythmic in animals maintained in constant darkness,[91,94,199,202,210] presumably because of delayed rhythmic signals received from the dorsomedial (shell) region. VIP is an inhibitory neurotransmitter within the SCN.[197,215] Administration of VIP *in vivo*[216] and *in vitro*[217] has been found to cause phase shifts of circadian rhythms similar to those caused by light pulses (i.e., delays in early subjective night and advances in late subjective night). Mice lacking the gene that encodes the major VIP receptor (*vipr2*) exhibit weak circadian rhythmicity of locomotor activity in constant darkness.[218] Synaptic input via VIP seems to be essential for many SCN cells to exhibit circadian rhythmicity and for many others to become synchronized and give

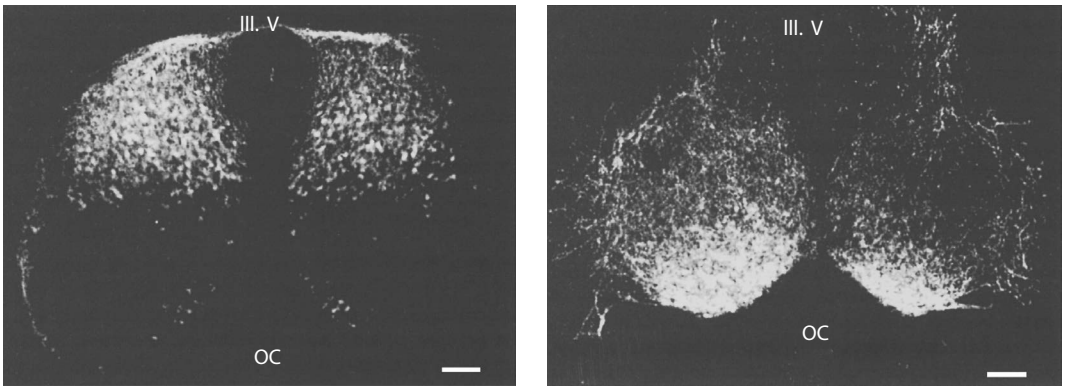


FIGURE 12.22 Location of neurotransmitters in the suprachiasmatic nucleus (SCN). These immunofluorochemistry images of the SCN of newborn rats (*Rattus norvegicus*) show the concentration of AVP-containing neurons in the dorsal region of the nucleus (A) and the concentration of VIP-containing somata in the ventral region with fibers extending dorsally (B). *Abbreviations:* III.V, third ventricle; OC, optic chiasm. The scale bar is 100 µm. (Reproduced from Nakamura, W. et al., *Eur. J. Neurosci.*, 14, 666, Copyright 2001, Blackwell Publishing. With permission.)

rise to the master circadian pacemaker.[109] Excessive VIP stimulation, however, can actually impair synchronization of SCN cells.[219]

In summary, cells in the ventrolateral (core) region are generally not intrinsically rhythmic and use VIP (in addition to GABA) as their main neurotransmitter, whereas cells in the dorsomedial (shell) region are intrinsically rhythmic and use AVP (in addition to GABA) as their main neurotransmitter. Evidence in support of other neurotransmitters utilized in the communication among SCN cells has been produced, although additional studies are necessary to better determine their function. These neurotransmitters include gastrin-releasing peptide,[90,155,184,199,200,203,210,216,220] calbindin,[65,203,204] somatostatin,[184,198] thyrotropin-releasing hormone,[221] angiotensin,[222] nitric oxide,[223] and neuromedin U.[224] Neurotransmitters used in the afferent pathways to the SCN will be discussed in Chapter 13.

12.3 MOLECULAR PROCESSES

Very little was known about molecular mechanisms of circadian rhythmicity until 1992. Since then, circadian physiology has benefited greatly from the explosion of findings in molecular biology that characterized the late twentieth century and early twenty-first century. Molecular biology is the star science of our time. The amazing applications of knowledge generated by molecular biologists are in the newspaper headlines and movie pictures. Steven Spielberg's four motion pictures based on Michael Crichton's book, *Jurassic Park*,[225] added fantasy to but did not create the fact that DNA (the nucleic acid that carries genetic information) can be retrieved from the remains of life forms that existed thousands or even millions of years in the past.[226–229] Dolly, the ewe, made the headlines as the first demonstration that a fully functional mammal could be cloned from a single adult cell.[230] The rate of genetic mutation has been used as a *molecular clock* to measure archeological time and to determine branching of species during evolution.[231,232] DNA fingerprinting was used to prove that the stain in Ms. Lewinsky's dress was semen from President Bill Clinton.[233] The Human Genome Project cataloged every gene in the human genome,[234] opening the doors for unlimited progress in human medicine. Craig Venter and colleagues demonstrated control of a bacterial cell by a chemically synthesized genome,[235] as if this were the "eight day of creation"—this time, by the hands of scientists.[236]

Just as impressive as the technical achievements of molecular biology are its philosophical implications. After all, molecular biology provides the physicochemical bases of biological processes. Behavioral characteristics of whole organisms can now be explained by the chemical mechanisms of gene activation and transcription. It is almost as if proof had finally been provided for the claims of *logical positivism* (in the first half of the twentieth century) that complex phenomena can be reduced to simple elements,[237] so that all sciences can ultimately be reduced to the physical sciences.[238] A natural expansion of this is the solution of the old *mind–body dichotomy* by the assertion that the mind is an expression of the operation of brain cells.[239–242] This *radical materialism* may be a little bit too challenging for conservative readers, but it is a natural implication of new findings in neuroscience and molecular biology and is fully consistent with the materialist worldview of science discussed in Chapter 2.

Before examining what molecular biology has to say about circadian rhythms, it is important to point out that the relative simplicity of the phenomena described here is the result of a careful presentation of the most significant features and does not reflect the complexity of actual research in the field. Because of the rapid advances in scientific research, basic college education cannot possibly prepare the average citizen to fully understand specialized research conducted

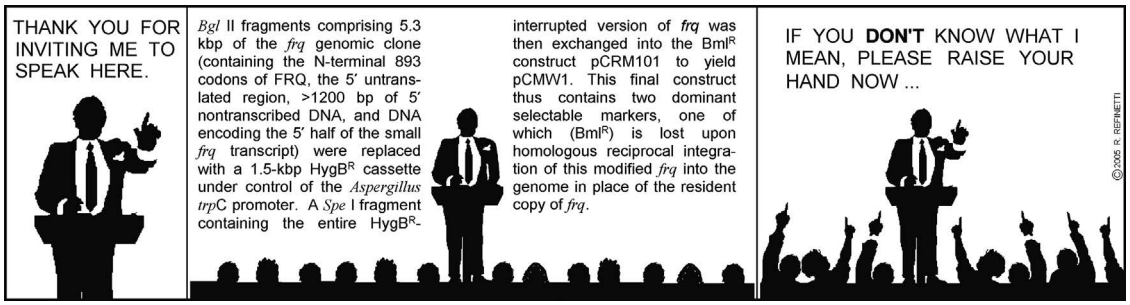


FIGURE 12.23 Raise your hand! This cartoon emphasizes the complexity of current research methods in molecular biology. (Lecture contents quoted literally from Aronson, B.D. et al. *Proc. Natl. Acad. Sci. USA*, 91, 7683, 1994.)

during his or her lifetime. Figure 12.23, which includes a literal transcript from a typical published article in the field, is a reminder of this simple fact.

12.3.1 Early Studies

Researchers originally assumed, very sensibly, that the molecular mechanisms of circadian rhythmicity would be more easily identified in simple life forms than in mammals. In Chapter 6, it was mentioned that a specific single-gene mutation had been identified in the fruit fly, *Drosophila melanogaster* (Figure 12.24), early in the 1970s.[243] The gene—called *per*, for *period*—is located in the X chromosome and exhibits multiple mutant alleles.[244] As any other gene, *per* is present in cells of all organs of the fly. Curiously, it is also activated in most organs, so that PER (the protein produced by the *per* gene) is produced rhythmically almost everywhere in the fly's body.[245,246] However, only *per* expression in nerve cells and glia of the brain is necessary for circadian rhythmicity of locomotor activity.[247] The *accessory medulla* (aMe) or a nearby group of cells called the *ventral lateral neurons* (LN_V) is considered to be the fly's equivalent of the mammalian SCN.[248] The function of the LN_V seems to be not quite to generate rhythmicity (because other organs can also generate molecular rhythmicity) but to synchronize the rhythmicity of multiple oscillators.[249]

In 1992, it was shown that the *per* gene in the fruit fly is expressed in a circadian manner, and that fluctuations in *per* mRNA abundance are influenced by its own translation product (the PER protein).[250] Accordingly, transient experimenter-induced increases in PER production cause phase shifts of the activity rhythm.[251] The exciting aspect of this is that the *per*–PER relationship constitutes the type of feedback loop that we briefly discussed in Chapter 6 as the basic mechanism of biological timing. As diagrammed in Figure 12.25, a biochemical process (*per* transcription) is regulated by its product (the PER protein) through a negative-feedback loop. Of course, there are thousands or millions of feedback loops in the body, but very few of them involve genes that are known to affect the circadian system. The idea that the *per*–PER loop might be the mechanism responsible for the generation of circadian rhythmicity stimulated many researchers to search for other genes that might also be involved in the process.

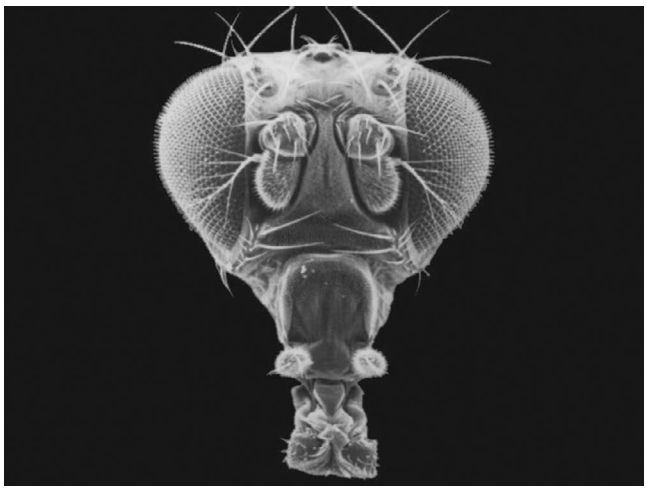


FIGURE 12.24 Close-up of a fly. This electron-microscope photograph shows the head of a fruit fly (*Drosophila melanogaster*) up close. (Courtesy of FlyBase, Indiana University, Bloomington, IN.)

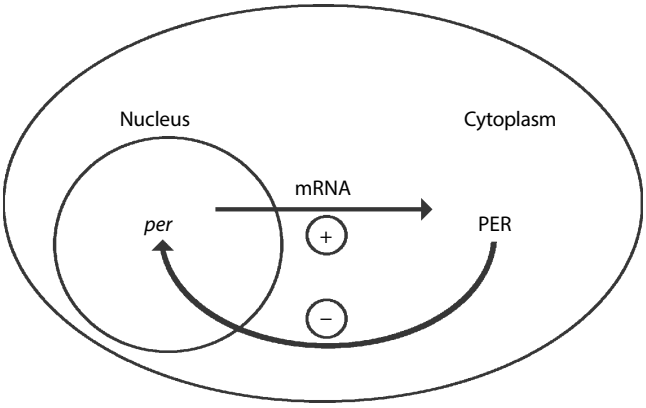


FIGURE 12.25 A simple molecular loop. This diagram of an eukaryotic cell illustrates the core mechanism of the circadian clock in animals: a feedback loop involving the *per* gene and its protein.

The *per* gene, as virtually all other genes, is not a single, uninterrupted sequence of bases. As shown in Figure 12.26, the coding region of the gene is fragmented, with alternating exons (protein-coding segments) and introns (noncoding segments). The PER protein produced by the *per* gene is a sequence of 1224 amino acids, as shown in Figure 12.27. In 1994, another *Drosophila* gene involved in the control of circadian rhythmicity—*tim*, short for *timeless*, located in chromosome 2—was identified.[252] Similarly to *per*, *tim* RNA accumulates rhythmically, and the rhythmicity is dependent on the presence of both TIM and PER.[253] Indeed, TIM binds to PER *in vitro*, and the TIM–PER complex migrates to the nucleus to inhibit *per* transcription.[254] So, the *per*–PER loop is not a one-gene loop. Still, a loop involving a few genes and a few proteins became the top candidate for the basis of the biochemical mechanism of circadian rhythmicity.

Curiously, research on mammals did not lag behind research on fruit flies. We saw in Chapter 6 that two single-gene mutations had been identified in mammals: the *tau* gene in the golden hamster[255] and the *clock* gene in the mouse.[256] Three mammalian homologs of the *per* gene were identified by other research teams: *per1*,[257] *per2*,[258] and *per3*.[259] RNA levels of these genes were found to exhibit circadian rhythmicity in the SCN. The *clock* gene in the mouse (a large transcription unit with 24 exons spanning approximately 100,000 base pairs of DNA) was characterized in 1997.[260,261] Soon afterward, another clock gene in the mouse was identified, *bmal1*.[262] It turned out that *bmal1*, *clock* (or just *clk*), and *per1* are coexpressed in the SCN, and the proteins encoded by the first two (BMAL1 and CLK) interact to drive the transcription of *per1*. Shortly thereafter, mammalian homologs of the fruit fly's *tim* gene were also identified and cloned, although mRNA levels of TIM were found not to oscillate in the mouse.[263,264]

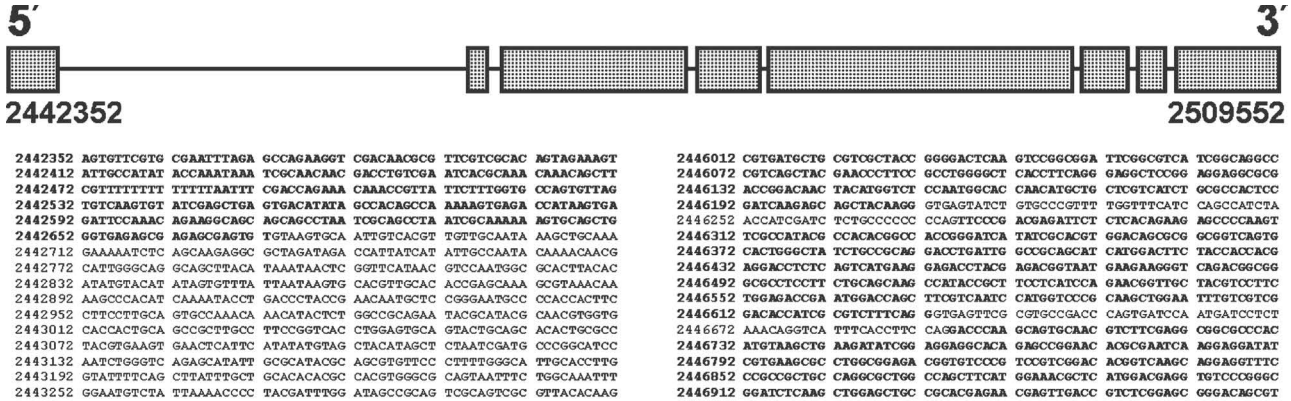


FIGURE 12.26 The *per* gene. Shown are a positional diagram and the full sequence of bases of the *per* gene in the fruit fly (*Drosophila melanogaster*). Coding regions of the gene are shown in boldface: A, adenine; C, cytosine; G, guanine; and T, thymine. (From U.S. National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, GenBank accession number AE003425.)

MEGGESTEST	HNTKVSDSAY	SNSCSNSQSQ	RSGSSKSRLS	GSHSSGSSGY	GGKPSTQASS	60
SDMIIKRNKD	KSRKKKKNKG	AGQGAGQAQT	LISASTSLEG	RDEEKPRPSG	TGCVEQQICR	120
ELQDQQHGED	HSEPQAIEQL	QQEEEEDQSG	SESEADRVEG	VAKSEAAQSF	PIPSPLSVTI	180
VPPSMGGCGG	VGHAAGLDSG	LAKFDKTWEA	GPGKLESMTG	VGAAAAGTGQ	RGERVKEDSF	240
CCVISMHDGI	VLYTTPSITD	VLGYPRDMWL	GRSFIDFVHL	KDRATFASQI	TTGIPIAESR	300
GSVPKDAKST	FCVMLRRYRG	LKSGGFGVIG	RPVSYEPFRL	GLTFREAPEE	ARPDNYMVSN	360
GTNMLLVICA	TPIKSSYKVP	DEILSQKSPK	FAIRHTATGI	ISHVDSAAVS	ALGYLPQDLI	420
GRSIMDFYHH	EDLSVMKETY	ETVMKKGQTA	GASFCSKPYR	FLIQNGCYVL	LETEWTSFVN	480
PWSRKLEFVV	GHHRVFQGPK	QCNVEEAAPT	CKLKISEEAQ	SRNTRIKEDI	VKRLAETVSR	540
PSDTVKQEVS	RRCQALASFM	ETLMDEVSRA	DLKLELPHEN	ELTVSERDSV	MLGEISPHHD	600
YYDSKSSTET	PPSYNQLNYN	ENLLRFFNSK	PVTAPAELDP	PKTEPPEPRG	TCVSGASGPM	660
SPVHEGSGGS	GSSGNFTTAS	NIHMSSVTNT	SIAGTGGTGT	GTGTGTGTGT	GTGTGTGTGT	720
GTGTGTGTGT	GTGTGTGTGT	GTGNGTNSGT	GTGTASSSKG	GTAAIPPVTL	TESLLNKHND	780
EMEKFMLKKH	RESRGRTGEK	SKKSANDTLK	MLEYSGPGHG	IKRGGSHSWE	GEANKPKQQL	840
TLGTDAIKGA	AGSAGGAVGT	GGVGSGGAGV	AGGGGSGTGV	AGTPEGRATT	TSGTGTPGGA	900
GGGGGAGAAA	AAGASSSVGS	STPGPSSYPT	CTQNINLWPP	FSVGITPPVH	STHTAMAQSS	960
FSSAGLFPTF	YYIPASLTPT	SPTRSPRMHK	HPHKGGTDMP	TTSQQAAAAA	AQAMPLQYMA	1020
GVMYPHPSLF	YTHPAAAAAT	AMMYQPMPFP	GMANALQIPE	RPLGSQSAYN	KSVYTTTPAS	1080
MTKKVPGAFH	SVTTPAQVQR	PSSQSASVKT	EPGSSAAVSD	PCKKEVPDSS	PIPSVMGDYN	1140
SDPPCSSSNP	ANNKKYTDSN	GNSDDMDGSS	FSSFYSSFIK	TTDGSESPPD	TEKDPKHRKL	1200
KSMSTSESKI	MEHPEEDQTQ	HGDG				1224

FIGURE 12.27 The PER protein. The PER protein in the fruit fly is made up of 1224 amino acids, as shown here. If you are not familiar with the one-letter abbreviations of amino acids, see Table 2.2. (Courtesy of U.S. National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, GenBank accession number P07663.)

12.3.2 Molecular Clock of the Fruit Fly

Soon after the *clk* gene was characterized in the mouse, an invertebrate homolog of the *clk* gene was identified in *Drosophila*.[265,266] As depicted in Figure 12.28, CLK and CYC (the *Drosophila* homolog of BMAL1) interact as a positive element in a circadian transcriptional loop by stimulating the expression of *per* and *tim*.[267,268] In more detail, *cyc* is constitutively (nonrhythmically) expressed, whereas *clk* is rhythmically expressed by a mechanism that can be described as follows. The products of *clk* and *cyc* (CLK and CYC) form a heterodimer that attaches to a particular site in the promoter regions of *per* and *tim* (a site identified by the nucleotide sequence CACGTG, which is called an E-box). Activation of *per* and *tim* drives the production of PER and TIM, which inhibit the CLK–CYC dimer, thus closing the feedback loop. The roles of other genes involved in the process (*dbt*, *vri*, *pdf*, and *ck2*) were identified later.[269–273] Although these other genes are not part of the main loop, they are necessary for the proper timing of the process. Because transcription and translation are relatively rapid processes, additional elements are necessary to slow the loop down to the circadian range (about 24 hours).

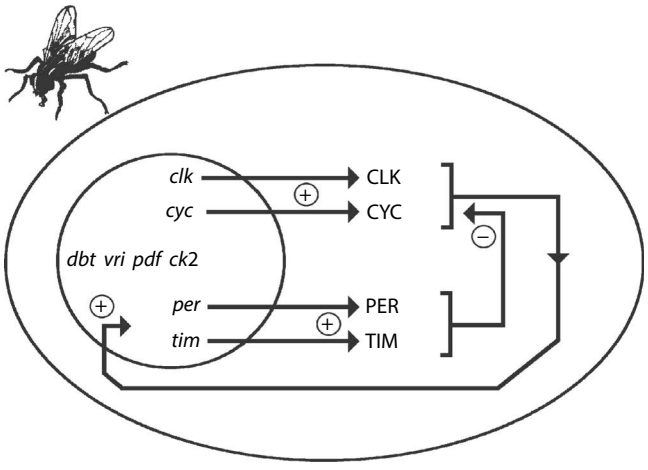


FIGURE 12.28 Molecular mechanism of the circadian clock in the fruit fly. This diagram illustrates the major players in the circadian clock of the fruit fly. For graphic purposes, the inhibition of CLK–CYC by PER–TIM is displayed in the cytoplasm although it actually takes place in the nucleus of the cell. (Data from Williams, J.A. and Sehgal, A., *Annu. Rev. Physiol.*, 63, 729, 2001; Devlin, P.F. and Kay, S.A., *Annu. Rev. Physiol.*, 63, 677, 2001; Lin, J.M. et al., *Nature*, 420, 816, 2002.)

Figure 12.29 shows the amino-acid sequences of the CLK protein in the fruit fly as well as in the zebra fish and the mouse. The sequences include a bHLH and two PAS domains. The acronym *bHLH* stands for "basic Helix-Loop-Helix," which is a designation of a motif characteristic of various gene transcription factors (that is, proteins that activate genes) whose structure resembles two helices connected by a loop. The *PAS domain* is so called because this sequence of approximately 270 amino acids was originally found in three distinct proteins: **P**ER, **A**RNT (aryl hydrocarbon receptor nuclear transporter), and **S**IM (single-minded protein).[274] All of the major clock proteins in animals (including CLK) contain at least one PAS domain. Nonetheless, notice in the figure that the CLK sequences for the three species are very similar but not identical. For instance, at position 40, all three species have arginine (R); at position 41, the fish and the mouse have valine (V) but the fly has lysine (K); at positions 42, 43, and 44, the sequences are identical for the three species; at position 45, the amino acids differ in all three species; and so on.

As research continues, more genes and proteins are being found to participate in the clock's core mechanism. At the present time, the transcriptional–translational loop in the fruit fly is believed to actually be a double loop, as diagrammed in Figure 12.30. Besides the PER–TIM and CLK–CYC loop, there seems to be a loop involving CLK, VRI (vrille), and PDP1ε (PAR domain protein 1ε), which provides for the rhythmic expression of *clk*.[271] In addition to the circadian transcriptional cycles, there are several

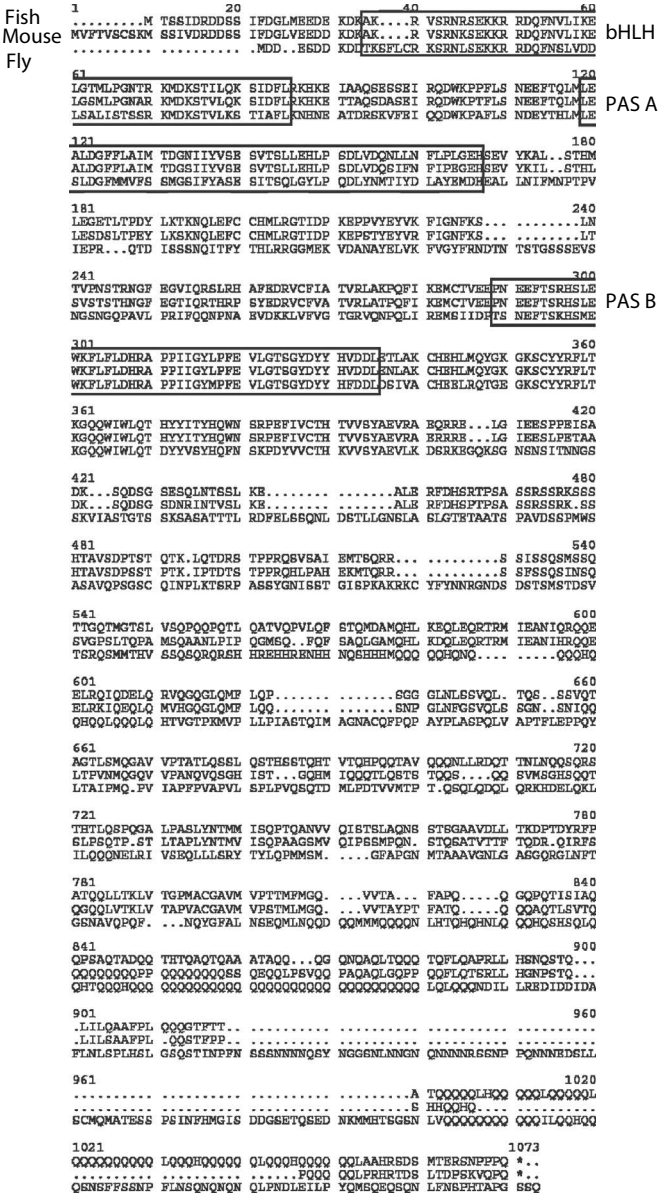


FIGURE 12.29 The CLOCK protein. The amino-acid sequence of the CLOCK protein is shown for the zebra fish (*Danio rerio*), the domestic mouse (*Mus musculus*), and the fruit fly (*Drosophila melanogaster*). The bHLH, PAS A, and PAS B regions are boxed and labeled. Dots indicate spaces inserted into the sequences to provide optimal alignment. The one-letter abbreviations of amino acids are listed in Table 2.2. (Adapted from Whitmore, D. et al., *Nat. Neurosci.*, 1, 701, 1998.)

points of posttranscriptional control, particularly a gradual phosphorylation of PER by the product of *dbt* (*double-time*).[270,272,273] Two new proteins involved in the core mechanism of the fruit fly clock have been recently described: JARID1a[275] and ATAXIN-2.[276] Additional genes and proteins that participate in the processing of afferent signals to the clock (and efferent signals from the clock) will be discussed in Chapter 13.

A lingering question is, of course, whether all clock genes have been identified. An obvious approach to answering the question is to perform DNA microarrays. As mentioned in Chapter 2, DNA microarrays allow the simultaneous monitoring of the expression of thousands of genes. Studies using RNA collected from fly heads at different times of the day have generally monitored about 14,000 genes (that is, the full genome of the fly), approximately 150 of which show a rhythmic pattern of expression under a light–dark cycle (and half as many in constant darkness).[277–280] Figure 12.31 shows the temporal pattern of expression of 72 genes that exhibit particularly robust rhythmicity under a light–dark cycle. Rhythmicity is indicated by three bands of light gray (denoting higher expression) over 3 days. The genes were arranged vertically in order of time of maximal expression. Notice that different genes peak at different times of the day across the whole day. A few of these genes are known clock genes (such as *per*, *tim*, and *clk*), but it is anyone's guess which of the other genes are novel clock genes and which are clock-controlled genes (i.e., genes involved in efferent processes). A major disappointment in microarray analyses has been the inconsistency of findings in different studies. Fewer than 20 genes have been consistently found to be rhythmically expressed in all the studies; the other 130 or so were detected in only one or two of the studies.[280] A research team reanalyzed the data from previous studies using its own data analysis procedure. The effort resulted in the identification of 81 rhythmic genes that had been previously identified as rhythmic and 133 rhythmic genes that none of the previous studies had identified as rhythmic.[281] Evidently, the data at hand do not yet provide a consistent description of circadian gene expression in the fruit fly. Lack of standards in microarray technology, rather than the complexity of genomes, may be to blame.[282]

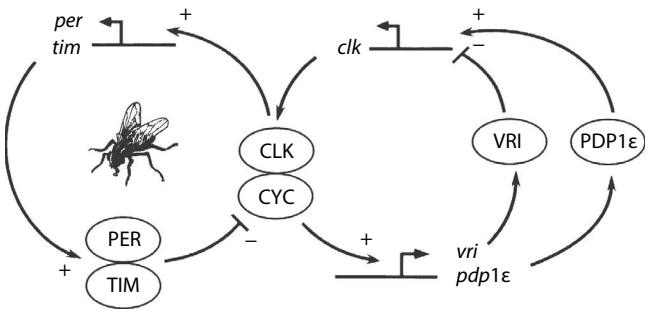


FIGURE 12.30 Molecular mechanism of the circadian clock in the fruit fly: double loop. If one looks closer at the core clock mechanism in the fruit fly, there are at least two feedback loops involved: the main one for *per* and *tim* activation and a secondary one for *clk* activation. (Adapted from Cyran, S.A. et al., *Cell*, 112, 329, 2003.)

12.3.3 Molecular Clock of Other Simple Organisms

Negative-feedback loops were found to be at the core of the circadian clock also in bacteria (*Synechococcus*), plants (the mustard plant), and fungi (bread mold), although the specific genes and proteins are not the same (Figure 12.32).

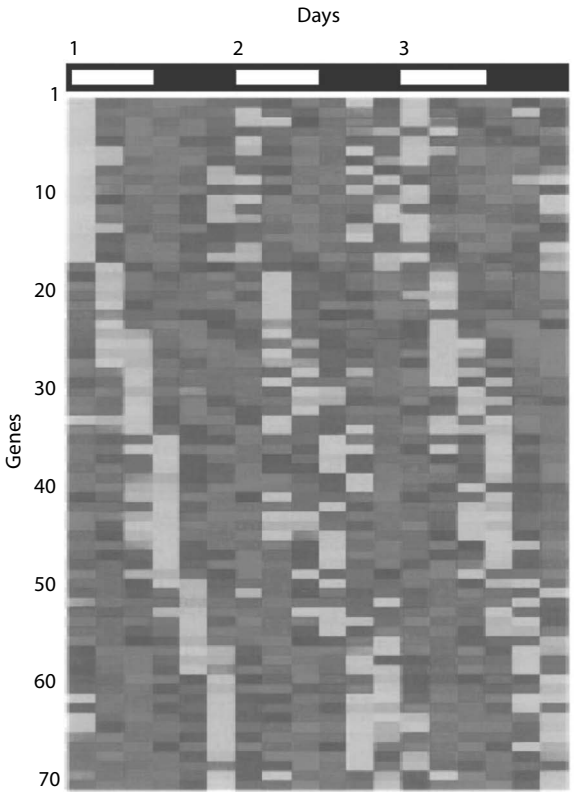


FIGURE 12.31 DNA array of *Drosophila* head. The graph shows a 72-gene segment of a DNA array study of genes expressed in the head of the fruit fly (*Drosophila melanogaster*). Higher levels of gene expression are indicated by lighter shades of gray. Notice that all genes depicted exhibit three bands of higher expression over the 3 days, thus indicating daily rhythmicity. The 72 genes were arranged vertically in order of time of maximal expression. Clearly, different genes are maximally expressed at different times of the day. (Adapted from Lin, Y. et al., *Proc. Natl. Acad. Sci. USA*, 99, 9562, 2002.)

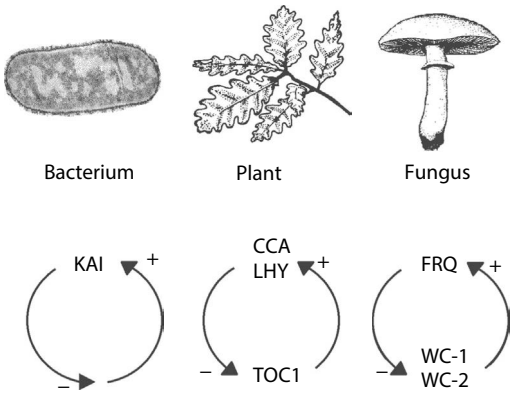


FIGURE 12.32 Molecular mechanisms of the circadian clock in nonanimals. The diagrams show the main proteins involved in the feedback loops that make up the circadian clock in bacteria, plants, and fungi. (Data from Johnson, C.H., *Annu. Rev. Physiol.*, 63, 695, 2001; Schultz, T.F. and Kay, S.A., *Science*, 301, 326, 2003; Cheng, P. et al., *Proc. Natl. Acad. Sci. USA*, 98, 7408, 2001.)

In the cyanobacterium (*Synechococcus elongatus*), the circadian clock involves a simple negative-feedback loop composed of the *kai* gene and its protein (KAI)—although there are three versions of *kai* (*kaiA*, *kaiB*, and *kaiC*), which may interact in a more complex manner.[283,284] More details about the bacterial clock will be discussed in Section 12.3.5.

In the mustard plant (*Arabidopsis thaliana*), the loop involves two sets of clock proteins: TOC1 (**T**iming **o**f **c**hlorophyll a/b-binding protein expression **1**) and CCA1-LHY (**C**ircadian **c**lock **a**ssociated **1** and **L**ate **e**longation **h**ypocotyl),[285,286] as diagrammed in Figure 12.32. Several other proteins affect the amplitude and period of the clock but are not part of the clock itself, including GI (**Gi**gantea),[287] ZTL (**Z**ei**tl**upe),[288] and TIC (**Ti**me for **c**offee).[289] Incidentally, if you feel that all these genes/proteins have very silly names, you are right! With thousands of genes to be named, researchers quickly run out of elegant names. As research progresses, additional proteins are being found to be involved in the clock of the mustard plant, most recently cADPR[290] and CHE.[291] To complicate the picture further, it seems that the transcriptional–translational loop found in the roots is different from the one found in the shoots.[292]

In bread mold, *Neurospora crassa*, the central clock gene is *frq* (short for *frequency*). Similarly to *per* in the fruit fly, the *frq* gene in bread mold encodes a product (the FRQ protein) that indirectly feeds back on the gene to regulate its transcription on a circadian time scale.[293,294] The abundance of *frq* mRNA increases in response to photic stimulation, and the magnitude of this increase correlates with the magnitude of phase shifts of the "activity" rhythm (production of asexual spores).[295] The loop involves two additional genes, called white collar (*wc-1* and *wc-2*), whose proteins (WC-1 and WC-2) form a complex that stimulates *frq* expression and is inhibited by FRQ[296–298] (Figure 12.32). A microarray study involving 1300 genes (14% of the genome) identified 145 cycling genes,[299] but there is no external criterion to judge how many of these are false positives, much less to determine how many genes are clock genes, and how many are clock-controlled genes. Although it was originally thought that phosphorylation of FRQ modulates the speed of the clock by modulating FRQ degradation,[300] it is now thought that the phosphorylation process itself, not the resulting degradation of FRQ, is what determines the speed of the clock.[301] Also, the single loop described in Figure 12.32 is likely to involve additional loops.[302,303]

12.3.4 Mammalian Molecular Clock

The main loop in the mouse has been found to be similar to the one in the fruit fly (Figure 12.33). BMAL1 (also known as MOP3 or ARNTL) is homologous to CYC in the fly and forms a heterodimer with CLK.[304] Although TIM has been shown to play a similar role in the rat as it does in the fly,[305] the bulk of the available evidence is that TIM is not an important player in the mammalian clock.[306] Instead of TIM, PER combines with CRY (cryptochrome).[307–309] To make things more complicated, there are three forms

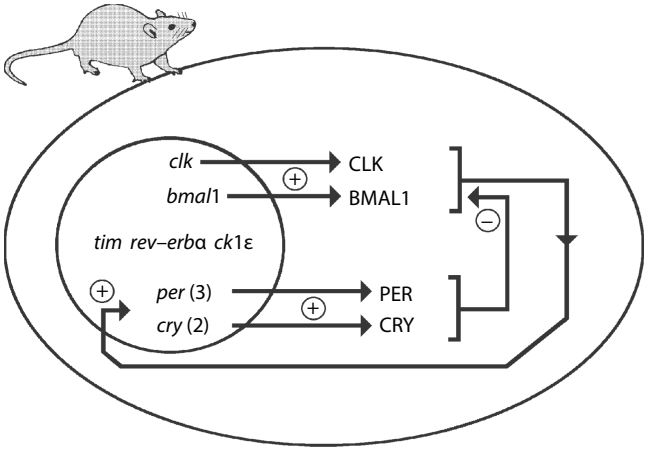


FIGURE 12.33 Molecular mechanism of the circadian clock in the mouse. This diagram illustrates the major players in the circadian clock of the mouse. For graphic purposes, the inhibition of CLK–BMAL1 by PER–CRY is displayed in the cytoplasm although it actually takes place in the nucleus of the cell. BMAL1 is also known as MOP3. (Data from Reppert, S.M. and Weaver, D.R., *Annu. Rev. Physiol.*, 63, 647, 2001; Devlin, P.F. and Kay, S.A., *Annu. Rev. Physiol.*, 63, 677, 2001; Barnes, J.W. et al., *Science*, 302, 439, 2003.)

of PER (PER1, PER2, and PER3) and two forms of CRY (CRY1 and CRY2).[310] The abundance of the various PER and CRY proteins has been shown to oscillate daily in the SCN.[204,311–325]

Figure 12.34 shows patterns of expression of three of the clock genes in the SCN of the mouse. The brain slices were processed by immunocytochemistry with antibodies against PER1 (which is labeled as mPER1 to specify that it is a mouse protein), CLOCK, and BMAL1. Notice that PER1 is present in much higher concentration between CT 10 and CT 18 than at other circadian times. CLOCK was constitutively expressed, and BMAL1 showed weak rhythmicity.[326] Constitutive expression of CLOCK and rhythmic expression of BMAL1 have been reported in various other studies in mice.[309,313,316] Similar results in sheep are shown in Figure 12.35.

As diagrammed in Figure 12.33, the feedback loop of the molecular clock in the mouse starts with *clk* and *bmal1* being transcribed in the nucleus and translated in the cytoplasm. A representation of the resulting proteins (CLK and BMAL1) is shown in Figure 12.36. After being synthesized in the cytoplasm, CLK and BMAL1 form a dimer, translocate to the nucleus, and activate the transcription of *per* and *cry*. PER and CRY are synthesized in the cytoplasm, accumulate to a critical level, heterodimerize, and then translocate to the nucleus. Once in the nucleus, the PER–CRY complex interacts negatively with the CLK–BMAL1 complex, thus closing the loop. We saw that, in the fly, CYC (the homolog of BMAL1) is constitutively expressed, whereas CLK is rhythmically expressed because of a second loop that involves *vri* and *pdp1* (see Figure 12.30). In the mouse, however, BMAL1 is rhythmically expressed, and this rhythmicity seems to be due to a second loop wherein REV–ERBα is stimulated by CLK–BMAL1 and later inhibits the transcription of *bmal1*.[327–329] As mentioned earlier, *clk* seems to be constitutively expressed in the mouse, but the situation may be more complicated. As indicated in Figure 12.37, the abundance of CLK *is* cyclic both in the nucleus and the cytoplasm of the cell; however, because the two cycles are in antiphase, the average abundance of CLK in the cell appears to be constitutive.[330] Evidently, more research is needed on this issue.

Confirmation that a particular gene is an essential component of the circadian clock can be obtained by the observation of loss of circadian rhythmicity in mice that had the gene knocked out. There was confusion at first when *clk* knockout mice were produced and tested. Given the preeminent position of *clk* in the transcriptional–translational loop, knockout mice were expected to be arrhythmic, just like SNC-lesioned animals are. Yet, *clk* knockout mice were found to exhibit clear circadian rhythmicity of

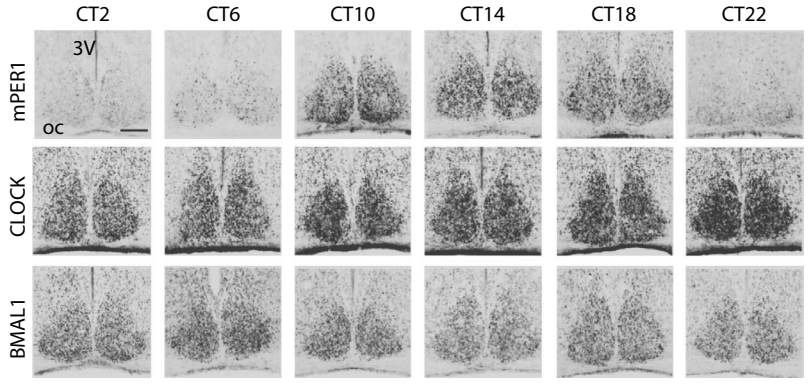


FIGURE 12.34 Tracking the molecular clock in mice. These series of coronal slices of mouse brain in the region of the suprachiasmatic nucleus show the circadian variation in abundance of three clock proteins: mPER1, CLOCK (CLK), and BMAL1. The mice were maintained in constant darkness (dim red light) on the day of euthanasia. The brain slices were stained by antibodies against the three proteins (with diaminobenzidine as the chromogen). Notice that abundance of PER1 varies according to circadian time, whereas abundance of CLK and BMAL1 is relatively constant throughout the circadian cycle. *Abbreviations:* 3V, third ventricle; CT, circadian time; OC, optic chiasm. The scale bar is 200 μm. (Adapted from von Gall, C. et al., *Eur. J. Neurosci.*, 18, 125, 2003.)

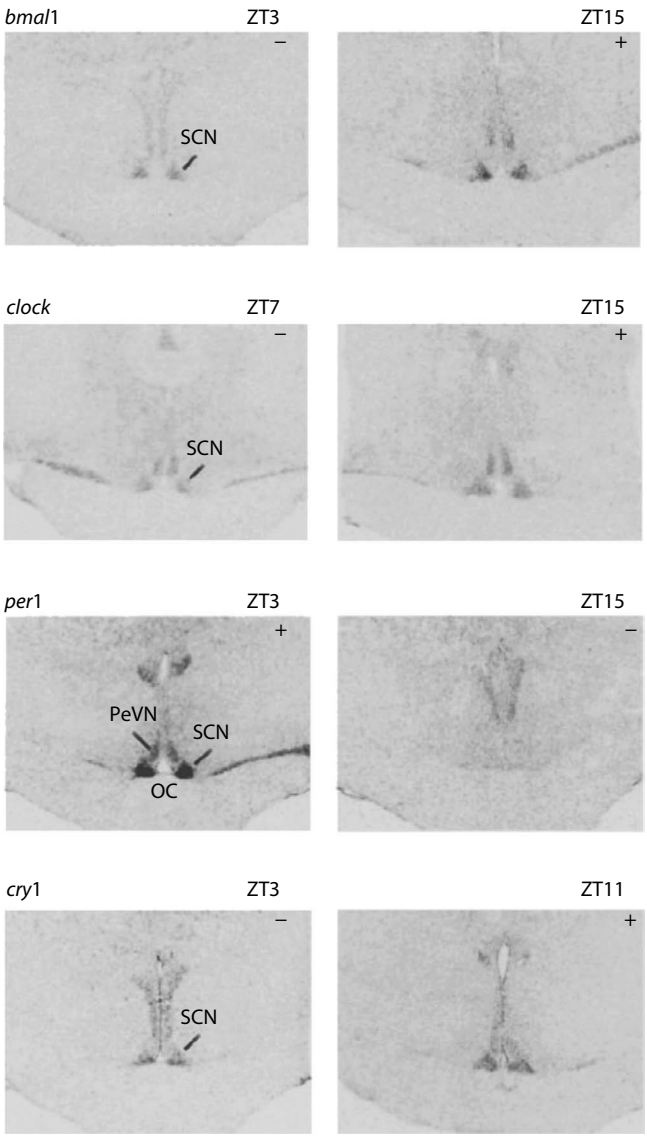


FIGURE 12.35 Tracking the molecular clock in sheep. These autoradiograms of coronal slices of sheep brain in the region of the suprachiasmatic nucleus show the daily variation in the expression of four clock genes: *bmal1*, *clock*, *per1*, and *cry1*. The sheep were maintained under a light–dark cycle (LD 16:8) prior to euthanasia. The brain slices were hybridized to radioactively labeled probes (*in situ* hybridization). Notice that *per1* is much more strongly expressed during the early part of the day (ZT 3) than later on (ZT 15), whereas expression of the other three genes shows much less daily variation. *Abbreviations:* OC, optic chiasm; PeVN, periventricular nucleus; SCN, suprachiasmatic nucleus; ZT, zeitgeber time. (Reproduced from Lincoln, G., Messager, S., Andersson, H., and Hazlerigg, D., Temporal expression of seven clock genes in the SCN and pars tuberalis of the sheep, *Proc. Natl. Acad. Sci. USA*, 99, 13890–13895, Copyright 2002, National Academy of Sciences USA. With permission.)

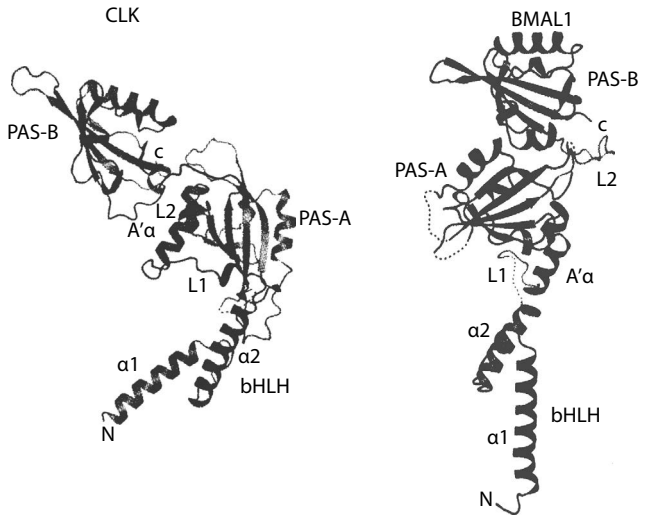


FIGURE 12.36 The proteins CLOCK (CLK) and BMAL1. The diagrams show the structure of two main proteins that operate in the mammalian molecular clock. (Adapted from Huang, N. et al., *Science*, 337, 189, 2012.)

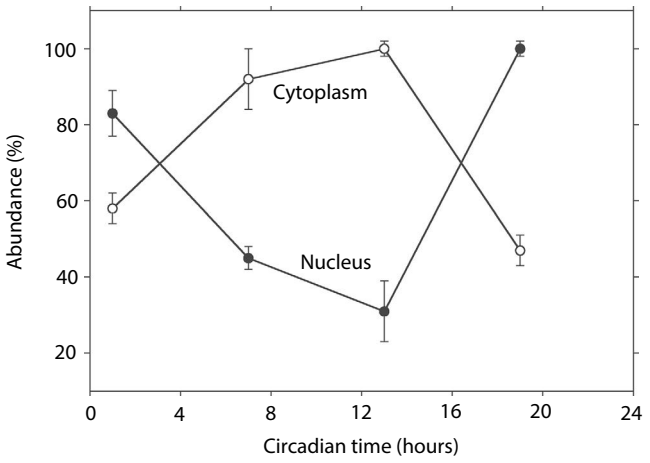


FIGURE 12.37 CLOCK in nucleus and cytoplasm of mouse cells. The graph shows that, in the suprachiasmatic nucleus (SCN) of the mouse, abundance of CLOCK protein does exhibit circadian oscillation if abundance is measured separately in the nucleus and cytoplasm of cells. Mice were maintained in constant darkness prior to euthanasia. SCN extracts were fractionated and analyzed by Western blotting using CLOCK antibodies. Each data point corresponds to the mean (± SE) of three mice. Notice that the rhythm of CLOCK abundance in the cytoplasm is in antiphase to the rhythm in the cell nucleus. (Data from Kondratov, R.V. et al., *Genes Dev.*, 17, 1921, 2003.)

behavior.[331,332] It turned out that, in the absence of CLK, BMAL1 can partner with NPAS2 (neuronal PAS domain protein 2, also known as MOP4), so that the *clk* knockout mice used NPAS2 instead of CLK to run their molecular clock.[331] Without surprises, mice with the *bmal1* gene knocked out were found to be arrhythmic in constant darkness.[304,333–335] Knocking out *per1* or *per2* separately was not sufficient to eliminate circadian rhythmicity, but *per1*/*per2* double knockouts were found to be arrhythmic, as the third *per* gene (*per3*) does not seem to be an important component of the mouse clock.[313,336] Similarly, *cry1*/*cry2* double knockouts were shown to be arrhythmic in constant darkness.[102,308,337]

As for the *tau* gene in the golden hamster, it was eventually found to be an allele of the *ck1ε* gene (*casein kinase 1ε*), a homolog of the *Drosophila*'s *dbt* gene.[338] In normal animals, CK1 is believed to accelerate the circadian clock by promoting degradation of PER proteins.[339–342] The mutated (*tau*) form of *ck1* is more effective than the wild-type form in destabilizing PER, thus speeding up the circadian clock (and, consequently, shortening the circadian period).[111] A demonstration of the role of CK1 in PER degradation is provided in Figure 12.38. To facilitate the monitoring of protein synthesis, the study was conducted on cell cultures (of mouse fibroblasts). The figure shows immunoblots for five proteins under two conditions: a control condition (left column) and a condition in which both CK1δ and CK1ε were chemically inactivated (right column). The blots in the *control* column show that CK1δ, CK1ε, and ACTIN (a control protein that is always expressed) were constitutively expressed, whereas PER1 and PER2 oscillated daily. The blots in the *inactivated* column show that CK1δ was inactivated (and an inactive form is present), CK1ε was also inactivated, ACTIN remained active, and PER1 and PER2 lost rhythmicity (because *per1* and *per2* were no longer subject to timed inhibition by CK1).[343] At first, it seemed that CK1δ was more effective than CK1ε in phosphorylating PER,[344,345] but it became clear later that the two CK1s are similarly effective although redundant.[343] CK2 may work in concert with CK1ε.[346]

As was the case in the fruit fly, it is very likely that more genes and proteins will be found to participate in the clock's core mechanism as further research is conducted. A diagram of the genes and proteins currently believed to make up the mammalian circadian clock is shown in Figure 12.39. A double loop, analogous to the double loop described for the fruit fly, can be seen here along with input from extracellular signals. Although the diagram indicates a few mechanisms of posttranslational modification of clock proteins (such as phosphorylation by CK1), it does not include all mechanisms of posttranslational modification that have been identified, which comprise not only phosphorylation but also dephosphorylation, ubiquitination, sumoylation, and acetylation.[347,348] Some of the proteins recently identified as potential participants in the mammalian molecular clock include PGC1α,[349] FBXL3,[350] cAMP,[351] RACK1,[352] PKCα,[352] PSF,[353] JARID1a,[275] CAVIN-3,[354] USP2,[355] NONO,[356] and TRAP150.[357] Additionally, it seems that micro-RNAs help generate the time delay needed for the 24-hour cycle of the circadian molecular loop.[358]

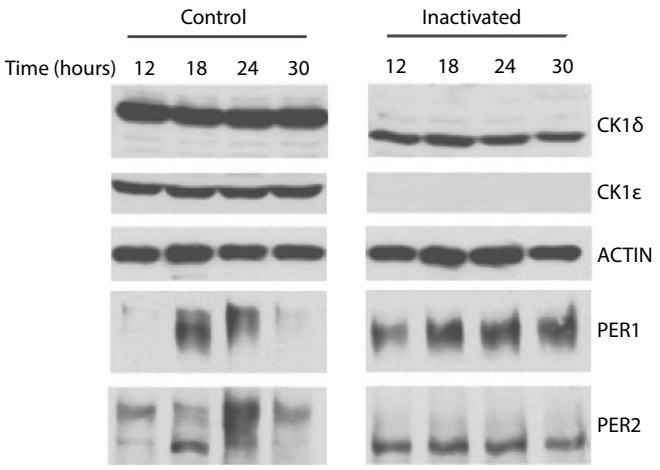


FIGURE 12.38 The role of casein kinase 1 (CK1). When CK1 is inactivated in mouse fibroblast cells, PER1 and PER2 stop oscillating. (Adapted from Lee, H. et al., *Proc. Natl. Acad. Sci. USA*, 108, 16451, 2011.)

A big surprise in research conducted during the first decade of the twenty-first century was that the elaborate mammalian molecular clock that we have just described is not found exclusively in SCN cells. As we will discuss in Section 12.4, the same molecular clock is found in cells of all other organs in the mammalian body. Because only a few other organs (such as the pineal gland and the eyes) possess circadian pacemaking capacity, the question arises about the relationship between molecular clocks and physiological clocks. It is possible that only the SCN has the ability to synchronize its own cells and generate a physiological pacemaker, whereas other organs have rhythmic cells that run at different speeds and cannot coalesce into a functional pacemaker, but evidence in support of this hypothesis is limited.[359] The remaining sections in this chapter will deal with this important issue in various ways.

12.3.5 Beyond the Loop

Figure 12.40 summarizes the clock loops in bacteria, the mustard plant, bread mold, the fruit fly, and the mouse. Although some form of negative-feedback loop is present in all clocks,[360,361] there are fundamental differences in the molecular machinery of circadian pacemakers between bacteria and eukaryotes, and the proteins involved in the loops are not homologous in plants, fungi, and animals.[362,363] Nonetheless, it has been pointed out that one small player (*casein kinase 2*) is conserved among all eukaryotic (animal, plant, and fungal) circadian systems.[270,364] Within the animal kingdom, different sets of clock genes are used by different taxa, but at least the *per* gene is found in all animal taxa studied so far,[365] including annelids,[366] mollusks,[367] insects,[368–372] reptiles,[373] birds,[9,374] and mammals.[257,375–381] *Per*, *clk*, and *cyc* (or *bmal1*) seem to be common to all arthropods and chordates, including insects,[267,372,382] fishes,[383] birds,[374,384,385] rodents,[380,386–391] ruminants,[392] and humans.[381,393,394] From a larger evolutionary perspective, a comparison of proteins found in bacteria, archaea, and eukarya revealed that *peroxiredoxins* (which are ubiquitous antioxidant enzymes) exhibit daily rhythmicity in all three domains of life, suggesting that metabolic rhythms are ancient and were likely responsible for the evolution of circadian clocks.[395]

Detailed comparisons of the molecular mechanisms of circadian rhythmicity in different species cannot be conducted until the mechanisms are fully understood in at least a few species. Yet, recent discoveries suggest that full understanding

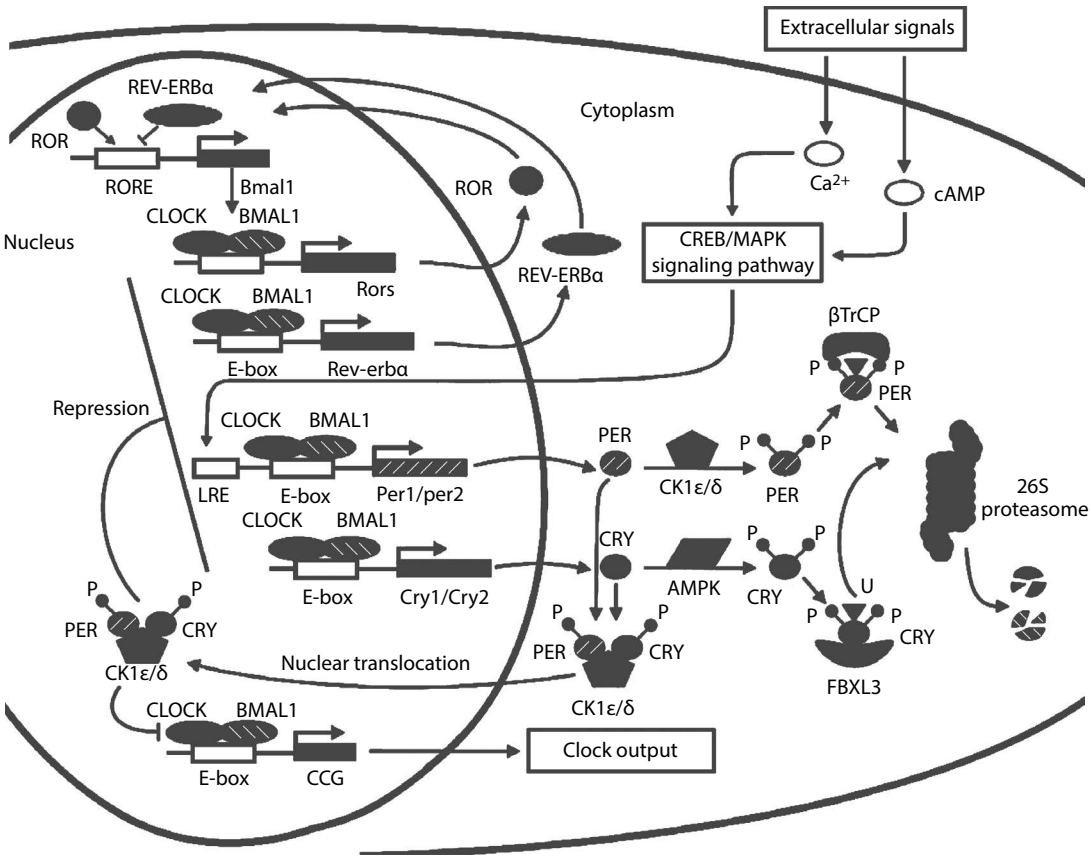


FIGURE 12.39 Model of the mammalian molecular clock. This diagram summarizes the major genes and proteins involved in the mechanism of the circadian molecular clock. *Abbreviations* (in addition to those introduced in the text): CCG, clock-controlled gene; P, phosphate; U, ubiquitin. (Adapted from Lowrey, P.L. and Takahashi, J.S., *Adv. Genet.*, 74, 175, 2011.)

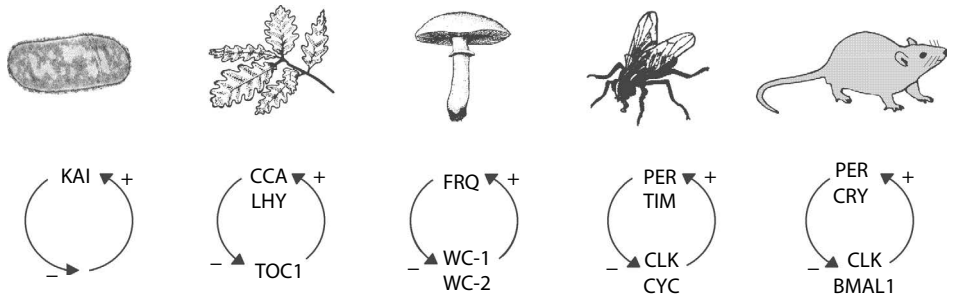


FIGURE 12.40 Summary of molecular mechanisms of the circadian clock. The diagrams show the main proteins involved in the feedback loops that make up the circadian clock in the five main groups of living beings (bacteria, plants, fungi, invertebrate animals, and vertebrate animals). (Data from Johnson, C.H., *Annu. Rev. Physiol.*, 63, 695, 2001; Devlin, P.F. and Kay, S.A., *Annu. Rev. Physiol.*, 63, 677, 2001.)

of the clock loops may be no more than a disappointingly small step in the path to the full understanding of the molecular machinery of circadian pacemakers. Because "clock genes are really housekeeping genes [that] are integral to the most basic functions of cells,"[396] it is extremely difficult to distinguish pacemaking features from ordinary life features.

The surprise came first from bacteria: a research team led by Takao Kondo at Nagoya University (in Japan) studied the transcriptional–translational loop in *S. elongatus*, which was believed to involve the three *kai* genes, as indicated earlier. They placed some KaiC protein in a test tube, added KaiA and KaiB in the same proportion found in live cells, and provided ATP as fuel. What happened? Considering that there were no genes to run the transcriptional–translational loop, we would expect no active process to ensue. Yet, Kondo found that KaiC phosphorylation oscillated robustly with a period of about 24 hours for at least three cycles.[397,398] He clearly had in his hands a circadian clock without a transcriptional–translational loop! His research team and other teams have examined this *posttranslational regulation* in some detail.[399–402]

In multicellular organisms, the downfall of the transcriptional–translational loop model could have been predicted. Because each cell in the body must undergo chromatin remodeling as part of its epigenetic differentiation from stem cells, it is evident that the protein-transcribing genes of the genome cannot tell the whole story. Furthermore, recent studies on human nerve cells indicate that cell differentiation may involve somatic genome diversification, in the sense that up to a quarter of nerve cells exhibit significant DNA copy number variation.[403] These considerations make it unreasonable to assume that circadian rhythmicity can be explained by the concerted action of a few protein-transcribing genes with equal representation in all cells of the body. Yet, most biologists retained faith in the model of the circadian clock as a transcriptional–translational feedback loop even when it became clear that cells in virtually every organ of the body express the transcriptional–translational loop, whereas most organs do not exhibit intrinsic circadian rhythmicity in their physiology.

The faith was challenged in 2007, when Ueli Schibler's research team at the University of Geneva (in Switzerland) reported the results of a microarray study of gene expression in tissue samples from mouse liver. The researchers used two groups of mice: a control group and a group in which *bmal1* expression was inhibited exclusively in the liver, thus effectively stopping the hepatic circadian clock in animals that otherwise exhibited circadian rhythmicity. Mice were sacrificed at various times of the day, and their livers were removed for analysis. A sample of the results is shown in Figure 12.41. Notice that, in mice with working hepatic clocks (A), all 400 or so genes are expressed differently across the day—that is, they are rhythmic. In mice without a clock (B), most genes are not rhythmic—but some genes *are* rhythmic, even though the transcriptional–translational loop has been stopped by inhibition of *bmal1* expression! Rhythmic genes can be seen around position 100 and around position 350, for example, and they include the *per2* gene. An inevitable conclusion is that peripheral cells need not have functional transcriptional–translational loops in order to exhibit circadian oscillation of "clock genes." Another conclusion is that, if rhythmicity is not produced by the local transcriptional–translational loop, then it must be imposed systemically—that is, it must come from other organs, presumably from the SCN.[404] Importantly, this is not just an instance of coordination of peripheral rhythmicity by the SCN (as we will discuss in Section 12.4.3), but *generation* of rhythmicity in a peripheral organ by the SCN. Using a different approach, another research team reached the conclusion that the SCN is not simply a synchronizer of peripheral oscillators but actually a pacemaker of peripheral oscillators.[18]

More dramatic results, which have not received the deserved attention so far, were obtained by Akhilesh Reddy's team at the University of Cambridge (in the United Kingdom) and Vincent Cassone's team at the University of Kentucky. Reddy's team studied human red blood cells, which do not have a nucleus (and, therefore, do not have DNA and are not capable of transcription). Even though the cells did not have transcriptional–translational loops, they exhibited circadian rhythmicity of cell metabolism.[405] The research team led by

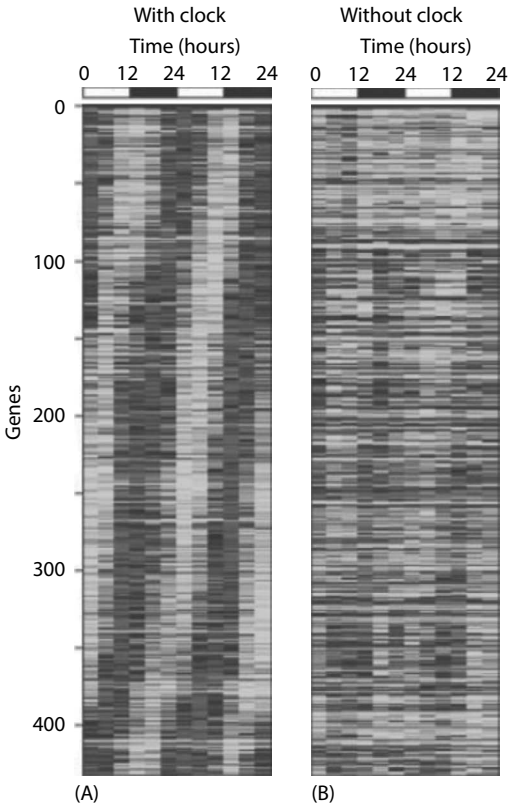


FIGURE 12.41 Keeping time without a clock. These gene microarrays show 24-hour oscillation of gene expression in normal liver cells (A) and reduced but still detectable oscillation in cells without a molecular clock (B). (Adapted from Kornmann, B. et al., *PLoS Biol.*, 5, art. e34, 2007.)

Vincent Cassone (Figure 12.42) investigated circadian rhythmicity in undifferentiated mouse embryonic stem cells and in cells allowed to differentiate. Undifferentiated stem cells did not exhibit the transcriptional–translational feedback loop, which is an interesting observation but not a ground-shaking finding in itself. What was ground shaking was the finding that undifferentiated stem cells expressed a self-sustained rhythm in glucose uptake despite the lack of a cellular clock.[406] Reddy's and Cassone's findings indicate that circadian rhythmicity in mammalian cells does not require a transcriptional–translational feedback loop. The condition is reminiscent of the circadian rhythmicity of Kai proteins in bacteria and puts into question the dogma of the mammalian circadian clock as a transcriptional–translational feedback loop. Regarding the bacterial clock, it seems that the transcriptional–translational loop is needed to fine-tune the operation of the protein-only clock.[407] It remains to be determined if a similar process is in operation in the mammalian clock.

Additional studies will be needed to confirm whether the description of transcriptional–translational loops is indeed no more than a very small step in the path to the full understanding of the molecular machinery of circadian pacemakers. It has been recently shown that the circadian transcriptional–translational loop is connected to mitochondrial oxidative metabolism,[408–410] so that there is at least a remote possibility



FIGURE 12.42 Vincent Cassone. This circadian physiologist from the University of Kentucky investigated circadian rhythmicity in embryonic stem cells and found that they uptake glucose rhythmically even though they lack a functional molecular clock. (Photo courtesy of Vincent Cassone.)

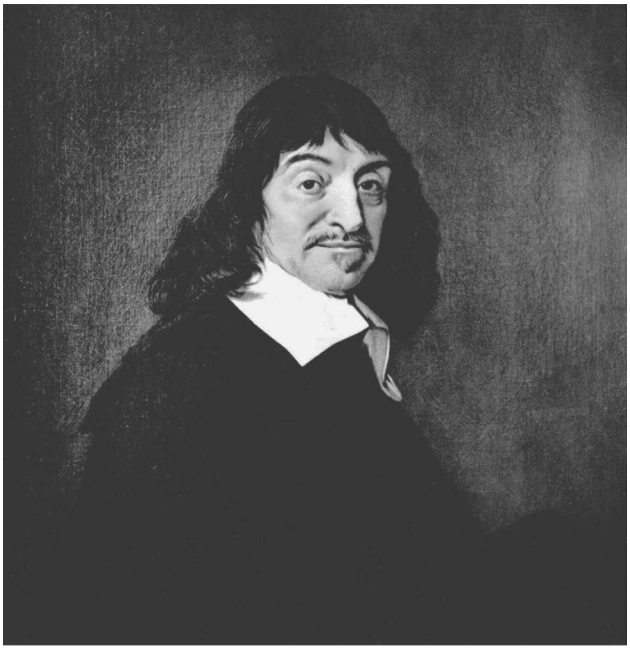


FIGURE 12.43 René Descartes (1596–1650). This French philosopher and mathematician, universally recognized as the father of modern rationalism, conjectured that the pineal gland might be the "seat of the soul" in the human brain. (Copyright ArtToday, Tucson, AZ.)

that what has been called the circadian molecular clock for the past 20 years is in reality only a secondary loop in the more general process of cell metabolism. This would explain why all mammalian cells exhibit this mechanism even though only neurons in the SCN are actual circadian pacemakers. If so, however, the molecular mechanism of circadian rhythmicity in the SCN would remain unidentified.

12.4 OTHER PACEMAKERS

As the master circadian pacemaker in mammals, the SCN deserved the attention it received in the preceding three sections of this chapter. However, the SCN is not the only circadian pacemaker in mammals, is perhaps not even the most important pacemaker in some nonmammalian vertebrates, and is nonexistent in invertebrates and nonanimals. Therefore, other circadian pacemakers must be discussed in this section. We saw that the accessory medulla (aMe) or the ventral lateral neurons (LN_V) are the fruit fly's equivalent of the mammalian SCN.[248] However, even in vertebrates—which posses an SCN—there are intrinsically rhythmic extra-SCN structures.

12.4.1 Pineal Gland

The seventeenth-century French philosopher René Descartes (Figure 12.43), who was also a skilled mathematician and anatomist, proposed that the pineal gland was the brain structure where the soul merged with the body.[411] Contemporary scientists do not deal with the concept of the *soul*, but, in a metaphorical sense, the pineal is the "seat of the soul" of the circadian system in some nonmammalian vertebrates. Consider Figure 12.44, which shows the perching activity records of a house sparrow (*Passer domesticus*) maintained in constant darkness. The rhythm clearly freeran for the first 2 weeks. However, when the bird's pineal gland was surgically ablated (arrow), circadian rhythmicity vanished. Ablation of the pineal gland has been shown to eliminate circadian rhythmicity in fishes,[412] lizards,[413,414] and birds.[415–417]

We do not need to go over the anecdote of the legless flea once again. The pineal gland of nonmammalian vertebrates could potentially be necessary for motor behavior and have nothing to do with circadian rhythmicity. So, let us move on to *in vitro* studies. Figure 12.45 shows the mean pattern of melatonin release from the pineal glands of house sparrows *in vitro*. Robust rhythmicity is evident. Of course, because the pineal gland is intrinsically photosensitive (see Chapter 11), the rhythm of melatonin release could be a simple reflection of the prevailing light–dark cycle. However, the study was conducted in constant darkness, so that the rhythmicity had to be endogenous. Intrinsic circadian rhythmicity of pineal glands in *vitro* has been demonstrated in fishes,[383,418,419] lizards,[420,421] and birds.[422,423] Rhythmic gene expression (using the luciferase-reporter-gene technique) has been documented *in vitro* in pineal glands from rats.[424]

Lest I give the impression that the pineal is more physiologically important than it really is regarding circadian rhythmicity, I should point out that its role as a circadian pacemaker is modest in many animals. In mammals, pineal ablation is inconsequential.[425–431] As a matter of fact, the armadillo (*Dasypus*

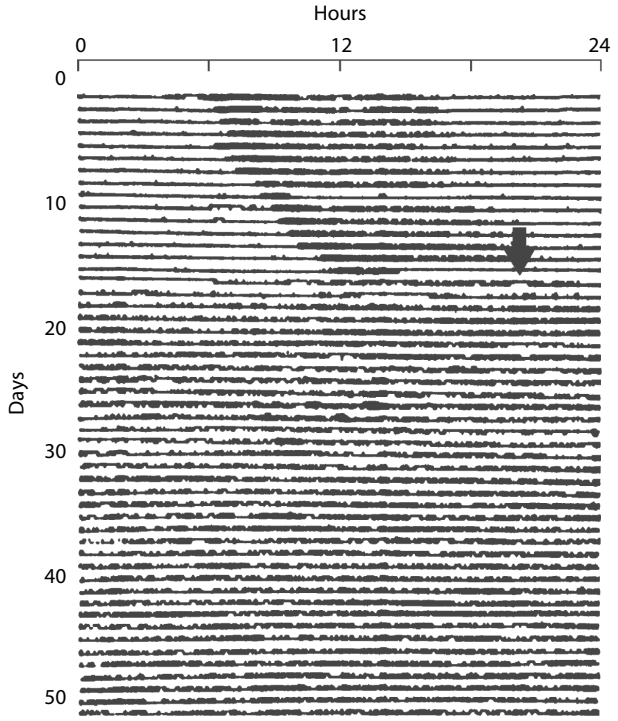


FIGURE 12.44 Sparrow needs the pineal gland to remain rhythmic. This actogram shows the rhythm of perching activity of a house sparrow (*Passer domesticus*) maintained in constant darkness. Pinealectomy was performed at the time indicated by the arrow. Notice that the circadian pattern of activity observed in the intact animal vanished after the pineal gland was removed. (Adapted from Gaston, S. and Menaker, M., *Science*, 160, 1125, 1968.)

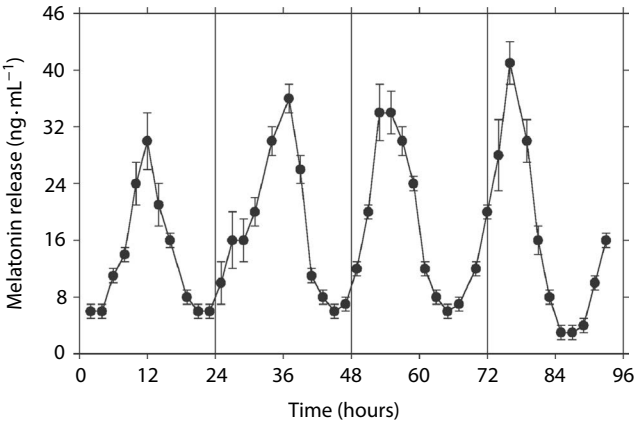


FIGURE 12.45 Sparrow's pineal gland is rhythmic *in vitro*. The graph shows the circadian oscillation in melatonin release from pineal glands *in vitro*. The data points correspond to the means (± SE) of 8 pineal glands from house sparrows (*Passer domesticus*). The glands were maintained in constant darkness. (Data from Brandstätter, R. et al., *Proc. Natl. Acad. Sci. USA*, 97, 12324, 2000.)

novemcinctus) is naturally devoid of the pineal gland and nevertheless exhibits circadian rhythmicity.[432] Dolphins also seem to lack a pineal gland.[433] In various studies on fishes,[434] lizards,[435–438] and birds,[439–442] pineal ablation disrupted behavioral rhythmicity but did not consistently abolish it. Thus, although the pineal may be an important component of the circadian system of some nonmammalian vertebrates,[443,444] it does not rival the SCN as the master pacemaker.

12.4.2 Eyes

The eyes (Figure 12.46) are, of course, photosensitive organs—even more so than the pineal gland. Like the pineal, they are also intrinsically rhythmic, even in mammals. Circadian rhythmicity in electrical activity or melatonin secretion of retinal tissue *in vitro* has been documented not only in invertebrates and lower vertebrates[383,420,445,446] but also in birds and rodents.[447–451] Figure 12.47 shows that cell cultures of the chick retina release melatonin rhythmically *in vitro*. Retinal cells were dissociated and then

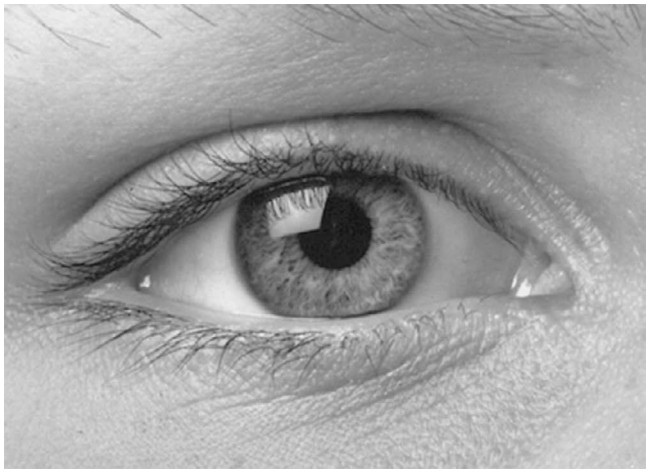


FIGURE 12.46 Timed look. In some organisms, the eyes contain circadian pacemaking cells. (Copyright ArtToday, Tucson, AZ.)

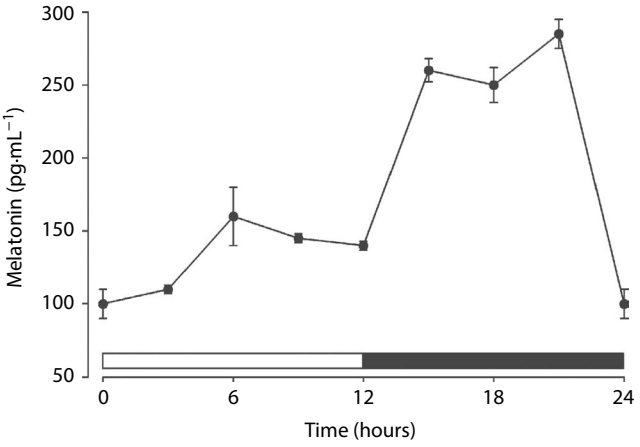


FIGURE 12.47 Chicken's retina is rhythmic *in vitro*. The graph shows daily rhythmicity in melatonin secretion of cultured retinal cells. The data points correspond to the means (± SE) of three cell cultures (retinospheroids) of chicken embryos (*Gallus domesticus*). Notice that much more melatonin is secreted during the dark phase than during the light phase of the light–dark cycle (indicated by the horizontal bar at the bottom of the graph). (Data from Willbold, E. et al., *Dev. Neurosci.*, 24, 504, 2002.)

allowed to reaggregate. By the 8th day in culture, they had differentiated and formed networks called *retinospheroids*. The supernatant of the culture medium was analyzed for melatonin content. As shown in the figure, melatonin secretion is clearly higher during the dark phase of the light–dark cycle. Although not shown in the figure, rhythmicity persisted in constant darkness.[447] More recently, intrinsic rhythmicity of clock gene expression of retinal or corneal tissue has been documented in birds[384,452] and rodents.[320,424,453–455]

In the quail (*Coturnix coturnix*), the intrinsic rhythmicity of the eyes seems to be very important for the organism's circadian system because ocular enucleation (i.e., removal of the eyes) abolishes the circadian rhythm of body temperature.[440,456] In the pigeon (*Columba livia*), ocular enucleation abolishes the rhythms of locomotor activity and feeding, but only if it is paired with ablation of the pineal gland.[439,457] In mammals, the eyes are not necessary for organismal rhythmicity,[23,45,427,458–461] although the absence of the eyes may have a small effect on the free-running period of the locomotor activity rhythm[462] and may affect cellular rhythmicity in a small region of the SCN.[463]

12.4.3 Liver

Great interest in the liver developed because of the liver's potential role as the food-entrainable oscillator (FEO). As we saw in Chapter 8, there is abundant evidence that there must be a damped extra-SCN pacemaker that responds to food restriction. Because no brain structure has been unequivocally identified as the site of the FEO,[76,464–466] and because the liver is directly involved in food processing, many researchers reasoned that the liver might be the sought-after FEO.

The first question, of course, is whether there is daily or circadian rhythmicity in liver function. The answer to this question is a straightforward *yes*. We saw in Chapter 5 that hepatic function, as measured by enzymatic activity and cholesterol synthesis, exhibits daily rhythmicity.[467–475] Also, the expression of clock genes in the livers of animals killed at different times of the day was shown to be rhythmic whether the animals were maintained under a light–dark cycle[37,386,476–484] or in constant darkness or constant dim light.[18,331,340,344,384,455,485–488] Microarray studies identified hundreds of cyclic genes in the livers of rats and mice.[329,489–495]

Of course, rhythmicity in the liver could be driven by the SCN. So, the next question is whether circadian rhythmicity in the liver can be intrinsically generated. In one gene expression study, ablation of the SCN several days before liver-tissue collection was found to severely damp mRNA cycling in the liver.[490] Examples for *per2* and *bmal1* are shown in Figure 12.48. Notice that, for both genes, livers from intact controls exhibit robust rhythmicity (lower panels), whereas livers from animals sustaining SCN ablation (SCN-X) express the genes constitutively (upper panels). However, in two other studies, SCN ablation was found to be inconsequential.[40,496] Evidently, *in vitro* studies are needed to settle the issue. At least seven studies have been conducted using the technique of genetically engineered bioluminescence (luciferase reporter gene). All seven studies identified intrinsic rhythmicity of gene expression in liver explants,[113,344,496–500] and an example

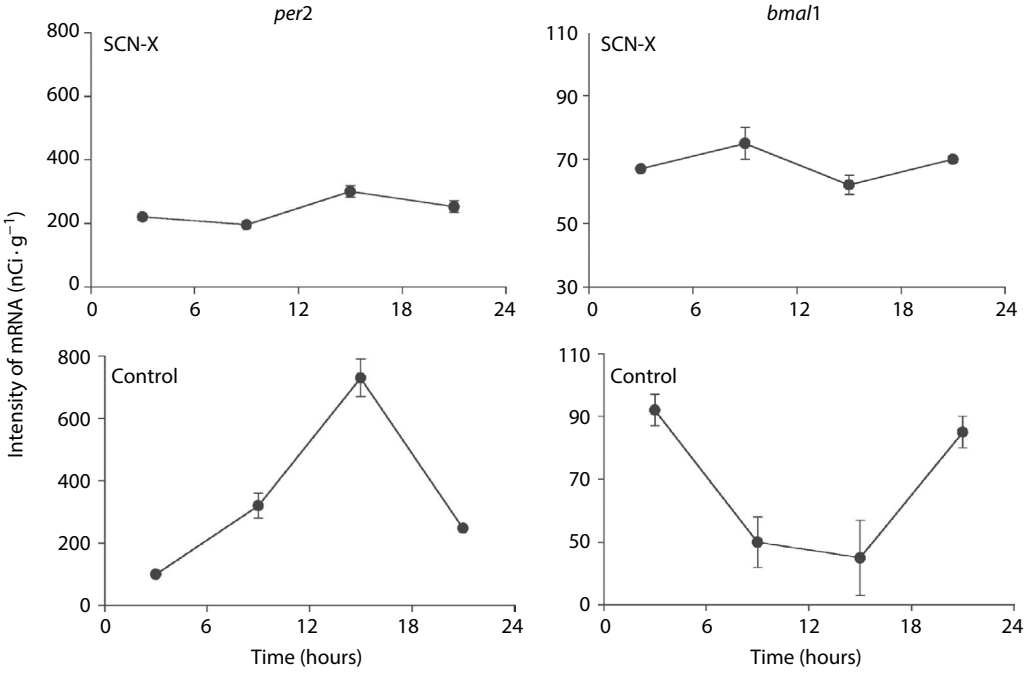


FIGURE 12.48 Liver's clock depends on suprachiasmatic nucleus (SCN) clock. The graphs show the daily variation in the expression of two clock genes (*per2* and *bmal1*) in the liver of previously intact mice (control) and of mice that had received SCN lesions many days earlier (SCN-X). Each data point corresponds to the mean (± SE) of three mice (*Mus musculus*). Notice that rhythmicity in gene expression in the liver is absent in mice devoid of SCN. (Data from Akhtar, R.A. et al., *Curr. Biol.*, 12, 540, 2002.)

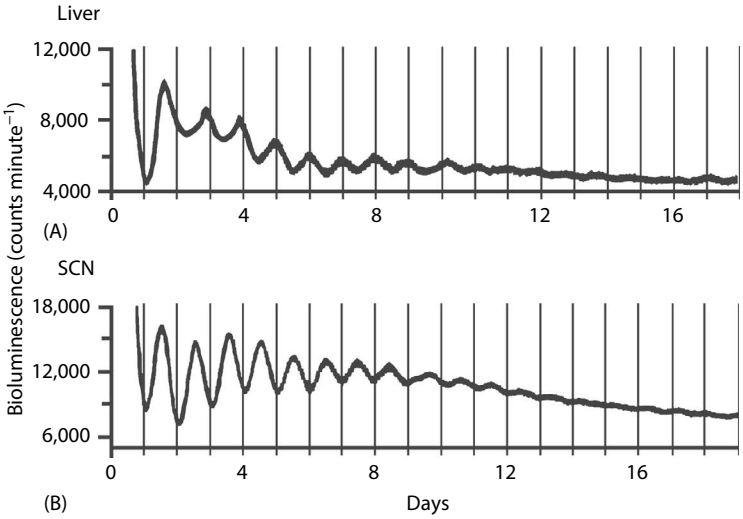


FIGURE 12.49 Is the suprachiasmatic nucleus (SCN) really necessary? By tracking the expression of the *per2* gene instead of the *per1* gene in mice (*Mus musculus*), investigators were able to measure persistent daily rhythmicity of liver tissue *in vitro*. Panel A shows that hepatic function—as estimated by variations in luminescence (glow) induced by an *mPer2*-promoter-driven luciferase reporter gene—is rhythmic for at least a week *in vitro*. Rhythmicity damps over time, but the damping is similar to that observed in SCN tissue (Panel B), suggesting that the liver tissue would have remained rhythmic if the culture conditions were more favorable. (Adapted from Yoo, S.H. et al., *Proc. Natl. Acad. Sci. USA*, 101, 5339, 2004.)

is shown in Figure 12.49. Because the culture conditions were not ideal, SCN data are shown for comparison. Notice that rhythmicity in the liver persists *in vitro* for as long as the rhythmicity in the SCN persists.

A third question is whether the liver responds to food restriction in a manner compatible with entrainment of organismal rhythms. We will discuss the afferent pathways of the circadian system in greater detail in Chapter 13, but we can discuss now how the rhythmicity in the liver is affected by food restriction. Several studies have addressed the issue of the dependence of the temporal pattern of gene expression in the liver on the light–dark cycle and the feeding schedule to which the animals are submitted prior to tissue collection. It has been consistently found that rhythms of gene expression in the liver can be shifted by shifts in the time of feeding but not by shifts in the light–dark cycle, whereas rhythms of gene expression in the SCN can be shifted by shifts in the light–dark cycle but not by shifts in the time of feeding.[37,41,314,337,482,495,497,498,501–504] An example is shown in Figure 12.50. Notice that when food is freely available (upper panels), *per1* expression peaks late during the light phase of the light–dark cycle both in the liver and the SCN; however, when food is available only in the middle of the light phase (when mice normally do not eat), peak gene expression is shifted in the liver but not in the SCN (lower panels). Phase shifting of the liver (but not of the SCN) in response to restricted feeding has been shown also in animals maintained in constant darkness.[314,501] This suits very well the distinction between FEO (liver) and light-entrainable oscillator (SCN). However, you will remember from Chapter 8 that there is behavioral evidence that the SCN can be entrained not only by photic stimuli but also by food restriction. Accordingly, in a study in which rats were fed by total parenteral nutrition (through an intravenous catheter), shifts in the time of feeding caused shifts in the rhythms of gene expression both in the liver and in the SCN.[505] It is possible that the failure to detect effects of restricted feeding on the SCN in the other studies was due to a masking effect of the disruption in behavior caused by feeding during the inactive phase of the circadian cycle. Under total parenteral nutrition, the effects of nutrient intake are isolated from the confounding effects of sleep disruption, mastication, and so on.

A fourth question—which has not been asked often enough—is whether the pattern of gene expression measured in the liver has anything to do with actual liver function. Of course, the expression of clock genes in the liver has *something* to do with liver function. Without gene expression, there is no protein synthesis; and, without protein synthesis, there are no enzymes to run the chemical reactions necessary for organ function. However, a multitude of posttranscriptional and posttranslational processes that are not yet understood might alter the ultimate effect of gene expression. We saw earlier in this chapter that, in the fruit fly, the *per* gene is expressed in virtually every organ of the body—and, yet, behavioral rhythmicity is dependent on *per* expression in the brain. Indeed, a study in rats revealed that, although the rhythm of gene expression in the liver is shifted by food restriction, the magnitude of the shift is not consistent with the pattern of behavioral food-anticipatory activity.[506] Also, rats with dysfunctional livers (chemically induced cirrhosis) exhibit normal anticipatory activity to a restricted-feeding schedule.[507] Normal mice fed a normal balanced diet under restricted feeding show food-anticipatory activity as well as entrainment of the gene expression rhythm in the liver,

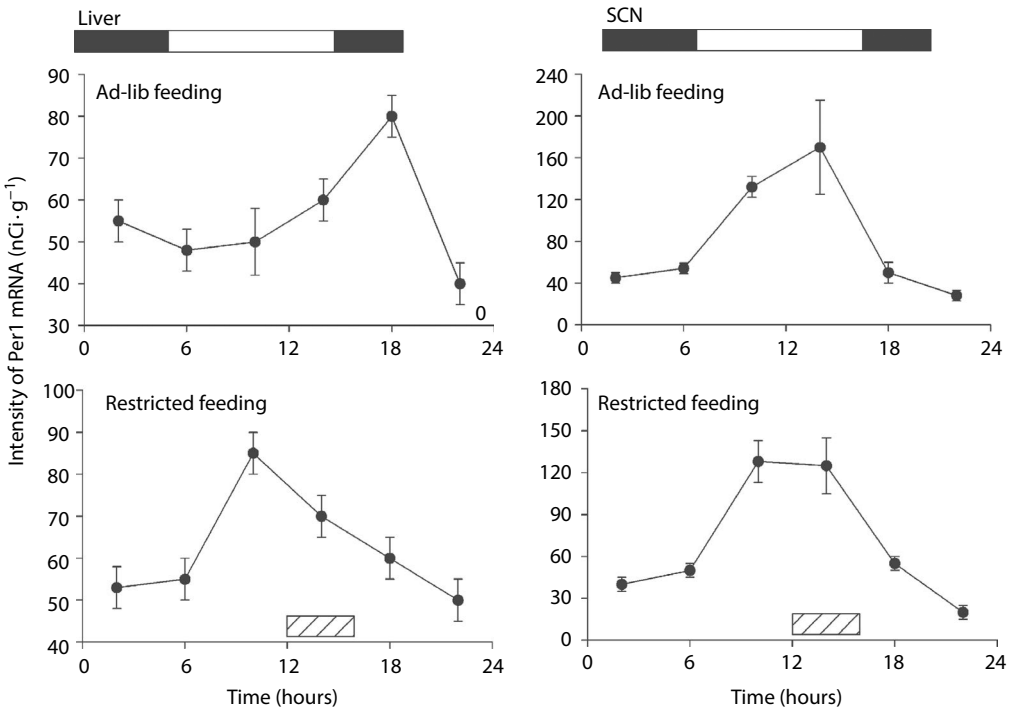


FIGURE 12.50 Is the liver the food-entrainable pacemaker? The graphs show the daily variation in the expression of a clock gene (*per1*) in the liver and the suprachiasmatic nucleus (SCN) of mice previously maintained under a light–dark cycle (indicated by the horizontal bars at the top) either with food freely available (ad-lib feeding) or with food access restricted to 4 hours per day (restricted feeding, indicated by the hatched bars at the bottom). Each data point corresponds to the mean (± SE) of 3 or 4 mice (*Mus musculus*). Notice that in the ad-lib feeding condition, the pattern of gene expression in the liver is similar to that in the SCN. The pattern of gene expression in the liver (but not in the SCN) is shifted under the restricted-feeding regime. (Data from Hara, R. et al., *Genes Cells*, 6, 269, 2001.)

but mice fed a diet composed of 100% glucose do not show entrainment of the gene expression rhythm in the liver even though they do show behavioral food-anticipatory activity.[508]

A true test of the responsiveness of liver function to restricted feeding involves the actual measurement of organ function, such as the synthesis of cholesterol. This is technically difficult in small rodents but can be easily accomplished in larger mammals. Data from a study conducted on goats are shown in Figure 12.51. Measurements were made of body core temperature, plasma urea concentration, and plasma cholesterol concentration. As mentioned in Chapter 9, the synthesis of urea by the liver is dependent on the digestive process, but the synthesis of cholesterol exhibits daily rhythmicity in food-deprived animals (and, therefore, is a reliable index of independent hepatic function). Notice that when the time of feeding was delayed by 6 hours (as indicated by the small triangles), the rhythm of urea production (as well as the rhythm of body temperature) followed along; however, the rhythm of cholesterol production was not affected by the shift in feeding time.[474] Thus, at least in goats, the liver does not seem to be entrained by restricted feeding and, therefore, is *not* the site of the FEO.

12.4.4 Other Peripheral Clocks

In addition to the SCN, the pineal, the eyes, and the liver, gene expression studies in vertebrates have described daily or circadian oscillation in a variety of peripheral organs, including the adrenal gland, brain, esophagus, heart, intestine, kidney, skeletal muscle, uterus, oral mucosa, salivary glands, pancreas, pituitary gland, spleen, stomach, testis, thyroid, and adipose tissue.[18,116,315,318,383,384,386,392,408,455,478–481,485,487,489,491,494,501,503,506,509–518] In principle, all of these organs could derive their rhythmicity from the SCN's rhythmicity. However, *in vitro* studies have described rhythmicity of gene expression in fibroblast (collagen) cell cultures[519–524] as well as in the kidney, pancreas, lung, skeletal muscle, olfactory bulb, pituitary gland, cortical glial cells, cerebellum, adipose tissue, and hair follicle cells.[34,114,408,496–498,500,525–531] It is clear, therefore, that peripheral organs can exhibit intrinsic rhythmicity. The question is, again, whether the rhythmicity in gene expression is reflected in rhythmicity of organ function. Organ function *in vitro* has been studied in only a few organs, but it has been shown that explanted adrenal glands secrete corticosteroids rhythmically in the circadian range,[532] that cultured pancreatic islets secrete insulin rhythmically in the circadian range,[533] that cultured muscle and fat cells consume glucose rhythmically in the circadian range,[534] and that explanted olfactory bulbs fire action potentials rhythmically in the circadian range.[535] Of course, the fact that an organ possesses intrinsic circadian rhythmicity does not imply that it has an impact on the operation of the organism. For instance, although the olfactory bulb is intrinsically rhythmic, its ablation has no effect on the circadian rhythm of locomotor activity of rats.[535]

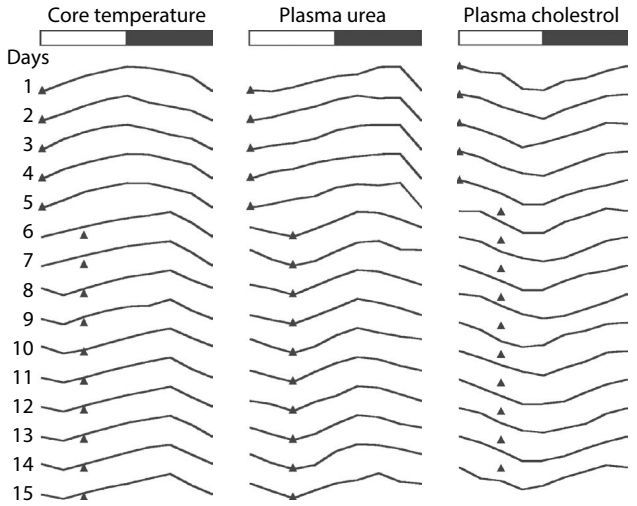


FIGURE 12.51 No evidence of liver clock in actual organ function. These actogram-style graphs show the average rhythms of body core temperature, plasma urea concentration, and plasma cholesterol concentration of five goats (*Capra hircus*) maintained under a light–dark cycle (as indicated by the horizontal bars at the top) and a restricted-feeding schedule (with food served once a day at the times indicated by the small triangles). On day 6, feeding time was delayed by 6 hours. The change in feeding time altered the shape of the temperature rhythm (perhaps as a masking effect) and caused a 6-hour delay in the rhythm of plasma urea concentration (as an indication of liver response to the delay in the digestive process). However, the rhythm of plasma cholesterol (which reflects digestion-independent liver function) was unaffected by the change in feeding time. (Adapted from Piccione, G. et al., *Comp. Biochem. Physiol. A*, 134, 563, 2003.)

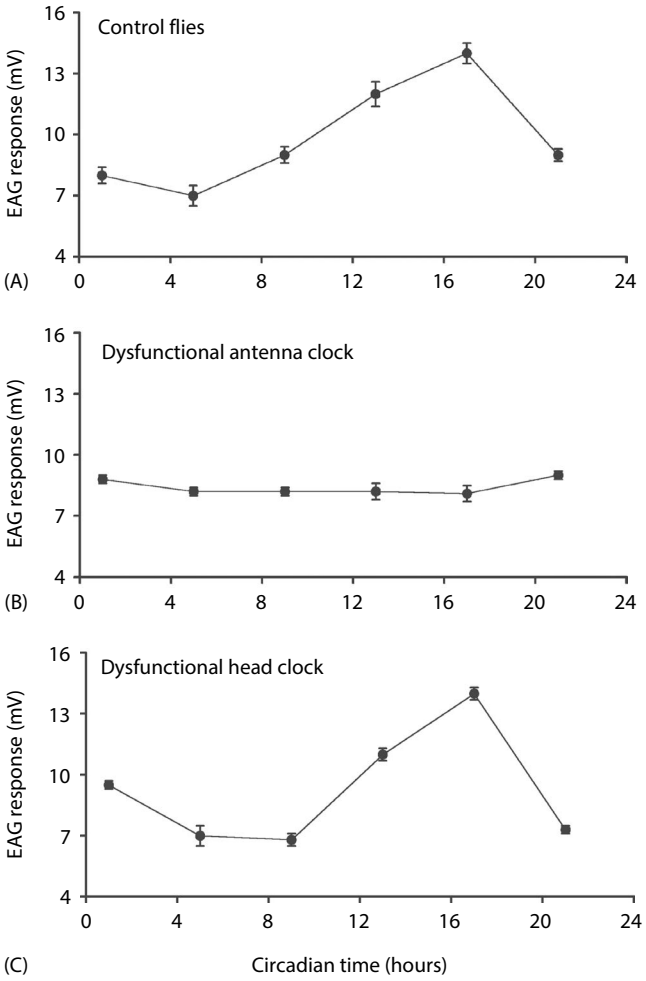


FIGURE 12.52 A true peripheral clock in the fruit fly. The graphs show the circadian variation in the electroantennogram (EAG) response of the fruit fly to an odor (ethyl acetate) presented to the antenna of control flies (A), flies with a dysfunctional molecular clock in the antenna (B), and flies with a dysfunctional molecular clock in the head (C). The data points correspond to the means (± SE) of approximately 24 flies. Notice that a dysfunctional antenna clock eliminates rhythmicity of the EAG response but a dysfunctional head clock does not. (Data from Tanoue, S. et al., *Curr. Biol.*, 14, 638, 2004.)

In invertebrates and plants, there are various reports of multiple pacemakers in individual organisms. For instance, in the common bean plant (*Phaseolus vulgaris*), individual plants maintained in constant light can exhibit 27-hour rhythmicity in leaflet movement at the same time as 24-hour rhythmicity in photosynthesis, which evidently requires the presence of at least two distinct circadian pacemakers.[536] In the mustard plant (*A. thaliana*), the epidermal cell layer and mesophyll layers express genes with periods differing by up to 1.5 hours.[537] In the red goosefoot herb (*Chenopodium rubrum*), individual plants can exhibit 30-hour rhythmicity in flowering response, 27-hour rhythmicity in dark respiration, 21-hour rhythmicity in photosynthesis, and 18-hour rhythmicity in dry weight.[538] The unicellular plankton *Lingulodinium polyedrum* (formerly, *Gonyaulax polyedra*) maintained in constant light exhibits a 22-hour rhythm of aggregation simultaneously with a 25-hour rhythm of bioluminescence.[539] As a matter of fact, the bioluminescence rhythm at 18°C exhibits a 22-hour glow component simultaneously with a 24-hour flash component.[540] Particularly interesting is the olfactory response of the fruit fly (*D. melanogaster*). Olfactory responses can be measured as the electrical activity of neurons in the antenna (*electroantennogram* [EAG]) following stimulation by an odorant substance (such as ethyl acetate). As shown in Panel A of Figure 12.52, normal flies maintained in constant darkness exhibit circadian rhythmicity in the EAG response. If the clock genes are selectively rendered dysfunctional in the antenna, the rhythmicity vanishes (Panel B). If, instead, the clock genes are rendered dysfunctional in the head, rhythmicity is not disturbed (Panel C). Thus, circadian gene expression in the antenna is both necessary and sufficient to produce circadian oscillation in the EAG response.[541] This means that olfactory rhythmicity can be maintained by a clock distinct from the clock that controls rhythmicity of locomotor behavior. We do not know whether the brain clock supervises the operation of the antennal clock in normal flies, but it is evident that the antenna can operate independently of the brain. We also do not know whether a similar autonomy of pacemakers can be found in mammals, although we do know that various autonomous processes in nonmammalian organisms

are *not* found in mammals. For instance, urodele amphibians can regenerate lost limbs, whereas mammals cannot.[542–544]

Four other candidates for the site of the mammalian FEO, this time in the brain, have been named. One of the candidates is the *paraventricular nucleus of the thalamus* (PVT). A study in rats has shown that Fos expression in the PVT (as well as in the SCN) peaks right before the beginning of anticipatory activity in animals subjected to a restricted-feeding schedule, and that anticipatory activity is eliminated after ablation of the PVT.[545] This does not prove that the PVT is the FEO, but it suggests that the PVT is at least a major component of the brain circuit responsible for food-anticipatory activity.

The forebrain is the second FEO candidate. You will remember that CLK is a major player in the mammalian molecular clock. The CLK paralog NPAS2 is expressed in the forebrain but not in the SCN,[546] and it interacts there with BMAL1 (or MOP3) just like CLK does in the SCN.[547,548] Interestingly, NPAS2 knockout mice respond faster to a shift in the light–dark cycle than wild-type mice do (as if the master pacemaker were no longer disrupted by the FEO) and respond very poorly to restricted feeding (as if the FEO had been eliminated).[549] In contrast, homozygous *clock*-mutant mice (with dysfunctional CLK) respond normally to food restriction.[550] Thus, the evidence suggests that the FEO is dependent on NPAS2 and, therefore, is located somewhere in the forebrain. If this is true, one can propose a dual brain mechanism involving the SCN and the forebrain clock.[551] As diagrammed in Figure 12.53, the SCN would be entrained by light (1) and would control the various rhythms exhibited by the organism, such as activity, sleep, and feeding (2). The forebrain clock would be entrained by the nonphotic feedback from activity, sleep, and feeding (3). Under normal circumstances, the forebrain clock would simply pass on the information to the SCN (4a). However, in animals with SCN ablation or in animals exposed to conflicting photic and nonphotic zeitgebers, the forebrain clock would directly modulate activity, sleep, and feeding (4b). This is quite a bit of speculation based on a single study of knockout mice, but it makes sense. One problem, of course, is the assumption that the SCN does not receive nonphotic input, but the scheme could be easily adapted to include a parallel feedback line from activity, sleep, and feeding to the SCN (5). Alternatively, entrainment of the SCN by food restriction might be achieved through input from the forebrain clock (4a). Some support for the proposal that NPAS2 cells are the seat of the FEO comes from a study in orexin knockout mice. Orexin (also called hypocretin) is a neurotransmitter used in brain circuits involved in the regulation of feeding and sleep.[552] Orexin knockout mice not only show reduced food-anticipatory activity under restricted-feeding conditions but also have reduced *npas2* expression in the forebrain.[553] This correlation between deficient *npas2* expression and deficient food-anticipatory activity is suggestive of a causal link.

The third FEO candidate is the dorsomedial hypothalamic nucleus (DMH). A research team led by Clifford Saper at Harvard Medical School conducted at least two major studies on this matter. In the first study, the researchers showed that food-anticipatory activity in rats can be eliminated by complete lesions of the DMH but not by lesions of cell groups surrounding the DMH.[554] In the second study, Saper and colleagues took advantage of the circadian arrhythmicity of *bmal1* knockout mice. The researchers injected a viral vector containing the *bmal1* gene exclusively in the SCN or in

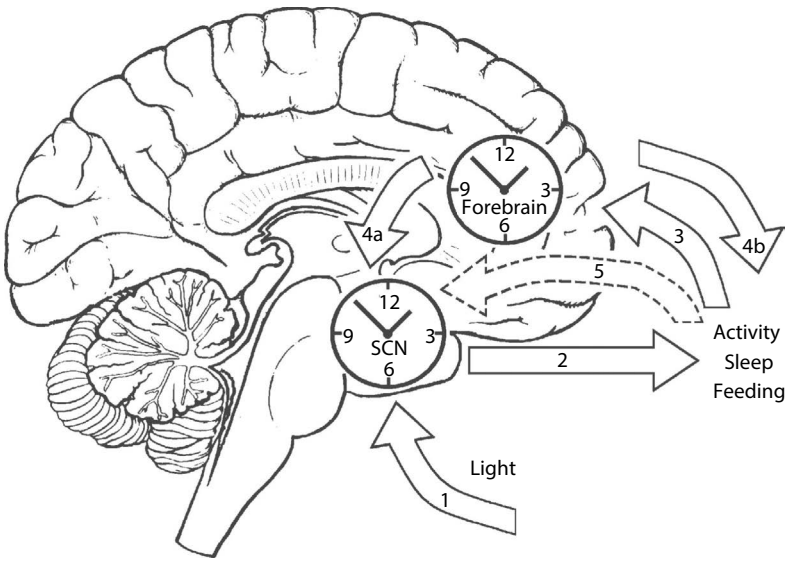


FIGURE 12.53 A circadian pacemaker in the forebrain? If the recent discovery of a molecular clock in the forebrain—which responds to restricted feeding—is confirmed at the organism level, a new conceptualization of the circadian system may be justified. As indicated in the diagram, it is possible that environmental light entrains the circadian clock in the suprachiasmatic nucleus (SCN) (1), the clock in the SCN controls effector organs (2), the feedback from the effector organs (including feeding) entrains the clock in the forebrain (3), and the clock in the forebrain modulates SCN activity under normal circumstances (4a) or directly controls the effector organs when the SCN is damaged or weakened (4b). Direct feedback from the effector organs to the SCN (5) may also occur. (Adapted from Green, C.B. and Menaker, M., *Science*, 301, 319, 2003.)

the DMH to restore rhythmicity exclusively in these two sites. *Bmal1* knockout mice injected in the SCN (and thus presumably having a functional SCN) exhibited entrainment of the body temperature rhythm by a 24-hour light–dark cycle but not by a cycle of food availability. Conversely, *bmal1* knockout mice injected in the DMH (and thus presumably having a functional DMH) exhibited entrainment of the body temperature rhythm by a cycle of food availability but not by a light–dark cycle.[333] These results provide very strong evidence that the DMH is the seat of the FEO. Unfortunately, the results of the first study are questionable because other teams failed to eliminate food-anticipatory activity by inflicting large DMH lesions in rats[555] or mice.[503] The results of the second study are partially misleading because, although *bmal1* knockout mice are arrhythmic in constant darkness, they do exhibit food-anticipatory activity when subjected to restricted feeding.[556,557] Thus, Saper's mice would have exhibited entrainment of the body temperature rhythm by a cycle of food availability even if they had not been injected with the viral vector containing the *bmal1* gene in the DMH. Consequently, the strong evidence in support of the DMH as the seat of the FEO evaporates.

The fourth FEO candidate is the cerebellum. A research team led by Etienne Challet at the University of Strasbourg (in France) showed that the cerebellum exhibits circadian rhythmicity of gene expression of various clock genes, that the temporal pattern of gene expression can be entrained by restricted feeding, and that the rhythmicity of gene expression is eliminated in rats with impaired cerebellar function produced by intraventricular injections of a neurotoxin that depletes Purkinje cells. Additionally, the researchers showed that mice with a mutation that impairs the cerebellar circuitry do not show food-anticipatory activity, whereas normal mice do.[530] Further research is needed to corroborate this suggestive evidence that the cerebellum houses the FEO.

As we close this chapter, a summarizing statement is in order. As in all other aspects of morphology and physiology, it is obvious that there is great variety in the organization of the circadian systems of different taxonomic groups. Within the vertebrates, and particularly mammals, there is compelling evidence that multiple organs exhibit intrinsic circadian rhythmicity but also that the circadian organization of the major physiological systems (and the coordinated function of the animal as a whole) is dependent on the healthy operation of the SCN. One may question whether the SCN is the "conductor of the circadian orchestra" or the "ringmaster of the circadian circus,"[558] but it would be inappropriate to deny its status as the master circadian pacemaker.

SUMMARY

1. The master circadian pacemaker in mammals is located in the suprachiasmatic nucleus (SCN) in the rostroventral hypothalamus. Lesion studies identified it in 1972, and *in vitro* and *in vivo* functional studies, as well as transplant studies, have repeatedly corroborated the finding since then.
2. The SCN has two main subdivisions: the ventrolateral (or core) region and the dorsomedial (or shell) region. Neurons in the ventrolateral region are generally not intrinsically rhythmic and use VIP (in addition to GABA) as their main neurotransmitter, whereas cells in the dorsomedial region are intrinsically rhythmic and use AVP (in addition to GABA) as their main neurotransmitter. The ventrolateral region projects heavily to the dorsomedial region.
3. Great progress has been made in the study of molecular mechanisms of circadian rhythmicity during the last two decades. The circadian clock seems to be made up of transcriptional–translational loops that, in most animals, involve the *per*, *clk*, and *cyc* (or *bmal1*) genes. In mammals, CLK and BMAL1 are transcription factors that regulate the expression of *per* and *cry*, whose products (PER and CRY) inhibit CLK and BMAL1 in a negative-feedback loop. Numerous posttranscriptional and posttranslational mechanisms are involved. Recent evidence raises questions, however, about whether the transcriptional–translational loop is indeed the molecular mechanism of the circadian clock.
4. The pineal gland, the eyes, and other organs exhibit intrinsic circadian rhythmicity, at least in some vertebrate species. These multiple pacemakers seem to be under the control of the master circadian pacemaker located in the SCN. The location of the alternate food-entrainable pacemaker has still not been determined with certainty.

EXERCISES

12.1 Simulation of free-running rhythms. The program **Free-run** (the 9th icon from the right in the Circadian banner) allows you to simulate two particular phenomena: the pooling of SCN cells with varying circadian periods to produce a single *organismal* period and the production of *temporal chimeras* by placing two distinct circadian pacemakers in the same animal (as in hamsters with partial SCN lesions receiving a hypothalamic graft from a genotypically distinct hamster). These two phenomena were described in Sections 12.1 and 12.2.

1. Start the program **Free-run**.
2. At the top panel, select *Two pacemakers*.
3. Set the *Period* and *Strength* of the two pacemakers at your discretion and click on *Plot*. For instance, if one pacemaker has a period of **24** hours and a strength of **60%**, while the other has a period of **20** hours and a strength of **40%**, the actogram of organismal activity will look like a series of horizontal lines dense at the center. If you look closely, you will be able to identify both the 24- and the 20-hour components.
4. Repeat the procedure with other combinations of period and strength. You may have a hard time

identifying the 24-hour component in the plot generated by a pacemaker with 24-hour period and 40% strength combined with a pacemaker with 22-hour period and 60% strength, for example.

5. After you have exhausted your curiosity, select *One pacemaker* at the top panel.
6. Let us start with all the default values, which are **1** for the *No. of cells*, **22** hours for the *Lowest period*, **26** hours for the *Highest period*, and *Oscillator* as the *Integration site*. Click on *Plot*.
7. The actogram shown will have a circadian period anywhere in the 22–26-hour range. Click on *Plot* many times and observe the various periods.
8. Change the *No. of cells* to **100** and run several simulations again. Notice how the range of periods is greatly reduced. Since the period of the individual cells ranges from 22 to 26 hours, the mean period of all 100 cells tends to 24.0 hours.
9. Change the *No. of cells* to **1000** and run a few more simulations. Notice how the range of periods is reduced even further (although not as dramatically as when you switched from 1 to 100 cells).
10. Because the *Integration site* was left at the default value (*Oscillator*), the reduction in the range of expressed periods was a natural consequence of the averaging process. That is, the period of the expressed rhythm was the mean of the periods of the various cells. Statisticians have long known that the standard deviation of a distribution of means is much smaller than the standard deviation of the distribution of the elements. However, it is theoretically possible that the integration of the period of individual SCN cells takes place not at the SCN itself (the *Oscillator*) but at the organs that receive the output of SCN cells (the *Effectors*). What would happen if the effector organs responsible for locomotor activity received multiple inputs from SCN cells with different circadian periods?
11. Select *Effectors* as the *Integration site*. Unless you want to spend several minutes waiting for the simulation to be completed, make sure to reduce the *No. of cells* to **2**. Then click on *Plot*.
12. The outcome does not look very natural. Run the simulation a few more times. Notice that none of them looks like the record of locomotor activity of a normal animal.
13. Change the *No. of cells* to **10** and run several more simulations. The output looks even more unusual. It would seem that integration at the effector organs is *not* what happens in real animals. Of course, you have not tried a larger number of cells yet, but I can tell you that the output is still abnormal for 1000 cells. You can try it for yourself. If your computer is slower than 1 GHz, the simulation may take over a minute. In faster computers, it should take a few seconds. Change the *No. of cells* to **1000** and click on *Plot*.

12.2 *In vitro* activity of suprachiasmatic neurons. Perhaps the most convincing evidence that the SCN contains a circadian pacemaker is the observation of circadian rhythmicity in isolated hypothalamic slices maintained in a dish (i.e., an *in vitro* preparation). The techniques to maintain a brain slice healthy *in vitro* and to record the activity of nerve cells in the preparation are quite sophisticated, so that you cannot conduct this exercise on your own. If you have access to a cellular electrophysiology laboratory, the main investigator (or one of his or her assistants) can show you that the activity of suprachiasmatic neurons (as assessed by the number of action potentials per unit time) oscillates daily (even if light, temperature, and nutrients are maintained constant). In contrast, an isolated slice of cortical tissue, for example, exhibits only random oscillations in activity.

SUGGESTIONS FOR FURTHER READING

Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M., and Allada, R. (2002). A role for casein kinase 2α in the *Drosophila* circadian clock. *Nature* 420: 816–820.

By now, you should be familiar with the idea that suggestions for further reading on specific topics can be gathered from the list of source articles in the "References" section and that only general readings are listed in this section. However, I would like to explicitly list this research article here because it is an exceptionally good example of a well-written article with rigorous research methods and clear-cut results.

Klein, D. C., Moore, R. Y., and Reppert, S. M. (Eds.) (1991). *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press.

An excellent collection of review articles written by experts in the anatomy, physiology, pharmacology, and molecular biology of the SCN. Research advances during the last two decades have refined but not contradicted the material contained in this book from the early 1990s.

Sehgal, A. (Ed.) (2003). *Molecular Biology of Circadian Rhythms.* New York: Wiley.

Written by specialists for specialists, this book provides a detailed account of molecular mechanisms in circadian rhythmicity. The authors of the various chapters are world-renowned researchers in their fields.

Sehgal, A. (Ed.) (2015a). *Circadian Rhythms and Biological Clocks Part A* (*Methods in Enzymology*, Vol. 551). San Diego, CA: Academic Press.

Also written by specialists for specialists and edited by Sehgal, this volume partially supersedes the one published 12 years earlier. Here again, the authors of the various chapters are world-renowned researchers in their fields.

Sehgal, A. (Ed.) (2015b). *Circadian Rhythms and Biological Clocks Part B* (*Methods in Enzymology*, Vol. 552). San Diego, CA: Academic Press.

This second part of the book edited by Sehgal in 2015 concentrates less on molecular mechanisms and more on neural and organismal mechanisms. Once again, the authors of the various chapters are world-renowned researchers in their fields.

WEBSITES TO EXPLORE

Biological Clocks Lectures (Howard Hughes Medical Institute): http://www.hhmi.org/biointeractive/clocks/lectures.html.

Brain Catalog: http://braininfo.rprc.washington.edu/.

Brain Facts: http://www.brainfacts.org/.

Carl Johnson's Laboratory (Vanderbilt University): http://as.vanderbilt.edu/johnsonlab/.

Circadian Expression Profiles Database: http://bioinf.itmat.upenn.edu/circa/.

Society for Neuroscience: http://www.sfn.org/.

REFERENCES

1. Olds, J. (1958). Self-stimulation of the brain. *Science* 127: 315–324.
2. Alleva, J. J., Waleski, M. V., and Alleva, F. R. (1971). A biological clock controlling the estrous cycle of the hamster. *Endocrinology* 88: 1368–1379.
3. Stephan, F. K. and Zucker, I. (1972). Circadian rhythm in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proceedings of the National Academy of Sciences of the United States of America* 69: 1583–1586.
4. Moore, R. Y. and Eichler, V. B. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Research* 42: 201–206.
5. Janik, D. S., Pickard, G. E., and Menaker, M. (1990). Circadian locomotor rhythms in the desert iguana. II. Effects of electrolytic lesions to the hypothalamus. *Journal of Comparative Physiology A* 166: 811–816.
6. Minutini, L., Innocenti, A., Bertolucci, C., and Foà, A. (1995). Circadian organization in the ruin lizard *Podarcis sicula*: The role of the suprachiasmatic nuclei of the hypothalamus. *Journal of Comparative Physiology A* 176: 281–288.
7. Bertolucci, C., Sovrano, V. A., Magnone, M. C., and Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
8. Takahashi, J. S. and Menaker, M. (1982). Role of the suprachiasmatic nuclei in the circadian system of the house sparrow, *Passer domesticus*. *Journal of Neuroscience* 2: 815–828.
9. Yoshimura, T., Yasuo, S., Suziki, Y., Makino, E., Yokota, Y., and Ebihara, S. (2001). Identification of the suprachiasmatic nucleus in birds. *American Journal of Physiology* 280: R1185–R1189.
10. Redlin, U. and Mrosovsky, N. (1999). Masking by light in hamsters with SCN lesions. *Journal of Comparative Physiology A* 184: 439–448.
11. Mistlberger, R. E. (1993). Circadian properties of anticipatory activity to restricted water access in suprachiasmatic-ablated hamsters. *American Journal of Physiology* 264: R22–R29.
12. Ralph, M. R., Foster, R. G., Davis, F. C., and Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247: 975–978.
13. Pickard, G. E. and Turek, F. W. (1982). Splitting of the circadian rhythm of activity is abolished by unilateral lesions of the suprachiasmatic nuclei. *Science* 215: 1119–1121.
14. Stetson, M. H. and Watson-Whitmyre, M. (1976). Nucleus suprachiasmaticus: The biological clock in the hamster? *Science* 191: 197–199.
15. Harrington, M. E., Rahmani, T., and Lee, C. A. (1993). Effects of damage to SCN neurons and efferent pathways on circadian activity rhythms of hamsters. *Brain Research Bulletin* 30: 655–669.
16. Refinetti, R., Kaufman, C. M., and Menaker, M. (1994). Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters. *Journal of Comparative Physiology A* 175: 223–232.
17. Aguilar-Roblero, R., Morin, L. P., and Moore, R. Y. (1994). Morphological correlates of circadian rhythm restoration induced by transplantation of the suprachiasmatic nucleus in hamsters. *Experimental Neurology* 130: 250–260.
18. Guo, H., Brewer, J. M., Lehman, M. N., and Bittman, E. L. (2006). Suprachiasmatic regulation of circadian rhythms of gene expression in hamster peripheral organs: Effects of transplanting the pacemaker. *Journal of Neuroscience* 26: 6406–6412.
19. Ruby, N. F. and Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *American Journal of Physiology* 263: R353–R362.
20. Paul, M. J., Kauffman, A. S., and Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
21. Fernandez, F., Lu, D., Ha, P., Costacurta, P., Chavez, R., Heller, H. C., and Ruby, N. F. (2014). Dysrhythmia in the suprachiasmatic nucleus inhibits memory processing. *Science* 346: 854–857.
22. Satinoff, E. and Prosser, R. A. (1988). Suprachiasmatic nuclear lesions eliminate circadian rhythms of drinking and activity, but not of body temperature, in male rats. *Journal of Biological Rhythms* 3: 1–22.
23. Ruis, J. F., Rietveld, W. J., and Buys, P. J. (1987). Effects of suprachiasmatic nuclei lesions on circadian and ultradian rhythms in body temperature in ocular enucleated rats. *Journal of Interdisciplinary Cycle Research* 18: 259–273.
24. Stephan, F. K. and Nunez, A. A. (1977). Elimination of circadian rhythms in drinking, activity, sleep, and temperature by isolation of the suprachiasmatic nuclei. *Behavioral Biology* 20: 1–16.
25. Honma, K., Honma, S., and Hiroshige, T. (1987). Activity rhythms in the circadian domain appear in suprachiasmatic nuclei lesioned rats given methamphetamine. *Physiology and Behavior* 40: 767–774.
26. Honma, S., Honma, K., Shirakawa, T., and Hiroshige, T. (1988). Rhythms in behavior, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. *Physiology and Behavior* 44: 247–255.
27. Stephan, F. K., Swann, J. M., and Sisk, C. L. (1979). Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. *Behavioral and Neural Biology* 25: 545–554.
28. Kurumiya, S. and Kawamura, H. (1991). Damped oscillation of the lateral hypothalamic multineuronal activity synchronized to daily feeding schedules in rats with suprachiasmatic nucleus lesions. *Journal of Biological Rhythms* 6: 115–127.
29. Warren, W. S., Champney, T. H., and Cassone, V. M. (1994). The suprachiasmatic nucleus controls the circadian rhythm of heart rate via the sympathetic nervous system. *Physiology and Behavior* 55: 1091–1099.

30. Sano, H., Hayashi, H., Makino, M., Takezawa, H., Hirai, M., Saito, H., and Ebihara, S. (1995). Effects of suprachiasmatic lesions on circadian rhythms of blood pressure, heart rate and locomotor activity in the rat. *Japanese Circulation Journal* 59: 565–573.
31. Wachulec, M., Li, H., Tanaka, H., Peloso, E., and Satinoff, E. (1997). Suprachiasmatic nuclei lesions do not eliminate homeostatic thermoregulatory responses in rats. *Journal of Biological Rhythms* 12: 226–234.
32. Scheer, F. A. J. L., Ter Horst, G. J., van der Vliet, J., and Buijs, R. M. (2001). Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *American Journal of Physiology* 280: H1391–H1399.
33. Ohtsuka-Isoya, M., Hayashi, H., and Shinoda, H. (2001). Effect of suprachiasmatic nucleus lesion on circadian dentin increment in rats. *American Journal of Physiology* 280: R1364–R1370.
34. Abraham, U., Prior, J. L., Granados-Fuentes, D., Piwnica-Worms, D. R., and Herzog, E. D. (2005). Independent circadian oscillations of *Per1* in specific brain areas *in vivo* and *in vitro*. *Journal of Neuroscience* 25: 8620–8626.
35. Chiesa, J. J., Cambras, T., Carpentieri, A. R., and Díez-Noguera, A. (2010). Arrhythmic rats after SCN lesions and constant light differ in short time scale regulation of locomotor activity. *Journal of Biological Rhythms* 25: 37–46.
36. Angeles-Castellanos, M., Salgado-Delgado, R., Rodriguez, K., Buijs, R. M., and Escobar, C. (2010). The suprachiasmatic nucleus participates in food entrainment: A lesion study. *Neuroscience* 165: 1115–1126.
37. Sabath, E., Salgado-Delgado, R., Guerrero-Vargas, N. N., Guzman-Ruiz, M. A., Basualdo, M. C., Escobar, C., and Buijs, R. M. (2014). Food entrains clock genes but not metabolic genes in the liver of suprachiasmatic nucleus lesioned rats. *FEBS Letters* 588: 3104–3110.
38. Marchant, E. G. and Mistlberger, R. E. (1997). Anticipation and entrainment to feeding time in intact and SCN-ablated C57BL/6J mice. *Brain Research* 765: 273–282.
39. Filipski, E., King, V. M., Li, X. M., Granda, T. G., Mormont, M. C., Liu, X. H., Claustrat, B., Hastings, M. H., and Lévi, F. (2002). Host circadian clock as a control point in tumor progression. *Journal of the National Cancer Institute* 94: 690–697.
40. Filipski, E., King, V. M., Etienne, M. C., Li, X. M., Claustrat, B., Granda, T. G., Milano, G., Hastings, M. H., and Lévi, F. (2004). Persistent twenty-four hour changes in liver and bone marrow despite suprachiasmatic nuclei ablation in mice. *American Journal of Physiology* 287: R844–R851.
41. Pezuk, P., Mohawk, J. A., Yoshikawa, T., Sellix, M. T., and Menaker, M. (2010). Circadian organization is governed by extra-SCN pacemakers. *Journal of Biological Rhythms* 25: 432–441.
42. Malloy, J. N., Paulose, J. K., Li, Y., and Cassone, V. M. (2012). Circadian rhythms of gastrointestinal function are regulated by both central and peripheral oscillators. *American Journal of Physiology* 303: G461–G473.
43. Mulder, C. K., Papantoniou, C., Gerkema, M. P., and van der Zee, E. A. (2014). Neither the SCN nor the adrenals are required for circadian time-place learning in mice. *Chronobiology International* 31: 1075–1092.
44. Mahoney, M., Bult, A., and Smale, L. (2001). Phase response curve and light-induced Fos expression in suprachiasmatic nucleus and adjacent hypothalamus of *Arvicanthis niloticus*. *Journal of Biological Rhythms* 16: 149–162.
45. Sato, T. and Kawamura, H. (1984). Effects of bilateral suprachiasmatic nucleus lesions on the circadian rhythms in a diurnal rodent, the Siberian chipmunk (*Eutamias sibiricus*). *Journal of Comparative Physiology A* 155: 745–752.
46. Gerkema, M. P., Groos, G. A., and Daan, S. (1990). Differential elimination of circadian and ultradian rhythmicity by hypothalamic lesions in the common vole, *Microtus arvalis*. *Journal of Biological Rhythms* 5: 81–95.
47. Zucker, I., Boshes, M., and Dark, J. (1983). Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. *American Journal of Physiology* 244: R472–R480.
48. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
49. Del Rosso, L. M., Hoque, R., James, S., Gonzalez-Toledo, E., and Chesson, A. L. (2014). Sleep-wake pattern following gunshot suprachiasmatic damage. *Journal of Clinical Sleep Medicine* 10: 443–445.
50. Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.
51. Osborne, A. R. and Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.
52. Ruby, N. F., Ibuka, N., Barnes, B. M., and Zucker, I. (1989). Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *American Journal of Physiology* 257: R210–R215.
53. Eastman, C. I., Mistlberger, R. E., and Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology and Behavior* 32: 357–368.
54. Liu, S., Chen, X. M., Yoda, T. Nagashima, K., Fukuda, Y., and Kanosue, K. (2002). Involvement of the suprachiasmatic nucleus in body temperature modulation by food deprivation in rats. *Brain Research* 929: 26–36.
55. Jansen, H. T., Sergeeva, A., Stark, G., and Sorg, B. A. (2012). Circadian discrimination of reward: Evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiology International* 29: 454–468.
56. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
57. Dunn, J. D., Castro, A. J., and McNulty, J. A. (1977). Effect of suprachiasmatic ablation on the daily temperature rhythm. *Neuroscience Letters* 6: 345–348.
58. Powell, E. W., Halberg, F., Pasley, J. N., Lubanovic, W., Ernsberger, P., and Scheving, L. E. (1980). Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *Journal of Thermal Biology* 5: 189–196.
59. Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N., and Scheving, L. E. (1979). Nomifensine chronopharmacology, schedule-shifts and circadian temperature rhythms in di-suprachiasmatically lesioned rats: Modeling emotional chronopathology and chronotherapy. *Chronobiologia* 6: 405–424.
60. Fuller, C. A., Lydic, R., Sulzman, F. M., Albers, H. E., Tepper, B., and Moore-Ede, M. C. (1981). Circadian rhythm of body temperature persists after suprachiasmatic lesions in the squirrel monkey. *American Journal of Physiology* 241: R385–R391.

61. Aalto, J. and Kiianmaa, K. (1984). Circadian rhythms of water and alcohol intake: Effect of REM-sleep deprivation and lesion of the suprachiasmatic nucleus. *Alcohol* 1: 403–407.
62. Weaver, D. R. and Reppert, S. M. (1989). Periodic feeding of SCN-lesioned pregnant rats entrains the fetal biological clock. *Developmental Brain Research* 46: 291–296.
63. Deboer, T., Overeem, S., Visser, N. A. H., Duindam, H., Frölich, M., Lammers, G. L., and Meijer, J. H. (2004). Convergence of circadian and sleep regulatory mechanisms on hypocretin-1. *Neuroscience* 129: 727–732.
64. LeSauter, J. and Silver, R. (1994). Suprachiasmatic nucleus lesions abolish and fetal grafts restore circadian gnawing rhythms in hamsters. *Restorative Neurology and Neuroscience* 6: 135–143.
65. Kriegsfeld, L. J., LeSauter, J., and Silver, R. (2004). Targeted microlesions reveal novel organization of the hamster suprachiasmatic nucleus. *Journal of Neuroscience* 24: 2449–2457.
66. Liu, J. H. K. and Shieh, B. E. (1995). Suprachiasmatic nucleus in the neural circuitry for the circadian elevation of intraocular pressure in rabbits. *Journal of Ocular Pharmacology and Therapeutics* 11: 379–388.
67. Mistlberger, R. E., Bergmann, B. M., Waldenar, W., and Rechtschaffen, A. (1983). Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep* 6: 217–233.
68. Trachsel, L., Edgar, D. M., Seidel, W. F., Heller, H. C., and Dement, W. C. (1992). Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: Effects of sleep deprivation and triazolam administration. *Brain Research* 589: 253–261.
69. Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J., and Buijs, R. M. (2000). Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology* 141: 3832–3841.
70. Palm, I. F., van der Beek, E. M., Swarts, H. J. M., van der Vliet, J., Wiegant, V. M., Buijs, R. M., and Kalsbeek, A. (2001). Control of the estradiol-induced prolactin surge by the suprachiasmatic nucleus. *Endocrinology* 142: 2296–2302.
71. Kalsbeek, A., Fliers, E., Romijn, J. A., la Fleur, S. E., Wortel, J., Bakker, O., Endert, E., and Buijs, R. M. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685.
72. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N., and Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
73. La Fleur, S. E., Kalsbeek, A., Wortel, J., and Buijs, R. M. (1999). A suprachiasmatic nucleus generated rhythm in basal glucose concentrations. *Journal of Neuroendocrinology* 11: 643–652.
74. Ruiter, M., La Fleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A., and Buijs, R. M. (2003). The daily rhythm in plasma glucagon concentrations in the rat is modulated by the biological clock and by feeding behavior. *Diabetes* 52: 1709–1715.
75. Hastings, M. H., Roberts, A. C., and Herbert, J. (1985). Neurotoxic lesions of the anterior hypothalamus disrupt the photoperiodic but not the circadian system of the Syrian hamster. *Neuroendocrinology* 40: 316–324.
76. Mistlberger, R. E. and Rusak, B. (1988). Food-anticipatory circadian rhythms in rats with paraventricular and lateral hypothalamic ablations. *Journal of Biological Rhythms* 3: 277–291.
77. Harrington, M. E., Eskes, G. A., Dickson, P., and Rusak, B. (1990). Lesions dorsal to the suprachiasmatic nuclei abolish split activity rhythms of hamsters. *Brain Research Bulletin* 24: 593–597.
78. Pieper, D. R. and Lobocki, C. A. (1991). Olfactory bulbectomy lengthens circadian period of locomotor activity in golden hamsters. *American Journal of Physiology* 261: R973–R978.
79. Goodless-Sanchez, N., Moore, R. Y., and Morin, L. P. (1991). Lateral hypothalamic regulation of circadian rhythm phase. *Physiology and Behavior* 49: 533–537.
80. Ruby, N. F. (1995). Paraventricular nucleus ablation disrupts daily torpor in Siberian Hamsters. *Brain Research Bulletin* 37: 193–198.
81. Marcilhac, A., Maurel, D., Anglade, G., Ixart, G., Mekaouche, M., Héry, F., and Siaud, P. (1997). Effects of bilateral olfactory bulbectomy on circadian rhythms of ACTH, corticosterone, motor activity and body temperature in male rats. *Archives of Physiology and Biochemistry* 105: 552–559.
82. Moore, R. Y. and Danchenko, R. L. (2002). Paraventricular-subparaventricular hypothalamic lesions selectively affect circadian function. *Chronobiology International* 19: 345–360.
83. Shibata, S. and Moore, R. Y. (1987). Development of neuronal activity in the rat suprachiasmatic nucleus. *Brain Research* 431: 311–315.
84. Satinoff, E., Li, H., Tcheng, T. K., Liu, C., McArthur, A. J., Medanic, M., and Gillette, M. U. (1993). Do the suprachiasmatic nuclei oscillate in old rats as they do in young ones? *American Journal of Physiology* 265: R1216–R1222.
85. Shibata, S. and Moore, R. Y. (1994). Calmodulin inhibitors produce phase shifts of circadian rhythms *in vivo* and *in vitro*. *Journal of Biological Rhythms* 9: 27–41.
86. Derambure, P. S. and Boulant, J. A. (1994). Circadian thermosensitive characteristics of suprachiasmatic neurons in vitro. *American Journal of Physiology* 266: R1876–R1884.
87. Meijer, J. H., Schaap, J., Watanabe, K., and Albus, H. (1997). Multiunit activity recordings in the suprachiasmatic nuclei: In vivo versus in vitro models. *Brain Research* 753: 322–327.
88. Prosser, R. A. (1998). In vitro circadian rhythms of the mammalian suprachiasmatic nuclei: Comparison of multi-unit and single-unit neuronal activity recordings. *Journal of Biological Rhythms* 13: 30–38.
89. Chen, D., Buchanan, G. F., Ding, J. M., Hannibal, J., and Gillette, M. U. (1999). Pituitary adenylyl cyclase-activating peptide: A pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 96: 13468–13473.
90. McArthur, A. J., Coogan, A. N., Ajpru, S., Sugden, D., Biello, S. M., and Piggins, H. D. (2000). Gastrin-releasing peptide phase-shifts suprachiasmatic nuclei neuronal rhythms *in vitro*. *Journal of Neuroscience* 20: 5496–5502.
91. Nakamura, W., Honma, S., Shirakawa, T., and Honma, K. (2001). Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *European Journal of Neuroscience* 14: 666–674.
92. Schaap, J., Albus, H., vander Leest, H. T., Eilers, P. H. C., Détári, L., and Meijer, J. H. (2003). Heterogeneity of rhythmic suprachiasmatic nucleus neurons: Implications for circadian waveform and photoperiodic encoding. *Proceedings of the National Academy of Sciences of the United States of America* 100: 15994–15999.

93. Zlomanczuk, P., Margraf, R. R., and Lynch, G. R. (1991). In vitro electrical activity in the suprachiasmatic nucleus following splitting and masking of wheel-running behavior. *Brain Research* 559: 94–99.
94. Shinohara, K., Honma, S., Katsuno, Y., Abe, H., and Honma, K. (1995). Two distinct oscillators in the rat suprachiasmatic nucleus *in vitro*. *Proceedings of the National Academy of Sciences of the United States of America* 92: 7396–7400.
95. Ruby, N. F. and Heller, H. C. (1996). Temperature sensitivity of the suprachiasmatic nucleus of ground squirrels and rats in vitro. *Journal of Biological Rhythms* 11: 126–136.
96. Hall, A. C., Hoffmater, R. M., Stern, E. L., Harrington, M. E., and Bickar, D. (1997). Suprachiasmatic nucleus neurons are glucose sensitive. *Journal of Biological Rhythms* 12: 388–400.
97. Earnest, D. J., Liang, F. Q., Ratcliff, M., and Cassone, V. M. (1999). Immortal time: Circadian clock properties of rat suprachiasmatic cell lines. *Science* 283: 693–695.
98. Abe, M., Herzog, E. D., and Block, G. D. (2000). Lithium lengthens the circadian period of individual suprachiasmatic nucleus neurons. *NeuroReport* 11: 3261–3264.
99. Jagota, A., de la Iglesia, H. O., and Schwartz, W. J. (2000). Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro*. *Nature Neuroscience* 3: 372–376.
100. Mrugala, M., Zlomanczuk, P., Jagota, A., and Schwartz, W. J. (2000). Rhythmic multiunit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *American Journal of Physiology* 278: R987–R994.
101. Allen, G., Rappe, J., Earnest, D. J., and Cassone, V. M. (2001). Oscillating on borrowed time: Diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts. *Journal of Neuroscience* 21: 7937–7943.
102. Albus, H., Bonnefont, X., Chaves, I., Yasui, A., Diczy, J., van der Horst, G. T. J., and Meijer, J. H. (2002). Cryptochrome-deficient mice lack circadian electrical activity in the suprachiasmatic nuclei. *Current Biology* 12: 1130–1133.
103. Quintero, J. E., Kuhlman, S. J., and McMahon, D. G. (2003). The biological clock nucleus: A multiphasic oscillator network regulated by light. *Journal of Neuroscience* 23: 8070–8076.
104. Herzog, E. D. and Huckfeldt, R. M. (2003). Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 763–770.
105. Sugiyama, T., Yoshioka, T., and Ikeda, M. (2004). mPer2 antisense oligonucleotides inhibit mPER2 expression but not circadian rhythms of physiological activity in cultured suprachiasmatic nucleus neurons. *Biochemical and Biophysical Research Communications* 323: 479–483.
106. Aton, S. J., Block, G. D., Tei, H., Yamazaki, S., and Herzog, E. D. (2004). Plasticity of circadian behavior and the suprachiasmatic nucleus following exposure to non-24-hour light cycles. *Journal of Biological Rhythms* 19: 198–207.
107. Burgoon, P. W., Lindberg, P. T., and Gillette, M. U. (2004). Different patterns of circadian oscillation in the suprachiasmatic nucleus of hamster, mouse, and rat. *Journal of Comparative Physiology A* 190: 167–171.
108. Ohta, H., Yamazaki, S., and McMahon, D. G. (2005). Constant light desynchronizes mammalian clock neurons. *Nature Neuroscience* 8: 267–269.
109. Aton, S. J., Colwell, C. S., Harmar, A. J., Waschek, J., and Herzog, E. D. (2005). Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nature Neuroscience* 8: 476–483.
110. Meredith, A. L., Wiler, S. W., Miller, B. H., Takahashi, J. S., Fodor, A. A., Ruby, N. F., and Aldrich, R. W. (2006). BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nature Neuroscience* 9: 1041–1049.
111. Meng, Q. J., Logunova, L., Maywood, E. S., Gallego, M., Yoo, S. H., Takahashi, J. S., Virshup, D. M., Boot-Handford, R. P., Hastings, M. H., and Loudon, A. S. I. (2008). Setting clock speed in mammals: The CK1ε *tau* mutation in mice accelerates circadian pacemakers by selectively destabilizing PERIOD proteins. *Neuron* 58: 78–88.
112. Van der Leest, H. T., Rohling, J. H. T., Michel, S., and Meijer, J. H. (2009). Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLOS ONE* 4: e4976.
113. Tahara, Y., Hirao, A., Moriya, T., Kudo, T., and Shibata, S. (2010). Effects of medial hypothalamic lesions on feeding-induced entrainment of locomotor activity and liver *per2* expression in *per2*::luc mice. *Journal of Biological Rhythms* 25: 9–18.
114. Wolff, G., Duncan, M. J., and Esser, K. A. (2013). Chronic phase advance alters circadian physiological rhythms and peripheral molecular clocks. *Journal of Applied Physiology* 115: 373–382.
115. Kuljis, D. A., Loh, D. H., Truong, D., Vosko, A. M., Ong, M. L., McClusky, R., Arnold, A. P., and Colwell, C. S. (2013). Gonadal- and sex-chromosome-dependent sex differences in the circadian system. *Endocrinology* 154: 1501–1512.
116. Husse, J., Leviavski, A., Tsang, A. H., Oster, H., and Eichele, G. (2014). The light-dark cycle controls peripheral rhythmicity in mice with a genetically ablated suprachiasmatic nucleus clock. *FASEB Journal* 28: 4950–4960.
117. Inouye, S. T. (1982). Restricted daily feeding does not entrain circadian rhythms of the suprachiasmatic nucleus in the rat. *Brain Research* 232: 194–199.
118. Kurumiya, S. and Kawamura, H. (1988). Circadian oscillation of the multiple unit activity in the guinea pig suprachiasmatic nucleus. *Journal of Comparative Physiology A* 162: 301–308.
119. Meijer, J. H., Watanabe, K., Détári, L., and Schaap, J. (1996). Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats. *Brain Research* 741: 352–355.
120. Meijer, J. H., Watanabe, K., Schaap, J., Albus, H., and Détári, L. (1998). Light responsiveness of the suprachiasmatic nucleus: Long-term multiunit and single-unit recordings in freely moving rats. *Journal of Neuroscience* 18: 9078–9087.
121. Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D., and Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus *in vivo*. *Journal of Neuroscience* 18: 10709–10723.
122. Deboer, T., Vansteensel, M. J., Détári, L., and Meijer, J. H. (2003). Sleep states alter activity of suprachiasmatic nucleus neurons. *Nature Neuroscience* 6: 1086–1090.
123. Miyamoto, H., Nakamura-Ogiso, E., Hamada, K., and Hensch, T. K. (2012). Serotonergic integration of circadian clock and ultradian sleep-wake cycles. *Journal of Neuroscience* 32: 14794–14803.
124. Antle, M. C., van Diepen, H. C., Deboer, T., Pedram, P., Pereira, R R., and Meijer, J. H. (2012). Methylphenidate modifies the motion of the circadian clock. *Neuropsychopharmacology* 37: 2446–2455.

125. Takasu, N. N., Pendergast, J. S., Olivas, C. S., Yamazaki, S., and Nakamura, W. (2013). *In vivo* monitoring of multi-unit neural activity in the suprachiasmatic nucleus reveals robust circadian rhythms in *Period1*$^{-/-}$ mice. *PLOS ONE* 8: e64333.
126. Coomans, C. P., van den Berg, S. A. A., Houben, T., van Klinken, J. B., van den Berg, R., Pronk, A. C. M., Havekes, L. M. et al. (2013). Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. *FASEB Journal* 27: 1721–1732.
127. Schwartz, W. J., Davidsen, L. C., and Smith, C. B. (1980). *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *Journal of Comparative Neurology* 189: 157–167.
128. Schwartz, W. J., Reppert, S. M., Eagan, S. M., and Moore-Ede, M. C. (1983). In vivo metabolic activity of the suprachiasmatic nuclei: A comparative study. *Brain Research* 274: 184–187.
129. McEachron, D. L., Kripke, D. F., Sharp, F. R., Lewy, A. J., and McClellan, D. E. (1985). Lithium effects on selected circadian rhythms in rats. *Brain Research Bulletin* 15: 347–350.
130. Room, P., Tielemans, A. J. P. C., De Boer, T., Tonnaer, J. A. D. M., Wester, J., Van den Broek, J. H. M., and Van Delft, A. M. L. (1989). Local cerebral glucose uptake in anatomically defined structures of freely moving rats. *Journal of Neuroscience Methods* 27: 191–202.
131. Room, P. and Tielemans, A. J. P. C. (1989). Circadian variations in local cerebral glucose utilization in freely moving rats. *Brain Research* 505: 321–325.
132. Servière, J., Gendrot, G., LeSauter, J., and Silver, R. (1994). Host resets phase of grafted suprachiasmatic nucleus: A 2-DG study of time course of entrainment. *Brain Research* 655: 168–176.
133. Sumová, A., Trávnícková, Z., Mikkelsen, J. D., and Illnerová, H. (1998). Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research* 801: 254–258.
134. Guido, M. E., Guido, L. B. de, Goguen, D., Robertson, H. A., and Rusak, B. (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *Journal of Biological Rhythms* 14: 275–280.
135. Nunez, A. A., Bult, A., McElhinny, T. L., and Smale, L. (1999). Daily rhythms of Fos expression in hypothalamic targets of the suprachiasmatic nucleus in diurnal and nocturnal rodents. *Journal of Biological Rhythms* 14: 300–306.
136. Sumová, A., Trávnícková, Z., and Illnerová, H. (2000). Spontaneous c-Fos rhythm in the rat suprachiasmatic nucleus: Location and effect of photoperiod. *American Journal of Physiology* 279: R2262–R2269.
137. Schwartz, M. D., Nunez, A. A., and Smale, L. (2004). Differences in the suprachiasmatic nucleus and lower subparaventricular zone of diurnal and nocturnal rodents. *Neuroscience* 127: 13–23.
138. Mendoza, J., Angeles-Castellanos, M., and Escobar, C. (2005). A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *European Journal of Neuroscience* 22: 2855–2862.
139. Romero, M. T., Lehman, M. N., and Silver, R. (1993). Age of donor influences ability of suprachiasmatic nucleus grafts to restore circadian rhythmicity. *Developmental Brain Research* 71: 45–52.
140. Viswanathan, N. and Davis, F. C. (1995). Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. *Brain Research* 686: 10–16.
141. Grosse, J. and Davis, F. C. (1998). Melatonin entrains the restored circadian activity rhythms of Syrian hamsters bearing fetal suprachiasmatic nucleus grafts. *Journal of Neuroscience* 18: 8032–8037.
142. Boer, G. J., van Esseveldt, K. E., van der Geest, B. A. M., Duindam, H., and Rietveld, W. J. (1999). Vasopressin-deficient suprachiasmatic nucleus grafts re-instate circadian rhythmicity in suprachiasmatic nucleus lesioned arrhythmic rats. *Neuroscience* 89: 375–385.
143. Zlomanczuk, P., Mrugala, M., de la Iglesia, H. O., Ourednik, V., Quesenberry, P. J., Snyder, E. Y., and Schwartz, W. J. (2002). Transplanted clonal neural stem-like cells respond to remote photic stimulation following incorporation within the suprachiasmatic nucleus. *Experimental Neurology* 174: 162–168.
144. Tousson, E. and Meissl, H. (2004). Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus. *Journal of Neuroscience* 24: 2983–2988.
145. Saitoh, Y., Matsui, Y., Nihonmatsu, I., and Kawamura, H. (1991). Cross-species transplantation of the suprachiasmatic nuclei from rats to Siberian chipmunks (*Eutamias sibiricus*) with suprachiasmatic lesions. *Neuroscience Letters* 123: 77–81.
146. Sollars, P. J., Kimble, D. P., and Pickard, G. E. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *Journal of Neuroscience* 15: 2109–2122.
147. Lehman. M. N., LeSauter, J., and Silver, R. (1998). Fiber outgrowth from anterior hypothalamic and cortical xenografts in the third ventricle. *Journal of Comparative Neurology* 391: 133–145.
148. Kaufman, C. M. and Menaker, M. (1993). Effect of transplanting suprachiasmatic nuclei from donors of different ages into completely SCN lesioned hamsters. *Journal of Neural Transplantation and Plasticity* 4: 257–265.
149. LeSauter, J. and Silver, R. (1999). Localization of a suprachiasmatic nucleus subregion regulating locomotor rhythmicity. *Journal of Neuroscience* 19: 5574–5585.
150. Vogelbaum, M. A. and Menaker, M. (1992). Temporal chimeras produced by hypothalamic transplants. *Journal of Neuroscience* 12: 3619–3627.
151. Vogelbaum, M. A., Galef, J., and Menaker, M. (1993). Factors determining the restoration of circadian behavior by hypothalamic transplants. *Journal of Neural Transplantation and Plasticity* 4: 239–256.
152. Low-Zeddies, S. S. and Takahashi, J. S. (2001). Chimera analysis of the *Clock* mutation in mice shows that complex cellular integration determines circadian behavior. *Cell* 105: 25–42.
153. Van den Pol, A. N. (1980). The hypothalamic suprachiasmatic nucleus of rat: Intrinsic anatomy. *Journal of Comparative Neurology* 191: 661–702.
154. Okamura, H., Tominaga, K., Ban, Y., Tanaka, M., Tamada, Y., Inouye, S. T., and Ibata, Y. (1994). Morphological survey of the suprachiasmatic nucleus in slice culture using roller tube method: 1. Architecture of the suprachiasmatic nucleus *in vitro*. *Acta Histochemica et Cytochemica* 27: 159–169.
155. Lahmam, M., El M'rabet, A., Ouarour, A., Pévet, P., Challet, E., and Vuillez, P. (2008). Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent *Lemniscomys barbarus*. *Chronobiology International* 25: 882–904.

156. Yan, L., Foley, N. C., Bobula, J. M., Kriegsfeld, L. J., and Silver, R. (2005). Two antiphase oscillations occur in each suprachiasmatic nucleus of behaviorally split hamsters. *Journal of Neuroscience* 25: 9017–9026.
157. Leak, R. K., Card, J. P., and Moore, R. Y. (1999). Suprachiasmatic pacemaker organization analyzed by viral transynaptic transport. *Brain Research* 819: 23–32.
158. Colwell, C. S. (2000). Rhythmic coupling among cells in the suprachiasmatic nucleus. *Journal of Neurobiology* 43: 379–388.
159. Jobst, E. E., Robinson, D. W., and Allen, C. N. (2004). Potential pathways for intercellular communication within the calbindin subnucleus of the hamster suprachiasmatic nucleus. *Neuroscience* 123: 87–99.
160. Long, M. A., Jutras, M. J., Connors, B. W., and Burnwell, R. D. (2005). Electrical synapses coordinate activity in the suprachiasmatic nucleus. *Nature Neuroscience* 8: 61–66.
161. Burgoon, P. W. and Boulant, J. A. (2001). Temperature-sensitive properties of rat suprachiasmatic nucleus neurons. *American Journal of Physiology* 281: R706–R715.
162. Pennartz, C. M. A., de Jeu, M. T. G., Bos, N. P. A., Schaap, J., and Geurtsen, A. M. S. (2002). Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. *Nature* 416: 286–290.
163. Saeb-Parsy, K. and Dyball, R. E. J. (2003). Defined cell groups in the rat suprachiasmatic nucleus have different day/night rhythms of single-unit activity in vivo. *Journal of Biological Rhythms* 18: 26–42.
164. Kuhlman, S. J. and McMahon, D. G. (2004). Rhythmic regulation of membrane potential and potassium current persists in SCN neurons in the absence of environmental input. *European Journal of Neuroscience* 20: 1113–1117.
165. Jackson, A. C., Yao, G. L., and Bean, B. P. (2004). Mechanism of spontaneous firing in dorsomedial suprachiasmatic nucleus neurons. *Journal of Neuroscience* 24: 7985–7998.
166. Kononenko, N. I. and Dudek, F. E. (2004). Mechanism of irregular firing of suprachiasmatic nucleus neurons in rat hypothalamic slices. *Journal of Neurophysiology* 91: 267–273.
167. Dowse, H. B. and Ringo, J. M. (1987). Further evidence that the circadian clock in *Drosophila* is a population of coupled ultradian oscillators. *Journal of Biological Rhythms* 2: 65–76.
168. Miller, J. D. and Fuller, C. A. (1992). Isoperiodic neuronal activity in suprachiasmatic nucleus of the rat. *American Journal of Physiology* 263: R51–R58.
169. Barrio, R. A., Zhang, L., and Maini, P. K. (1997). Hierarchically coupled ultradian oscillators generating robust circadian rhythms. *Bulletin of Mathematical Biology* 59: 517–532.
170. Welsh, D. K., Logothetis, D. E., Meister, M., and Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14: 697–706.
171. Liu, C., Weaver, D. R., Strogatz, S. H., and Reppert, S. M. (1997). Cellular construction of a circadian clock: Period determination in the suprachiasmatic nuclei. *Cell* 91: 855–860.
172. Honma, S., Shirakawa, T., Katsuno, Y., Namihira, M., and Honma, K. (1998). Circadian periods of single suprachiasmatic neurons in rats. *Neuroscience Letters* 250: 157–160.
173. Honma, S., Nakamura, W., Shirakawa, T., and Honma, K. (2004). Diversity in the circadian periods of single neurons of the rat suprachiasmatic nucleus depends on nuclear structure and intrinsic period. *Neuroscience Letters* 358: 173–176.
174. Herzog, E. D., Aton, S. J., Numano, R., Sakaki, Y., and Tei, H. (2004). Temporal precision in the mammalian circadian system: A reliable clock from less reliable neurons. *Journal of Biological Rhythms* 19: 35–46.
175. Díez-Noguera, A. (1994). A functional model of the circadian system based on the degree of intercommunication in a complex system. *American Journal of Physiology* 267: R1118–R1135.
176. Bouskila, Y. and Dudek, F. E. (1995). Can a population of suprachiasmatic nucleus neurons with different period lengths produce a stable circadian rhythm? *Brain Research* 670: 333–336.
177. Kunz, H. and Achermann, P. (2003). Simulation of circadian rhythm generation in the suprachiasmatic nucleus with locally coupled self-sustained oscillators. *Journal of Theoretical Biology* 224: 63–78.
178. Antle, M. C., Foley, D. K., Foley, N. C., and Silver, R. (2003). Gates and oscillators: A network model of the brain clock. *Journal of Biological Rhythms* 18: 339–350.
179. Shirakawa, T., Honma, S., Katsuno, Y., Oguchi, H., and Honma, K. (2000). Synchronization of circadian firing rhythms in cultured rat suprachiasmatic neurons. *European Journal of Neuroscience* 12: 2833–2838.
180. Honma, S., Shirakawa, T., Nakamura, W., and Honma, K. (2000). Synaptic communication of cellular oscillations in the rat suprachiasmatic neurons. *Neuroscience Letters* 294: 113–116.
181. Yamaguchi, S., Isejima, H., Matsuo, T., Okura, R., Yagita, K., Kobayashi, M., and Okamura, H. (2003). Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302: 1408–1412.
182. Prosser, R. A., Edgar, D. M., Heller, H. C., and Miller, J. D. (1994). A possible glial role in the mammalian circadian clock. *Brain Research* 643: 296–301.
183. Bennett, M. R. and Schwartz, W. J. (1994). Are glia among the cells that express immunoreactive c-Fos in the suprachiasmatic nucleus? *NeuroReport* 5: 1737–1740.
184. Van den Pol, A. N. (1986). Gamma-aminobutyrate, gastrin releasing peptide, serotonin, somatostatin, and vasopressin: Ultrastructural immunocytochemical localization in presynaptic axons in the suprachiasmatic nucleus. *Neuroscience* 17: 643–659.
185. Moore, R. Y. and Speh, J. C. (1993). GABA is the principal neurotransmitter of the circadian system. *Neuroscience Letters* 150: 112–116.
186. Morin, L. P. and Blanchard, J. H. (2001). Neuromodulator content of hamster intergeniculate leaflet neurons and their projection to the suprachiasmatic nucleus or visual midbrain. *Journal of Comparative Neurology* 437: 79–90.
187. Belenky, M. A. and Pickard, G. E. (2001). Subcellular distribution of 5-HT_{1B} and 5-HT_7 receptors in the mouse suprachiasmatic nucleus. *Journal of Comparative Neurology* 432: 371–388.
188. Liou, S. Y., Shibata, S., Albers, H. E., and Ueki, S. (1990). Effects of GABA and anxiolytics on the single unit discharge of suprachiasmatic neurons in rat hypothalamic slices. *Brain Research Bulletin* 25: 103–107.
189. Wagner, S., Castel, M., Gainer, H., and Yarom, Y. (1997). GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature* 387: 598–603.
190. Liu, C. and Reppert, S. M. (2000). GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron* 25: 123–128.

191. Belenky, M. A., Sagiv, N., Fritschy, J. M., and Yarom, Y. (2003). Presynaptic and postsynaptic $GABA_A$ receptors in rat suprachiasmatic nucleus. *Neuroscience* 118: 909–923.
192. Biggs, K. R. and Prosser, R. A. (1998). $GABA_B$ receptor stimulation phase-shifts the mammalian circadian clock in vitro. *Brain Research* 807: 250–254.
193. Tominaga, K., Shibata, S., Hamada, T., and Watanabe, S. (1994). $GABA_A$ receptor agonist muscimol can reset the phase of neural activity rhythm in the rat suprachiasmatic nucleus in vitro. *Neuroscience Letters* 166: 81–84.
194. Novak, C. M. and Albers, H. E. (2004). Novel phase-shifting effects of $GABA_A$ receptor activation in the suprachiasmatic nucleus of a diurnal rodent. *American Journal of Physiology* 286: R820–R825.
195. Novak, C. M. and Albers, H. E. (2004). Circadian phase alteration by GABA and light differs in diurnal and nocturnal rodents during the day. *Behavioral Neuroscience* 118: 498–504.
196. Strecker, G. J., Wuarin, J. P., and Dudek, F. E. (1997). $GABA_A$-mediated local synaptic pathways connect neurons in the rat suprachiasmatic nucleus. *Journal of Neurophysiology* 78: 2217–2220.
197. Itri, J. and Colwell, C. S. (2003). Regulation of inhibitory synaptic transmission by vasoactive intestinal peptide (VIP) in the mouse suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 1589–1597.
198. Okamura, H., Tominaga, K., Ban, Y., Fukuhara, C., Yanaihara, N., Ibata, Y., and Inouye, S. T. (1994). Morphological survey of the suprachiasmatic nucleus in slice culture using roller tube method: 2. Peptidergic neurons. *Acta Histochemica et Cytochemica* 27: 171–179.
199. Schilling, J. and Nürnberger, F. (1998). Dynamic changes in the immunoreactivity of neuropeptide systems of the suprachiasmatic nuclei in golden hamsters during the sleep-wake cycle. *Cell Tissue Research* 294: 233–241.
200. Smale, L. and Boverhof, J. (1999). The suprachiasmatic nucleus and intergeniculate leaflet of *Arvicanthis niloticus*, a diurnal murid rodent from East Africa. *Journal of Comparative Neurology* 403: 190–208.
201. Van der Zee, E. A., Oklejewicz, M., Jansen, K., Daan, S., and Gerkema, M. P. (2002). Vasopressin immunoreactivity and release in the suprachiasmatic nucleus of wild-type and *tau* mutant Syrian hamsters. *Brain Research* 936: 38–46.
202. Hofman, M. A. (2003). Circadian oscillations of neuropeptide expression in the human biological clock. *Journal of Comparative Physiology A* 189: 823–831.
203. Cohen, R., Kronfeld-Schor, N., Ramanathan, C., Baumgras, A., and Smale, L. (2010). The substructure of the suprachiasmatic nucleus: Similarities between nocturnal and diurnal spiny mice. *Brain, Behavior and Evolution* 75: 9–22.
204. Rocha, V. A., Frazão, R., Campos, L. M. G., Mello, P., Donato, J., Cruz-Rizzolo, R. J., Nogueira, M. I., and Pinato, L. (2014). Intrinsic organization of the suprachiasmatic nucleus in the capuchin monkey. *Brain Research* 1543: 65–72.
205. Murakami, N., Takamure, M., Takahashi, K., Utunomiya, K., Kuroda, H., and Etoh, T. (1991). Long-term cultured neurons from rat suprachiasmatic nucleus retain the capacity for circadian oscillation of vasopressin release. *Brain Research* 545: 347–350.
206. Hofman, M. A. and Swaab, D. F. (1993). Diurnal and seasonal rhythms of neuronal activity in the suprachiasmatic nucleus of humans. *Journal of Biological Rhythms* 8: 283–295.
207. Tominaga, K., Shinohara, K., Otori, Y., Fukuhara, C., and Inouye, S. T. (1992). Circadian rhythms of vasopressin content in the suprachiasmatic nucleus of the rat. *NeuroReport* 3: 809–812.
208. Kalsbeek, A., van Heerikhuize, J. J., Wortel, J., and Buijs, R. M. (1998). Restricted daytime feeding modifies suprachiasmatic nucleus vasopressin release in rats. *Journal of Biological Rhythms* 13: 18–29.
209. Watanabe, K., Vanecek, J., and Yamaoka, S. (2000). In vitro entrainment of the circadian rhythm of vasopressin-releasing cells in suprachiasmatic nucleus by vasoactive intestinal polypeptide. *Brain Research* 877: 361–366.
210. Dardente, H., Menet, J. S., Challet, E., Tournier, B. B., Pévet, P., and Masson-Pévet, M. (2004). Daily and circadian expression of neuropeptides in the suprachiasmatic nuclei of nocturnal and diurnal rodents. *Molecular Brain Research* 124: 143–151.
211. Noguchi, T., Watanabe, K., Ogura, A., and Yamaoka, S. (2004). The clock in the dorsal suprachiasmatic nucleus runs faster than that in the ventral. *European Journal of Neuroscience* 20: 3199–3202.
212. Gerkema, M. P., van der Zee, E. A., and Feistma, L. E. (1994). Expression of circadian rhythmicity correlates with the number of arginine-vasopressin-immunoreactive cells in the suprachiasmatic nucleus of common voles, *Microtus arvalis*. *Brain Research* 639: 93–101.
213. Kaufman, C. M. and Menaker, M. (1994). Ontogeny of light-induced Fos-like immunoreactivity in the hamster suprachiasmatic nucleus. *Brain Research* 633: 162–166.
214. Abizaid, A., Horvath, B., Keefe, D. L., Leranth, C., and Horvath, T. L. (2004). Direct visual and circadian pathways target neuroendocrine cells in primates. *European Journal of Neuroscience* 20: 2767–2776.
215. Reed, H. E., Cutler, D. J., Brown, T. M., Coen, C. W., and Piggins, H. D. (2002). Effects of vasoactive intestinal polypeptide on neurons of the rat suprachiasmatic nuclei *in vitro*. *Journal of Neuroendocrinology* 14: 639–646.
216. Piggins, H. D., Antle, M. C., and Rusak, B. (1995). Neuropeptides phase shift the mammalian circadian pacemaker. *Journal of Neuroscience* 15: 5612–5622.
217. Reed, H. E., Meyer-Spasche, A., Cutler, D. J., Coen, C. W., and Piggins, H. D. (2001). Vasoactive intestinal polypeptide (VIP) phase-shifts the rat suprachiasmatic nucleus clock *in vitro*. *European Journal of Neuroscience* 13: 839–843.
218. Hughes, A. T., Fahey, B., Cutler, D. J., Coogan, A. N., and Piggins, H. D. (2004). Aberrant gating of photic input to the suprachiasmatic circadian pacemaker of mice lacking the $VPAC_2$ receptor. *Journal of Neuroscience* 24: 3522–3526.
219. An, S., Harang, R., Meeker, K., Granados-Fuentes, D., Tsai, C. A., Mazuski, C., Kim, J., Doyle, F. J., Petzold, L. R., and Herzog, E. D. (2013). A neuropeptide speeds circadian entrainment by reducing intercellular synchrony. *Proceedings of the National Academy of Sciences of the United States of America* 110: E4355–E4361.
220. Tanaka, M., Hayashi, S., Tamada, Y., Ikeda, T., Hisa, Y., Takamatsu, T., and Ibata, Y. (1997). Direct retinal projections to GRP neurons in the suprachiasmatic nucleus of the rat. *NeuroReport* 8: 2187–2191.
221. Gary, K. A., Sollars, P. J., Lexow, N., Winokur, A., and Pickard, G. E. (1996). Thyrotropin-releasing hormone phase shifts circadian rhythms in hamsters. *NeuroReport* 7: 1631–1634.
222. Thomas, M. A., Fleissner, G., Stöhr, M., Hauptfleisch, S., and Lemmer, B. (2004). Localization of components of the renin-angiotensin system in the suprachiasmatic nucleus of normotensive Sprague-Dawley rats. Part A. Angiotensin I/II, a light and electron microscopic study. *Brain Research* 1008: 212–223.

223. Mitome, M., Shirakawa, T., Oshima, S., Nakamura, W., and Oguchi, H. (2001). Circadian rhythm of nitric oxide production in the dorsal region of the suprachiasmatic nucleus in rats. *Neuroscience Letters* 303: 161–164.
224. Nakahara, K., Hanada, R., Murakami, N., Teranishi, H., Ohgusu, H., Fukushima, N., Moriyama, M., Ida, T., Kangawa, K., and Kojima, M. (2004). The gut-brain peptide neuromedin U is involved in the mammalian circadian oscillator system. *Biochemical and Biophysical Research Communications* 318: 156–161.
225. Crichton, M. (1990). *Jurassic Park*. New York: Knopf.
226. Cherfas, J. (1991). Ancient DNA: Still busy after death. *Science* 253: 1354–1356.
227. Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D. A., and Cooper, A. (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300: 791–795.
228. Noonan, J. P., Hofreiter, M., Smith, D., Priest, J. R., Rohland, N., Rabeder, G., Krause, J., Detter, J. C., Pääbo, S., and Rubin, E. M. (2005). Genomic sequencing of Pleistocene cave bears. *Science* 309: 597–600.
229. Noonan, J. P., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Chen, F. et al. (2006). Sequencing and analysis of Neanderthal genomic DNA. *Science* 314: 1113–1118.
230. Krauthammer, C. (1997). A special report on cloning. *Time* 149 (March 10).
231. Doolittle, R. F., Feng, D. F., Tsang, S., Cho, G., and Little, E. (1996). Determining divergence times of the major kingdoms of living organisms with a protein clock. *Science* 271: 470–477.
232. Wray, G. A., Levinton, J. S., and Shapiro, L. H. (1996). Molecular evidence for Deep Precambrian divergences among metazoan phyla. *Science* 274: 568–573.
233. Starr, K. (1998). *The Starr Report: The Official Report of the Independent Counsel's Investigation of the President*. Rocklin, CA: Prima.
234. International Human Genome Sequencing Consortium (2001). Initial sequencing and analysis of the human genome. *Nature* 409: 860–921.
235. Gibson, D. G., Glass, J. I., Lartigue, C., Noskov, V. N., Chuang, R. Y., Algire, M. A., Benders, G. A. et al. (2010). Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 329: 52–56.
236. Venter, J. C. (2011). Synthesizing life: Designing genomes from scratch will be the next revolution in biology. *Scientist* 25(10): 60.
237. Ayer, A. J. (1959). *Logical Positivism*. Chicago, IL: Free Press.
238. Carnap, R. (1966). *Philosophical Foundations of Physics*. New York: Basic Books.
239. Quine, W. V. (1995). *From Stimulus to Science*. Cambridge, MA: Harvard University Press.
240. Crick, F. (1995). *The Astonishing Hypothesis: The Scientific Search for the Soul*. New York: Simon & Schuster.
241. Metzinger, T. (2003). *Being No One: The Self-Model Theory of Subjectivity*. Cambridge, MA: MIT Press.
242. Koch, C. (2004). *The Quest for Consciousness: A Neurobiological Approach*. Englewood, CO: Roberts & Company.
243. Konopka, R. J. and Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* 68: 2112–2116.
244. Hall, J. C. (1990). Genetics of circadian rhythms. *Annual Review of Genetics* 24: 659–697.
245. Plautz, J. D., Kaneko, M., Hall, J. C., and Kay, S. A. (1997). Independent photoreceptive circadian clocks throughout *Drosophila*. *Science* 278: 1632–1635.
246. Cheng, Y., Gvakharia, B., and Hardin, P. E. (1998). Two alternatively spliced transcripts from the *Drosophila period* gene rescue rhythms having different molecular and behavioral characteristics. *Molecular and Cellular Biology* 18: 6505–6514.
247. Ewer, J., Frisch, B., Hamblen-Coyle, M. J., Rosbash, M., and Hall, J. C. (1992). Expression of the *period* clock gene within different cell types in the brain of *Drosophila* adults and mosaic analysis of these cells' influence on circadian behavioral rhythms. *Journal of Neuroscience* 12: 3321–3349.
248. Helfrich-Förster, C. (2004). The circadian clock in the brain: A structural and functional comparison between mammals and insects. *Journal of Comparative Physiology A* 190: 601–613.
249. Peng, Y., Stoleru, D., Levine, J. D., Hall, J. C., and Rosbash, M. (2003). *Drosophila* free-running rhythms require intercellular communication. *PLoS Biology* 1: 32–40.
250. Hardin, P. E., Hall, J. C., and Rosbash, M. (1992). Circadian oscillations in period gene mRNA levels are transcriptionally regulated. *Proceedings of the National Academy of Sciences of the United States of America* 89: 11711–11715.
251. Edery, I., Rutila, J. E., and Rosbash, M. (1994). Phase shifting of the circadian clock by induction of the *Drosophila period* protein. *Science* 263: 237–240.
252. Sehgal, A., Price, J. L., Man, B., and Young, M. W. (1994). Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. *Science* 263: 1603–1606.
253. Sehgal, A., Rothenfluh-Hilfiker, A., Hunter-Ensor, M., Chen, Y., Myers, M. P., and Young, M. W. (1995). Rhythmic expression of *timeless*: A basis for promoting circadian cycles in *period* gene autoregulation. *Science* 270: 808–810.
254. Gekakis, N., Saez, L., Delahaye-Brown, A. M., Myers, M. P., Sehgal, A., Young, M. W., and Weitz, C. J. (1995). Isolation of *timeless* by PER protein interaction: Defective interaction between *timeless* protein and long-period mutant PER. *Science* 270: 811–815.
255. Ralph, M. R. and Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
256. Vitaterna, M. H., King, D. P., Chang, A. M., Kornhauser, J. M., Lowrey, P. L., McDonald, J. D., Dove, W. F., Pinto, L. H., Turek, F. W., and Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264: 719–725.
257. Tei, H., Okamura, H., Shigeyoshi, Y., Fukuhara, C., Ozawa, R., Hirose, M., and Sakaki, Y. (1997). Circadian oscillation of a mammalian homologue of the *Drosophila period* gene. *Nature* 389: 512–516.
258. Shearman, L. P., Zylka, M. J., Weaver, D. R., Kolakowski, L. F., and Reppert, S. M. (1997). Two *period* homologs: Circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron* 19: 1261–1269.
259. Takumi, T., Taguchi, K., Miyake, S., Sakakida, Y., Takashima, N., Matsubara, C., Maebayashi, Y. et al. (1998). A light-independent oscillatory gene *mPer3* in mouse SCN and OVLT. *EMBO Journal* 17: 4753–4759.
260. King, D. P., Zhao, Y., Sangoram, A. M., Wilsbacher, L. D., Tanaka, M., Antoch, M. P., Steeves, T. D. L. et al. (1997). Positional cloning of the mouse circadian *Clock* gene. *Cell* 89: 641–653.

261. Antoch, M. P., Song, E. J., Chang, A. M., Vitaterna, M. H., Zhao, Y., Wilsbacher, L. D., Sangoram, A. M., King, D. P., Pinto, L. H., and Takahashi, J. S. (1997). Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. *Cell* 89: 655–667.
262. Gekakis, N., Staknis, D., Nguyen, H. B., Davis, F. C., Wilsbacher, L. D., King, D. P., Takahashi, J. S., and Weitz, C. J. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. *Science* 280: 1564–1569.
263. Koike, N., Hida, A., Numano, R., Hiose, M., Sakaki, Y., and Tei, H. (1998). Identification of the mammalian homologues of the *Drosophila timeless* gene, *Timeless1*. *FEBS Letters* 441: 427–431.
264. Zylka, M. J., Shearman, L. P., Levine, J. D., Jin, X., Weaver, D. R., and Reppert, S. M. (1998). Molecular analysis of mammalian *Timeless*. *Neuron* 21: 1115–1122.
265. Bae, K., Lee, C., Sidote, D., Chuang, K., and Edery, I. (1998). Circadian regulation of a *Drosophila* homolog of the mammalian *Clock* gene: PER and TIM function as positive regulators. *Molecular and Cellular Biology* 18: 6142–6151.
266. Lee, C., Bae, K., and Edery, I. (1998). The *Drosophila* CLOCK protein undergoes daily rhythms in abundance, phosphorylation, and interactions with the PER-TIM complex. *Neuron* 21: 857–867.
267. Glossop, N. R. J., Lyons, L. C., and Hardin, P. E. (1999). Interlocked feedback loops within the *Drosophila* circadian oscillator. *Science* 286: 766–768.
268. Bae, K., Lee, C., Hardin, P. E., and Edery, I. (2000). dCLOCK is present in limiting amounts and likely mediates daily interactions between the dCLOCK-CYC transcription factor and the PER-TIM complex. *Journal of Neuroscience* 20: 1746–1753.
269. Renn, S. C. P., Park, J. H., Rosbash, M., Hall, J. C., and Taghert, P. H. (1999). A pdf neuropeptide gene mutation and ablation of PDF neurons each cause severe abnormalities of behavioral circadian rhythms in *Drosophila*. *Cell* 99: 791–802.
270. Akten, B., Jauch, E., Genova, G. K., Kim, E. Y., Edery, I., Raabe, T., and Jackson, F. R. (2003). A role for CK2 in the *Drosophila* circadian oscillator. *Nature Neuroscience* 6: 251–257.
271. Cyran, S. A., Buchsbaum, A. M., Reddy, K. L., Lin, M. C., Glossop, N. R. J., Hardin, P. E., Young, M. W., Storti, R. V., and Blau, J. (2003). *Vrille*, *Pdp1*, and *dClock* form a second feedback loop in the *Drosophila* circadian clock. *Cell* 112: 329–341.
272. Preuss, F., Fan, J. Y., Kalive, M., Bao, S., Schuenemann, E., Bjes, E. S., and Price, J. L. (2004). *Drosophila doubletime* mutations which either shorten or lengthen the period of circadian rhythms decrease the protein kinase activity of casein kinase I. *Molecular and Cellular Biology* 24: 886–898.
273. Nawathean, P. and Rosbash, M. (2004). The Doubletime and CKII kinases collaborate to potentiate Drosophila PER transcriptional repressor activity. *Molecular Cell* 13: 213–223.
274. Takahashi, J. S. (1992). Circadian clock genes are ticking. *Science* 258: 238–239.
275. DiTacchio, L., Le, H. D., Vollmers, C., Hatori, M., Witcher, M., Secombe, J., and Panda, S. (2011). Histone lysine demethylase JARID1a activates CLOCK-BMAL1 and influences the circadian clock. *Science* 333: 1881–1885.
276. Lim, C. and Allada, R. (2013). ATAXIN-2 activates PERIOD translation to sustain circadian rhythms in *Drosophila*. *Science* 340: 875–879.
277. McDonald, M. J. and Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* 107: 567–578.
278. Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N., and Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32: 657–671.
279. Ceriani, M. F., Hogenesch, J. B., Yanovsky, M., Panda, S., Straume, M., and Kay, S. A. (2002). Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *Journal of Neuroscience* 22: 9305–9319.
280. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N., and Taghert, P. H. (2002). Influence of the *period*-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America* 99: 9562–9567.
281. Keegan, K. P., Pradhan, S., Wang, J. P., and Allada, R. (2007). Meta-analysis of *Drosophila* circadian microarray studies identifies a novel set of rhythmically expressed genes. *PLoS Computational Biology* 3: e208.
282. Marshall, E. (2004). Getting the noise out of gene arrays. *Science* 306: 630–631.
283. Kitayama, Y., Iwasaki, H., Nishiwaki, T., and Kondo, T. (2003). KaiB functions as an attenuator of KaiC phosphorylation in the cyanobacterial circadian clock system. *EMBO Journal* 22: 2127–2134.
284. Nakahira, Y., Katayama, M., Miyashita, H., Kutsuna, S., Iwasaki, H., Oyama, T., and Kondo, T. (2004). Global gene repression by KaiC as a master process of prokaryotic circadian system. *Proceedings of the National Academy of Sciences of the United States of America* 101: 881–885.
285. Schultz, T. F. and Kay, S. A. (2003). Circadian clocks in daily and seasonal control of development. *Science* 301: 326–328.
286. Salomé, P. A. and McClung, C. R. (2004). The *Arabidopsis thaliana* clock. *Journal of Biological Rhythms* 19: 425–435.
287. Park, D. H., Somers, D. E., Kim, Y. S., Choy, Y. H., Lim, H. K., Soh, M. S., Kim, H. J., Kay, S. A., and Nam, H. G. (1999). Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285: 1579–1582.
288. Más, P., Kim, W. Y., Somers, D., and Kay, S. A. (2003). Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. *Nature* 426: 567–570.
289. Hall, A., Bastow, R. M., Davis, S. J., Hanano, S., McWatters, H. G., Hibberd, V., Doyle, M. R. et al. (2003). The *TIME FOR COFFEE* gene maintains the amplitude and timing of Arabidopsis circadian clocks. *Plant Cell* 15: 2719–2729.
290. Dodd, A. N., Gardner, M. J., Hotta, C. T., Hubbard, K. E., Dalchau, N., Love, J., Assie, J. M. et al. (2007). The *Arabidopsis* circadian clock incorporates a cADPR-based feedback loop. *Science* 318: 1789–1792.
291. Pruneda-Paz, J. L., Breton, G., Para, A., and Kay, S. A. (2009). A functional genomics approach reveals CHE as a component of the *Arabidopsis* circadian clock. *Science* 323: 1481–1485.
292. James, A. B., Monreal, J. A., Nimmo, G. A., Kelly, C. L., Herzyk, P., Jenkins, G. I., and Nimmo, H. G. (2008). The circadian clock in *Arabidopsis* roots is a simplified slave version of the clock in shoots. *Science* 322: 1832–1835.
293. Aronson, B. D., Johnson, K. A., Loros, J. L., and Dunlap, J. C. (1994). Negative feedback defining a circadian clock: Autoregulation of the clock gene *frequency*. *Science* 263: 1578–1584.
294. Aronson, B. D., Johnson, K. A., and Dunlap, J. C. (1994). Circadian clock locus frequency: Protein encoded by a single open reading frame defines period length and temperature compensation. *Proceedings of the National Academy of Sciences of the United States of America* 91: 7683–7687.

295. Crosthwaite, S. K., Loros, J. J., and Dunlap, J. C. (1995). Light-induced resetting of a circadian clock is mediated by a rapid increase in *frequency* transcript. *Cell* 81: 1003–1012.
296. Crosthwaite, S. K., Dunlap, J. C., and Loros, J. J. (1997). *Neurospora wc-1* and *wc-2*: Transcription, photoresponses, and the origins of circadian rhythmicity. *Science* 276: 763–769.
297. Refinetti, R. (1999). Amplitude of the daily rhythm of body temperature in eleven mammalian species. *Journal of Thermal Biology* 24: 477–481.
298. Cheng, P., Yang, Y., and Liu, Y. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 98: 7408–7413.
299. Correa, A., Lewis, Z. A., Greene, A. V., March, I. J., Gomer, R. H., and Bell-Pedersen, D. (2003). Multiple oscillators regulate circadian gene expression in *Neurospora*. *Proceedings of the National Academy of Sciences of the United States of America* 100: 13597–13602.
300. Liu, Y., Loros, J., and Dunlap, J. C. (2000). Phosphorylation of the *Neurospora* clock protein FREQUENCY determines its degradation rate and strongly influences the period length of the circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 97: 234–239.
301. Larrondo, L. F., Olivares-Yañez, C., Baker, C. L., Loros, J. J., and Dunlap, J. C. (2015). Decoupling circadian clock protein turnover from circadian period determination. *Science* 347: 518.
302. de Paula, R. M., Lewis, Z. A., Greene, A. V., Seo, K. S., Morgan, L. W., Vitalini, M. W., Bennett, L., Gomer, R. H., and Bell-Pedersen, D. (2006). Two circadian timing circuits in *Neurospora crassa* cells share components and regulate distinct rhythmic processes. *Journal of Biological Rhythms* 21: 159–168.
303. Xue, Z., Ye, Q., Anson, S. R., Yang, J., Xiao, G., Kowbel, D., Glass, N. L., Crosthwaite, S. K., and Liu, Y. (2014). Transcriptional interference by antisense RNA is required for circadian clock function. *Nature* 514: 650–653.
304. Bunger, M. K., Wilsbacher, L. D., Moran, S. M., Clendenin, C., Radcliffe, L. A., Hogenesch, J. B., Simon, M. C., Takahashi, J. S., and Bradfield, C. A. (2000). *Mop3* is an essential component of the master circadian pacemaker in mammals. *Cell* 103: 1009–1017.
305. Barnes, J. W., Tischkau, S. A., Barnes, J. A., Mitchell, J. W., Burgoon, P. W., Hickok, J. R., and Gillette, M. U. (2003). Requirement of mammalian *timeless* for circadian rhythmicity. *Science* 302: 439–442.
306. Hardin, P. E. (2004). Transcription regulation within the circadian clock: The E-box and beyond. *Journal of Biological Rhythms* 19: 348–360.
307. Van der Horst, G. T. J., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J. et al. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* 398: 627–630.
308. Okamura, H., Miyake, S., Sumi, Y., Yamaguchi, S., Yasui, A., Muijtjens, M., Hoeijmakers, J. H. J., and van der Horst, G. T. J. (1999). Photic induction of *mPer1* and *mPer2* in *Cry*-deficient mice lacking a biological clock. *Science* 286: 2531–2534.
309. Shearman, L. P., Sriram, S., Weaver, D. R., Maywood, E. S., Chaves, I., Zheng, B., Kume, K. et al. (2000). Interacting molecular loops in the mammalian circadian clock. *Science* 288: 1013–1019.
310. Reppert, S. M. and Weaver, D. R. (2001). Molecular analysis of mammalian circadian rhythms. *Annual Review of Physiology* 63: 647–676.
311. Miyamoto, Y. and Sancar, A. (1998). Vitamin B_2-based blue-light photoreceptors in the retinohypothalamic tract as the photoactive pigments for setting the circadian clock in mammals. *Proceedings of the National Academy of Sciences of the United States of America* 95: 6097–6102.
312. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S., and Honma, K. (2001). Clock gene expressions in the suprachiasmatic nucleus and other areas of the brain during rhythm splitting in CS mice. *Molecular Brain Research* 87: 92–99.
313. Bae, K., Jin, X., Maywood, E. S., Hastings, M. H., Reppert, S. M., and Weaver, D. R. (2001). Differential functions of *mPer1*, *mPer2*, and *mPer3* in the SCN circadian clock. *Neuron* 30: 525–536.
314. Hara, R., Wan, K., Wakamatsu, H., Aida, R., Moriya, T., Akiyama, M., and Shibata, S. (2001). Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes to Cells* 6: 269–278.
315. Abe, H., Honma, S., Namihira, M., Masubuchi, S., and Honma, K. (2001). Behavioural rhythm splitting in the CS mouse is related to clock gene expression outside the suprachiasmatic nucleus. *European Journal of Neuroscience* 14: 1121–1128.
316. Maywood, E. S., O'Brien, J. A., and Hastings, M. H. (2003). Expression of mCLOCK and other circadian clock-relevant proteins in the mouse suprachiasmatic nuclei. *Journal of Neuroendocrinology* 15: 329–334.
317. Butler, M. P., Honma, S., Fukumoto, T., Kawamoto, T., Fujimoto, K., Noshiro, M., Kato, Y., and Honma, K. (2004). *Dec1* and *Dec2* expression is disrupted in the suprachiasmatic nuclei of *Clock* mutant mice. *Journal of Biological Rhythms* 19: 126–134.
318. Bittman, E. L., Doherty, L., Huang, L., and Paroskie, A. (2003). *Period* gene expression in mouse endocrine tissues. *American Journal of Physiology* 285: R561–R569.
319. Nagano, M., Adachi, A., Nakahama, K., Nakamura, T., Tamada, M., Meyer-Bernstein, E., Sehgal, A., and Shigeyoshi, Y. (2003). An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *Journal of Neuroscience* 23: 6141–6151.
320. Park, K. and Kang, H. M. (2004). Cloning and circadian expression of rat *cry1*. *Molecules and Cells* 18: 256–260.
321. Hanai, S., Masuo, Y., Shirai, H., Oishi, K., Saida, K., and Ishida, N. (2005). Differential circadian expression of endothelin-1 mRNA in the rat suprachiasmatic nucleus and peripheral tissues. *Neuroscience Letters* 377: 65–68.
322. Mendoza, J., Graff, C., Dardente, H., Pévet, P., and Challet, E. (2005). Feeding cues alter clock gene oscillations and photic responses in the suprachiasmatic nuclei of mice exposed to a light-dark cycle. *Journal of Neuroscience* 25: 1514–1522.
323. Fukuhara, C., Aguzzi, J., Bullock, N., and Tosini, G. (2005). Effect of long-term exposure to constant dim light on the circadian system of rats. *Neurosignals* 14: 117–125.
324. LeGates, T. A., Altimus, C. M., Wang, H., Lee, H. K., Yang, S., Zhao, H., Kirkwood, A., Weber, E. T., and Hattar, S. (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature* 491: 594–598.
325. Yamaguchi, Y., Suzuki, T., Mizoro, Y., Kori, H., Okada, K., Chen, Y., Fustin, J. M. et al. (2013). Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. *Science* 342: 85–90.
326. Von Gall, C., Noton, E., Lee, C., and Weaver, D. R. (2003). Light does not degrade the constitutively expressed BMAL1 protein in the mouse suprachiasmatic nucleus. *European Journal of Neuroscience* 18: 125–133.

327. Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U., and Schibler, U. (2002). The orphan nuclear receptor REV-ERBα controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110: 251–260.
328. Yin, L., Wu, N., Curtin, J. C., Qatanani, M., Szwergold, N. R., Reid, R. A., Waitt, G. M. et al. (2007). Rev-erbα, a heme sensor that coordinates metabolic and circadian pathways. *Science* 318: 1786–1789.
329. Cho, H., Zhao, X., Hatori, M., Yu, R. T., Barish, G. D., Lam, M. T., Chong, L. W. et al. (2012). Regulation of circadian behaviour and metabolism by *Rev-erb*α and *Rev-erb*β. *Nature* 485: 123–127.
330. Kondratov, R. V., Chernov, M. V., Kondratova, A. A., Gorbacheva, V. Y., Gudkov, A. V., and Antoch, M. P. (2003). BMAL1-dependent circadian oscillation of nuclear CLOCK: Posttranslational events induced by dimerization of transcriptional activators of the mammalian clock system. *Genes and Development* 17: 1921–1932.
331. DeBruyne, J. P., Noton, E., Lambert, C. M., Maywood, E. S., Weaver, D. R., and Reppert, S. M. (2006). A clock shock: Mouse CLOCK is not required for circadian oscillator function. *Neuron* 50: 465–477.
332. Dallmann, R., DeBruyne, J. P., and Weaver, D. R. (2011). Photic resetting and entrainment in CLOCK-deficient mice. *Journal of Biological Rhythms* 26: 390–401.
333. Fuller, P. M., Lu, J., and Saper, C. B. (2008). Differential rescue of light- and food-entrainable circadian rhythms. *Science* 320: 1074–1077.
334. Shi, S., Hida, A., McGuinness, O. P., Wasserman, D. H., Yamazaki, S., and Johnson, C. H. (2010). Circadian clock gene *Bmal1* is not essential after all: Functional replacement with its paralog, *Bmal2*. *Current Biology* 20: 316–321.
335. Takasu, N. N., Kurosawa, G., Tokuda, I. T., Mochizuki, A., Todo, T., and Nakamura, W. (2012). Circadian regulation of food-anticipatory activity in molecular clock-deficient mice. *PLOS ONE* 7: e48892.
336. Pendergast, J. S., Oda, G. A., Niswender, K. D., and Yamazaki, S. (2012). *Period* determination in the food-entrainable and methamphetamine-sensitive circadian oscillator(s). *Proceedings of the National Academy of Sciences of the United States of America* 109: 14218–14223.
337. Vollmers, C., Gill, S., DiTacchio, L., Pulivarthy, S. R., Le, H. D., and Panda, S. (2009). Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proceedings of the National Academy of Sciences of the United States of America* 106: 21453–21458.
338. Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamazaki, S., Zemenides, P. D., Ralph, M. R., Menaker, M., and Takahashi, J. S. (2000). Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* 288: 483–491.
339. Eide, E. J., Woolf, M. F., Kang, H., Woolf, P., Hurst, W., Camacho, F., Vielhaber, E. L., Giovanni, A., and Virshup, D. M. (2005). Control of mammalian circadian rhythm by CKIε-regulated proteasome-mediated PER2 degradation. *Molecular and Cellular Biology* 25: 2795–2807.
340. Xu, Y., Toh, K. L., Jones, C. R., Shin, J. Y., Fu, Y. H., and Ptácek, L. J. (2007). Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell* 128: 59–70.
341. Isojima, Y., Nakajima, M., Ukai, H., Fujishima, H., Yamada, R. G., Masumoto, K. H., Kiuchi, R. et al. (2009). CKIε/δ-dependent phosphorylation is a temperature-insensitive, period-determining process in the mammalian circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 106: 15744–15749.
342. Lee, H., Chen, R., Lee, Y., Yoo, S., and Lee, C. (2009). Essential roles of CKIδ and CKIε in the mammalian circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 106: 21359–21364.
343. Lee, H., Chen, R., Kim, H., Etchegaray, J. P., Weaver, D. R., and Lee, C. (2011). The period of the circadian oscillator is primarily determined by the balance between casein kinase 1 and protein phosphatase 1. *Proceedings of the National Academy of Sciences of the United States of America* 108: 16451–16456.
344. Etchegaray, J. P., Machida, K. K., Noton, E., Constance, C. M., Dallmann, R., Di Napoli, M. N. et al. (2009). Casein kinase 1δ regulates the pace of the mammalian circadian clock. *Molecular and Cellular Biology* 29: 3853–3866.
345. Walton, K. M., Fisher, K., Rubitski, D., Marconi, M., Meng, Q. J., Sládek, M., Adams, J. et al. (2009). Selective inhibition of casein kinase 1 ε minimally alters circadian clock period. *Journal of Pharmacology and Experimental Therapeutics* 330: 430–439.
346. Tsuchiya, Y., Akashi, M., Matsuda, M., Goto, K., Miyata, Y., Node, K., and Nishida, E. (2009). Involvement of the protein kinase CK2 in the regulation of mammalian circadian rhythms. *Science Signaling* 2: ra26.
347. Ukai, H. and Ueda, H. R. (2010). Systems biology of mammalian circadian clocks. *Annual Review of Physiology* 72: 579–603.
348. Lowrey, P. L. and Takahashi, J. S. (2011). Genetics of circadian rhythms in mammalian model organisms. *Advances in Genetics* 74: 175–230.
349. Liu, C., Li, S., Liu, T., Borjigin, J., and Lin, J. D. (2007). Transcriptional coactivator PGC-1α integrates the mammalian clock and energy metabolism. *Nature* 447: 477–481.
350. Busino, L., Bassermann, F., Maiolica, A., Lee, C., Nolan, P. M., Godinho, S. I., Draetta, G. F., and Pagano, M. (2007). SCF-Fbxl3 controls the oscillation of the circadian clock by directing the degradation of cryptochrome proteins. *Science* 316: 900–904.
351. O'Neill, J. S., Maywood, E. S., Chesham, J. E., Takahashi, J. S., and Hastings, M. H. (2008). cAMP-dependent signaling as a core component of the mammalian circadian pacemaker. *Science* 320: 949–953.
352. Robles, M. S., Boyault, C., Knutti, D., Padmanabhan, K., and Weitz, C. J. (2010). Identification of RACK1 and protein kinase cα as integral components of the mammalian circadian clock. *Science* 327: 463–466.
353. Duong, H. A., Robles, M. S., Knutti, D., and Weitz, C. J. (2011). A molecular mechanism for circadian clock negative feedback. *Science* 332: 1436–1439.
354. Schneider, K., Köcher, T., Andersin, T., Kurzchalia, T., Schibler, U., and Gatfield, D. (2012). CAVIN-3 regulates circadian period length and PER:CRY protein abundance and interactions. *EMBO Reports* 13: 1138–1144.
355. Yang, Y., Duguay, D., Bédard, N., Rachalski, A., Baquiran, G., Na, C. H., Fahrenkrug, J. et al. (2012). Regulation of behavioral circadian rhythms and clock protein PER1 by the deubiquitinating enzyme USP2. *Biology Open* 1: 789–801.
356. Kowalska, E., Ripperger, J. A., Hoegger, D. C., Bruegger, P., Buch, T., Birchler, T., Mueller, A., Albrecht, U., Contaldo, C., and Brown, S. A. (2013). NONO couples the circadian clock to the cell cycle. *Proceedings of the National Academy of Sciences of the United States of America* 110: 1592–1599.

357. Lande-Diner, L., Boyault, C., Kim, J. Y., and Weitz, C. J. (2013). A positive feedback loop links circadian clock factor CLOCK-BMAL1 to the basic transcriptional machinery. *Proceedings of the National Academy of Sciences of the United States of America* 110: 16021–16026.
358. Chen, R., D'Alessandro, M., and Lee, C. (2013). miRNAs are required for generating a time delay critical for the circadian oscillator. *Current Biology* 23: 1959–1968.
359. Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annual Review of Physiology* 72: 517–549.
360. Poon, A. C. and Ferrell, J. E. (2007). A clock with a flip switch. *Science* 318: 757–758.
361. Brown, S. A., Kowalska, E., and Dallmann, R. (2012). (Re) inventing the circadian feedback loop. *Developmental Cell* 22: 477–487.
362. Hardin, P. E. (2000). From biological clock to biological rhythms. *Genome Biology* 1: art. 1023.
363. Rosbash, M. (2009). The implications of multiple circadian clock origins. *PLoS Biology* 7: e1000062.
364. Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M., and Allada, R. (2002). A role for casein kinase 2 α in the *Drosophila* circadian clock. *Nature* 420: 816–820.
365. Van Gelder, R. N., Herzog, E. D., Schwartz, W. J., and Taghert, P. H. (2003). Circadian rhythms: In the loop at last. *Science* 300: 1534–1535.
366. Zantke, J., Ishikawa-Fujiwara, T., Arboleda, E., Lohs, C., Schipany, K., Hallay, N., Straw, A. D., Todo, T., and Tessmar-Raible, K. (2013). Circadian and circalunar clock interactions in a marine annelid. *Cell Reports* 5: 99–113.
367. Constance, C. M., Green, C. B., Tei, H., and Block, G. D. (2002). *Bulla gouldiana period* exhibits unique regulation at the mRNA and protein levels. *Journal of Biological Rhythms* 17: 413–427.
368. Regier, J. C., Fang, Q. Q., Mitter, C., Peigler, R. S., Friedlander, T. P., and Solis, M. A. (1998). Evolution and phylogenetic utility of the *period* gene in Lepidoptera. *Molecular Biology and Evolution* 15: 1172–1182.
369. Toma, D. P., Bloch, G., Moore, D., and Robinson, G. E. (2000). Changes in *period* mRNA levels in the brain and division of labor in honey bee colonies. *Proceedings of the National Academy of Sciences of the United States of America* 97: 6914–6919.
370. Shimizu, I., Kawai, Y., Taniguchi, M., and Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
371. Závodská, R., Sauman, I., and Sehnal, F. (2003). Distribution of PER protein, pigment-dispersing hormone, prothoracicotropic hormone, and eclosion hormone in the cephalic nervous system of insects. *Journal of Biological Rhythms* 18: 106–122.
372. Merlin, C., Gegear, R. J., and Reppert, S. M. (2009). Antennal circadian clocks coordinate sun compass orientation in migratory monarch butterflies. *Science* 325: 1700–1704.
373. Magnone, M. C., Jacobmeier, B., Bertolucci, C., Foà, A., and Albrecht, U. (2005). Circadian expression of the clock gene *Per2* is altered in the ruin lizard (*Podarcis sicula*) when temperature changes. *Molecular Brain Research* 133: 281–285.
374. Yoshimura, T., Suzuki, Y., Makino, E., Suzuki, T., Kuroiwa, A., Matsuda, Y., Namikawa, T., and Ebihara, S. (2000). Molecular analysis of avian circadian clock genes. *Molecular Brain Research* 78: 207–215.
375. Messager, S., Ross, A. W., Barrett, P., and Morgan, P. J. (1999). Decoding photoperiodic time through *Per1* and ICER gene amplitude. *Proceedings of the National Academy of Sciences of the United States of America* 96: 9938–9943.
376. Mrosovsky, N., Edelstein, K., Hastings, M. H., and Maywood, E. S. (2001). Cycle of *period* gene expression in a diurnal mammal (*Spermophilus tridecemlineatus*): Implications for nonphotic phase shifting. *Journal of Biological Rhythms* 16: 471–478.
377. Oster, H., Avivi, A., Joel, A., Albrecht, U., and Nevo, E. (2002). A switch from diurnal to nocturnal activity in *S. ehrenbergi* is accompanied by an uncoupling of light input and the circadian clock. *Current Biology* 12: 1919–1922.
378. Sumová, A., Sládek, M., Jác, M., and Illnerová, H. (2002). The circadian rhythm of Per1 gene product in the rat suprachiasmatic nucleus and its modulation by seasonal changes in daylength. *Brain Research* 947: 260–270.
379. Beaulé, C., Houle, L. M., and Amir, S. (2003). Expression profiles of PER2 immunoreactivity within the shell and core regions of the rat suprachiasmatic nucleus. *Journal of Molecular Neuroscience* 21: 133–147.
380. van der Veen, D. R., Le Minh, N., Gos, P., Arneric, M., Gerkema, M. P., and Schibler, U. (2006). Impact of behavior on central and peripheral circadian clocks in the common vole *Microtus arvalis*, a mammal with ultradian rhythms. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3393–3398.
381. Li, J. Z., Bunney, B. G., Meng, F., Hagenauer, M. H., Walsh, D. M., Vawter, M. P., Evans, S. J. et al. (2013). Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proceedings of the National Academy of Sciences of the United States of America* 110: 9950–9955.
382. Chang, D. C., McWatters, H. G., Williams, J. A., Gotter, A. L., Levine, J. D., and Reppert, S. M. (2003). Constructing a feedback loop with circadian clock molecules from the silkmoth, *Antheraea pernyi*. *Journal of Biological Chemistry* 278: 38149–38158.
383. Whitmore, D., Foulkes, N. S., Strähle, U., and Sassone-Corsi, P. (1998). Zebrafish *Clock* rhythmic expression reveals independent peripheral circadian oscillators. *Nature Neuroscience* 1: 701–707.
384. Chong, N. W., Chaurasia, S. S., Haque, R., Klein, D. C., and Iuvone, P. M. (2003). Temporal-spatial characterization of chicken clock genes: Circadian expression in retina, pineal gland, and peripheral tissues. *Journal of Neurochemistry* 85: 851–860.
385. Fidler, A. E. and Gwinner, E. (2003). Comparative analysis of avian BMAL1 and CLOCK protein sequences: A search for features associated with owl nocturnal behavior. *Comparative Biochemistry and Physiology B* 136: 861–874.
386. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U., and Nevo, E. (2002). Circadian genes in a blind subterranean mammal. II. Conservation and uniqueness of the three *Period* homologs in the blind subterranean mole rat, *Spalax ehrenbergi* superspecies. *Proceedings of the National Academy of Sciences of the United States of America* 99: 11718–11723.
387. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U., and Nevo, E. (2004). Circadian genes in a blind subterranean mammal. III. Molecular cloning and circadian regulation of cryptochrome genes in the blind subterranean mole rat, *Spalax ehrenbergi* superspecies. *Journal of Biological Rhythms* 19: 22–34.
388. Sumová, A., Jác, M., Sládek, M., Sauman, I., and Illnerová, H. (2003). Clock gene daily profiles and their phase relationship in the rat suprachiasmatic nucleus are affected by photoperiod. *Journal of Biological Rhythms* 18: 134–144.

389. Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H., and Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *Journal of Biological Rhythms* 18: 159–169.
390. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P., and Challet, E. (2003). Circadian profile and photic regulation of clock genes in the suprachiasmatic nucleus of a diurnal mammal, *Arvicanthis ansorgei*. *Neuroscience* 116: 583–591.
391. Shieh, K. R. (2003). Distribution of the rhythm-related genes *rPeriod1*, *rPeriod2*, and *rClock* in the rat brain. *Neuroscience* 118: 831–843.
392. Lincoln, G., Messager, S., Andersson, H., and Hazlerigg, D. (2002). Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: Evidence for an internal coincidence timer. *Proceedings of the National Academy of Sciences of the United States of America* 99: 13890–13895.
393. Griffin, E. A., Staknis, D., and Weitz, C. J. (1999). Light-independent role of CRY1 and CRY2 in the mammalian clock. *Science* 286: 768–771.
394. Chen, Y. and Tan, E. C. (2004). Identification of human *Clock* gene variants by denaturing high-performance liquid chromatography. *Journal of Human Genetics* 49: 209–214.
395. Edgar, R. S., Green, E. W., Zhao, Y., van Ooijen, G., Olmedo, M., Maywood, E. S., Hastings, M. H. et al. (2012). Peroxiredoxins are conserved markers of circadian rhythms. *Nature* 485: 459–464.
396. Takahashi, J. S., Shimomura, K., and Kumar, V. (2008). Searching for genes underlying behavior: Lessons from circadian rhythms. *Science* 322: 909–912.
397. Tomita, J., Nakajima, M., Kondo, T., and Iwasaki, H. (2005). No transcription-translation feedback in circadian rhythm of KaiC phosphorylation. *Science* 307: 251–254.
398. Nakajima, M., Imai, K., Ito, H., Nishiwaki, T., Murayama, Y., Iwasaki, H., Oyama, T., and Kondo, T. (2005). Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* 308: 414–415.
399. Kageyama, H., Nishiwaki, T., Nakajima, M., Iwakasi, H., Oyama, T., and Kondo, T. (2006). Cyanobacterial circadian pacemaker: Kai protein complex dynamics in the KaiC phosphorylation cycle in vitro. *Molecular Cell* 23: 161–171.
400. Mehra, A., Hong, C. I., Shi, M., Loros, J. J., Dunlap, J. C., and Ruoff, P. (2006). Circadian rhythmicity by autocatalysis. *PLoS Computational Biology* 2: 816–823.
401. Rust, M. J., Markson, J. S., Lane, W. S., Fisher, D. S., and O'Shea, E. K. (2007). Ordered phosphorylation governs oscillation of a three-protein circadian clock. *Science* 318: 809–812.
402. Johnson, C. H., Egli, M., and Stewart, P. L. (2008). Structural insights into a circadian oscillator. *Science* 322: 697–701.
403. McConnell, M. J., Lindberg, M. R., Brennand, K. J., Piper, J. C., Voet, T., Cowing-Zitron, C., Shumilina, S. et al. (2013). Mosaic copy number variation in human neurons. *Science* 342: 632–637.
404. Kornmann, B., Schaad, O., Bujard, H., Takahashi, J. S., and Schibler, U. (2007). System-driven and oscillator-dependent circadian transcription in mice with a conditionally active liver clock. *PLoS Biology* 5: e34.
405. O'Neill, J. S. and Reddy, A. B. (2011). Circadian clocks in human red blood cells. *Nature* 469: 498–503.
406. Paulose, J. K., Rucker, E. B., and Cassone, V. M. (2012). Toward the beginning of time: Circadian rhythms in metabolism precede rhythms in clock gene expression in mouse embryonic stem cells. *PLOS ONE* 7: e49555.
407. Teng, S. W., Mukherji, S., Moffitt, J. R., de Buyl, S., and O'Shea, E. K. (2013). Robust circadian oscillations in growing cyanobacteria require transcriptional feedback. *Science* 340: 737–740.
408. Ramsey, K. M., Yoshino, J., Brace, C. S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H. K. et al. (2009). Circadian clock feedback cycle through NAMPT-mediated NAD^+ biosynthesis. *Science* 324: 651–654.
409. Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., and Sassone-Corsi, P. (2009). Circadian control of the NAD^+ salvage pathway by CLOCK-SIRT1. *Science* 324: 654–657.
410. Peek, C. B., Affinati, A. H., Ramsey, K. M., Kuo, H., Yu, W., Sena, L. A., Ilkayeva, O. et al. (2013). Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. *Science* 342: 591.
411. Carlson, N. R. (1994). *Physiology of Behavior*, 5th edn. Boston, MA: Allyn & Bacon.
412. Garg, S. K. and Sundararaj, B. I. (1986). Role of pineal in the regulation of some aspects of circadian rhythmicity in the catfish, *Heteropneustes fossilis* (Bloch). *Chronobiologia* 13: 1–11.
413. Underwood, H. (1983). Circadian organization of the lizard *Anolis carolinensis*: A multioscillator system. *Journal of Comparative Physiology* 152: 265–274.
414. Molina-Borja, M. (1996). Pineal gland and circadian locomotor activity rhythm in the lacertid *Gallotia eisentrauti*: Pinealectomy induces arrhythmicity. *Biological Rhythm Research* 27: 1–11.
415. Gaston, S. and Menaker, M. (1968). Pineal function: The biological clock in the sparrow? *Science* 160: 1125–1127.
416. Binkley, S., Kluth, E., and Menaker, M. (1971). Pineal function in sparrows: Circadian rhythms and body temperature. *Science* 174: 311–314.
417. Heigl, S. and Gwinner, E. (1994). Periodic melatonin in the drinking water synchronizes circadian rhythms in sparrows. *Naturwissenschaften* 81: 83–85.
418. Iigo, M., Fujimoto, Y., Gunji-Suzuki, M., Yokosuka, M., Hara, M., Ohtani-Kaneko, R., Tabata, M., Aida, K., and Hirata, K. (2004). Circadian rhythm of melatonin release from the photoreceptive pineal organ of a teleost, ayu (*Plecoglossus altivelis*) in flow-through culture. *Journal of Neuroendocrinology* 16: 45–51.
419. Debruyne, J., Hurd, M. W., Gutiérrez, L., Kaneko, M., Tan, Y., Wells, D. E., and Cahill, G. M. (2004). Isolation and phenogenetics of a novel circadian rhythm mutant in zebrafish. *Journal of Neurogenetics* 18: 403–428.
420. Tosini, G. and Menaker, M. (1998). Multioscillatory circadian organization in a vertebrate, *Iguana iguana*. *Journal of Neuroscience* 18: 1105–1114.
421. Bertolucci, C., Wagner, G., Foà, A., Gwinner, E., and Brandstätter, R. (2003). Photoperiod affects amplitude but not duration of in vitro melatonin production in the ruin lizard (*Podarcis sicula*). *Journal of Biological Rhythms* 18: 63–70.
422. Brandstätter, R., Kumar, V., Abraham, U., and Gwinner, E. (2000). Photoperiodic information acquired and stored *in vivo* retained *in vitro* by a circadian oscillator, the avian pineal gland. *Proceedings of the National Academy of Sciences of the United States of America* 97: 12324–12328.
423. Schenda, J. and Vollrath, L. (2000). Single-cell recordings from chick pineal glands in vitro reveal ultradian and circadian oscillations. *Cellular and Molecular Life Sciences* 57: 1785–1792.
424. Yoshikawa, T., Yamazaki, S., and Menaker, M. (2005). Effects of preparation time on phase of cultured tissues reveal complexity of circadian organization. *Journal of Biological Rhythms* 20: 500–512.

425. Cheung, P. W. and McCormack, C. E. (1982). Failure of pinealectomy or melatonin to alter circadian activity rhythm of the rat. *American Journal of Physiology* 242: R261–R264.
426. Spencer, F., Shirer, H. W., and Yochim, J. M. (1976). Core temperature in the female rat: Effect of pinealectomy or altered lighting. *American Journal of Physiology* 231: 355–360.
427. Bobbert, A. C. and Riethoven, J. J. (1991). Feedback in the rabbit's central circadian system, revealed by the changes in its free-running food intake pattern induced by blinding, cervical sympathectomy, pinealectomy, and melatonin administration. *Journal of Biological Rhythms* 6: 263–278.
428. Warren, W. S. and Cassone, V. M. (1995). The pineal gland: Photoreception and coupling of behavioral, metabolic, and cardiovascular circadian outputs. *Journal of Biological Rhythms* 10: 64–79.
429. Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *Journal of Biological Rhythms* 8: 189–197.
430. Schuhler, S., Pitrosky, B., Kirsch, R., and Pévet, P. (2002). Entrainment of locomotor activity rhythm in pinealectomized adult Syrian hamsters by daily melatonin infusion. *Behavioural Brain Research* 133: 343–350.
431. Redman, J. R. and Francis, A. J. P. (1998). Entrainment of rat circadian rhythms by the melatonin agonist S-20098 requires intact suprachiasmatic nuclei but not the pineal. *Journal of Biological Rhythms* 13: 39–51.
432. Harlow, H. J., Phillips, J. A., and Ralph, C. L. (1982). Circadian rhythms and the effects of exogenous melatonin in the nine-banded armadillo, *Dasypus novemcinctus*: A mammal lacking a distinct pineal gland. *Physiology and Behavior* 29: 307–313.
433. Panin, M., Gabai, G., Ballarin, C., Peruffo, A., and Cozzi, B. (2012). Evidence of melatonin secretion in cetaceans: Plasma concentration and extrapineal HIOMT-like presence in the bottlenone dolphin *Tursiops truncatus*. *General and Comparative Endocrinology* 177: 238–245.
434. Sánchez-Vázquez, F. J., Iigo, M., Madrid, J. A., and Tabata, M. (2000). Pinealectomy does not affect the entrainment to light nor the generation of the circadian demand-feeding rhythms of rainbow trout. *Physiology and Behavior* 69: 455–461.
435. Underwood, H. (1983). Circadian pacemakers in lizards: Phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
436. Innicenti, A., Minutini, L., and Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
437. Tosini, G. and Menaker, M. (1996). The pineal complex and melatonin affect the expression of the daily rhythm of behavioral thermoregulation in the green iguana. *Journal of Comparative Physiology A* 179: 135–142.
438. Bartell, P. A., Miranda-Anaya, M., and Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
439. Oshima, I., Yamada, H., Goto, M., Sato, K., and Ebihara, S. (1989). Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. *Journal of Comparative Physiology A* 166: 217–226.
440. Underwood, H. (1994). The circadian rhythm of thermoregulation in Japanese quail. I. Role of the eyes and pineal. *Journal of Comparative Physiology A* 175: 639–653.
441. Underwood, H. and Edmonds, K. (1995). The circadian rhythm of thermoregulation in Japanese quail. II. Multioscillator control. *Journal of Biological Rhythms* 10: 234–247.
442. Heigl, S. and Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
443. Menaker, M., Moreira, L. F., and Tosini, G. (1997). Evolution of circadian organization in vertebrates. *Brazilian Journal of Medical and Biological Research* 30: 305–313.
444. Underwood, H. (2001). Circadian organization in nonmammalian vertebrates. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 111–140.
445. Michel, S., Geusz, M. E., Zaritsky, J. J., and Block, G. D. (1993). Circadian rhythm in membrane conductance expressed in isolated neurons. *Science* 259: 239–241.
446. Page, T. L., Wassmer, G. T., Fletcher, J., and Block, G. D. (1997). Aftereffects of entrainment on the period of the pacemaker in the eye of the mollusk *Bulla gouldiana*. *Journal of Biological Rhythms* 12: 218–225.
447. Willbold, E., Huhn, J., Korf, H. W., Voisin, P., and Layer, P. G. (2002). Light-dark and circadian melatonin rhythms are established de novo in re-aggregates of the embryonic chicken retina. *Developmental Neuroscience* 24: 504–511.
448. Zhang, Y., Coleman, J. E., Fuchs, G. E., and Semple-Rowland, S. L. (2003). Circadian oscillator function in embryonic retina and retinal explant cultures. *Molecular Brain Research* 114: 9–19.
449. Tosini, G. and Menaker, M. (1996). Circadian rhythms in cultured mammalian retina. *Science* 272: 419–421.
450. Tosini, G. and Menaker, M. (1998). The *tau* mutation affects temperature compensation of hamster retinal circadian oscillators. *NeuroReport* 9: 1001–1005.
451. Sakamoto, K., Liu, C., and Tosini, G. (2004). Circadian rhythms in the retina of rats with photoreceptor degeneration. *Journal of Neurochemistry* 90: 1019–1024.
452. Bailey, M. J., Beremand, P. D., Hammer, R., Reidel, E., Thomas, T. L., and Cassone, V. M. (2004). Transcriptional profiling of circadian patterns of mRNA expression in the chick retina. *Journal of Biological Chemistry* 279: 52247–52254.
453. Numano, R., Yamazaki, S., Umeda, N., Samura, T., Sujino, M., Takahashi, R., Ueda, M. et al. (2006). Constitutive expression of the Period1 gene impairs behavioral and molecular circadian rhythms. *Proceedings of the National Academy of Sciences of the United States of America* 103: 3716–3721.
454. Ruan, G. X., Zhang, D. Q., Zhou, T., Yamazaki, S., and McMahon, D. G. (2006). Circadian organization of the mammalian retina. *Proceedings of the National Academy of Sciences of the United States of America* 103: 9703–9708.
455. Peirson, S. N., Butler, J. N., Duffield, G. E., Takher, S., Sharma, P., and Foster, R. G. (2006). Comparison of clock gene expression in SCN, retina, heart, and liver of mice. *Biochemical and Biophysical Research Communications* 351: 800–807.
456. Steele, C. T., Zivkovic, B. D., Siopes, T., and Underwood, H. (2003). Ocular clocks are tightly coupled and act as pacemakers in the circadian system of Japanese quail. *American Journal of Physiology* 284: R208–R218.
457. Chabot, C. C. and Menaker, M. (1992). Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons. *Journal of Comparative Physiology A* 170: 615–622.

458. Halberg, F. (1953). Beobachtungen über 24 Stunden-Periodik in standardisierter Versuchsanordnung vor und nach Epinephrektomie und bilateraler optischer Enucleation. *Berichte über die Gesamte Physiologie und Experimentelle Pharmakologie* 162: 354–355.
459. Richter, C. P. (1978). "Dark-active" rat transformed into "light-active" rat by destruction of 24-hr clock: Function of 24-hr clock and synchronizers. *Proceedings of the National Academy of Sciences of the United States of America* 75: 6276–6280.
460. Yamazaki, S., Goto, M., and Menaker, M. (1999). No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *Journal of Biological Rhythms* 14: 197–201.
461. Freedman, M. S., Lucas, R. J., Soni, B., von Schantz, M., Muñoz, M., David-Gray, Z., and Foster, R. (1999). Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* 284: 502–504.
462. Yamazaki, S., Alones, V., and Menaker, M. (2002). Interaction of the retina with suprachiasmatic pacemakers in the control of circadian behavior. *Journal of Biological Rhythms* 17: 315–329.
463. Lee, H. S., Nelms, J. L., Nguyen, M., Silver, R., and Lehman, M. N. (2003). The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. *Nature Neuroscience* 6: 111–112.
464. Wakamatsu, H., Yoshinobu, Y., Aida, R., Moriya, T., Akiyama, M., and Shibata, S. (2001). Restricted-feeding-induced anticipatory activity rhythm is associated with a phase shift of the expression of mPer1 and mPer2 mRNA in the cerebral cortex and hippocampus but not in the suprachiasmatic nucleus of mice. *European Journal of Neuroscience* 13: 1190–1196.
465. Angeles-Castellanos, M., Aguilar-Roblero, R., and Escobar, C. (2004). c-Fos expression in hypothalamic nuclei of food-entrained rats. *American Journal of Physiology* 286: R158–R165.
466. Mendoza, J. (2007). Circadian clocks: Setting time by food. *Journal of Neuroendocrinology* 19: 127–137.
467. Ho, K. J. (1979). Circadian rhythm of cholesterol biosynthesis: Dietary regulation in the liver and small intestine of hamsters. *International Journal of Chronobiology* 6: 39–50.
468. Edwards, P. A., Muroya, H., and Gould, R. G. (1972). In vivo demonstration of the circadian rhythm of cholesterol biosynthesis in the liver and intestine of the rat. *Journal of Lipid Research* 13: 396–401.
469. Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C., and Porter, J. W. (1972). The effects of fasting, refeeding, and time of day on the levels of enzymes effecting the conversion of β-hydroxy-β-methylglutaryl coenzyme A to squalene. *Journal of Biological Chemistry* 247: 3014–3022.
470. Dugan, R. E., Slakey, L. L., Briedis, A. V., and Porter, J. W. (1972). Factors affecting the diurnal variation in the level of β-hydroxy-β-methylglutaryl coenzyme A reductase and cholesterol-synthesizing activity in rat liver. *Archives of Biochemistry and Biophysics* 152: 21–27.
471. Lanza-Jacoby, S., Stevenson, N. R., and Kaplan, M. L. (1986). Circadian changes in serum and liver metabolites and liver lipogenic enzymes in ad libitum- and meal-fed, lean and obese Zucker rats. *Journal of Nutrition* 116: 1798–1809.
472. Wasan, K. M., Brunner, L. J., Berens, K. L., Meltzer, A. A., and Luke, D. R. (1989). Circadian assessment of lipids in the hyperphagic obese rat compared with lean litter-mates. *Chronobiology International* 6: 223–228.
473. Jurevics, H., Hostettler, J., Barrett, C., Morell, P., and Toews, A. D. (2000). Diurnal and dietary-induced changes in cholesterol synthesis correlate with levels of mRNA for HMG-CoA reductase. *Journal of Lipid Research* 41: 1048–1053.
474. Piccione, G., Caola, G., and Refinetti, R. (2003). Circadian rhythms of body temperature and liver function in fed and food-deprived goats. *Comparative Biochemistry and Physiology A* 134: 563–572.
475. Piccione, G., Caola, G., and Refinetti, R. (2007). Daily rhythms of liver-function indicators in rabbits. *Journal of Physiological Sciences* 57: 101–105.
476. Brown, S. A., Zumbrunn, G., Fleury-Olela, F., Preitner, N., and Schibler, U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Current Biology* 12: 1574–1583.
477. Cailotto, C., La Fleur, S. E., Van Heijningen, C., Wortel, J., Kalsbeek, A., Feenstra, M., Pévet, P., and Buijs, R. M. (2005). The suprachiasmatic nucleus controls the daily variation of plasma glucose via the autonomic output to the liver: Are the clock genes involved? *European Journal of Neuroscience* 22: 2531–2540.
478. Kohsaka, A., Laposky, A. D., Ramsey, K. M., Estrada, C., Joshu, C., Kobayashi, Y., Turek, F. W., and Bass, J. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metabolism* 6: 414–421.
479. Tsuchiya, Y., Minami, I., Kadotani, H., and Nishida, E. (2005). Resetting of peripheral circadian clock by prostaglandin E_2. *EMBO Reports* 6: 256–261.
480. Zvonic, S., Ptitsyn, A. A., Conrad, S. A., Scott, L. K., Floyd, Z. E., Kilroy, G., Wu, X., Goh, B. C., Mynatt, R. L., and Gimble, J. M. (2006). Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* 55: 962–970.
481. Guo, H., Brewer, J. M., Champhekar, A., Harris, R. B. S., and Bittman, E. L. (2005). Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. *Proceedings of the National Academy of Sciences of the United States of America* 102: 3111–3116.
482. Feillet, C. A., Ripperger, J. A., Magnone, M. C., Dulloo, A., Albrecht, U., and Challet, E. (2006). Lack of food anticipation in *Per2* mutant mice. *Current Biology* 16: 2016–2022.
483. Lamia, K. A., Sachdeva, U. M., DiTacchio, L., Williams, E. C., Alvarez, J. G., Egan, D. F., Vasquez, D. S. et al. (2009). AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* 326: 437–440.
484. Andersson, H., Johnston, J. D., Messager, S., Hazlerigg, D., and Lincoln, G. (2005). Photoperiod regulates clock gene rhythms in the ovine liver. *General and Comparative Endocrinology* 142: 357–363.
485. Tong, Y., Guo, H., Brewer, J. M., Lee, H., Lehman, M. N., and Bittman, E. L. (2004). Expression of *haPer1* and *haBmal1* in Syrian hamsters: Heterogeneity of transcripts and oscillations in the periphery. *Journal of Biological Rhythms* 19: 113–125.
486. Lee, C., Weaver, D. R., and Reppert, S. M. (2004). Direct association between mouse PERIOD and CKIε is critical for a functional circadian clock. *Molecular and Cellular Biology* 24: 584–594.
487. Yamamoto, T., Nakahata, Y., Soma, H., Akashi, M., Mamine, T., and Takumi, T. (2004). Transcriptional oscillation of canonical clock genes in mouse peripheral tissues. *BMC Molecular Biology* 5: art. 18.

488. Sheward, W. J., Maywood, E. S., French, K. L., Horn, J. M., Hastings, M. H., Seckl, J. R., Holmes, M. C., and Harmar, A. J. (2007). Entrainment to feeding but not to light: Circadian phenotype of $VPAC_2$ receptor-null mice. *Journal of Neuroscience* 27: 4351–4358.
489. Kita, Y., Shiozawa, M., Jin, W., Majewski, R. R., Besharse, J. C., Greene, A. S., and Jacob, H. J. (2002). Implications of circadian gene expression in kidney, liver and the effects of fasting on pharmacogenomic studies. *Pharmacogenetics* 12: 55–65.
490. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H., and Kyriacou, C. P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Current Biology* 12: 540–550.
491. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H., and Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.
492. Oishi, K., Miyazaki, K., Kadota, K., Kikuno, R., Nagase, T., Atsumi, G., Ohkura, N. et al. (2003). Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. *Journal of Biological Chemistry* 278: 41519–41527.
493. Desai, V. G., Moland, C. L., Branham, W. S., Delongchamp, R. R., Fang, H., Duffy, P. H., Peterson, C. A., Beggs, M. L., and Fuscoe, J. C. (2004). Changes in expression level of genes as a function of time of day in the liver of rats. *Mutation Research* 549: 115–129.
494. Ptitsyn, A. A., Zvonic, S., Conrad, S. A., Scott, L. K., Mynatt, R. L., and Gimble, J. M. (2006). Circadian clocks are resounding in peripheral tissues. *PLoS Computational Biology* 2(3): e16.
495. Hirao, J., Arakawa, S., Watanabe, K., Ito, K., and Furukawa, T. (2006). Effects of restricted feeding on daily fluctuations of hepatic functions including P450 monooxygenase activities in rats. *Journal of Biological Chemistry* 281: 3165–3171.
496. Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M. et al. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences of the United States of America* 101: 5339–5346.
497. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M., and Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685.
498. Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y., and Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science* 291: 490–493.
499. Terazono, H., Mutoh, T., Yamaguchi, S., Kobayashi, M., Akiyama, M., Udo, R., Ohdo, S., Okamura, H., and Shibata, S. (2003). Adrenergic regulation of clock gene expression in mouse liver. *Proceedings of the National Academy of Sciences of the United States of America* 100: 6795–6800.
500. Yamanaka, Y., Honma, S., and Honma, K. (2008). Scheduled exposures to a novel environment with a running-wheel differentially accelerate re-entrainment of mice peripheral clocks to new light-dark cycles. *Genes to Cells* 13: 497–507.
501. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes and Development* 14: 2950–2961.
502. Ohta, H., Xu, S., Moriya, T., Iigo, M., Watanabe, T., Nakahata, N., Chisaka, H. et al. (2008). Maternal feeding controls fetal biological clock. *PLOS ONE* 3: e2601.
503. Moriya, T., Aida, R., Kudo, T., Akiyama, M., Doi, M., Hayasaka, N., Nakahata, N., Mistlberger, R., Okamura, H., and Shibata, S. (2009). The dorsomedial hypothalamic nucleus is not necessary for food-anticipatory circadian rhythms of behavior, temperature or clock gene expression in mice. *European Journal of Neuroscience* 29: 1447–1460.
504. Saini, C., Liani, A., Curie, T., Gos, P., Kreppel, F., Emmenegger, Y., Bonacina, L. et al. (2013). Real-time recording of circadian liver gene expression in freely moving mice reveals the phase-setting behavior of hepatocyte clocks. *Genes and Development* 27: 1526–1536.
505. Miki, H., Yano, M., Iwanaga, H., Tsujinaka, T., Nakayama, M., Kobayashi, M., Oishi, K. et al. (2003). Total parenteral nutrition entrains the central and peripheral circadian clocks. *NeuroReport* 14: 1457–1461.
506. Davidson, A. J., Poole, A. S., Yamazaki, S., and Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain and Behavior* 2: 32–39.
507. Escobar, C., Mendoza, J. Y., Salazar-Juárez, A., Ávila, J., Hernández-Muñoz, R., Díaz-Muñoz, M., and Aguilar-Roblero, R. (2002). Rats made cirrhotic by chronic CCl_4 treatment still exhibit anticipatory activity to a restricted feeding schedule. *Chronobiology International* 19: 1073–1086.
508. Hirao, A., Tahara, Y., Kimura, I., and Shibata, S. (2009). A balanced diet is necessary for proper entrainment signals of the mouse liver clock. *PLOS ONE* 4: e6909.
509. Sakamoto, K. and Ishida, N. (2000). Light-induced phase-shifts in the circadian expression rhythm of mammalian *Period* genes in the mouse heart. *European Journal of Neuroscience* 12: 4003–4006.
510. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M., and Honma, K. (2000). Clock genes outside the suprachiasmatic nucleus involved in manifestation of locomotor activity rhythm in rats. *European Journal of Neuroscience* 12: 4206–4214.
511. Mühlbauer, E., Wolgast, S., Finckh, U., Peschke, D., and Peschke, E. (2004). Indication of circadian oscillations in the rat pancreas. *FEBS Letters* 564: 91–96.
512. Bjarnason, G. A., Jordan, R. C. K., Wood, P. A., Li, Q., Lincoln, D. W., Sothern, R. B., Hrushesky, W. J. M., and Ben-David, Y. (2001). Circadian expression of clock genes in human oral mucosa and skin. *American Journal of Pathology* 158: 1793–1801.
513. Sakamoto, K., Kadota, K., and Oishi, K. (2004). Light-induced phase-shifting of the peripheral circadian oscillator in the hearts of food-deprived mice. *Experimental Animals* 53: 471–474.
514. Oster, H., Damerow, S., Hut, R. A., and Eichele, G. (2006). Transcriptional profiling in the adrenal gland reveals circadian regulation of hormone biosynthesis genes and nucleosome assembly genes. *Journal of Biological Rhythms* 21: 350–361.
515. Dolatshad, H., Campbell, E. A., O'Hara, L., Maywood, E. S., Hastings, M. H., and Johnson, M. H. (2006). Developmental and reproductive performance in circadian mutant mice. *Human Reproduction* 21: 68–79.
516. Zheng, L., Seon, Y. J., McHugh, J., Papagerakis, S., and Papagerakis, P. (2012). Clock genes show circadian rhythms in salivary glands. *Journal of Dental Research* 91: 783–788.
517. Wu, T., Ni, Y., Dong, Y., Xu, J., Song, X., Kato, H., and Fu, Z. (2010). Regulation of circadian gene expression in the kidney by light and food cues in rats. *American Journal of Physiology* 298: R635–R641.

518. Wunderer, F., Kühne, S., Jilg, A., Ackermann, K., Sebesteny, T., Maronde, E., and Stehle, J. H. (2013). Clock gene expression in the human pituitary gland. *Endocrinology* 154: 2046–2057.
519. Yagita, K., Tamanini, F., van der Horst, G. T. J., and Okamura, H. (2001). Molecular mechanisms of the biological clock in cultured fibroblasts. *Science* 292: 278–281.
520. Grundschober, C., Delaunay, F., Pühlhofer, A., Triqueneaux, G., Laudet, V., Bartfai, T., and Nef, P. (2001). Circadian regulation of diverse gene products revealed by mRNA expression profiling of synchronized fibroblasts. *Journal of Biological Chemistry* 276: 46751–46758.
521. Duffield, G. E., Best, J. D., Meurers, B. H., Bittner, A., Loros, J. J., and Dunlap, J. C. (2002). Circadian programs of transcriptional activation, signaling, and protein turnover revealed by microarray analysis of mammalian cells. *Current Biology* 12: 551–557.
522. Tamaru, T., Isojima, Y., van der Horst, G. T. J., Takei, K., Nagai, K., and Takamatsu, K. (2003). Nucleocytoplasmic shuttling and phosphorylation of BMAL1 are regulated by circadian clock in cultured fibroblasts. *Genes to Cells* 8: 973–983.
523. Izumo, M., Johnson, C. H., and Yamazaki, S. (2003). Circadian gene expression in mammalian fibroblasts revealed by real-time luminescence reporting: Temperature compensation and damping. *Proceedings of the National Academy of Sciences of the United States of America* 100: 16089–16094.
524. Welsh, D. K., Yoo, S. H., Liu, A. C., Takahashi, J. S., and Kay, S. A. (2004). Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Current Biology* 14: 2289–2295.
525. Granados-Fuentes, D., Prolo, L. M., Abraham, U., and Herzog, E. D. (2004). The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *Journal of Neuroscience* 24: 615–619.
526. Prolo, L. M., Takahashi, J. S., and Herzog, E. D. (2005). Circadian rhythm generation and entrainment in astrocytes. *Journal of Neuroscience* 25: 404–408.
527. Collaco, A. M., Rahman, S., Dougherty, E. J., Williams, B. B., and Geusz, M. E. (2005). Circadian regulation of a viral gene promoter in live transgenic mice expressing firefly luciferase. *Molecular Imaging and Biology* 7: 342–350.
528. Buhr, E. D., Yoo, S. H., and Takahashi, J. S. (2010). Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* 330: 379–385.
529. Akashi, M., Soma, H., Yamamoto, T., Tsugitomi, A., Yamashita, S., Yamamoto, T., Nishida, E., Yasuda, A., Liao, J. K., and Node, K. (2010). Noninvasive method for assessing the human circadian clock using hair follicle cells. *Proceedings of the National Academy of Sciences of the United States of America* 107: 15643–15648.
530. Mendoza, J., Pévet, P., Felder-Schmittbuhl, M. P., Bailly, Y., and Chalett, E. (2010). The cerebellum harbors a circadian oscillator involved in food anticipation. *Journal of Neuroscience* 30: 1894–1904.
531. Watts, L. M., Browne, J. A., and Murphy, B. A. (2012). Investigation of a non-invasive method of assessing the equine circadian clock using hair follicle cells. *Journal of Circadian Rhythms* 10: art. 7.
532. Andrews, R. V. (1968). Temporal secretory responses of cultured hamster adrenals. *Comparative Biochemistry and Physiology* 26: 179–193.
533. Peschke, E. and Peschke, D. (1998). Evidence for a circadian rhythm of insulin release from perifused rat pancreatic islets. *Diabetologia* 41: 1085–1092.
534. Feneberg, R. and Lemmer, B. (2004). Circadian rhythm of glucose uptake in cultures of skeletal muscle cells and adipocytes in Wistar-Kyoto, Wistar, Goto-Kakizaki, and Spontaneously Hypertensive rats. *Chronobiology International* 21: 521–538.
535. Granados-Fuentes, D., Saxena, M. T., Prolo, L. M., Aton, S. J., and Herzog, E. D. (2004). Olfactory bulb neurons express functional, entrainable circadian rhythms. *European Journal of Neuroscience* 19: 898–906.
536. Hennessey, T. L. and Field, C. B. (1992). Evidence of multiple circadian oscillators in bean plants. *Journal of Biological Rhythms* 7: 105–113.
537. Thain, S. C., Murtas, G., Lynn, J. R., McGrath, R. B., and Millar, A. J. (2002). The circadian clock that controls gene expression in Arabidopsis is tissue specific. *Plant Physiology* 130: 102–110.
538. Chia-Looi, A. and Cumming, B. G. (1972). Circadian rhythms of dark respiration, flowering, net photosynthesis, chlorophyll content, and dry weight changes in *Chenopodium rubrum*. *Canadian Journal of Botany* 50: 2219–2226.
539. Roenneberg, T. and Morse, D. (1993). Two circadian oscillators in one cell. *Nature* 362: 362–364.
540. Von der Heide, F., Wilkens, A., and Rensing, L. (1992). The effects of temperature on the circadian rhythms of flashing and glow in *Gonyaulax polyedra*: Are the two rhythms controlled by two oscillators? *Journal of Biological Rhythms* 7: 115–123.
541. Tanoue, S., Krishnan, P., Krishnan, B., Dryer, S. E., and Hardin, P. E. (2004). Circadian clocks in antennal neurons are necessary and sufficient for olfaction rhythms in *Drosophila*. *Current Biology* 14: 638–649.
542. Dinsmore, C. E. (1996). Urodele limb and tail regeneration in early biological thought: An essay on scientific controversy and social change. *International Journal of Developmental Biology* 40: 621–627.
543. Nye, H. L., Cameron, J. A., Chernoff, E. A., and Stocum, D. L. (2003). Regeneration of the urodele limb: A review. *Developmental Dynamics* 226: 280–294.
544. Brockes, J. P. and Kumar, A. (2005). Appendage regeneration in adult vertebrates and implications for regenerative medicine. *Science* 310: 1919–1923.
545. Nakahara, K., Fukui, K., and Murakami, N. (2004). Involvement of thalamic paraventricular nucleus in the anticipatory reaction under food restriction in the rat. *Journal of Veterinary Medical Science* 66: 1297–1300.
546. Garcia, J. A., Zhang, D., Estill, S. J., Michnoff, C., Rutter, J., Reick, M., Scott, K., Diaz-Arrastia, R., and McKnight, S. L. (2000). Impaired cued and contextual memory in NPAS2-deficient mice. *Science* 288: 2226–2230.
547. Reick, M., Garcia, J. A., Dudley, C., and McKnight, S. L. (2001). NPAS2: An analog of Clock operative in the mammalian forebrain. *Science* 293: 506–509.
548. Rutter, J., Reick, M., Wu, L. C., and McNight, S. L. (2001). Regulation of Clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293: 510–514.
549. Dudley, C. A., Erbel-Sieler, C., Estill, S. J., Reick, M., Franken, P., Pitts, S., and McKnight, S. L. (2003). Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science* 301: 379–383.
550. Pitts, S., Perone, E., and Silver, R. (2003). Food-entrained circadian rhythms are sustained in arrhythmic *Clk/Clk* mutant mice. *American Journal of Physiology* 285: R57–R67.
551. Green, C. B. and Menaker, M. (2003). Clocks on the brain. *Science* 301: 319–320.

552. Willie, J. T., Chemelli, R. M., Sinton, C. M., and Yanagisawa, M. (2001). To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annual Review of Neuroscience* 24: 429–458.
553. Akiyama, M., Yuasa, T., Hayasaka, N., Horikawa, K., Sakurai, T., and Shibata, S. (2004). Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. *European Journal of Neuroscience* 20: 3054–3062.
554. Gooley, J. J., Schomer, A., and Saper, C. B. (2006). The dorsomedial hypothalamic nucleus is critical for the expression of food-entrainable circadian rhythms. *Nature Neuroscience* 9: 398–407.
555. Acosta-Galvan, G., Yi, C. X., van der Vliet, J., Jhamandas, J. H., Panula, P., Angeles-Castellanos, M., Basualdo, M. C., Escobar, C., and Buijs, R. M. (2011). Interaction between hypothalamic dorsomedial nucleus and the suprachiasmatic nucleus determines intensity of food anticipatory behavior. *Proceedings of the National Academy of Sciences of the United States of America* 108: 5813–5818.
556. Mistlberger, R. E., Yamazaki, S., Pendergast, J. S., Landry, G. J., Takumi, T., and Nakamura, W. (2008). Comment on "Differential rescue of light- and food-entrainable circadian rhythms". *Science* 322: 675.
557. Mistlberger, R. E., Buijs, R. M., Challet, E., Escobar, C., Landry, G. J., Kalsbeek, A., Pévet, P., and Shibata, S. (2009). Standards of evidence in chronobiology: Critical review of a report that restoration of *Bmal1* expression in the dorsomedial hypothalamus is sufficient to restore circadian food anticipatory rhythms in *Bmal1*$^{-/-}$ mice. *Journal of Circadian Rhythms* 7: art. 3.
558. Davidson, A. J., Yamazaki, S., and Menaker, M. (2003). SCN: Ringmaster of the circadian circus or conductor of the circadian orchestra? *Novartis Foundation Symposia* 253: 110–121.

13 Afference and Efference

13.1 AFFERENT PATHWAYS

In Chapter 11, we discussed the sensory *receptors* that provide environmental information to the circadian system. In Chapter 12, we discussed the cells and molecules that make up the master *pacemaker* and the multiple peripheral slave pacemakers. In this chapter, we will discuss the pathways through which the receptors send information to the master pacemaker (the *afference*) and the pathways through which the pacemaker sends information to the various effector organs (the *efference*). We will make frequent use of some technical terms that are reviewed in Figure 13.1. For conduction of nerve impulses, the term "orthodromic" refers to the movement of impulses in the natural direction of the nerve (that is, from the cell body to the axon), whereas the term "antidromic" refers to the movement of impulses in the opposite direction (that is, from the axon to the cell body). Notice that the physical direction of movement is reversed in the efferent pathway as compared to the afferent pathway. For transport of practically everything else (including substances moving along the axon of a neuron), the terms "anterograde" and "retrograde" are used instead of *orthodromic* and *antidromic*. Anterograde transport refers to movement of substances from dendrites (or cell body) to the axon of a neuron, whereas retrograde transport refers to movement of substances from the axon to the cell body.

13.1.1 Anatomy

We saw in Chapter 11 that, in mammals, all photic input to the circadian system comes from the eyes. The eyes actually provide input to four different systems (Figure 13.2). Information destined to the visual system proceeds to the lateral geniculate nucleus of the thalamus (LGN), and from there to the visual cortex in the occipital lobe of the brain. Information used for the pupillary reflex proceeds to the pretectal area (PT). Information used for other eye reflexes (such as saccadic eye movements) proceeds to the superior colliculus in the midbrain (SC).[1,2] Finally, photic information destined to the circadian system (for entrainment and possibly also masking) proceeds mainly to the suprachiasmatic nucleus (SCN), although an alternate route through the thalamus also exists, as we will discuss shortly.[3,4]

Ganglion cells in the eyes make monosynaptic contact with the SCN through the *retino-hypothalamic tract* (RHT). Originally described in the rat,[5] the RHT has been characterized in various other studies in rats,[6–12] golden hamsters,[13–19] moles,[20] Nile grass rats,[21–24] squirrels,[25] tree shrews,[26,27] rabbits,[28] various species of monkeys,[27,29–31] and humans.[32] The various studies consistently indicate that the retinal fibers arrive predominantly in the ventral (core) region of the SCN.[5–7,12,21,23,26,28–30,32] As you will remember, visual pathways (like the pathways for most other senses) cross the body's midline as they ascend to the brain. The optic nerves cross at the optic chiasm, most fibers proceeding to the opposite (*contralateral*) side of the brain in rodents. The afference to the SCN, however, is consistently described as *bilateral*—with only a small bias toward the contralateral side—in mammals of various orders.[5,6,8,12,18,21,23,25,28–31] Bias toward *ipsilateral* afference has been described in tree shrews (*Tupaia belangeri*),[26] but the usual bias toward *contralateral* afference was described in a similar species (*Tupaia glis*).[27]

Neuroanatomists make use of special dyes to trace neural pathways in the central nervous system. Standard histological procedures that stain all neurons indiscriminately are of no use for the tracing of neural pathways because they reveal only a tangled mass of neurons. In order to visualize neural pathways, neuroanatomists use a few especially chosen substances that are picked up by nerve cells and transported along the axon. If the tissue is then stained to highlight the tracer, the neuroanatomist can actually see where the axon is going to or where it is coming from in sequential tissue slices. The recently developed technique of tissue preservation called CLARITY may soon allow anatomists to track neural pathways in a single tissue block, as CLARITY makes neural tissue become permeable to visible spectrum photons.[33]

A protein derived from the bean plant (*Phaseolus vulgaris* leucoagglutinin) is commonly used for *anterograde* tracing. For *retrograde* tracing, FluoroGold is the substance of choice these days. A third very popular substance is *cholera toxin subunit B* (CTB), which can be used for either anterograde or retrograde tracing. An example of the use of CTB for tracing the RHT is shown in Figure 13.3. CTB was injected in one eye and allowed to travel along the axons of retinal ganglion cells before the animal (a mouse) was killed so that its brain could be sliced and stained for microscopic examination. The side of the brain contralateral to the injected eye appears on the right side of the figure. Notice that CTB advanced through the optic chiasm and invaded both the contralateral and the ipsilateral SCN. Another example, this one in a tree shrew, is shown in Figure 13.4. The top panels (A and B) show brain sections at the region of the anterior SCN. The bottom panels (C and D) show sections at the region of the medial SCN. The sections on the left panels (A and C) were stained with cresyl violet to reveal the borders of the SCN (small arrowheads). The sections on the right panels (B and D) were treated for CTB immunohistochemistry. Injection of CTB in one eye resulted in bilateral staining of the SCN with slightly higher ipsilateral staining (right side of the sections). A third example, this one in a rat, is shown in Figure 13.5. CTB was injected in the

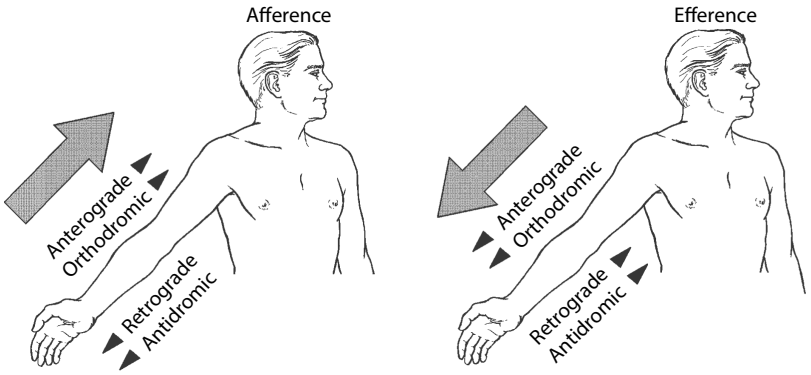


FIGURE 13.1 A few more technical terms. Afference refers to the flow of information from the periphery of the body to the central nervous system, whereas efference refers to the flow of information from the central nervous system to the periphery. Flow in the normal direction is called orthodromic (for nerve impulses) or anterograde (for everything else), whereas flow in a reverse direction is called antidromic (for nerve impulses) or retrograde (for everything else).

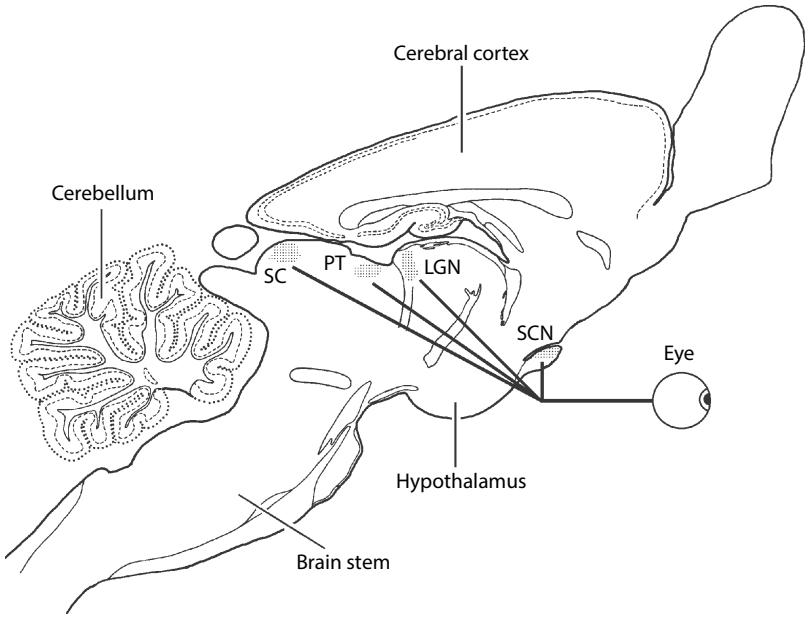


FIGURE 13.2 Nervous pathways from the eye to the brain. In mammals, retinal ganglion cells have four major projections to the brain, which correspond to the four sensory systems served by the eye. The visual afferent pathway goes through the lateral geniculate nucleus of the thalamus (LGN). The pupillary reflex relies on projection to the pretectal area (PT). Other eye reflexes are processed through the superior colliculus (SC). Photic afference for the circadian system goes directly to the suprachiasmatic nucleus in the hypothalamus (SCN) and indirectly through the thalamus (pathway not shown). (Data from Kelly, J.P., Visual system II: Anatomy of the central visual pathways, in: Kandel, E.R. and Schwartz, J.H., eds., *Principles of Neural Science*, Elsevier, New York, 1981, pp. 226–235; Card, J.P. and Moore, R.Y., The organization of visual circuits influencing the circadian activity of the suprachiasmatic nucleus, in: Klein, D.C. et al., *Suprachiasmatic Nucleus: The Mind's Clock*, Oxford University Press, New York, 1991, pp. 51–76; Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979.)

right eye (left side of the figure). Projection to the SCN was heavier to the left SCN (right side of the figure), but there was also strong ipsilateral projection. Contralaterally, the staining extended into the ventral subparaventricular zone ($_v$SPV), indicating unilateral innervation of this area. The brain slice was double stained for vasoactive intestinal polypeptide (VIP), and this is visible as sparse staining of the right $_v$SPV (left side of the figure) that corresponds to dorsal projections of the SCN (which will be discussed in Section 13.2).

Besides its direct retinal afference, the SCN receives photic information through an indirect pathway called the *geniculo-hypothalamic tract* (GHT). Within the LGN of the thalamus (the main destination of the visual afference) lies a small structure called the *intergeniculate leaflet* (IGL), which receives direct projection from the retina[7,8,11,12,17,18,25,26,34] and projects directly to the SCN.[35,36] The projection from the IGL to the SCN is the GHT. The pregeniculate nucleus in primates is equivalent to the IGL in rodents.[31]

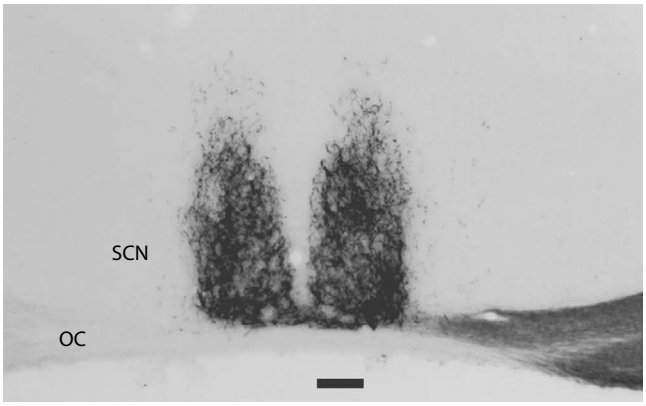


FIGURE 13.3 From the eye to the SCN: mouse. This microphotograph of the ventral region of a coronal section of the brain of a domestic mouse (*Mus musculus*) shows a typical result of anterograde tracing studies using cholera toxin subunit B (CTB). Injection of CTB in one eye results in staining of both the ipsilateral and the contralateral SCN (the right side of the section is the contralateral side). *Abbreviations:* OC, optic chiasm; SCN, suprachiasmatic nucleus. Scale bar: 100 μm. (Image courtesy of Daniela Lupi, Department of Visual Neuroscience, Imperial College London, London, U.K.)

Whereas the RHT and the GHT are the pathways of photic input to the SCN, the SCN must receive *nonphotic* input through other pathways. We know that information coming from all regions of the body ascends to the brain through a variety of nerves (Figure 13.6), but we do not know the exact pathways through which nonphotic information reaches the SCN. Candidates identified in functional studies will be described shortly. One pathway identified with CTB tracing is a projection from the arcuate nucleus (in the ventral hypothalamus caudal to the SCN) to the medial and dorsal SCN, which is thought to convey feeding-related information to the SCN.[37]

Detailed studies using both anterograde tracing to the SCN and retrograde tracing back from the SCN have mapped the SCN's afferent pathways in rats.[7] A summary of the findings is shown in Figure 13.7. In addition to the retina and IGL, the SCN receives afference from the limbic system, the hypothalamus, the pretectum, the paraventricular thalamus, and the raphe nuclei. The limbic system projects primarily to the dorsal SCN. The hypothalamus also projects mostly to the dorsal SCN but to various other brain sites as well. The pretectum projects mostly to the anterior hypothalamic area adjacent to the SCN and only sparsely to the SCN itself. The paraventricular portion of the thalamus projects to both the dorsal and ventral sections of the SCN, as well as to other structures dorsally. Finally, the raphe nuclei project primarily to the ventral SCN. The projections from the raphe nuclei (particularly the median raphe nucleus) to the SCN have been thoroughly investigated by a different group of researchers.[38]

13.1.2 Function

Of the seven sources of input to the SCN that were identified in anatomical studies (Figure 13.7), three have been shown to be functionally significant: the retina, the IGL,

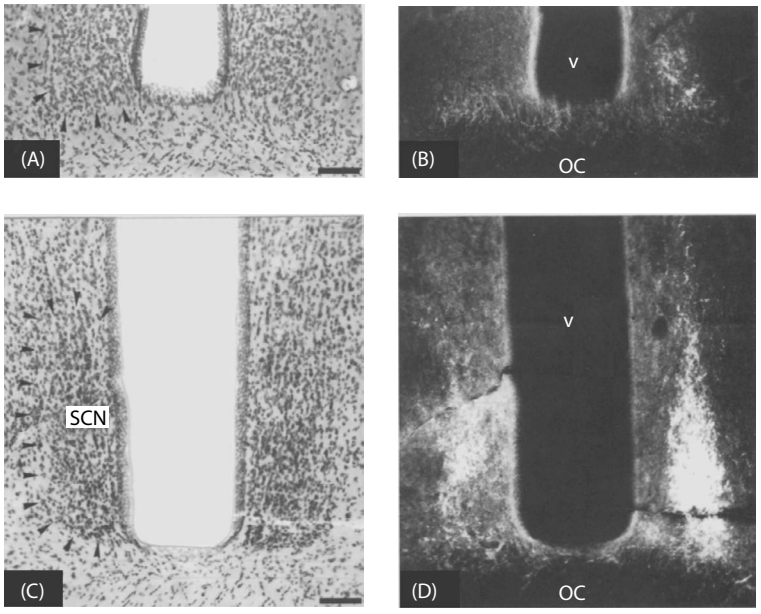


FIGURE 13.4 From the eye to the SCN: tree shrew. These microphotographs of the ventral region of coronal sections of the brain of a tree shrew (*Tupaia belangeri*) show the results of anterograde tracing studies using cholera toxin subunit B (CTB). The top panels (A and B) show sections from the anterior SCN. The bottom panels (C and D) show sections from the medial SCN. The sections on the left panels (A and C) were stained with cresyl violet to reveal the borders of the SCN (small arrowheads). The sections on the right panels (B and D) were treated for CTB immunohistochemistry. Injection of CTB in one eye resulted in bilateral staining of the SCN with slightly higher ipsilateral staining (right side of the sections). *Abbreviations:* OC, optic chiasm; SCN, suprachiasmatic nucleus; V, third ventricle. Scale bar: 100 μm. (Reproduced from *Brain Res.*, 874, Reuss, S. and Fuchs, E., Anterograde tracing of retinal afferents to the tree shrew hypothalamus and raphe, 66–74, Copyright 2000, with permission from Elsevier.)

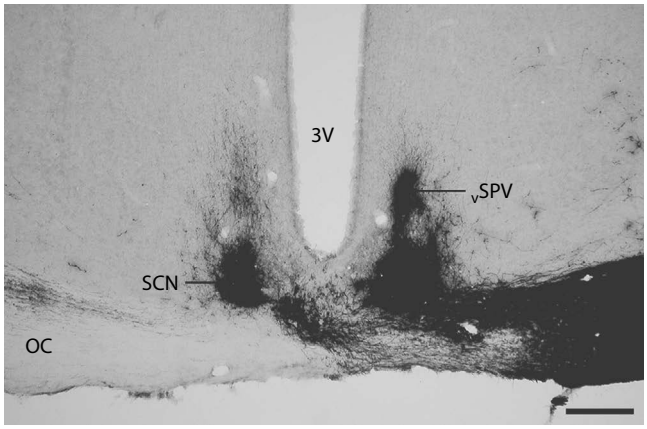


FIGURE 13.5 From the eye to the SCN: rat. This microphotograph of the ventral region of a coronal section of the brain of a laboratory rat (*Rattus norvegicus*) shows the result of an anterograde tracing study using cholera toxin subunit B (CTB). Injection of CTB in one eye resulted in bilateral staining of the SCN with slightly higher contralateral staining (right side of the section). Contralaterally, the staining extended into the $_{v}$SPV, indicating unilateral projection to this area. The section was double stained for VIP, and this is visible as sparse staining dorsal to the SCN on the left side of the section. *Abbreviations:* 3V, third ventricle; OC, optic chiasm; SCN, suprachiasmatic nucleus; $_{v}$SPV, ventral subparaventricular zone. Scale bar: 200 μm. (Reproduced from Gooley, J.J. et al., *J. Neurosci*., 23, 7093, Copyright 2003, Society for Neuroscience. With permission.)

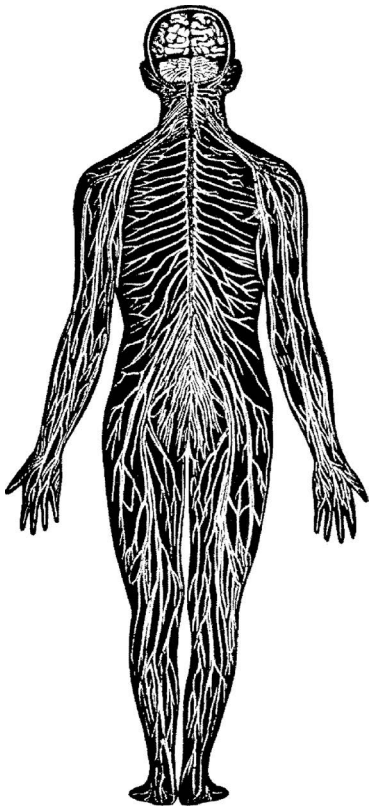


FIGURE 13.6 Nervous man. Information flows to and from the brain via a complex network of nerves. (Copyright ArtToday, Tucson, AZ.)

and the raphe nuclei. These functional connections correspond to the RHT, the GHT, and the raphe-hypothalamic pathway—all three of which terminate in the ventral SCN. The input–output arrangement of the SCN can be diagrammed as in Figure 13.8. The diagram does not include a fourth input pathway described in a single study in rats that documented SCN responses to stimulation of the paraventricular thalamus.[39]

Electrophysiological recording of neuronal activity in the SCN indicated that SCN cells respond to photic stimulation of the eyes[40–49] as well as to electrical stimulation of the optic nerve.[50–53] It has been shown that at least one out of four SCN cells responding to photic stimulation of the retina in mice is color sensitive, exhibiting blue-yellow antagonism.[54]

Figure 13.9 shows the response of SCN neurons to electrical stimulation of the optic nerve. Instead of traditional electrical recording, optical recording was conducted (that is, the investigators used a voltage-sensitive dye that changes the amount of light transmitted proportionally to the change in voltage in the neuron). When the optic nerve is electrically stimulated (S), a response of the SCN is observed within about 20 milliseconds. The response includes an early, small component due to conduction along myelinated fibers of the optic tract (OT) followed by a large component due to monosynaptic activation of SCN cells (SCN_1) and a third component due to synaptic activation of other neurons within the SCN (SCN_2).[55] The short latency of the response indicates that the afference is mediated by the direct RHT instead of by an indirect pathway such as the GHT. That the RHT is indeed the main pathway of photic information to the SCN was demonstrated in a study where minute knife cuts were used to selectively transect retinal axons innervating the SCN while sparing the projections to the midbrain and thalamus. Golden hamsters receiving such knife cuts lost the ability to be entrained by light–dark cycles.[14] Electrical stimulation of the optic nerve has also been shown to cause phase shifts of the rhythm of firing rate of rat SCN cells[56] as well as of the rhythm of locomotor activity of golden hamsters.[57] Genetically engineered atrophy of the optic nerve in mice prevented behavioral entrainment to light–dark cycles.[58]

The action potentials reaching the SCN through the RHT have been consistently found to increase the firing rate of the majority of responsive SCN cells in rats, mice, and hamsters.[40,41,43,45,46,48,49] In contrast, studies on degus (*Octodon degus*) found that the majority of responsive SCN cells were inhibited by impulses arriving through the RHT.[44,53] Because degus are crepuscular rather than nocturnal, it has been suggested that the RHT stimulates SCN cells in nocturnal animals and inhibits them in diurnal animals. Further studies are needed to confirm this generalization.

Many studies have made use of the Fos methodology (see Chapter 2) to investigate the SCN's photic afference. As you remember, Fos immunoreactivity is an index of cellular activity. Figure 13.10 exemplifies the increase in Fos immunoreactivity in the SCN and retina of naked-soled gerbils (*Taterillus petteri*) evoked by a 15-minute light pulse.

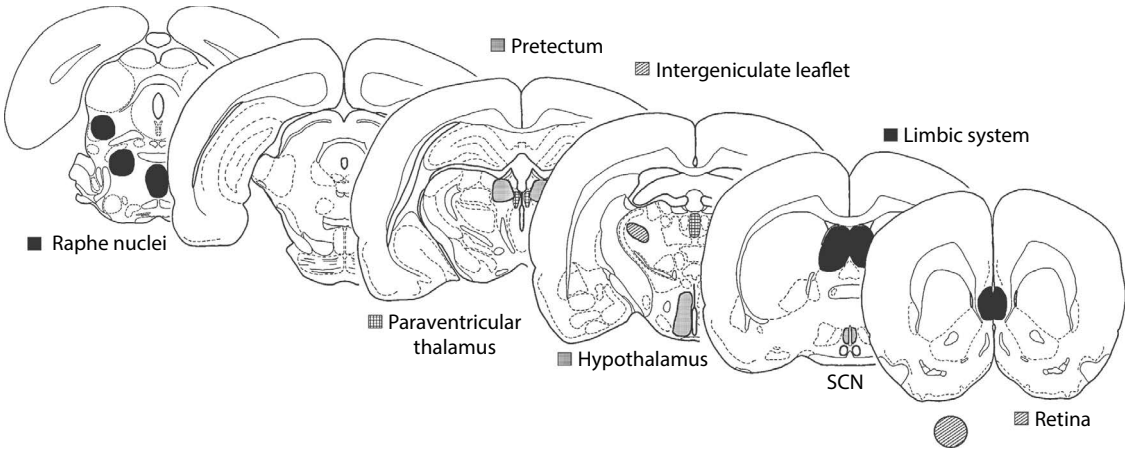


FIGURE 13.7 Brain afferents to the suprachiasmatic nucleus (SCN). The diagrams identify the seven major brain structures with terminal fields in the SCN of the laboratory rat (*Rattus norvegicus*). (Brain diagrams adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of structures after Moga, M.M. and Moore, R.Y., *J. Comp. Neurol.*, 389, 508, 1997.)

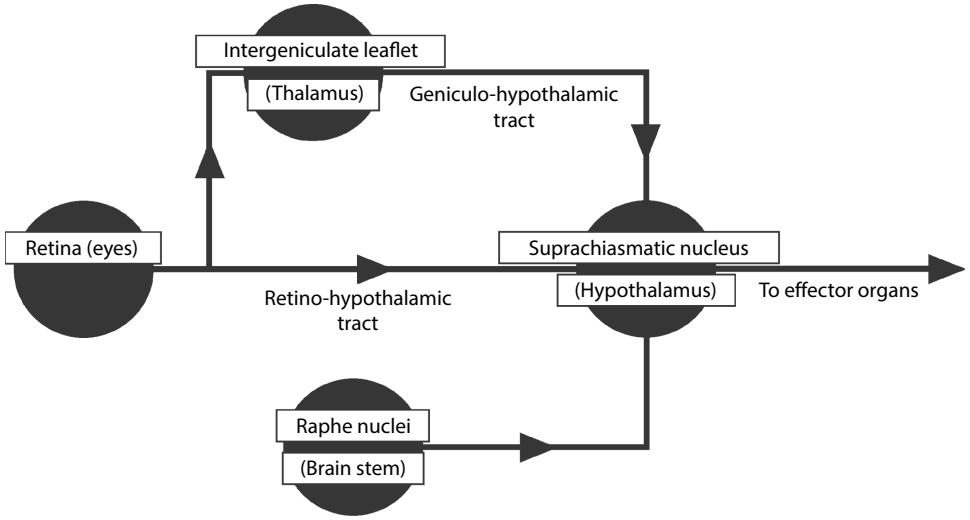


FIGURE 13.8 Functional afference to the circadian system. Functional studies have identified three main neural pathways to the suprachiasmatic nucleus: the retino-hypothalamic tract, the geniculo-hypothalamic tract, and a raphe-hypothalamic pathway.

The animals were killed, and their brains processed, 1 hour after the light pulse. Panels A and B show the brain and retina of control gerbils that received no light pulse. Panels C and D show the brain and retina of a gerbil that received the light pulse at circadian time sixteen (CT 16). Clearly, the light pulse elicited activity both in the SCN and in the retina. Light pulses presented to the eyes have been shown to evoke Fos expression in the SCN in numerous studies in various rodent species.[59–79] Most of these studies demonstrated that light-evoked Fos expression is phase dependent (i.e., that Fos expression is evoked only at particular circadian times, usually during subjective night) in accordance with the phase–response curve for behavioral phase shifts evoked by equivalent light pulses. In many—although not all—studies, the ventral region of the SCN was found to respond to photic stimulation of the eyes much more strongly than the dorsal region did.[59,60,63,67,69,72,74] This is in agreement with the anatomical data described earlier and contrasts with the intrinsic rhythmicity data presented in Chapter 12. That is, cells in the ventral (core) region tend *not* to be intrinsically rhythmic but to be responsive to remote photic stimulation, whereas cells in the dorsal (shell) region of the SCN tend to be intrinsically rhythmic but *not* to be responsive to remote photic stimulation.[67,80–82] One research team found that the dorsal region is actually inhibited by light pulses that activate the ventral region,[83,84] although the findings have not been confirmed in other laboratories.

The GHT has also been shown to be functionally involved in the afference to the SCN. In hamsters, electrical stimulation of the IGL (the origin of the GHT) was reported to cause phase-dependent phase shifts of the running-wheel activity rhythm,[85] although a later study showed that stimulation of the IGL may activate the SCN via the RHT (because of antidromic activation of the retino-genicular projection followed by orthodromic activation of the retino-suprachiasmatic projection).[86] Studies involving surgical ablation of the

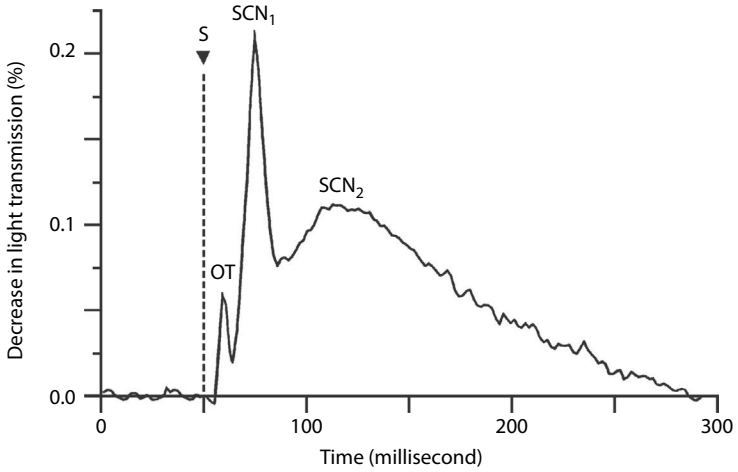


FIGURE 13.9 Recording afferent signals. The graph is a representative recording of neural activity in brain slices of golden hamsters (*Mesocricetus auratus*) with optic nerves attached. Optical, rather than electrical, recording was used (that is, the investigators used a voltage-sensitive dye that changes the amount of light transmitted as the voltage in the neuron changes). When the optic nerve is electrically stimulated (S), a response of the suprachiasmatic nucleus (SCN) is observed within about 20 ms. The response includes an early, small component due to conduction along myelinated fibers of the optic tract (OT), followed by a large component due to monosynaptic activation of SCN cells (SCN_1) and a third component due to synaptic activation of other neurons within the SCN (SCN_2). (Adapted from Senseman, D.M. and Rea, M.A., *Neuroimage*, 1, 247, 1994.)

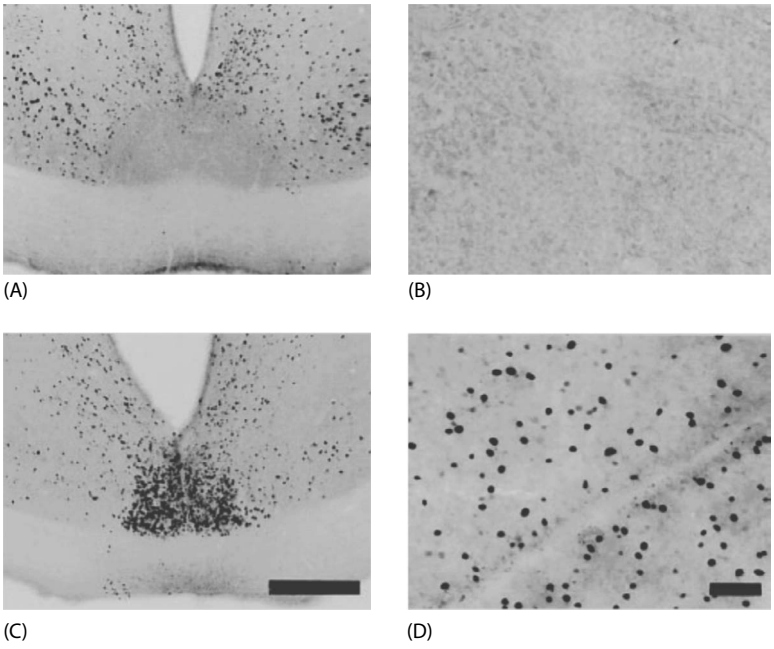


FIGURE 13.10 Photic stimulation induces Fos expression in the suprachiasmatic nucleus. These microphotographs show sections of the brain (left) and retina (right) of small naked-soled gerbils (*Taterillus petteri*) processed for Fos immunoreactivity. The top panels (A and B) refer to a control animal, whereas the bottom panels (C and D) refer to an animal that received a 15-minute light pulse at circadian time sixteen (CT 16). Both animals were killed at CT 17. Notice robust Fos immunoreactivity in the SCN and retina of the animal that received the light pulse. Scale bars: 200 μm (left) and 40 μm (right). (Reproduced from Dkhissi-Benyahya, O. et al., *J. Neurosci.*, 20, 7790, Copyright 2000, Society for Neuroscience. With permission.)

IGL did *not* provide evidence for a major role of the GHT in the photic afference to the SCN. Only small alterations in free-running period and responsiveness to light have been reported in animals with intergeniculate lesions.[87–94] The main effect of intergeniculate lesions seems to be a 50% reduction in the lengthening of circadian period caused by exposure to constant light in nocturnal rodents (that is, a lessening of Aschoff's rule).[95] In studies using the Fos methodology, light pulses were found to evoke Fos expression in the IGL, as they do in the SCN, except that the response was not phase dependent.[96–101] Conversely, nonphotic stimuli that cause phase shifts of behavioral rhythms did not evoke Fos

expression in the SCN, but did in the IGL.[102–104] This suggests that nonphotic afference to the SCN may travel through the GHT, whereas photic information travels primarily through the RHT. We shall return to this issue shortly.

13.1.3 Pharmacology

Much of the evidence for the involvement of the three pathways (RHT, GHT, and raphe-hypothalamic pathway) in the functional afference to the SCN comes from pharmacological studies. Four substances (three neurotransmitters and a hormone) have received particular attention: the excitatory amino acid *glutamate*, the pancreatic polypeptide called *neuropeptide Y*, the pervasive monoamine *serotonin* (also known as 5-hydroxytryptamine, or 5-HT), and the hormone *melatonin* (secreted primarily by the pineal gland). The structural formulas of these four substances are shown in Figure 13.11.

Glutamate seems to be the major neurotransmitter produced by RHT neurons (Figure 13.12). Optic nerve stimulation causes the release of glutamate from hypothalamic explants containing the SCN.[105] Stimulation of glutamatergic synapses by microinjection of glutamate or glutamatergic agonists has been shown to cause phase shifts both in the rhythm of neuronal activity of SCN cells *in vitro*[56,106–111] and in behavioral rhythms *in vivo*.[112–115] Glutamatergic stimulation mimics photic stimulation, in the sense that phase delays are evoked during early subjective night, phase advances are evoked during late subjective night, and no shifts are evoked during most of subjective day. Conversely, administration of glutamatergic antagonists suppresses the activation of SCN cells caused by electrical stimulation of the optic nerve *in vitro*[50,53,55,56] and attenuates behavioral phase shifts evoked by photic stimulation *in vivo*.[57,116,117] Figure 13.13 compares the phase–response curves for the resetting of the rhythm of locomotor activity of Nile grass rats caused by normal photic stimulation (Panel B)

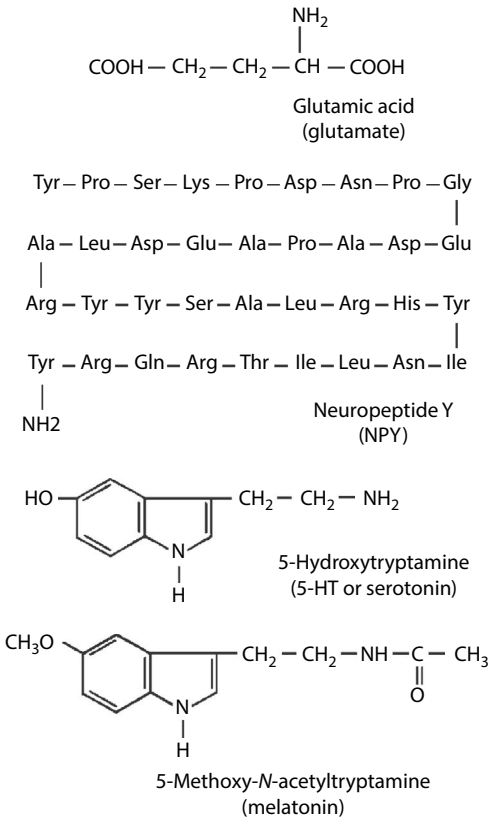


FIGURE 13.11 Main neurotransmitters in the afference to the suprachiasmatic nucleus (SCN). Four main chemicals are used by afferent pathways to the SCN. Three of them are neurotransmitters: glutamate, neuropeptide Y (NPY), and serotonin (5-HT). The fourth chemical is a hormone (melatonin). (Formulas from Feldman, R.S. and Quenzer, L.F., *Fundamentals of Neuropsychopharmacology*, Sinauer, Sunderland, MA, 1984; Cooper, J.R. et al., *The Biochemical Basis of Neuropharmacology*, 6th edn., Oxford University Press, New York, 1991.)

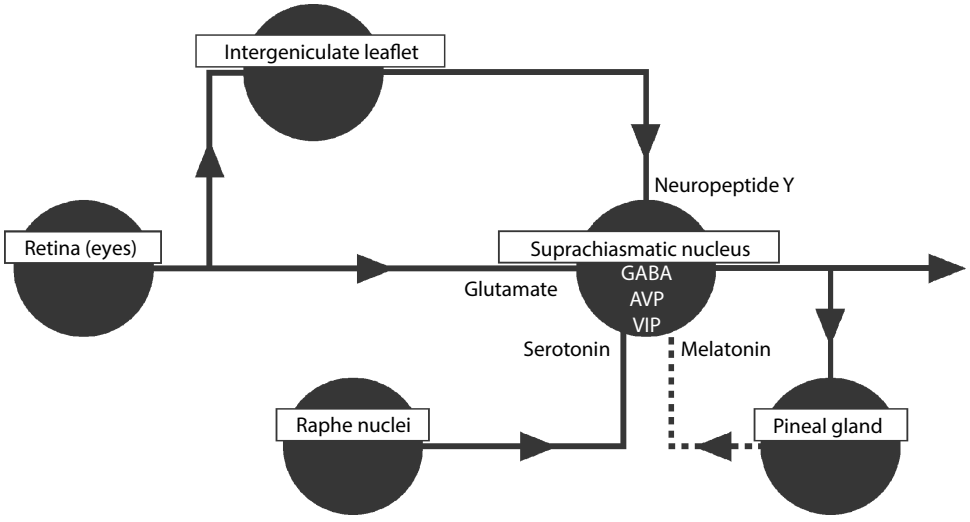


FIGURE 13.12 Neurochemistry of the suprachiasmatic nucleus (SCN). The diagram illustrates the four main afferent pathways to the SCN (as determined by functional studies) and the chemicals that they use for signal transmission. As detailed in Chapter 12, three main neurotransmitters are involved in signal transmission within the SCN: γ-aminobutyric acid (GABA), arginine vasopressin (AVP), and vasoactive intestinal polypeptide (VIP).

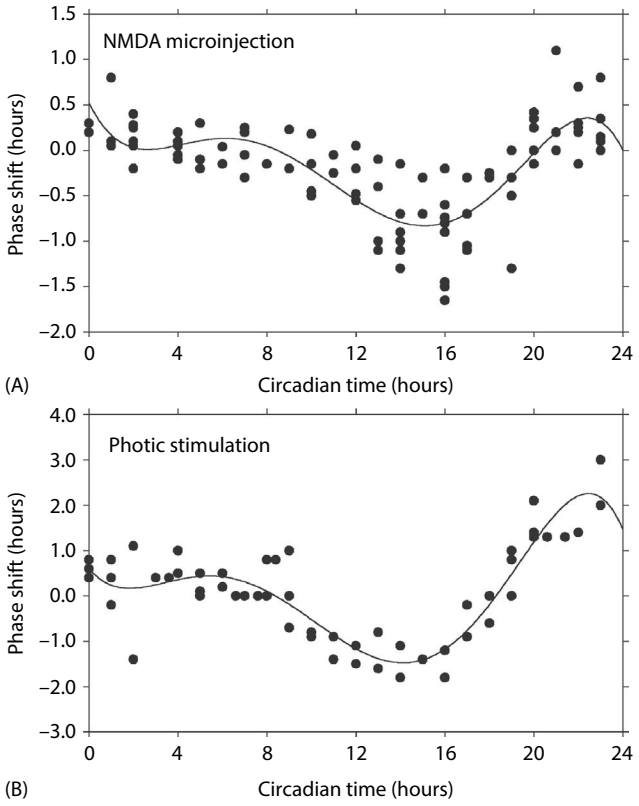


FIGURE 13.13 Glutamate and photic resetting. The graphs depict phase–response curves (PRCs) for the resetting of the rhythm of running-wheel activity of the Nile grass rat (*Arvicanthis niloticus*). Phase shifts were evoked either by microinjection of *N*-methyl-D-aspartate (NMDA, a glutamate agonist) into the suprachiasmatic nucleus (Panel A) or by the presentation of 4-hour-long light pulses (Panel B). Although the amplitude of the PRC for NMDA microinjection is smaller than that for photic stimulation, the shape of the two PRCs is very similar, which suggests that activation of glutamate receptors is the mechanism by which photic stimulation resets the circadian clock. (Data from Novak, C.M. and Albers, H.E., *Brain Res.*, 951, 255, 2002; Refinetti, R., *Acta Sci. Vet.*, 32, 1, 2004.)

and by microinjection of *N*-methyl-D-aspartate (NMDA, a glutamate agonist) into the SCN (Panel A). Although the amplitude of the phase–response curve for NMDA microinjection is smaller than that of the phase–response curve for photic stimulation (probably because of unequal doses), the shapes of the curves are practically identical in the two conditions, which reinforces the inference that glutamate is the neurotransmitter of the RHT.

Research conducted by Jens Hannibal (Figure 13.14) and his colleagues at the University of Copenhagen (in Denmark) has identified another neurotransmitter in the RHT: pituitary adenylate cyclase–activating polypeptide (PACAP). By performing double immunostaining for PACAP and CTB (the aforementioned anterograde tracer), the team found that PACAP-containing retinal ganglion cells project preferentially to the SCN in rats.[12,118] Furthermore, PACAP is coexpressed with melanopsin (the preferential circadian photopigment) in retinal ganglion cells of hamsters and humans.[119,120] PACAP



FIGURE 13.14 Jens Hannibal. This circadian physiologist from the University of Copenhagen (Denmark) is the leading researcher of the role of pituitary adenylate cyclase–activating polypeptide in the retino-hypothalamic tract. (Photo courtesy of Jens Hannibal.)

microinjected *in vivo* into the lateral ventricle of the hamster brain causes a small but significant phase shift of the behavioral activity rhythm.[119] The extent to which PACAP is actually involved in the operation of the circadian system is not clear, however. Another research group found that PACAP-deficient mice exhibit only minor impairment in circadian photic sensitivity.[121] A third group found that PACAP administration in the cerebral ventricles causes only modest phase shifts of the behavioral rhythm in mice, and the shifts can be suppressed by administration of MK-801 (a glutamatergic antagonist).[122] These latter results suggest that the small effect of PACAP may be exerted through glutamatergic receptors. An alternative explanation, as suggested by Hannibal's team, is that PACAP, acting through its preferential receptor (PAC1), modulates the action of glutamatergic circuits.[108] Thus, the action of PACAP would be to enhance or attenuate the signals traveling through the glutamatergic pathway rather than to actually convey the signals.

The neurotransmitter produced by GHT neurons seems to be *neuropeptide Y* (NPY). SCN cells have receptors for NPY,[15,94,123,124] and immunocytochemical studies have shown that NPY-containing cells from the IGL project to the ventral SCN.[21,36] Microinjection of NPY into the SCN (*in vivo* or *in vitro*) evokes phase shifts during subjective day.[125–129] Notice that this is the circadian phase when nonphotic stimuli cause behavioral phase shifts. Because NPY administration causes phase shifts in accordance with the *nonphotic* phase–response curve (in contrast to glutamate injections, which cause phase shifts in accordance with the photic phase–response curve), it is possible that the GHT serves as the terminal link in the pathway responsible for entrainment by

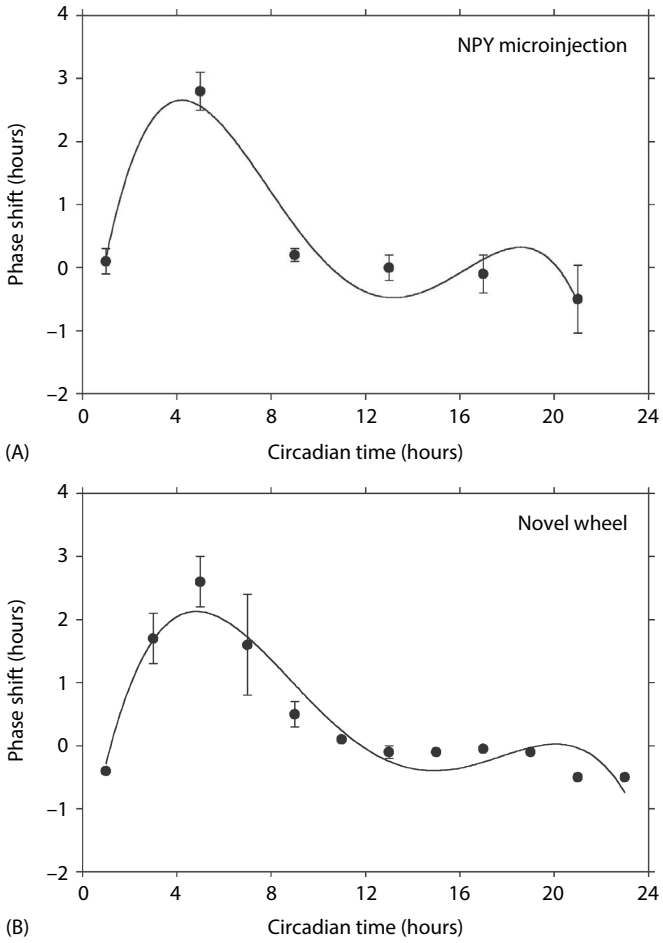


FIGURE 13.15 Neuropeptide Y (NPY) and nonphotic resetting. The graphs depict phase–response curves (PRCs) for the resetting of the rhythm of running-wheel activity of the golden hamster (*Mesocricetus auratus*). Phase shifts were evoked either by microinjection of NPY into the suprachiasmatic nucleus (Panel A) or by 3-hour-long access to a novel wheel (Panel B). The two PRCs are very similar, which suggests that activation of NPY receptors is the mechanism by which nonphotic stimulation resets the circadian clock. (Data from Biello, S.M. and Mrosovsky, N., *J. Biol. Rhythms*, 11, 27, 1996; Mrosovsky, N. et al., *J. Biol. Rhythms*, 7, 41, 1992.)

nonphotic stimuli. Figure 13.15 compares the phase–response curves for the resetting of the rhythm of locomotor activity of golden hamsters caused by exercise in a novel wheel (Panel B) or by microinjection of NPY into the SCN (Panel A). The shapes of the two phase–response curves are very similar, which reinforces the inference that NPY (released from the terminals of the GHT) may be the neurotransmitter associated with nonphotic resetting. In addition, you may remember from Chapter 8 that nonphotic stimuli presented during subjective night can suppress phase shifts evoked by light pulses. Accordingly, NPY administration blocks phase shifts evoked by photic or glutamatergic stimulation *in vivo* and *in vitro*.[109,115,123,130] Thus, NPY (through the GHT) may act by modulating the operation of the RHT.

The third major afferent pathway to the SCN, the *raphe-hypothalamic pathway*, may also be involved in the communication of nonphotic signals (Figure 13.12). This afference is part of the serotonergic pathways that originate in the raphe nuclei and project to various parts of the brain, including the SCN. Immunocytochemical studies have identified a dense network of serotonergic terminals, as well as specific serotonergic receptors, in the ventral SCN.[131–138] Accordingly, electrical stimulation of the raphe nuclei increases serotonin release in the SCN.[139] Administration of serotonin or serotonergic agonists evokes phase shifts during subjective day both *in vivo*[128,140–145] and *in vitro*.[133,146] Figure 13.16 shows the phase–response curve for the response of SCN cells to brief pulses of serotonin. The study was conducted in slices of mouse brain, and the serotonin pulses were administered by transient addition of serotonin to the medium. Phase advances in the rhythm of firing rate were observed during the middle of subjective day. Thus, serotonergic activation causes phase shifts according to the nonphotic phase–response curve, and the raphe-hypothalamic pathway might be a terminal link in the pathway responsible for entrainment by nonphotic stimuli. Against this possibility is the finding that four different serotonergic antagonists failed to block phase shifts caused by nonphotic stimuli in golden hamsters.[147] On the other hand, there is considerable evidence that the raphe-hypothalamic pathway can modulate activity in the RHT. Serotonergic agonists have been found to attenuate[148,149] or enhance[150,151] behavioral phase shifts evoked by photic stimuli. Serotonergic antagonists have been found to potentiate photically evoked shifts[152,153] and to prevent the attenuation of photically evoked shifts caused by

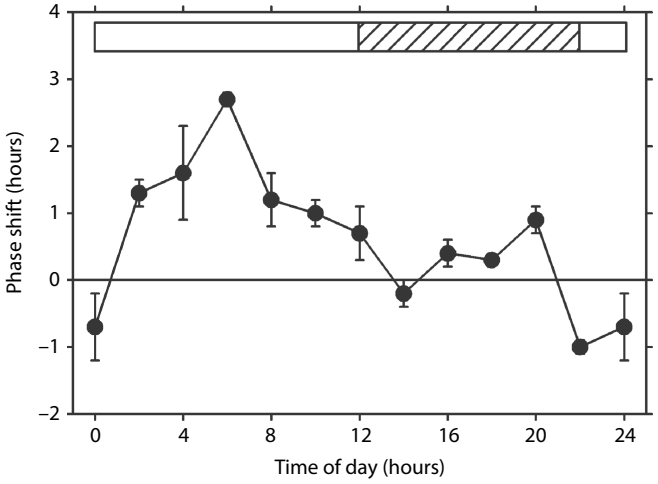


FIGURE 13.16 Serotonin and phase resetting *in vitro*. The graph depicts a phase–response curve for the resetting of the rhythm of electrical activity of single cells in cultured brain slices from domestic mice (*Mus musculus*). Phase shifts were evoked by addition of serotonin (10 μM) to the culture medium for 10 minutes. The light and dark phases of the light–dark cycle to which the animals were exposed before being euthanized are indicated by the horizontal light and hatched bars at the top of the graph. Each data point corresponds to the mean (± SE) of 3 cells. Serotonin pulses clearly evoke phase advances during the middle of subjective day and modest phase delays during early subjective day. (Data from Prosser, R.A., *Brain Res.*, 966, 110, 2003.)

simultaneous nonphotic stimulation.[154] Serotonergic activation *in vitro* was found to reduce the response of SCN cells to optic nerve stimulation.[55,134] Various other effects of serotonergic activation on photic input have been reported as well.[155–158] Overall, the data suggest that the raphe-hypothalamic pathway modulates activity in the RHT and that this modulation is dependent on nonphotic stimulation. However, the raphe-hypothalamic pathway is not necessarily responsible for the signals involved in nonphotic entrainment.

Figure 13.17 summarizes the actions of the three neurotransmitters discussed so far. During subjective night, glutamate released from the RHT is responsible for the process that leads to phase delays and phase advances evoked by photic stimulation. At this phase of the circadian cycle, both NPY (released from the GHT) and serotonin (released from the raphe-hypothalamic pathway) inhibit photically induced phase shifts. During subjective day, when glutamate does not mediate phase shifts, NPY (and perhaps serotonin) can effect phase shifts evoked by nonphotic stimuli. At this phase of the circadian cycle, activation of the RHT by photic stimulation inhibits nonphotically induced phase shifts mediated by NPY or serotonin.

Melatonin (which should not be confused with *melanopsin*) is also a major player in the afference to the SCN (Figure 13.12). The SCN modulates melatonin secretion by the pineal gland (see Section 13.2), and melatonin feedbacks on the SCN via the bloodstream. Figure 13.18 shows that the circadian system of the rat can be entrained by timed infusion of melatonin. The animal was maintained in constant darkness and was infused with melatonin for 1 hour each day through a subcutaneous catheter. Notice that the running-wheel activity rhythm was freerunning before the infusion protocol was initiated. The rhythm entrained to the zeitgeber a few weeks after the protocol was initiated, and it freeran from the appropriate phase when the infusion protocol was discontinued. Although a few researchers have failed to demonstrate entrainment by timed administration of melatonin,[159–162] entrainment has been documented in amphibians,[163] birds,[164–168] rodents,[169–179] monkeys,[180,181] and humans.[182–187] Presence of the pineal gland was found not to be required for the entraining effect of exogenous melatonin,[165,166,169,172,174,176] and immunocytochemical and autoradiographical studies demonstrated the presence of melatonin receptors in the SCN.[188–192] Various studies documented also phase shifts evoked by discrete pulses of melatonin administration,[193–202] and the phase–response curve was found to be of the nonphotic type (that is, with phase advances during subjective day).[193,194,197]

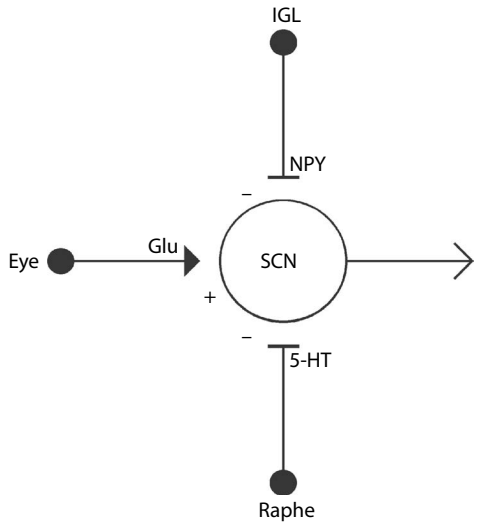


FIGURE 13.17 Neurotransmitters in action. This diagram summarizes the action of the three main neurotransmitters involved in the afferent pathways to the suprachiasmatic nucleus. See text for details. *Abbreviations:* 5-HT, serotonin; Glu, glutamate; IGL, intergeniculate leaflet; NPY, neuropeptide Y.

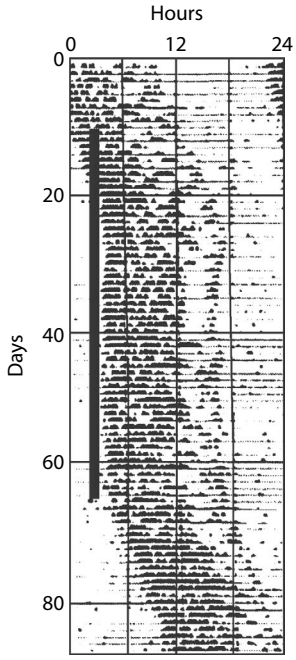


FIGURE 13.18 Melatonin and phase resetting *in vivo*. This actogram shows the rhythm of running-wheel activity of a laboratory rat (*Rattus norvegicus*) maintained in constant darkness and infused with melatonin subcutaneously for 1 hour each day (thick vertical bar). Notice that the daily cycle of melatonin administration entrained the activity rhythm. When the daily infusions stopped, the rhythm freeran from the appropriate phase. (Adapted from Slotten, H.A. et al., *Brain Res.*, 876, 10, 2000.)

Hormones aside from melatonin have been shown to affect circadian rhythmicity, although much less is known about their mechanisms of action in the circadian system. Small alterations in free-running period and circadian photic sensitivity have been reported in animals deprived of, or overstimulated by, reproductive hormones,[203–206] thyroid hormones,[207–209] corticosterone,[210] leptin,[211] and other hormones. Additionally, several neurotransmitters that have not been mentioned yet have been implicated in the afference to the circadian system. They include enkephaline,[212–214] histamine,[215,216] adenosine,[217,218] substance P,[56] nociceptin,[111] and norepinephrine.[219] In particular, *acetylcholine* (the main neurotransmitter of the peripheral nervous system that is also found abundantly in the central nervous system) has been shown to mimic the

phase-shifting effect of light pulses when administered into the cerebral ventricles or directly into the SCN. More precisely, administration of the cholinergic agonist *carbachol* causes phase shifts according to the photic phase–response curve,[220–224] and treatment with a cholinergic toxin attenuates photically induced phase shifts.[225] As was the case for neuropeptide Y and serotonin, acetylcholine seems to modulate the action of glutamate, as NMDA receptor antagonists block phase shifts induced by carbachol.[226]

13.1.4 Molecular Mechanisms

Most organisms (including invertebrate animals) are small enough to be translucent. This means that light can act directly on cellular processes of the molecular clock. Even in animals with fully developed visual systems, such as the fruit fly, the circadian system does not require specialized afferent pathways. We discussed the molecular mechanism of circadian rhythmicity in Chapter 12, and the diagram in Figure 13.19 shows how the mechanism is affected by light in the fruit fly. You will remember that the PER–TIM complex normally inhibits the CLK–CYC complex in the negative arm of the circadian loop. When TIM was identified in 1996, researchers already noticed that light exposure degrades TIM and decreases its association with PER.[227,228] Soon afterward, CRY (cryptochrome) was identified in the fruit fly and was found to be a functioning photoreceptor (regulated by light at the translational or posttranslational level) that blocks the function of the PER–TIM complex by pairing with TIM.[229–232] Thus, light can phase shift the pacemaker by facilitating the binding of CRY to TIM, which prevents the PER–TIM complex from inhibiting the CLK–CYC complex, thus allowing CLK–CYC to continue stimulating the transcription of *per* and *tim* for longer (Figure 13.19). An additional protein, JET (the product of the *jetlag* gene), seems to mediate the degradation of TIM.[233]

The fruit fly's circadian system receives photic information also through the eyes.[234] As a matter of fact, the fruit fly has three to five different photic afferent pathways.[235,236] Although these pathways seem to affect different aspects of photic entrainment, only the molecular mechanisms of the CRY pathway (described earlier) are known in detail at this time. In contrast, plants and fungi have no eyes, and mammals

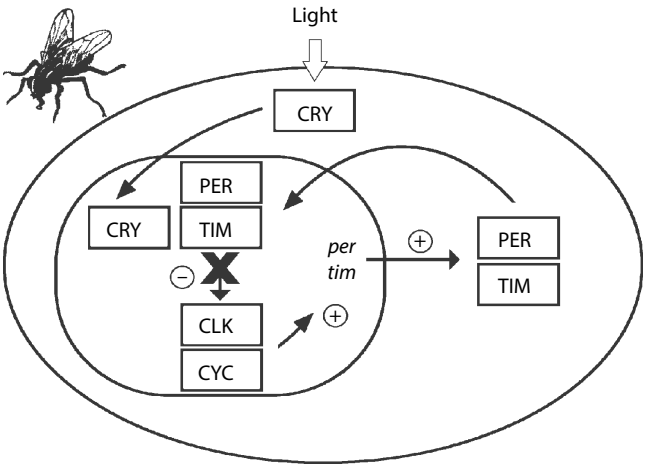


FIGURE 13.19 Molecular mechanism of photic resetting in the fruit fly. This simplified diagram illustrates the molecular mechanism of photic resetting in the fruit fly. Light promotes the binding of CRY with TIM. This reduces the inhibitory action of the PER–TIM dimer on CLK–CYC, which alters the timing of activation of the genes *per* and *tim*. (Data from Williams, J.A. and Sehgal, A., *Annu. Rev. Physiol.*, 63, 729, 2001; Devlin, P.F. and Kay, S.A., *Annu. Rev. Physiol.*, 63, 677, 2001.)

can perceive light only through the eyes, as was discussed in Chapter 11. In bacteria, where the molecular clock can run without gene translation and transcription, light can synchronize the clock by changes in the ATP/ADP ratio, which is a sufficient process in these obligate phototrophs that change the rate of ATP consumption when the lights are turned off or on.[237]

Figure 13.20 summarizes the molecular mechanisms of photic entrainment in plants, fungi, flies, and mice.[238] In plants (the mustard plant, *Arabidopsis thaliana*), a family of four red-light-activated phytochromes (PHY) and two blue-light-activated cryptochromes (CRY) act as photoreceptors that affect the clock loop by inhibiting the transcription of *cca* and *lhy*.[239] In fungi (bread mold, *Neurospora crassa*), it was observed early on that light interrupts the *frq* negative autoregulation[240] and that *wc-1* is needed for photoinduction.[241] A few years later, it was discovered that WC-1 is a blue-light photoreceptor that modulates photic input to the clock by binding (along with WC-2) to the *frq* promoter.[242,243] Thus, the

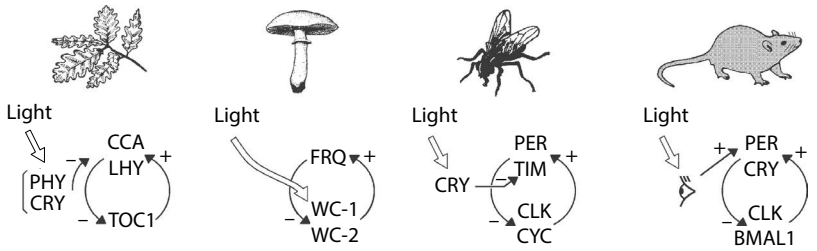


FIGURE 13.20 Molecular mechanisms of photic resetting in plants, fungi, and animals. These diagrams illustrate the basic mechanisms of photic resetting in plants, fungi, invertebrate animals, and vertebrate animals. See text for details. (Data from Schultz, T.F. and Kay, S.A., *Science*, 301, 326, 2003; Devlin, P.F. and Kay, S.A., *Annu. Rev. Physiol.*, 63, 677, 2001; Reppert, S.M. and Weaver, D.R., *Annu. Rev. Physiol.*, 63, 647, 2001.)

Neurospora clock is a neat clock where light acts directly on one of the main components of the feedback loop.

Also shown in Figure 13.20 is the molecular mechanism of photic resetting in mammals (the mouse, *Mus musculus*). Light is sensed in the eye, and the information is sent to the SCN via the RHT. The release of glutamate in the terminals of the RHT initiates a process that apparently involves calcium/calmodulin kinase and mitogen-activated protein kinase[244] and that eventually increases *per* transcription in the nucleus of SCN cells. The resulting transient overexpression of *per* alters the duration of the clock loop, thus effecting a phase shift. Of course, the process is actually much more complex because, as we saw in Chapter 12, there are three different versions of PER. When the mouse *per* genes were first identified, it was already apparent that *per1* and *per2*, but not *per3*, are overexpressed in SCN cells following photic stimulation during subjective night.[245–253] This was later found to be true also in laboratory rats, golden hamsters, grass rats, and molerats.[254–257] Research in mice, conducted by the research team led by Rae Silver at Columbia University (in New York), has revealed an intriguing scheme of the interplay of *per1* and *per2* in the generation of phase advances and phase delays.[258,259]

As diagrammed in Figure 13.21, Silver's team found that photic stimulation of the eyes induces *per1* expression, but not *per2* expression, in the ventral SCN at any time of the day. Thus, *per1* expression in the ventral SCN reflects merely the processing of photic information regardless of its impact on the circadian system. Photic stimulation during late subjective night (which causes *phase advances* of the pacemaker) induces *per1* expression in the *dorsal* SCN (in addition to the circadian-unrelated expression in the ventral SCN). Photic stimulation during early subjective night (which causes *phase delays* of the pacemaker) induces *per2* expression in the dorsal (and ventral) SCN. To avoid confusion, I must point out that this pattern of activation is given in relative terms. We saw in Chapter 12 that the *per* genes are expressed rhythmically in the SCN, the expression being higher during subjective day than during subjective night. The pattern of activation indicated in Figure 13.21 refers to *increases* in gene expression above the baseline (i.e., above the expression levels in constant darkness), not to absolute expression levels. Associations between *per1* expression and phase advances and between *per2* expression and phase delays were observed also by a different research team.[260] What is particularly noteworthy about Silver's study is that the expression pattern of *per1* and *per2* is different in the two subdivisions of the SCN. Although it is well known that different tissues express different genes, circadian physiologists had generally assumed that the basic mechanism of the circadian clock can be described within a single cell. Whereas this must necessarily be true in unicellular organisms, Silver's study shows that it is not true in mammals. We saw in Chapter 12 that individual SCN cells are intrinsically rhythmic but that intercellular communication is necessary for the coherent operation of the circadian pacemaker. Evidently, resetting of the pacemaker requires not only a network of cells but also the differentiation of two networks of cells.

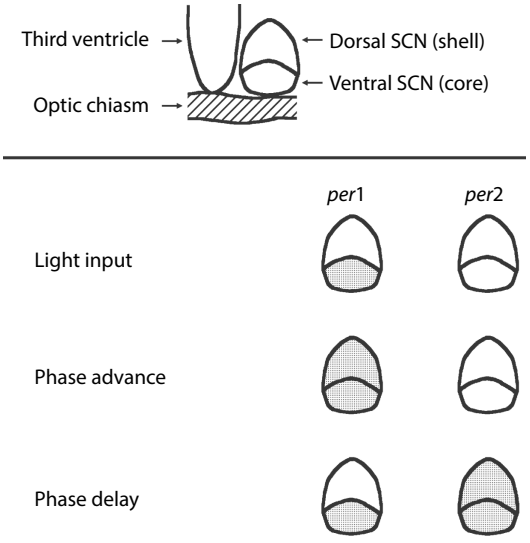


FIGURE 13.21 Molecular differentiation of phase advances and phase delays. These diagrams illustrate the finding that in the domestic mouse (*Mus musculus*), photic stimulation affects differently the expression of the genes *per1* and *per2* in different subdivisions of the suprachiasmatic nucleus (SCN). Photic stimulation, regardless of circadian phase, leads to enhanced *per1* expression in the ventral SCN. Photic stimulation that evokes phase advances leads to enhanced *per1* expression in the dorsal SCN. Photic stimulation that evokes phase delays leads to enhanced *per2* expression in both the ventral and dorsal SCNs. (Data from Yan, L. and Silver, R., *Eur. J. Neurosci.*, 16, 1531, 2002.)

Several attempts have been made to foster understanding of the molecular mechanisms of photic resetting by the use of mathematical models. A schematic representation of a model developed by Jaap van Pelt and colleagues at the Netherlands Institute for Brain Research (in Amsterdam) in 1999 is shown in Figure 13.22. At that time, most of the details about the feedback loop of the fruit fly's molecular clock were not known and, therefore, the model assumes the existence of a

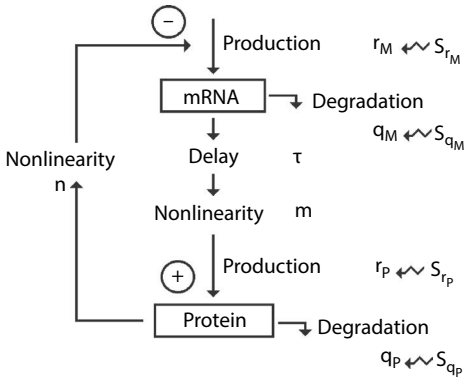


FIGURE 13.22 An early molecular model of the circadian clock. This diagram describes a simple mathematical model of the molecular mechanism of the circadian clock, including phase resetting by an environmental stimulus (S). The model assumes the involvement of only one gene and its protein, an unknown delay mechanism (τ), and unspecified sources of nonlinearity (n and m). (Adapted from Olde Scheper, T. et al., *J. Neurosci.*, 19, 40, 1999.)

single-gene loop (such as *per*). Various parameters of gene transcription and translation (*r*), degradation (*q*), and photic stimulation (*S*) are included. Considering the simplicity of the model, excellent results were obtained in the simulation of freeruns and phase shifts.[261] Today we know that light stimulates the equivalent of parameter r_M in the model (that is, light removes the PER–TIM-mediated repression of *per*). The photic phase–response curve generated by the model under this assumption (Figure 13.23, Panel A) does not closely approximate the actual phase–response curve of the fruit fly (Panel C). A better approximation is obtained by inhibition of parameter r_P (Panel B), but it has little biological significance. Evidently, a more sophisticated model is needed. Various models have been developed,[262–269] and one that deserves special mention is the one proposed by Daniel Forger and Charles Peskin (from New York University) in 2003.[270] This model, proposed as a detailed predictive model of the mammalian circadian clock, has 74 variables corresponding to various chemical reactions in an SCN cell of the mouse. The model generates impressively good predictions of daily oscillation in the intracellular concentration of circadian clock proteins, as exemplified in Figure 13.24. The system has an autonomous period of 24.3 hours and can be entrained by a 24-hour light–dark cycle through the modulation of PER1 and PER2 abundance. The issue of how the multiple proteins interact to ultimately drive organismal rhythms is not addressed by the model.

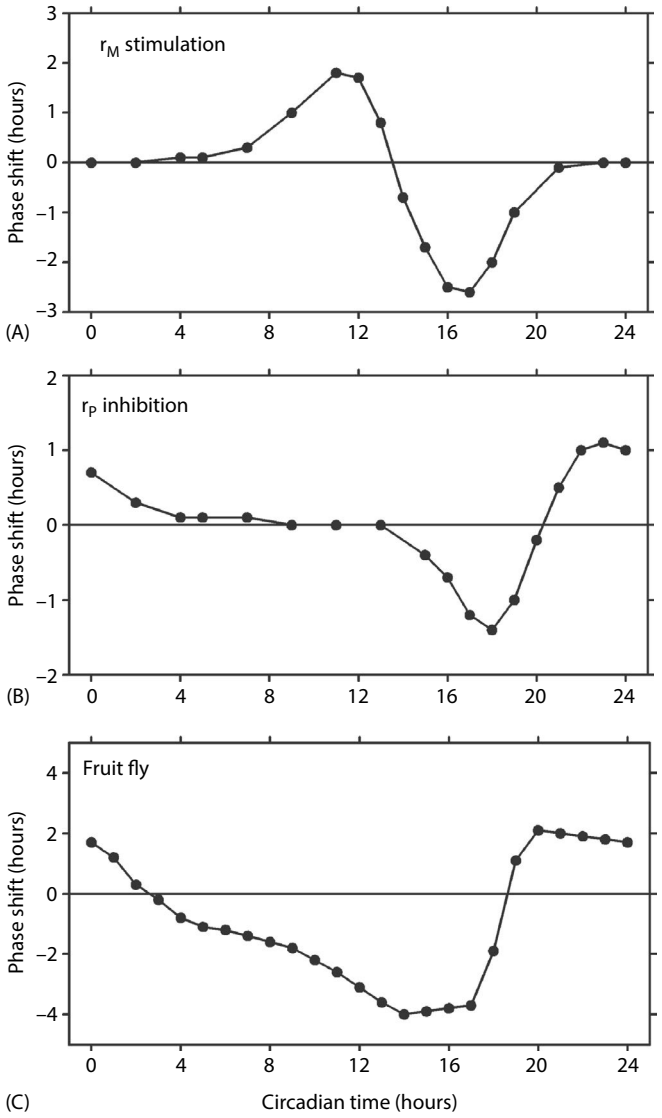


FIGURE 13.23 Testing the model. The graphs show two simulations of the photic phase–response curve of the fruit fly based on the model described in Figure 13.22 and a phase–response curve obtained from actual flies (as averaged from two independent studies). Notice that the simulation based on r_M stimulation is very unsatisfactory. The simulation based on r_P inhibition is better but not very accurate. (r_M and r_P data from Olde Scheper, T. et al., *J. Neurosci.*, 19, 40, 1999; Fruit fly data from Myers, M.P. et al., *Science*, 271, 1736, 1996; Rothenfluh, A. et al., *Genetics*, 156, 665, 2000.)

13.2 EFFERENT PATHWAYS

A circadian pacemaker and its afferent pathways would be of very little use if there were no efferent pathways to take the pacemaker's commands to the various organs in the body. Until recently, much less was known about circadian efferent mechanisms than about sensory processes and afferent mechanisms. In the last 15 years, considerable knowledge has been gained. In this section, we will review the available knowledge about circadian efferent mechanisms.

13.2.1 Anatomy

Ultimately, efferent organs are either *muscles* or *glands*—or, in the case of organisms that have a developed central nervous system, the various sections of the *brain* that generate thought, emotions, awareness, and so on (Table 13.1). The circadian system communicates with these organs in the same way that other physiological systems do—that is, through central nerve tracts or through peripheral nerves (although local diffusible substances may also be used, as we will see later). In mammals and many other vertebrates, peripheral nerves are either part of the *somatic nervous system*, which controls striated muscles responsible for movement of body parts, or of the *autonomic nervous system*, which controls smooth muscles, cardiac muscle, and glands responsible for vegetative functions[1,2] (Figure 13.25). Fibers from the *somatic* motor system usually originate in the cerebral cortex (the origination depicted in the figure) or in the cerebellar cortex and proceed directly (in the case of the so-called pyramidal system) or indirectly (in the case of the extrapyramidal system) to the spinal cord (or brain stem). There, the fibers make synaptic contact with somatic motor neurons, which innervate striated muscles. Fibers from the *autonomic* motor system usually originate in the hypothalamus (the origination depicted in the figure), the limbic system, or the prefrontal area. They make synaptic contact with preganglionic neurons in the spinal cord (or brain stem), which send fibers to autonomic ganglia. The postganglionic neurons innervate smooth muscles and glands.

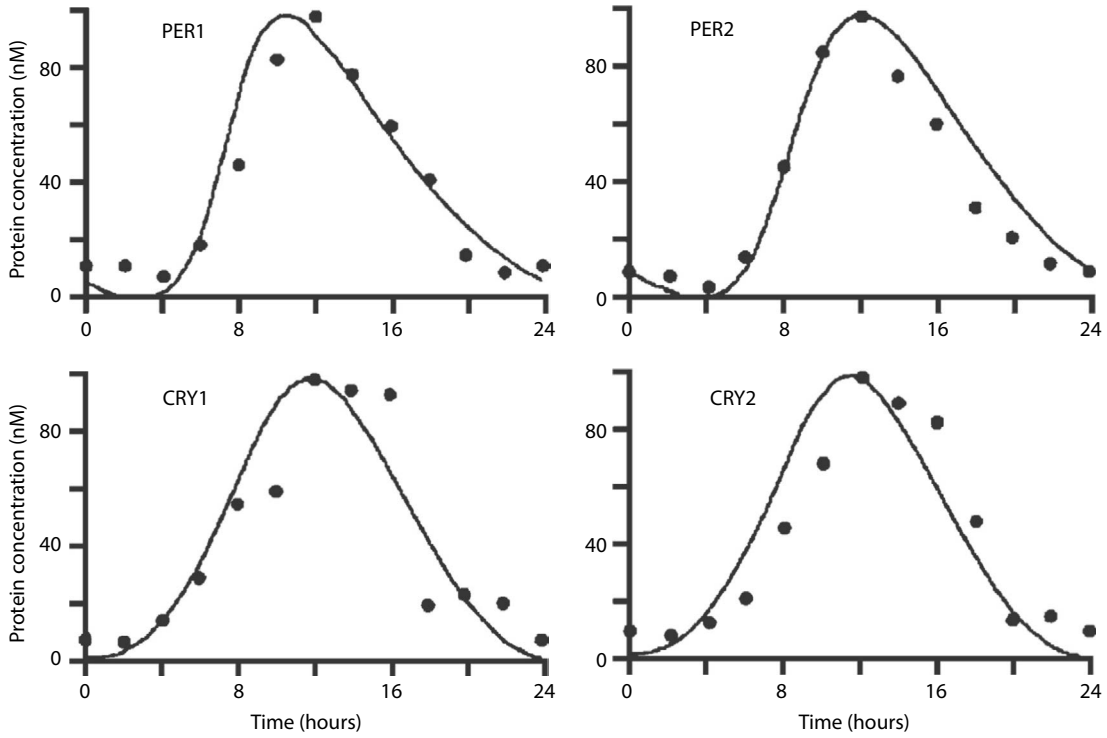


FIGURE 13.24 A later molecular model of the circadian clock. The graphs illustrate the performance of a model of the circadian clock with 74 variables corresponding to chemical reactions in a cell of the suprachiasmatic nucleus, including processes of translation, transcription, degradation, and dimerization. The curves are generated by the model. The data points correspond to actual measurements in real animals. Notice that the model generates very good predictions of daily oscillation in the concentration of circadian clock proteins. (Adapted from Forger, D.B. and Peskin, C.S., *Proc. Natl. Acad. Sci. USA*, 100, 14806, 2003.)

Table 13.1
Mammalian Effector Organs

Organ	Function	Example
Muscles		
Striated muscles	Control of voluntary movement	Biceps muscle (arm flexing)
Smooth muscles	Control of visceral movement	Intestinal muscle (peristalsis)
Cardiac muscle	Heart beating	Heart (blood circulation)
Glands		
Endocrine glands	Secretion of hormones	Pancreas (insulin)
Exocrine glands	Secretion of fluids	Sweat glands (sweat)
Brain[a]		
Limbic system	Emotions	Happiness, sadness
Reticular formation	Awareness	Wake, sleep
Hypothalamus	Appetite, sex drive	Hunger, lust
Cerebral cortex	Thought	$E = m \cdot c^2$

[a] The brain organs shown in the table are illustrative only. They are not the only parts of the brain that perform the described functions, and the described functions are only a small fraction of the functions performed by the brain. Also, it is arguable whether mammals other than humans exhibit thought and emotions.

The autonomic nervous system is traditionally subdivided into the *sympathetic nervous system* and the *parasympathetic nervous system* (Figure 13.26). Most organs are innervated by both systems, but some are not. For instance, the pineal gland has exclusively sympathetic innervation. The sympathetic nervous system is generally involved in energy-spending processes (elevated heart rate, increased blood flow to skeletal muscles, stimulation of adrenal gland, etc.), whereas the parasympathetic nervous system is involved in restorative processes (reduced heart rate, active salivation, increased blood flow to gastrointestinal system, etc.). Both systems use acetylcholine (through nicotinic receptors) as the neurotransmitter at the ganglionic synapses, but the sympathetic nervous system generally uses norepinephrine at the postganglionic synapses, whereas the parasympathetic nervous system uses acetylcholine (through muscarinic receptors) at the postganglionic synapses. The sympathetic ganglia are generally arranged in a row of ganglia parallel to the spinal cord, whereas the parasympathetic ganglia are located close to each target organ. An additional difference between the two systems is that sympathetic fibers leave the spinal cord at the thoracic or lumbar levels, whereas parasympathetic fibers leave the central nervous system at the cranial or sacral levels.

How the SCN connects to the somatic and autonomic nervous systems is not thoroughly known. As we will discuss

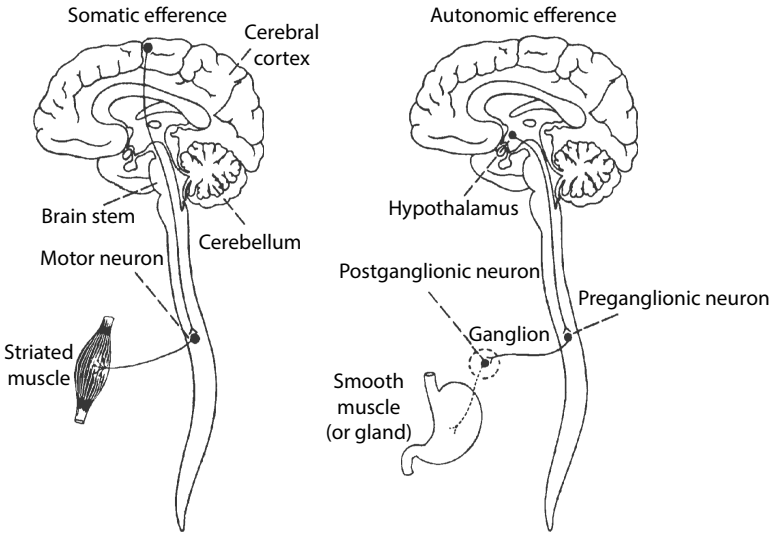


FIGURE 13.25 Efference. The major efferent pathways in higher vertebrates are either part of the somatic motor system (controlling striated muscles responsible for movement of body parts) or of the autonomic motor system (controlling smooth muscles, cardiac muscle, and glands responsible for vegetative functions). Autonomic efferent pathways include a ganglion outside the central nervous system. (Adapted from Machado, A., *Neuroanatomia Funcional*, Atheneu, Rio de Janeiro, Brazil, 1977.)

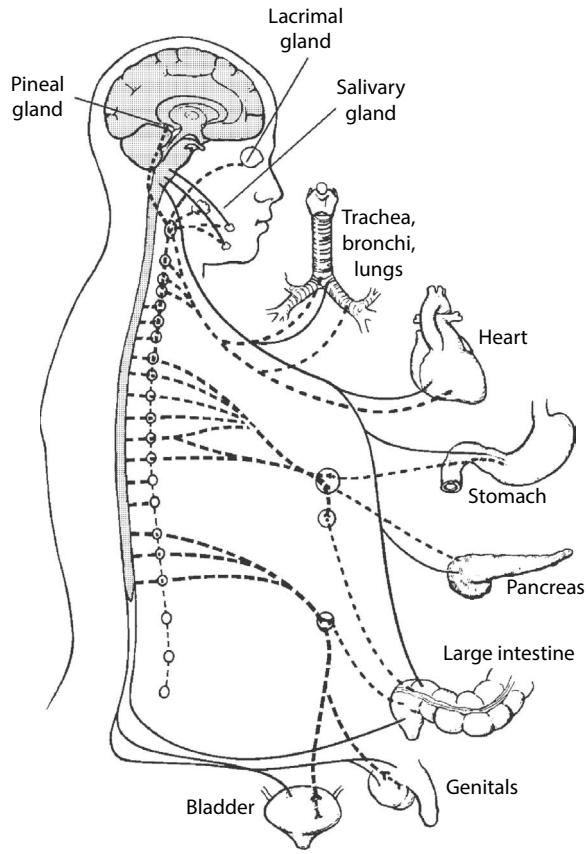


FIGURE 13.26 Autonomic nervous system. The diagram illustrates the innervation of major organs by the two branches of the autonomic nervous system: the sympathetic (dashed lines) and the parasympathetic (continuous lines). The sympathetic nervous system mobilizes the body's resources for action, whereas the parasympathetic nervous system promotes the conservation of bodily resources. (Adapted from *Medical Illustration Library*, Williams & Wilkins, Baltimore, MD, 1994.)

shortly, various studies conducted by the research team led by Ruud Buijs, then at the Netherlands Institute for Brain Research and now at the National Autonomous University of Mexico, have elucidated much of the SCN efference to the autonomic nervous system.[271–275] Much less is known about the somatic efference.

Detailed studies using retrograde tracing back from potential targets have mapped the SCN's efferent pathways in rats.[276] A summary of the findings is shown in Figure 13.27. The SCN projects to three main areas: the hypothalamus, the thalamus, and the septal area. The ventrolateral (core) SCN projects mainly to the dorsomedial (shell) region of the nucleus and to hypothalamic areas in the vicinity of the SCN, particularly the lateral subparaventricular zone ($_{L}$SPV).[276,277] The dorsomedial (shell) SCN projects more widely to various areas of the brain, particularly the paraventricular nucleus of the thalamus (PVT), the paraventricular nucleus of the hypothalamus (PVN), the medial subparaventricular zone ($_{M}$SPV), the preoptic area (POA), and the dorsomedial hypothalamic nucleus (DMH).[24,276–279] Combined anterograde–retrograde studies in the golden hamster identified greater hypothalamic projections from the ventrolateral (core) SCN than in rats, but the overall pattern of efference found was comparable.[280] Anterograde studies in vervet monkeys concurred in identifying SCN projections to the PVN, SPV, POA, and DMH.[29]

Figure 13.28 shows the ventral area of a section of mouse brain immunostained for peptide histidine isoleucine (PHI). PHI is structurally similar to VIP, which, as we saw in Chapter 12, is a major neurotransmitter used by cells in the ventral (core) SCN. The staining shows dorsal projections to the SCN shell and to the SPV. The diagram in Figure 13.29 illustrates these and several other monosynaptic efferent routes from the ventral and dorsal SCN. The projection from the SCN to the PVN has been particularly well documented.

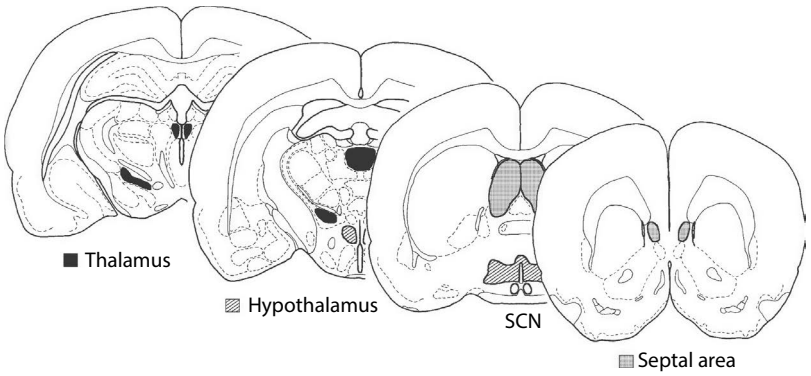


FIGURE 13.27 Suprachiasmatic nucleus (SCN) efference within the brain. The diagrams identify the three major brain structures to which the SCN projects in the laboratory rat (*Rattus norvegicus*). (Brain diagrams adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of structures after Leak, R.K. and Moore, R.Y., *J. Comp. Neurol.*, 433, 312, 2001.)

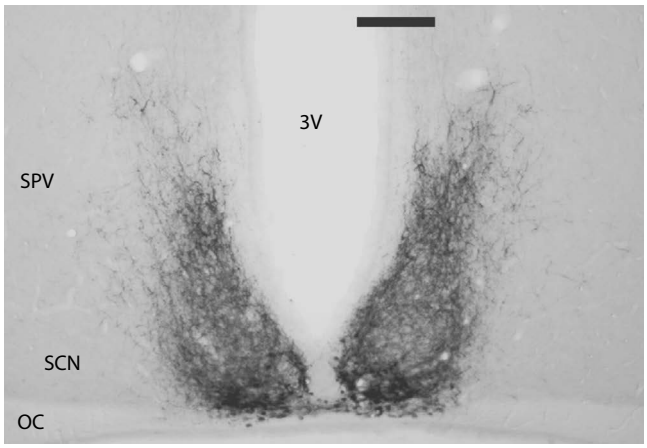


FIGURE 13.28 From SCN to SPV. This microphotograph of a coronal section of the brain of a domestic mouse (*Mus musculus*) shows SCN neurons that project dorsally to the SPV. The section was stained by immunohistochemistry for peptide histidine isoleucine. *Abbreviations:* 3V, third ventricle; OC, optic chiasm; SCN, suprachiasmatic nucleus; SPV, subparaventricular zone. Scale bar: 100 μm. (Image courtesy of Daniela Lupi, Department of Visual Neuroscience, Imperial College London, London, U.K.)

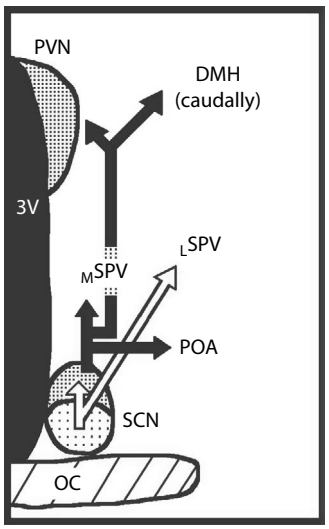


FIGURE 13.29 Out of the SCN. This diagram of a coronal section of the rodent brain in the vicinity of the SCN shows the major hypothalamic efferent routes from the ventral (core) and dorsal (shell) SCN. *Abbreviations:* 3V, third ventricle; DMH, dorsomedial hypothalamic nucleus; OC, optic chiasm; POA, preoptic area; PVN, paraventricular nucleus of the hypothalamus; SCN, suprachiasmatic nucleus; $_{L}$SPV, lateral subparaventricular zone; $_{M}$SPV, medial subparaventricular zone.

In some studies, the projection was found to involve predominantly VIP cells,[271,281] which indicates efference from the ventral SCN, but in others, it was found to involve predominantly arginine vasopressin (AVP) cells,[272,274] which is consistent with the view of efference from the dorsal SCN.

Retrograde tracing studies using FluoroGold and, in particular, the transsynaptic tracer *pseudorabies virus* (PRV), which gradually advances from one neuron to the next in a multineuron path, have allowed identification of the neural circuits that connect the SCN to the autonomic nervous system. By injecting PRV into autonomically innervated organs (liver and adrenal gland) and following the progress of retrograde infection of the nerves, Buijs' team found that the SCN has separate projections to the PVN for the sympathetic and parasympathetic branches[275] (Figure 13.30). Projections from the dorsal SCN proceed monosynaptically to the PVN; the PVN projects either to the dorsal motor nucleus of the vagus nerve (DMV), which projects to the parasympathetic ganglia, or to the intermediolateral column of the spinal cord (IML), which projects to the sympathetic ganglia. Figure 13.31 shows cells in the PVN that were retrogradely labeled after injection of FluoroGold in the upper thoracic spinal cord (IML). Although the SCN is not seen in the figure, some of its dorsal projections to the PVN (stained for VIP) are visible. Buijs' team traced also the efferent pathways from the dorsal SCN (through the PVN) to other organs, including the heart,[274] the pineal gland,[272] and the thyroid gland.[273] A different research group mapped the efference related to the autonomic innervation of the eyes and found, in agreement with Buijs' team, that the sympathetic efference involves projections from the SCN

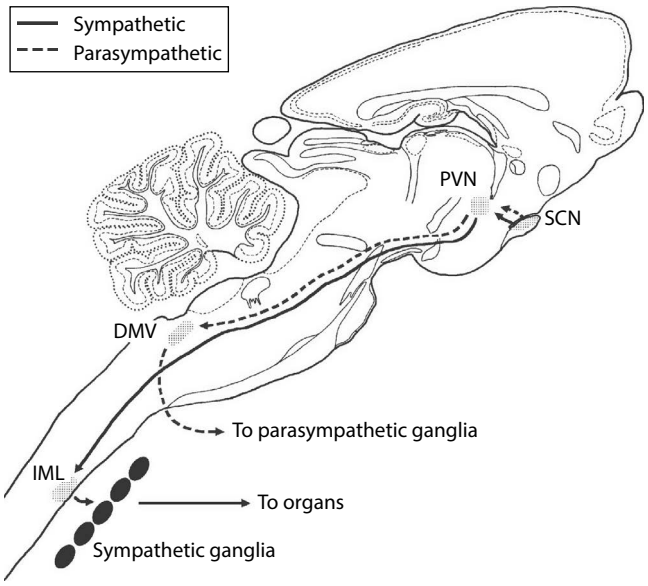


FIGURE 13.30 SCN and autonomic nervous system. This diagram of a sagittal section of the rat brain indicates that the SCN has separate projections to the PVN for sympathetic and parasympathetic efferences. *Abbreviations:* DMV, dorsal motor nucleus of the vagus nerve; IML, intermediolateral column of the spinal cord; PVN, paraventricular nucleus of the hypothalamus; SCN, suprachiasmatic nucleus. (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of structures after Buijs, R.M. et al., *J. Comp. Neurol.*, 464, 36, 2003.)

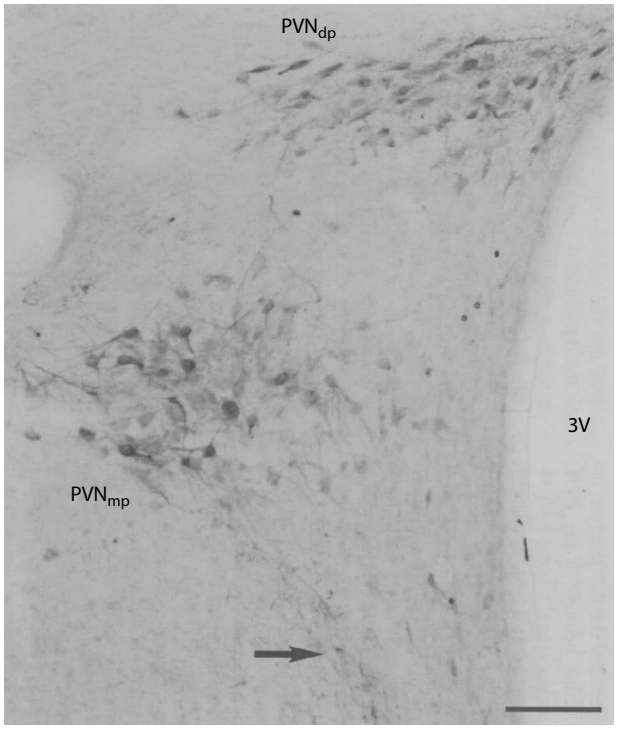


FIGURE 13.31 From paraventricular nucleus (PVN) to spinal cord. This microphotograph of a coronal section of the brain of a laboratory rat (*Rattus norvegicus*) shows cells in the PVN that project to the spinal cord. Large cell bodies and thick fibers were retrogradely labeled after injection of FluoroGold in the upper thoracic spinal cord. Thin fibers (indicated by the arrow) are VIP-immunoreactive fibers projecting dorsally from the suprachiasmatic nucleus to the PVN. *Abbreviations*: 3V, third ventricle; PVN_{dp}, dorsal paraventricular nucleus; PVN_{mp}, medial paraventricular nucleus. Scale bar: 200 μm. (Reproduced from *Brain Res.*, 748R, Teclemariam-Mesbah, R., Kalsbeek, A., Pévet, P., and Buijs, R.M., Direct vasoactive intestinal polypeptide-containing projection from the suprachiasmatic nucleus to spinal projecting hypothalamic paraventricular neurons, 71–76, Copyright 1997, with permission from Elsevier.)

to the PVN, from the PVN to the IML, and from the IML to the sympathetic ganglia.[282]

Several other research teams investigated the SCN efference to areas of the brain involved in the control of sleep and wakefulness.[283] Two of these areas are the ventrolateral preoptic area (VLPO) and the nucleus of the locus coeruleus (LC). The SCN does not project directly to the VLPO,[284] but it does project to the DMH, which on its turn projects to the VLPO.[278,285,286] The connection between the SCN and the LC is also disynaptic, involving a relay at the DMH.[287] Figure 13.32 shows the gradual retrograde infection of the SCN after pseudorabies virus injection into the LC. Although neither the DMH nor the LC is visible in the figure, the virus advanced progressively from the LC to the DMH, and from the DMH to the SCN.

13.2.2 Function

With small deviations, functional studies have generally agreed with anatomical studies of the SCN efference. For instance, functional connection between the ventrolateral (core) and dorsomedial (shell) SCN was demonstrated in cultured hypothalamic slices of rat brain. Release of AVP into the culture medium was recorded both before and after knife cuts separating the dorsal SCN from the ventral SCN. Intact SCNs released AVP rhythmically with a period of 23.8 hours. After the knife cuts, the ventral SCN continued to release AVP with a period of 23.8 hours, but the dorsal SCN started to release AVP much more rapidly (with a period of 23.2 hours).[288] This indicates that activity in the dorsal SCN is modulated by the ventral SCN.

The efferent pathway from the SCN to the nucleus of the LC via the DMH was confirmed by studies involving discrete lesions of the various nuclei. Lesions of the DMH not only attenuated the retrograde infection of the SCN by pseudorabies virus injected into the LC but also eliminated the circadian rhythmicity of firing rate in LC neurons.[287] Thus, it can be proposed that the rhythm of sleep and wakefulness is at least partially controlled by a neural circuit in which the SCN generates the rhythmic signal, the DMH conveys the signal to the LC, and the LC activates brain areas specifically involved in the modulation of sleep and wakefulness (Figure 13.33).

Regarding the SCN efference to the ventrolateral preoptic area (VLPO), a study of Fos expression in the VLPO (used as an index of cellular activity) showed that VLPO cells exhibit daily rhythmicity (which is not surprising) and that the rhythm of Fos expression peaks during the *day* in rats.[289]

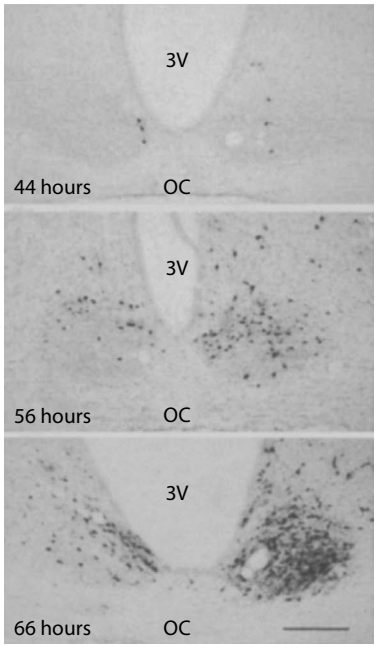


FIGURE 13.32 Backward from nucleus locus coeruleus. These microphotographs of coronal sections of the brain of a laboratory rat (*Rattus norvegicus*) show the temporal progression of suprachiasmatic nucleus (SCN) labeling after injection of pseudorabies virus (PRV) in the nucleus of the locus coeruleus (LC, a brain area involved in the control of arousal). PRV, which is transported transsynaptically and retrogradely, advanced through the dorsomedial hypothalamic nucleus (not shown) to the SCN, particularly to the ipsilateral SCN (right side of the section). *Abbreviations:* 3V, third ventricle; OC, optic chiasm. Scale bar: 200 μm. (Reproduced by permission from Macmillan Publishers Ltd. *Nat. Neurosci.*, Aston-Jones, G., Chen, S., Zhu, Y., Oshinsky, M.L., A neural circuit for circadian regulation of arousal, 4, 732–738, Copyright 2001, Nature Publishing Group.)

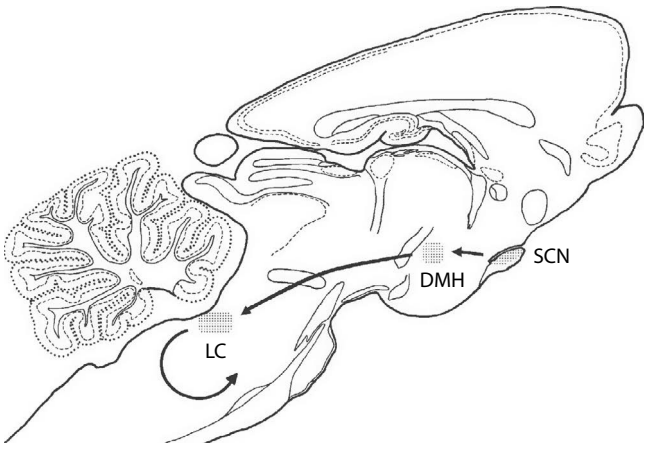


FIGURE 13.33 Suprachiasmatic nucleus (SCN) efference to locus coeruleus. This diagram of a sagittal section of the rat brain illustrates the finding that the SCN sends signals to the locus coeruleus (LC) through the dorsomedial hypothalamic nucleus (DMH). The locus coeruleus is involved in the control of arousal. (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of structures after Aston-Jones, G. et al., *Nat. Neurosci.*, 4, 732, 2001.)

The fact that neural activity peaks during the day in a nocturnal animal *is* surprising. You will remember from Chapter 12 that the activity rhythm of SCN cells does peak during the day (regardless of whether the animal is diurnal or nocturnal) but that the activity rhythms of cells in other parts of the brain and in other organs peak during the *night* in nocturnal animals. The fact that activity in the VLPO peaks during the day in rats suggests that the VLPO is very closely connected to the SCN functionally, perhaps being a first-order efferent target. Indeed, a different group of researchers conducted electrophysiological recordings in *in vitro* brain slices and found that about 30% of VLPO cells respond monosynaptically to electrical stimulation of the SCN.[290] A third team, led by Clifford Saper at Harvard Medical School, conducted functional studies involving the observation of organismal variables after discrete neurotoxic lesions of various hypothalamic nuclei as well as the analysis of anterograde and retrograde neural tracing.[286] As diagrammed in Figure 13.34, the researchers found that the VLPO does receive minor input directly from the SCN but that its main input is polysynaptic, involving relays at the $_{V}$SPV and the DMH. According to Saper's team, the efferent pathway to the VLPO is associated with the control of sleep, the pathway to the lateral hypothalamic area (LHA) is associated with the control of feeding and activity, and the pathway to the paraventricular nucleus (PVN) is associated with the control of corticosteroid secretion. They were unable to identify the efferent pathways associated with the rhythmic secretion of melatonin or with the rhythm of body temperature. Notice, however, that all main pathways that they identified pass through the DMH, which, accordingly, they consider to be a major hub in SCN efference.[286]

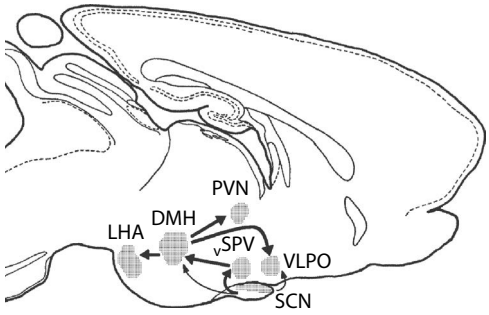


FIGURE 13.34 Functional role of dorsomedial hypothalamic nucleus. This diagram of a sagittal section of the rat brain illustrates the role of the dorsomedial hypothalamic nucleus in the functional efferent pathways of the SCN. Thick arrows represent main pathways. Thin arrows represent secondary pathways. The VLPO is involved in the control of sleep, the LHA in the control of feeding and activity, and the PVN in the control corticosteroid release. *Abbreviations:* DMH, dorsomedial hypothalamic nucleus; LHA, lateral hypothalamic area; PVN, paraventricular nucleus; SCN, suprachiasmatic nucleus; VLPO, ventrolateral preoptic area; $_{V}$SPV, ventral subparaventricular zone. (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of structures after Chou, T.C. et al., *J. Neurosci.*, 23, 10691, 2003.)

There is some disagreement among the results of functional studies of dorsal SCN projections to the paraventricular nucleus of the hypothalamus (PVN) and the SPV. In a study conducted almost 40 years ago, isolation of the SCN from the dorsal brain by means of knife cuts around the borders of the nucleus was found to eliminate circadian rhythmicity of drinking, sleep, and brain temperature—just as if the SCN itself had been ablated.[291] Seven years later, a separate research group reported that isolation of the SCN resulted in loss of rhythmicity of locomotor activity but not of corticosterone secretion.[292] Separate efferent routes for behavioral and endocrine processes would not be surprising, but a third research group reported that isolation of the SCN resulted in loss of rhythmicity of locomotor activity only if the knife cuts inadvertently damaged the SCN itself.[293] Evidently, these early studies were not conclusive. Studies involving small lesions targeted at the PVN and SPV provided some clarity to the issue. Lesions of the PVN were found to abolish rhythmicity of melatonin secretion but not to affect rhythmicity of locomotor activity or body temperature.[294–296] Lesions of the ventral SPV were found to disrupt rhythmicity of locomotor activity (but not of melatonin secretion or body temperature).[295,297,298] Whereas one group of researchers observed disruption of the body temperature rhythm after lesions of the dorsal SPV,[297] another did not.[295]

The notion that the SPV plays some role in the SCN efference is reinforced by a study of Fos expression whose results are shown in Figure 13.35. As expected in rats, Fos expression was found to be higher during the day than during the night in the SCN. Higher expression during the night than during the day in the PVN and supraoptic nucleus was also expected. Notice, however, that Fos expression in the SPV matched that of the SCN, not that of the other extra-SCN sites.[299] This suggests that the SPV is very closely connected to the SCN functionally and is perhaps a first-order efferent target.

Regarding the PVN, the SCN efference has been documented by electrophysiological recordings. Electrical stimulation of the SCN in brain slices *in vitro* was shown to inhibit (through GABA) or excite (through glutamate) cells in the PVN.[300–302] Response latencies in the order of 10 milliseconds suggested a monosynaptic connection, which is consistent with the anatomical data discussed previously. As a matter of fact, various functional studies have confirmed the full pathway from the SCN to the pineal gland through the PVN.[294,296,303] As diagrammed in Figure 13.36, the SCN and the pineal, which are located relatively close to each other, are connected by a long pathway that includes relays in the PVN, in the intermediolateral column of the thoracic spinal cord, and in the superior cervical sympathetic ganglion.

The efferent pathway responsible for the rhythm of body temperature has not been identified. We saw in Chapter 11 that there are temperature receptors in the brain, the spinal cord, the skin, and many internal organs. The *preoptic area* (POA) plays a double role as a temperature sensor and as a control center for thermoregulatory responses. The role of the POA as an integration center has been demonstrated in studies

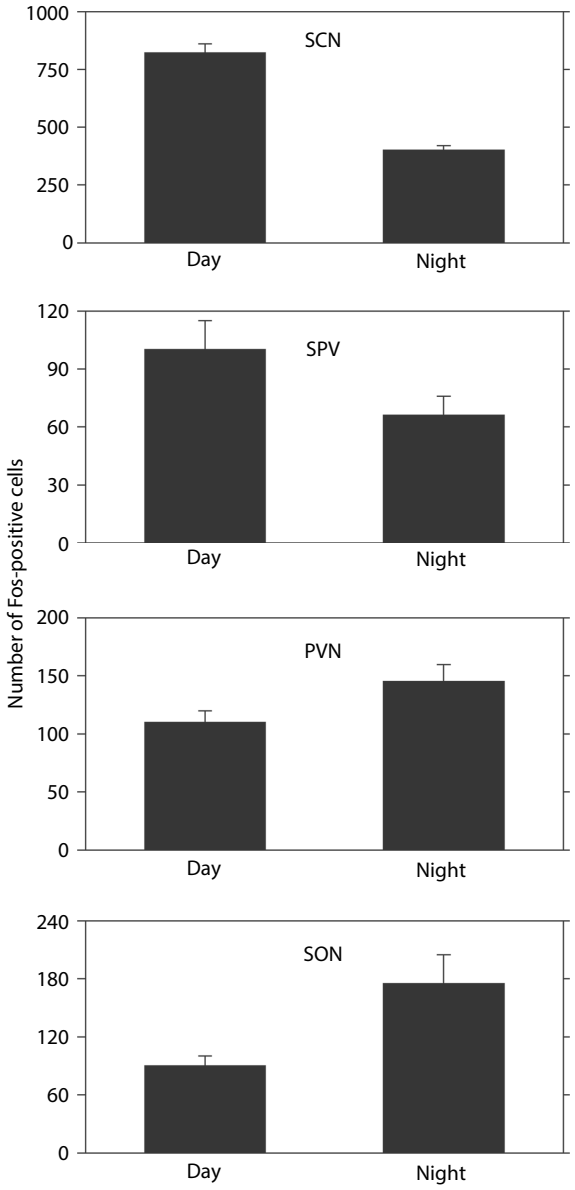


FIGURE 13.35 Functional role of subparaventricular zone. The graphs show the mean day–night differences in Fos immunoreactivity (which is an index of cellular activity) of neurons in four areas of the rat brain: suprachiasmatic nucleus (SCN), subparaventricular zone (SPV), paraventricular nucleus (PVN), and supraoptic nucleus (SON). The bars represent the means (± SE) of cell counts in 16 rats killed during the day and 16 rats killed during the night. Notice that the SPV, like the SCN, is more active during the day. (Data from Nunez, A.A. et al., *J. Biol. Rhythms*, 14, 300, 1999.)

in which POA lesions caused serious impairment of thermoregulatory responses.[304–311] In addition, glucose utilization and Fos expression were observed to be increased in the POA during thermoregulation.[312–314] POA lesions impair autonomic but not behavioral thermoregulatory responses,[304,306–308,310,315] which implies that another brain site must control behavior.[316,317] However, the fact that homeothermic animals exhibit rhythmicity of body temperature even when deprived of opportunities for behavioral action indicates that behavioral

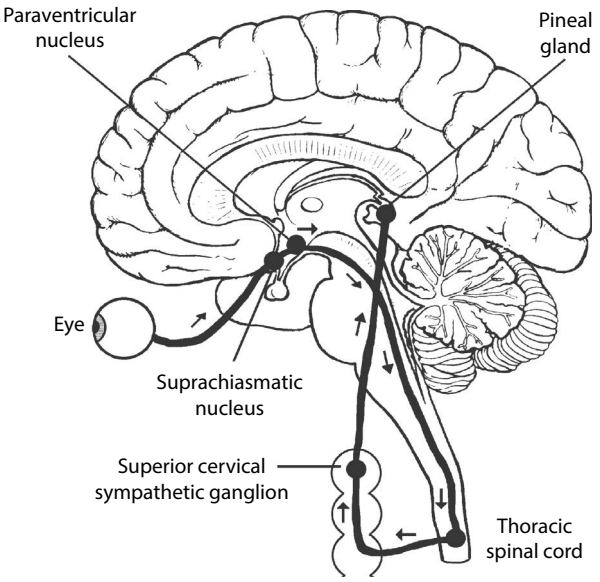


FIGURE 13.36 Retinal–pineal pathway. This diagram of the brain illustrates the long circadian-controlled pathway from the eyes to the pineal gland. Photic information is sent from the retina to the suprachiasmatic nucleus. The efferent pathway includes the paraventricular nucleus and the sympathetic branch of the autonomic nervous system. (Data from Klein, D.C. et al., *Brain Res. Bull.*, 10, 647, 1983; Buijs, R.M. et al., *J. Comp. Neurol.*, 464, 36, 2003.)

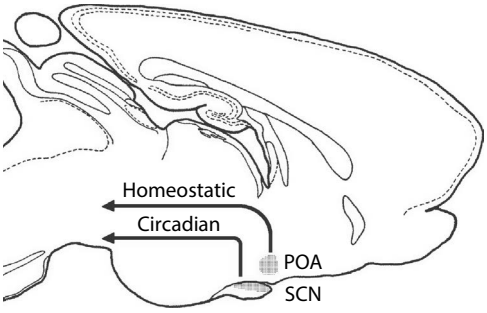


FIGURE 13.37 Efferent pathways for the control of body temperature. This diagram of a sagittal section of the rat brain illustrates that, although the anatomical details about the efferent pathways for the control of body temperature are not known yet, functional studies indicate that the homeostatic and circadian components of temperature regulation are separately controlled by the preoptic area (POA) and suprachiasmatic nucleus (SCN), respectively. (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of structures after Osborne, A.R. and Refinetti, R., *NeuroReport*, 6, 2187, 1995.)

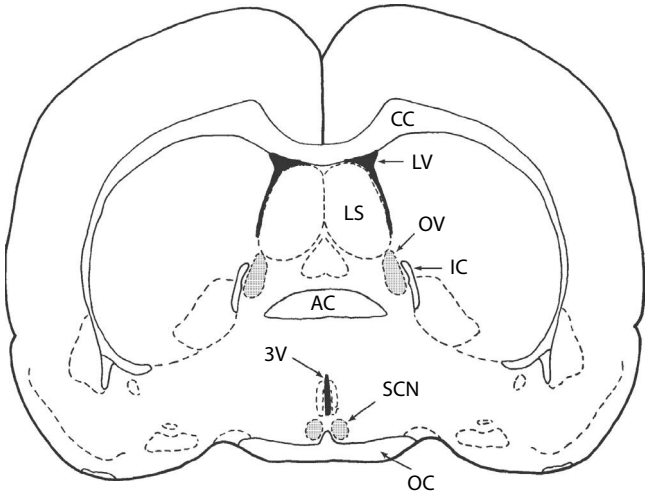


FIGURE 13.38 Oval nucleus of the bed nucleus of the stria terminalis (BST). This diagram of a coronal section of the rat brain shows the location of the oval nucleus of the BST in reference to other major structures of the rat brain. *Abbreviations:* 3V, third ventricle; AC, anterior commissure; CC, corpus callosum; IC, internal capsule; LS, lateral septum; LV, lateral ventricle; OV, oval nucleus of the bed nucleus; SCN, suprachiasmatic nucleus. (Brain diagram adapted from Pellegrino, L.J. et al., *A Stereotaxic Atlas of the Rat Brain*, 2nd edn., Plenum, New York, 1979; Identification of oval nucleus after Amir, S. et al., *J. Neurosci*., 24, 781, 2004.)

mechanisms are not necessary for the generation or modulation of the body temperature rhythm.

As previously discussed in Chapter 12, ablation of the SCN has been shown to eliminate circadian rhythmicity of body temperature in numerous studies on a variety of rodents.[291,318–332] Although *rhythmicity* is eliminated, *homeostatic control* is not affected.[318,319,328] That is, animals with full SCN lesions can thermoregulate normally. On the other hand, homeostatic impairment (without loss of rhythmicity) results from lesions of the POA.[320,333,334] That is, animals with POA lesions exhibit impaired regulation of body temperature but are still rhythmic. This dichotomy suggests that the circadian and homeostatic mechanisms of temperature control are independent, as previously discussed in Chapter 10. Thus, until a crucial experiment proves it otherwise, it seems reasonable to conclude that projections from the SCN to the POA are not relevant in the control of body temperature and that the POA and the SCN have separate functional projections to the pertinent efferent organs, as diagrammed in Figure 13.37.

Functional studies have provided suggestive evidence for at least one more SCN target, the bed nucleus of the stria terminalis (BST). In one study, the researchers recorded the electrical activity of several brain nuclei in freely moving golden hamsters. As expected, they found that the activity rhythm of the SCN peaked during the day and that the rhythms of other brain areas peaked during the night, except that the rhythm of the BST peaked during the day.[335] Of course, this phase coincidence suggests a close functional connection between the SCN and the BST. A separate study by another group identified a subnucleus within the BST that seems to be the actual target: the oval nucleus of the BST (Figure 13.38). This research group measured *per2* expression in rat brains at different times of the circadian cycle and, in agreement with the other study, found that the rhythms of *per2* expression in the SCN and in the oval nucleus were in phase (Figure 13.39).

After reviewing all of the preceding evidence for anatomical and functional connections of the SCN with other brain areas, I must now introduce a surprise. We saw in Chapter 12

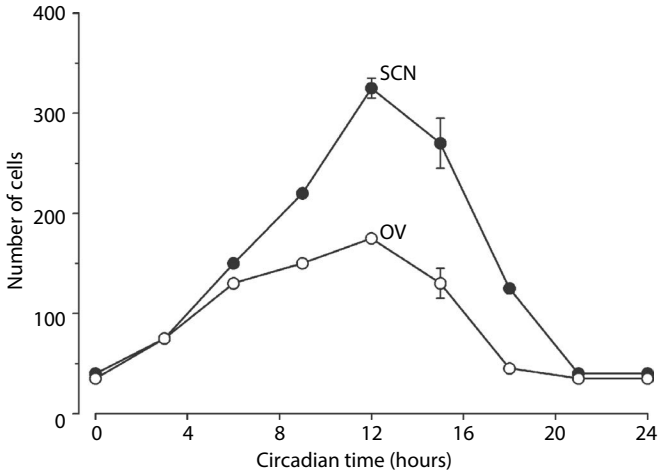


FIGURE 13.39 Cycling in phase with the suprachiasmatic nucleus (SCN). The graph shows that activity in the oval nucleus of the bed nucleus of the stria terminalis (OV), as assessed by Per2 immunoreactivity, oscillates daily in phase with activity in the SCN. Each data point corresponds to the mean cell count (± SE) in the brains of 4 rats maintained in constant darkness before euthanasia. (Data from Amir, S. et al., *J. Neurosci.*, 24, 781, 2004.)

that animals rendered arrhythmic by SCN ablation can regain circadian rhythmicity if they receive transplants of fetal hypothalamic tissue containing the SCN.[336–346] At that time, we did not address the issue of tissue regeneration. I allowed readers to make the very reasonable assumption that rhythmicity was regained because the grafted tissue developed the necessary neural connections with the host's brain. Although reasonable, the assumption is incorrect. SCN grafts good enough to restore rhythmicity of the locomotor activity rhythm show very limited integration with the host's tissue.[347–349] An example is shown in Figure 13.40, where fibers from the adjacent preoptic area are seen to project contralaterally without penetrating the grafted tissue in the third ventricle. As a matter of fact, it has been shown that outgrowth from the SCN grafts is equivalent to the outgrowth of control cortical grafts (which do not restore behavioral rhythmicity).[348] More impressively, behavioral rhythmicity *is* restored by SCN grafts encapsulated in a semipermeable container that prevents neural outgrowth but allows the diffusion of hormones.[350] Evidently, the restoration of behavioral rhythmicity must depend not on neural connections but on the diffusion of some unknown hormone produced by the SCN!

In vitro studies have added more excitement to the story. One group of researchers cultured SCN cells and fibroblast (collagen) cells. Not surprisingly, the SCN culture—but not the fibroblast culture—exhibited intrinsic circadian rhythmicity in glucose uptake and *per* expression. However, when the two cell lines were cocultured in the same medium but physically separated, the fibroblast cells became rhythmic.[351] Evidently, some diffusible substance from the SCN cells must have conferred rhythmicity to the fibroblast cells. Another research group studied the firing rates of SCN and PVN cells in mouse brain slices. Under normal conditions, the firing-rate rhythm of PVN cells was synchronized to the rhythm of SCN cells. In some slices, the SCN was ablated, and—not surprisingly—the PVN cells stopped being rhythmic. However, when a tiny slice containing a healthy SCN was placed next to the slice missing the SCN, the PVN cells became rhythmic again.[346] Examples of the recordings are shown in Figure 13.41. In the upper graph, PVN cells were arrhythmic until the tiny slice containing a healthy SCN (*SCN graft*) was added to the preparation. In the lower graph, PVN cells lost rhythmicity when the graft was removed. In both cases, the main slice and the graft were in the same medium but were not physically in contact (and, therefore, were unable to make synaptic contact).

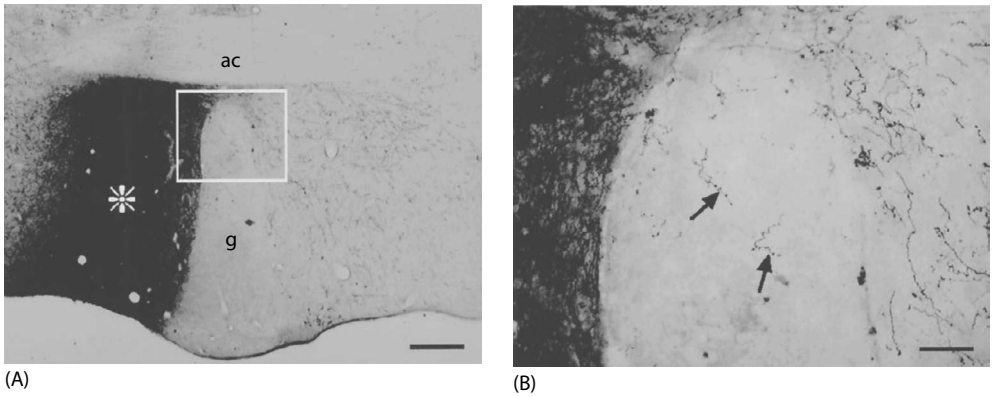


FIGURE 13.40 Alive and well, but not connected. These microphotographs of a coronal section of the brain of a golden hamster (*Mesocricetus auratus*) show a graft of fetal hypothalamic tissue transplanted into the third ventricle after ablation of the SCN. As seen several months after the transplant, the graft (g) is well integrated into the hamster's brain but receives only sparse input from the adjacent preoptic area (POA). (A) A low power microphotograph showing an injection (*) of the anterograde tracer *Phaseolus vulgaris* leucoagglutinin into the POA adjacent to the SCN graft. The boxed area in A is shown in B at higher magnification. Labeled fibers course around the graft to innervate the contralateral POA, but relatively few fibers actually enter the graft (arrows). *Abbreviations:* ac, anterior commissure; g, graft. Scale bars: 100 µm (A) and 50 µm (B). (Reproduced from Meyer-Bernstein, E.L. et al., *Endocrinology*, 140, 207, Copyright 1999, The Endocrine Society. With permission.)

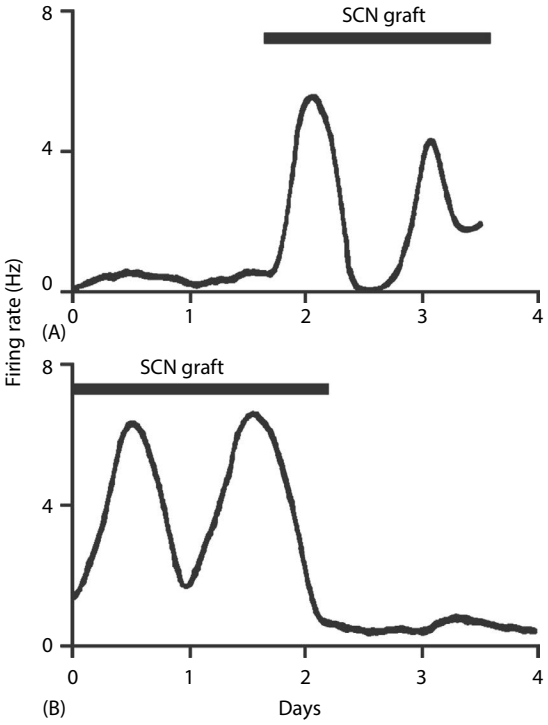


FIGURE 13.41 Suprachiasmatic nucleus (SCN) efference: neural or humoral? The graphs show representative records of the electrical activity of single neurons in the paraventricular nucleus (PVN) as recorded in mouse brain slices. The slices did not contain the SCNs and did not exhibit circadian rhythmicity unless an SCN graft was added to the preparation (heavy horizontal bars). Although no neural connection was established between the SCN and the PVN, PVN cells started exhibiting circadian rhythmicity shortly after the addition of the SCN graft (A) and lost rhythmicity when the graft was removed (Panel B). (Adapted from Tousson, E. and Meissl, H., *J. Neurosci.*, 24, 2983, 2004.)

Although neural efference from the SCN is likely to be important in intact animals, the evidence supports the conclusion that the SCN can communicate with peripheral organs through a diffusible substance. For a diffusible substance to travel long distances within the body, transportation via the blood stream is a necessity. Thus, a team of researchers surgically joined the circulatory systems of pairs of mice, creating parabiotic pairs. One member of each pair had its SCN lesioned. The pairs were kept under a light–dark cycle for many days and then transferred to constant darkness for 3 days and sacrificed. Expression of core clock genes was measured in various organs. Organs from nonparabiotic SCN-lesioned mice exhibited no circadian rhythmicity of gene expression, as expected. Heart, skeletal muscle, and spleen of parabiotic SCN-lesioned mice also failed to exhibit rhythmicity, but liver and kidney exhibited rhythmicity.[352] Evidently, the liver and kidneys of the SCN-lesioned mouse were synchronized by some blood-borne substance secreted by the SCN of the other mouse. The fact that the heart, skeletal muscle, and spleen were not synchronized suggests that they require signals sent by the SCN through the nervous system.

Although more research is needed to confirm the role of blood-borne substances in the communication of the SCN with peripheral organs, it is unlikely that the SCN has direct neural connections with every organ in the body. Once the SCN signal leaves the brain, it is likely that hormones are used to spread the signal through the body. Glucorticoids (cortisol and corticosterone being two of them) are hormones secreted by the adrenal glands under modulation by the SCN, as mentioned in Chapter 5. Because glucocorticoids are blood-borne substances secreted in abundance in a circadian fashion, and because glucocorticoid receptors are found in peripheral organs but not in the adult SCN, glucocorticoids are good candidates for the role of carriers of instructions from the SCN to peripheral organs. Studies in mice and rats have shown that glucocorticoids can phase-shift rhythms of gene expression in peripheral organs,[353,354] which provides additional support for the candidacy of glucocorticoids as blood-borne substances connecting the SCN with the rest of the body. This matter deserves further research.

As mentioned in Chapter 10, it has been suggested that the oscillation in body temperature of homeothermic animals might serve as a signal for the entrainment of peripheral organs.[355–360] A major problem with the hypothesis of body temperature serving as an entraining agent of peripheral organs is that body temperature is an unreliable variable in the majority of living beings, given that most living beings are poikilothermic. Only in homeotherms, whose body temperatures are independent of ambient temperature, would the body temperature rhythm be a reliable means to synchronize peripheral organs.

Finally, a few words must be added regarding the molecular mechanisms of efference. In Chapter 12, we discussed the feedback loops of the molecular clock and saw that microarray studies had identified about 100 or so genes that are rhythmically expressed in fruit flies[361–364] and rodents.[365–372] Most of these genes are probably *clock-controlled genes*, although some of them may actually be clock genes that have not been characterized yet. Unfortunately, the lack of replicability of results in different studies[364,373] prevents us from making any reliable conclusion about the identity of clock-controlled genes. Genes that are expressed rhythmically but that have not been previously identified as clock genes are usually assumed to be clock-controlled genes. However, to the extent that interarray comparisons within the same study can be trusted, it seems that the molecular clock has adapted its output functions to the needs of each particular tissue or organ.[363] Thus, the task of identifying and characterizing clock-controlled genes promises to be a long and arduous one.

SUMMARY

1. Three main neural pathways provide afference to the suprachiasmatic nucleus (SCN). The *retinohypothalamic tract* (RHT) connects retinal ganglion cells to the SCN via a monosynaptic pathway that uses glutamate as its main neurotransmitter.

The *geniculo-hypothalamic tract* (GHT) connects the intergeniculate islet of the thalamus to the SCN via a monosynaptic pathway that uses neuropeptide Y as its main neurotransmitter. The *raphe-hypothalamic pathway* connects raphe nuclei to the SCN and uses serotonin as its neurotransmitter. The RHT is the main carrier of photic information, whereas the two other pathways carry nonphotic information.

2. The SCN efference relies on neural connections (primarily to other hypothalamic sites) as well as on an as-yet unidentified diffusible substance. The hypothalamic *paraventricular nucleus* (PVN) is a main target of the SCN efference associated with circadian rhythmicity of the autonomic nervous system. The *dorsomedial hypothalamic nucleus* (DMH) seems to be a major target for efference associated with behavioral and endocrine rhythms. Many of the SCN projections identified in anatomical studies have not yet been investigated in functional studies.

EXERCISES

13.1 Body dissection. It is practically impossible to appreciate the importance of afferent and efferent pathways without having a basic knowledge of the internal structure of the body. If you have not conducted a whole-body dissection lately, it is important that you conduct one now.

1. Cats and rats are usually the most convenient subjects because of their moderate size and easy availability. You may use a recently deceased animal or a preserved one. Preserved specimens can be dissected more easily but are less similar to a live animal (for one thing, they do not bleed!). Depending on where you live, you may need a permit to euthanize mammals, in which case a preserved specimen will be more convenient. Live laboratory rats are sold by several animal suppliers, such as Charles River Laboratories (Wilmington, MA), Harlan (Indianapolis, IN), Hilltop Lab Animals (Scottdale, PA), Simonsen Laboratories (Gilroy, CA), Taconic (Germantown, NY), and Zivic Laboratories (Zelienople, PA). Preserved cats and rats are sold by major suppliers of biological education materials, such as Carolina Biological Supply Company (Burlington, NC) and WARD's Natural Science (Rochester, NY).
2. To conduct the dissection, you will need rubber gloves, scissors, a scalpel (or a sharp knife), and a forceps (or tweezers). To open the rib cage of a rat, regular scissors are sufficient. For a cat, bone-cutting forceps may be needed. In case you want to inspect the brain and spinal cord, you will need also bone rongeurs to break off the skull and spinal vertebrae.
3. If you use a preserved specimen, you may want to wear a facemask to reduce the odor of formaldehyde. This will probably not be necessary in the United States, where all major suppliers currently use an odorless preservative instead of formalin.
4. To serve as a guide for the dissection, Figure 13.42 provides a diagrammatic view of the major internal structures of the cat. At the level of detail presented, the diagram is useful for the dissection of almost any mammal. You should try to identify all the structures shown in the diagram. Of course, you

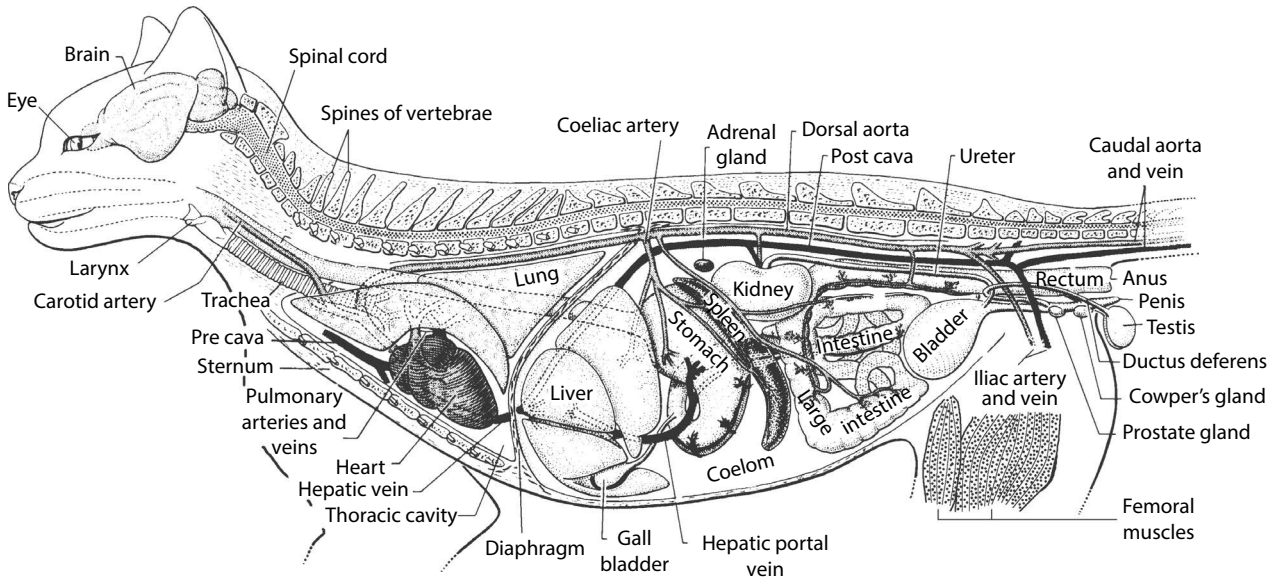


FIGURE 13.42 The inner cat. This diagram of the internal structure of a male cat can serve as a template for gross dissection of mammalian specimens. (Adapted from Storer, T.I. et al., *Elements of Zoology*, 4th edn., McGraw-Hill, New York, 1977.)

should not waste time searching for a penis and testicles if you have a female specimen! In this case, look for the uterus (which is bifurcated in rodents) and ovaries.

5. Start by laying the animal on its back (supine position) and gently cutting the abdominal skin and underlying muscle to expose the visceral organs. From then on, you are on your own! Have fun exploring the beauty and complexity of the animal body! (Having a knowledgeable person around to answer potential questions would be helpful, but you can learn a great deal on your own. Remember: no matter how inept you are, you cannot kill an animal that is already dead!)

13.2 Brain dissection. In the previous exercise, you performed a whole-body dissection. You probably did not have time to look at the brain in detail (and, if you used a rat, the brain was too small to allow easy inspection). In this exercise, you will dissect a brain.

1. Although you could use a human brain, it would be a waste to use a human brain in your first (or second, or third) brain dissection. The brain of a sheep or a cow is similar enough to the human brain to provide you with a valuable learning experience. You can buy a fresh sheep brain or cow brain at your local butcher shop. However, brain tissue is very soft and not suitable for didactic dissection. Sheep brains preserved in odorless fixatives are sold by major suppliers of biological education materials, such as Carolina Biological Supply Company (Burlington, NC) and WARD's Natural Science (Rochester, NY). Alternatively, you can prepare a fresh brain for dissection by freezing it in a freezer and later thawing it in a formalin solution.
2. To conduct the dissection, all you will need are rubber gloves, a scalpel (or a sharp knife), and a forceps (or tweezers). If you prepare the brain in formalin, you may want to wear a facemask to reduce the odor of formaldehyde.
3. To serve as a guide for the dissection, Figure 13.43 provides three diagrammatic views of the ruminant brain (i.e., cow, sheep, or goat). You should try to identify all the structures shown in the diagrams. Start with the lateral view and then move to the ventral view. Be forewarned that the brain that you are dissecting may still have meninges, blood vessels, and even pieces of bone attached to it. Gently peel them off (cutting them if needed) before trying to identify the brain structures.
4. After you have inspected the external surface of the brain, perform a midsagittal cut to identify the structures indicated in the *midsagittal view* diagram. In rare cases, you may find the pituitary gland (also called the hypophysis) at the ventral surface of the brain. Usually, the pituitary stays attached

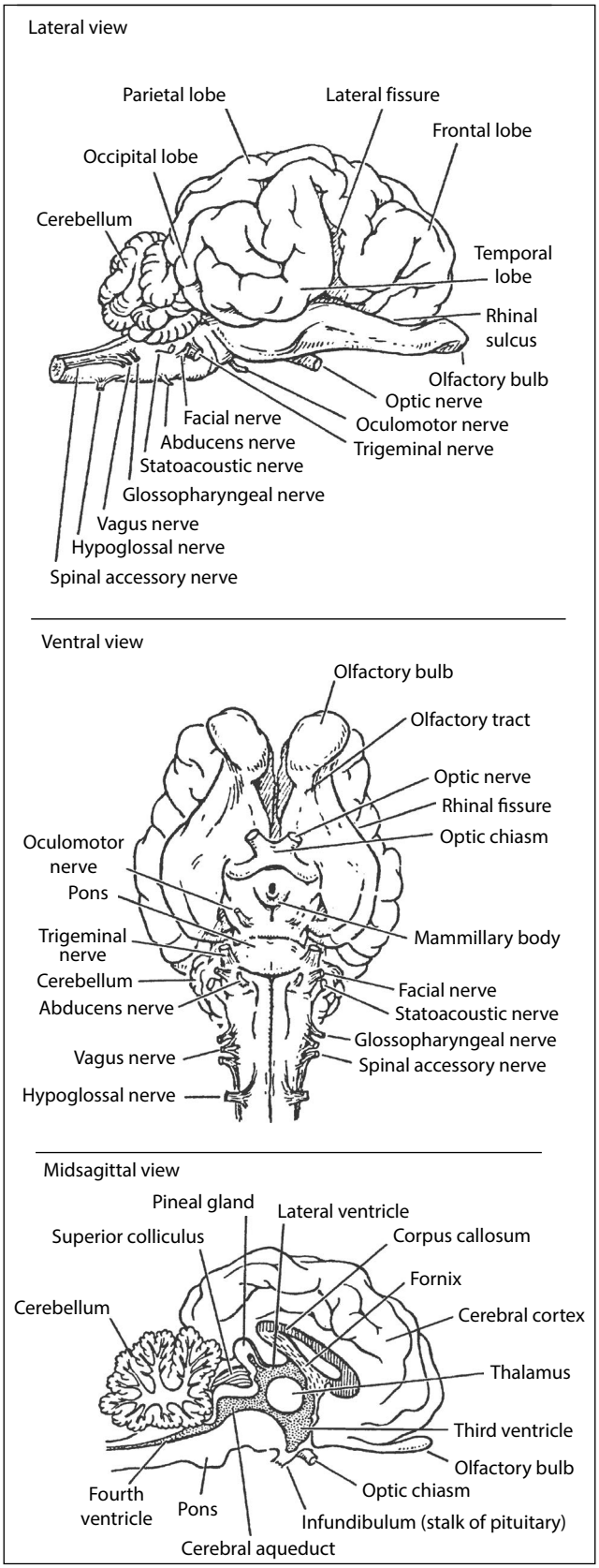


FIGURE 13.43 Ruminant brain. These diagrams of the ruminant brain can serve as templates for gross dissection of cow or sheep brains. (Adapted from Hart, B.L., *Experimental Neuropsychology: A Laboratory Manual*, W.H. Freeman, San Francisco, CA, 1969.)

to the skull when the brain is removed, and only its stalk is present.

5. Once you have identified all structures shown in Figure 13.43, you can do some exploration on your own. Particularly interesting is the performance of multiple lateral sagittal cuts to reveal central structures in the temporal lobe such as the hippocampus and the caudate. Regardless of how well you slice the brain, you will probably not be able to see the hypothalamus—and definitely not the SCN—in this crude preparation, but you may search for it just lateral to the third ventricle at the base of the brain (dorsal and caudal to the optic chiasm).

SUGGESTIONS FOR FURTHER READING

Butler, A. B. and Hodos, W. (2005). *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*, 2nd edn. New York: Wiley.

There is no book specialized in afferent and efferent pathways of the circadian system. However, this book provides excellent background by covering the organization of the nervous system in mammalian and nonmammalian vertebrates and its relationship with behavior, adaptation, and survival.

Fix, J. D. (2007). *Neuroanatomy*, 4th edn. Baltimore, MD: Williams & Wilkins.

A review of human neuroanatomy for medical students. It covers afferent and efferent pathways as well as all that lies in between.

Dixon, A. K., Bowden, D. J., Ellis, H., and Logan, B. M. (2015). *Human Sectional Anatomy: Atlas of Body Sections, CT and MRI Images*, 4th edn. Boca Raton, FL: CRC Press.

A unique book with full-color human cadaver sections shown alongside CT and MRI images, with accompanying labeled line diagrams.

Janig, W. and Habler, H. J. (2003). Neurophysiological analysis of target-related sympathetic pathways from animal to human: Similarities and differences. *Acta Physiologica Scandinavica* 177: 255–274.

An interesting review article dealing specifically with the autonomic efferent pathways in mammals.

WEBSITES TO EXPLORE

Allen Brain Atlas: http://www.brain-map.org/.

Fundamentals of Human Anatomy: http://www.innerbody.com/.

Neuroscience Information Framework: http://neuinfo.org/.

Neuroscience: Motor Systems (University of Kansas): http://www.kumc.edu/AMA-MSS/Study/neuro3.htm.

Visible Human Project at the U.S. National Library of Medicine: http://www.nlm.nih.gov/research/visible/visible_human.html.

Whole Brain Atlas (Harvard University): http://www.med.harvard.edu/AANLIB/home.html.

REFERENCES

1. Kandel, E. R., Schwartz, J. H., and Jessell, T. M. (2000). *Principles of Neural Science*, 4th edn. New York: McGraw-Hill.
2. Barrett, K. E., Barman, S. M., Boitano, S., and Brooks, H. L. (2012). *Ganong's Review of Medical Physiology*, 24th edn. New York: McGraw-Hill.
3. Card, J. P. and Moore, R. Y. (1991). The organization of visual circuits influencing the circadian activity of the suprachiasmatic nucleus. In: Klein, D. C., Moore, R. Y., and Reppert, S. M. (Eds.), *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press, pp. 51–76.
4. Golombek, D. A. and Rosenstein, R. E. (2010). Physiology of circadian entrainment. *Physiological Reviews* 90: 1063–1102.
5. Moore, R. Y. and Lenn, N. J. (1972). A retinohypothalamic projection in the rat. *Journal of Comparative Neurology* 146: 1–14.
6. Tanaka, M., Hayashi, S., Tamada, Y., Ikeda, T., Hisa, Y., Takamatsu, T., and Ibata, Y. (1997). Direct retinal projections to GRP neurons in the suprachiasmatic nucleus of the rat. *NeuroReport* 8: 2187–2191.
7. Moga, M. M. and Moore, R. Y. (1997). Organization of neural inputs to the suprachiasmatic nucleus in the rat. *Journal of Comparative Neurology* 389: 508–534.
8. Hattar, S., Liao, H. W., Takao, M., Berson, D. M., and Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science* 295: 1065–1069.
9. Berson, D. M., Dunn, F. A., and Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073.
10. Warren, E. J., Allen, C. N., Brown, R. L., and Robinson, D. W. (2003). Intrinsic light responses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735.
11. Gooley, J. J., Lu, J., Fischer, D., and Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience* 23: 7093–7106.
12. Hannibal, J. and Fahrenkrug, J. (2004). Target areas innervated by PACAP-immunoreactive retinal ganglion cells. *Cell Tissue Research* 316: 99–113.
13. Pickard, G. E. and Zucker, I. (1986). Influence of deuterium oxide on circadian activity rhythms of hamsters: Role of the suprachiasmatic nuclei. *Brain Research* 376: 149–154.
14. Johnson, R. F., Moore, R. Y., and Morin, L. P. (1988). Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Research* 460: 297–313.
15. Stopa, E. G., Johnson, J. K., Friedman, D. I., Ryer, H. I., Reidy, J., Kuo-LeBlanc, V., and Albers, H. E. (1995). Neuropeptide Y receptor distribution and regulation in the suprachiasmatic nucleus of the Syrian hamster (*Mesocricetus auratus*). *Peptide Research* 8: 95–100.
16. Bryant, D. N., LeSauter, J., Silver, R., and Romero, M. T. (2000). Retinal innervation of calbindin-D_{28K} cells in the hamster suprachiasmatic nucleus: Ultrastructural characterization. *Journal of Biological Rhythms* 15: 103–111.
17. Morin, L. P., Blanchard, J. H., and Provencio, I. (2003). Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: Bifurcation and melanopsin immunoreactivity. *Journal of Comparative Neurology* 465: 401–416.

18. Muscat, L., Huberman, A. D., Jordan, C. L., and Morin, L. P. (2003). Crossed and uncrossed retinal projections to the hamster circadian system. *Journal of Comparative Neurology* 466: 513–524.
19. Sollars, P. J., Smeraski, C. A., Kaufman, J. D., Ogilvie, M. D., Provencio, I., and Pickard, G. E. (2003). Melanopsin and non-melanopsin expressing retinal ganglion cells innervate the hypothalamic suprachiasmatic nucleus. *Visual Neuroscience* 20: 601–610.
20. Kudo, M., Yamamoto, M., and Nakamura, Y. (1991). Suprachiasmatic nucleus and retinohypothalamic projections in moles. *Brain, Behavior and Evolution* 38: 332–338.
21. Smale, L. and Boverhof, J. (1999). The suprachiasmatic nucleus and intergeniculate leaflet of *Arvicanthis niloticus*, a diurnal murid rodent from East Africa. *Journal of Comparative Neurology* 403: 190–208.
22. Novak, C. M., Smale, L., and Nunez, A. A. (1999). Fos expression in the sleep-active cell group of the ventrolateral preoptic area in the diurnal murid rodent, *Arvicanthis niloticus*. *Brain Research* 818: 375–382.
23. Gaillard, F., Karten, H. J., and Sauvé, Y. (2013). Retinorecipient areas in the diurnal murine rodent *Arvicanthis niloticus*: A disproportionally large superior colliculus. *Journal of Comparative Neurology* 521: 1699–1726.
24. Todd, W. D., Gall, A. J., Weiner, J. A., and Blumberg, M. S. (2012). Distinct retinohypothalamic innervation patterns predict the developmental emergence of species-typical circadian phase preference in nocturnal Norway rats and diurnal Nile grass rats. *Journal of Comparative Neurology* 520: 3277–3292.
25. Major, D. E., Rodman, H. R., Libedinsky, C., and Karten, H. J. (2003). Pattern of retinal projections in the California ground squirrel (*Spermophilus beecheyi*): Anterograde tracing study using cholera toxin. *Journal of Comparative Neurology* 463: 317–340.
26. Reuss, S. and Fuchs, E. (2000). Anterograde tracing of retinal afferents to the tree shrew hypothalamus and raphe. *Brain Research* 874: 66–74.
27. Murakami, D. M. and Fuller, C. A. (1990). The retinohypothalamic projection and oxidative metabolism in the suprachiasmatic nucleus of primates and tree shrews. *Brain, Behavior and Evolution* 35: 302–312.
28. Juárez, C., Morgado, E., Meza, E., Waliszewski, S. M., Aguilar-Roblero, R., and Caba, M. (2013). Development of retinal projections and response to photic input in the suprachiasmatic nucleus of New Zealand white rabbits. *Brain Research* 1499: 21–28.
29. Abizaid, A., Horvath, B., Keefe, D. L., Leranth, C., and Horvath, T. L. (2004). Direct visual and circadian pathways target neuroendocrine cells in primates. *European Journal of Neuroscience* 20: 2767–2776.
30. Costa, M. S. M., Santee, U. R., Cavalcante, J. S., Moraes, P. R. A., Santos, N. P., and Britto, L. R. G. (1999). Retinohypothalamic projections in the common marmoset (*Callithrix jacchus*): A study using cholera toxin subunit B. *Journal of Comparative Neurology* 415: 393–403.
31. Lima, R. R. M., Pinato, L., Nascimento, R. B. S., Engelberth, R. C. G., Nascimento, E. S., Cavalcante, J. C., Britto, L. R. G., Costa, M. S. M., and Cavalcante, J. S. (2012). Retinal projections and neurochemical characterization of the pregeniculate nucleus of the common marmoset (*Callithrix jacchus*). *Journal of Chemical Neuroanatomy* 44: 34–44.
32. Dai, J., van der Vliet, J., Swaab, D. F., and Buijs, R. M. (1998). Human retinohypothalamic tract as revealed by in vitro post-mortem tracing. *Journal of Comparative Neurology* 397: 357–370.
33. Chung, K. and Deisseroth, K. (2013). CLARITY for mapping the nervous system. *Nature Methods* 10: 508–513.
34. Vrang, N., Mrosovsky, N., and Mikkelsen, J. D. (2003). Afferent projections to the hamster intergeniculate leaflet demonstrated by retrograde and anterograde tracing. *Brain Research Bulletin* 59: 267–288.
35. Moore, R. Y., Weis, R., and Moga, M. M. (2000). Efferent projections of the intergeniculate leaflet and the ventral lateral geniculate nucleus in the rat. *Journal of Comparative Neurology* 420: 398–418.
36. Morin, L. P. and Blanchard, J. H. (2001). Neuromodulator content of hamster intergeniculate leaflet neurons and their projection to the suprachiasmatic nucleus or visual midbrain. *Journal of Comparative Neurology* 437: 79–90.
37. Yi, C. X., van der Vliet, J., Dai, J., Yin, G., Ru, L., and Buijs, R. M. (2006). Ventromedial arcuate nucleus communicates peripheral metabolic information to the suprachiasmatic nucleus. *Endocrinology* 147: 283–294.
38. Hay-Schmidt, A., Vrang, N., Larsen, P. J., and Mikkelsen, J. D. (2003). Projections from the raphe nuclei to the suprachiasmatic nucleus of the rat. *Journal of Chemical Neuroanatomy* 25: 293–310.
39. Alamilla, J. and Aguilar-Roblero, R. (2010). Glutamate and GABA neurotransmission from the paraventricular thalamus to the suprachiasmatic nuclei in the rat. *Journal of Biological Rhythms* 25: 28–36.
40. Meijer, J. H., Rusak, B., and Gänshirt, G. (1992). The relation between light-induced discharge in the suprachiasmatic nucleus and phase shifts of hamster circadian rhythms. *Brain Research* 598: 257–263.
41. Meijer, J. H., Watanabe, K., Détári, L., and Schaap, J. (1996). Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats. *Brain Research* 741: 352–355.
42. Roig, J. A., Granados-Fuentes, D., and Aguilar-Roblero, R. (1997). Neuronal subpopulations in the suprachiasmatic nuclei based on their response to retinal and intergeniculate leaflet stimulation. *NeuroReport* 8: 885–890.
43. Meijer, J. H., Watanabe, K., Schaap, J., Albus, H., and Détári, L. (1998). Light responsiveness of the suprachiasmatic nucleus: Long-term multiunit and single-unit recordings in freely moving rats. *Journal of Neuroscience* 18: 9078–9087.
44. Jiao, Y. Y., Lee, T. M., and Rusak, B. (1999). Photic responses of suprachiasmatic area neurons in diurnal degus (*Octodon degus*) and nocturnal rats (*Rattus norvegicus*). *Brain Research* 817: 93–103.
45. Schaap, J. and Meijer, J. H. (2001). Opposing effects of behavioural activity and light on neurons of the suprachiasmatic nucleus. *European Journal of Neuroscience* 13: 1955–1962.
46. Kuhlman, S. J., Silver, R., LeSauter, J., Bult-Ito, A., and McMahon, D. G. (2003). Phase resetting light pulses induce *Per1* and persistent spike activity in a subpopulation of biological clock neurons. *Journal of Neuroscience* 23: 1441–1450.
47. Nakamura, T. J., Fujimura, K., Ebihara, S., and Shinohara, K. (2004). Light response of the neuronal firing activity in the suprachiasmatic nucleus of mice. *Neuroscience Letters* 371: 244–248.
48. Wong, K. Y., Graham, D. M., and Berson, D. M. (2007). The retina-attached SCN slice preparation: An in vitro mammalian circadian visual system. *Journal of Biological Rhythms* 22: 400–410.

49. Van Oosterhout, F., Fisher, S. P., van Diepen, H. C., Watson, T. S., Houben, T., van der Leest, H. T., Thompson, S., Peirson, S. N., Foster, R. G., and Meijer, J. H. (2012). Ultraviolet light provides a major input to non-image-forming light detection in mice. *Current Biology* 22: 1397–1402.
50. Shibata, S., Liou, S. Y., and Ueki, S. (1986). Influence of excitatory amino acid receptor antagonists and of baclofen on synaptic transmission in the optic nerve to the suprachiasmatic nucleus in slices of rat hypothalamus. *Neuropharmacology* 25: 403–409.
51. Kim, Y. I. and Dudek, F. E. (1992). Intracellular electrophysiological study of suprachiasmatic nucleus neurons in rodents: Inhibitory synaptic mechanisms. *Journal of Physiology* 458: 247–260.
52. Pennartz, C. M. A., Hamstra, R., and Geurtsen, A. M. S. (2001). Enhanced NMDA receptor activity in retinal inputs to the rat suprachiasmatic nucleus during the subjective night. *Journal of Physiology* 532: 181–194.
53. Jiao, Y. Y. and Rusak, B. (2003). Electrophysiology of optic nerve input to suprachiasmatic nucleus neurons in rats and degus. *Brain Research* 960: 142–151.
54. Walmsley, L., Hanna, L., Mouland, J., Martial, F., West, A., Smedley, A. R., Bechtold, D. A., Webb, A. R., Lucas, R. J., and Brown, T. M. (2015). Colour as a signal for entraining the mammalian circadian clock. *PLoS Biology* 13: e1002127.
55. Senseman, D. M. and Rea, M. A. (1994). Fast multisite optical recording of mono- and polysynaptic activity in the hamster suprachiasmatic nucleus evoked by retinohypothalamic tract stimulation. *Neuroimage* 1: 247–263.
56. Kim, D. Y., Kang, H. C., Shin, H. C., Lee, K. J., Yoon, Y. W., Han, H. C., Na, H. S., Hong, S. K., and Kim, Y. I. (2001). Substance P plays a critical role in photic resetting of the circadian pacemaker in the rat hypothalamus. *Journal of Neuroscience* 21: 4026–4031.
57. De Vries, M. J., Treep, J. A., de Pauw, E. S. D., and Meijer, J. H. (1994). The effects of electrical stimulation of the optic nerves and anterior optic chiasm on the circadian activity rhythm of the Syrian hamster: Involvement of excitatory amino acids. *Brain Research* 642: 206–212.
58. Wee, R., Castrucci, A. M., Provencio, I., Gan, L., and Van Gelder, R. N. (2002). Loss of photic entrainment and altered free-running circadian rhythms in *math5*$^{-/-}$ mice. *Journal of Neuroscience* 22: 10427–10433.
59. Sutin, E. L. and Kilduff, T. S. (1992). Circadian and light-induced expression of immediate early gene mRNAs in the rat suprachiasmatic nucleus. *Molecular Brain Research* 15: 281–290.
60. Kaufman, C. M. and Menaker, M. (1994). Ontogeny of light-induced Fos-like immunoreactivity in the hamster suprachiasmatic nucleus. *Brain Research* 633: 162–166.
61. Teclemariam-Mesbah, R., Vuillez, P., van Rossum, A., and Pévet, P. (1995). Time course of neuronal sensitivity to light in the circadian timing system of the golden hamster. *Neuroscience Letters* 201: 5–8.
62. Sumová, A., Trávnícková, Z., and Illnerová, H. (1995). Memory on long but not on short days is stored in the rat suprachiasmatic nucleus. *Neuroscience Letters* 200: 191–194.
63. Grosse, J., Loudon, A. S. I., and Hastings, M. H. (1995). Behavioural and cellular responses to light of the circadian system of *tau* mutant and wild-type Syrian hamsters. *Neuroscience* 65: 587–597.
64. Vuillez, P., Jacob, N., Teclemariam-Mesbah, R., and Pévet, P. (1996). In Syrian and European hamsters, the duration of sensitive phase to light of the suprachiasmatic nuclei depends on the photoperiod. *Neuroscience Letters* 208: 37–40.
65. Shimomura, K., Kornhauser, J. M., Wisor, J. P., Umezu, T., Yamazaki, S., Ihara, N. L., Takahashi, J. S., and Menaker, M. (1998). Circadian behavior and plasticity of light-induced *c-fos* expression in SCN of *tau* mutant hamsters. *Journal of Biological Rhythms* 13: 305–314.
66. Morris, M. E., Viswanathan, N., Kuhlman, S., Davis, F. C., and Weitz, C. J. (1998). A screen for genes induced in the suprachiasmatic nucleus by light. *Science* 279: 1544–1547.
67. Sumová, A., Trávnícková, Z., Mikkelsen, J. D., and Illnerová, H. (1998). Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research* 801: 254–258.
68. Edelstein, K. and Amir, S. (1998). Glutamatergic antagonists do not attenuate light-induced Fos protein in rat intergeniculate leaflet. *Brain Research* 810: 264–268.
69. Guido, M. E., Guido, L. B. de, Goguen, D., Robertson, H. A., and Rusak, B. (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *Journal of Biological Rhythms* 14: 275–280.
70. Best, J. D., Maywood, E. S., Smith, K. L., and Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *Journal of Neuroscience* 19: 828–835.
71. Dkhissi-Benyahya, O., Sicard, B., and Cooper, H. M. (2000). Effects of irradiance and stimulus duration on early gene expression (Fos) in the suprachiasmatic nucleus: Temporal summation and reciprocity. *Journal of Neuroscience* 20: 7790–7797.
72. Amy, S. P., Chari, R., and Bult, A. (2000). Fos in the suprachiasmatic nucleus of house mouse lines that reveal a different phase-delay response to the same light pulse. *Journal of Biological Rhythms* 15: 95–102.
73. Kriegsfeld, L. J., Drazen, D. L., and Nelson, R. J. (2001). Circadian organization in male mice lacking the gene for endothelial nitric oxide synthase (eNOS$^{-/-}$). *Journal of Biological Rhythms* 16: 142–148.
74. Mahoney, M., Bult, A., and Smale, L. (2001). Phase response curve and light-induced Fos expression in suprachiasmatic nucleus and adjacent hypothalamus of *Arvicanthis niloticus*. *Journal of Biological Rhythms* 16: 149–162.
75. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J., and Mrosovsky, M. (2003). Behavioral arousal blocks light-induced phase advances in locomotor rhythmicity but not light-induced Perl and Fos expression in the hamster suprachiasmatic nucleus. *Neuroscience* 118: 253–261.
76. Muscat, L. and Morin, L. P. (2005). Binocular contributions to the responsiveness and integrative capacity of the circadian rhythm system to light. *Journal of Biological Rhythms* 20: 513–525.
77. Fite, K. V., Wu, P. S., and Bellemer, A. (2005). Photostimulation alters c-Fos expression in the dorsal raphe nucleus. *Brain Research* 1031: 245–252.
78. Zhang, Y., Kornhauser, J. M., Zee, P. C., Mayo, K. E., Takahashi, J. S., and Turek, F. W. (1996). Effects of aging on light-induced phase-shifting of circadian behavioral rhythms, Fos expression and CREB phosphorylation in the hamster suprachiasmatic nucleus. *Neuroscience* 70: 951–961.
79. Bedrosian, T. A., Vaughn, C. A., Galan, A., Daye, G., Weil, Z. M., and Nelson, R. J. (2013). Nocturnal light exposure impairs affective responses in a wavelength-dependent manner. *Journal of Neuroscience* 33: 13081–13087.
80. Derambure, P. S. and Boulant, J. A. (1994). Circadian thermosensitive characteristics of suprachiasmatic neurons in vitro. *American Journal of Physiology* 266: R1876–R1884.

81. Nakamura, W., Honma, S., Shirakawa, T., and Honma, K. (2001). Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *European Journal of Neuroscience* 14: 666–674.
82. Sumová, A., Trávnícková, Z., and Illnerová, H. (2000). Spontaneous c-Fos rhythm in the rat suprachiasmatic nucleus: Location and effect of photoperiod. *American Journal of Physiology* 279: R2262–R2269.
83. Beaulé, C., Arvanitogiannis, A., and Amir, S. (2001). Light suppresses Fos expression in the shell region of the suprachiasmatic nucleus at dusk and dawn: Implications for photic entrainment of circadian rhythms. *Neuroscience* 106: 249–254.
84. Beaulé, C., Barry-Shaw, J., and Amir, S. (2004). Fos expression in the suprachiasmatic nucleus during photic entrainment of circadian rhythms in retinally damaged rats. *Journal of Molecular Neuroscience* 22: 223–230.
85. Rusak, B., Meijer, J. H., and Harrington, M. E. (1989). Hamster circadian rhythms are phase-shifted by electrical stimulation of the geniculo-hypothalamic tract. *Brain Research* 493: 283–291.
86. Treep, J. A., Abe, H., Rusak, B., and Goguen, D. M. (1995). Two distinct retinal projections to the hamster suprachiasmatic nucleus. *Journal of Biological Rhythms* 10: 299–307.
87. Harrington, M. E. and Rusak, B. (1988). Ablation of the geniculo-hypothalamic tract alters circadian activity rhythms of hamsters housed under constant light. *Physiology and Behavior* 42: 183–189.
88. Kuroda, H., Fukushima, M., Nakai, M., Katayama, T., and Murakami, N. (1997). Daily wheel running activity modifies the period of free-running rhythms in rats via intergeniculate leaflet. *Physiology and Behavior* 61: 633–637.
89. Redlin, U., Vrang, N., and Mrosovsky, N. (1999). Enhanced masking response to light in hamsters with IGL lesions. *Journal of Comparative Physiology A* 184: 449–456.
90. Jacob, N., Vuillez, P., Lakdhar-Ghazal, N., and Pévet, P. (1999). Does the intergeniculate leaflet play a role in the integration of the photoperiod by the suprachiasmatic nucleus? *Brain Research* 828: 83–90.
91. Edelstein, K. and Amir, S. (1999). The role of the intergeniculate leaflet in entrainment of circadian rhythms to a skeleton photoperiod. *Journal of Neuroscience* 19: 372–380.
92. Goel, N., Governale, M. M., Jechura, T. J., and Lee, T. M. (2000). Effects of intergeniculate leaflet lesions on circadian rhythms in *Octodon degus*. *Brain Research* 877: 306–313.
93. Menet, J., Vuillez, P., Jacob, N., and Pévet, P. (2001). Intergeniculate leaflets lesion delays but does not prevent the integration of photoperiodic change by the suprachiasmatic nuclei. *Brain Research* 906: 176–179.
94. Gall, A. J., Smale, L., Yan, L., and Nunez, A. A. (2013). Lesions of the intergeniculate leaflet lead to a reorganization in circadian regulation and a reversal in masking responses to photic stimuli in the Nile grass rat. *PLOS ONE* 8: e67387.
95. Morin, L. P. and Pace, L. (2002). The intergeniculate leaflet, but not the visual midbrain, mediates hamster circadian rhythm response to constant light. *Journal of Biological Rhythms* 17: 217–226.
96. Zhang, Y., Van Reeth, O., Zee, P. C., Takahashi, J. S., and Turek, F. W. (1993). Fos protein expression in the circadian clock is not associated with phase shifts induced by a nonphotic stimulus, triazolam. *Neuroscience Letters* 164: 203–208.
97. Edelstein, K., Pfaus, J. G., Rusak, B., and Amir, S. (1995). Neonatal monosodium glutamate treatment prevents effects of constant light on circadian temperature rhythms of adults rats. *Brain Research* 675: 135–142.
98. Krajnak, K., Dickenson, L., and Lee, T. M. (1997). The induction of Fos-like proteins in the suprachiasmatic nuclei and intergeniculate leaflet by light pulses in degus (*Octodon degus*) and rats. *Journal of Biological Rhythms* 12: 401–412.
99. Amir, S. and Stewart, J. (1998). Conditioned fear suppresses light-induced resetting of the circadian clock. *Neuroscience* 86: 345–351.
100. Caldelas, I., Salazar-Juárez, A., Granados-Fuentes, D., Escobar, C., and Aguilar-Roblero, R. (1998). Circadian modulation of c-Fos expression occurs only in the SCN and not in other visual projection areas in the rat. *Biological Rhythm Research* 29: 494–500.
101. Marchant, E. G. and Morin, L. P. (1999). The hamster circadian rhythm system includes nuclei of the subcortical visual shell. *Journal of Neuroscience* 19: 10482–10493.
102. Janik, D. and Mrosovsky, N. (1992). Gene expression in the geniculate induced by a nonphotic circadian phase shifting stimulus. *NeuroReport* 3: 575–578.
103. Mead, S., Ebling, F. J. P., Maywood, E. S., Humbly, T., Herbert, J., and Hastings, M. H. (1992). A nonphotic stimulus causes instantaneous phase advances of the light-entrainable circadian oscillator of the Syrian hamster but does not induce the expression of *c-fos* in the suprachiasmatic nuclei. *Journal of Neuroscience* 12: 2516–2522.
104. Mikkelsen, J. D., Vrang, N., and Mrosovsky, N. (1998). Expression of Fos in the circadian system following nonphotic stimulation. *Brain Research Bulletin* 47: 367–376.
105. Liou, S. Y., Shibata, S., Iwasaki, K., and Ueki, S. (1986). Optic nerve stimulation-induced increase of release of ^{3}H-glutamate and ^{3}H-aspartate but not ^{3}H-GABA from the suprachiasmatic nucleus in slices of the rat hypothalamus. *Brain Research Bulletin* 16: 527–531.
106. Shirakawa, T. and Moore, R. Y. (1994). Glutamate shifts the phase of the circadian neuronal firing rhythm in the rat suprachiasmatic nucleus in vitro. *Neuroscience Letters* 178: 47–50.
107. Franken, P., Cao, V., Heller, H. C., and Miller, J. D. (1999). The glutamate induced phase shift in the SCN slice: A two pulse study. *Brain Research* 818: 34–40.
108. Chen, D., Buchanan, G. F., Ding, J. M., Hannibal, J., and Gillette, M. U. (1999). Pituitary adenylate cyclase-activating peptide: A pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 96: 13468–13473.
109. Yannielli, P. C. and Harrington, M. E. (2001). The neuropeptide Y Y5 receptor mediates the blockade of "photic-like" NMDA-induced phase shifts in the golden hamster. *Journal of Neuroscience* 21: 5367–5373.
110. Van der Leest, H. T., Rohling, J. H. T., Michel, S., and Meijer, J. H. (2009). Phase shifting capacity of the circadian pacemaker determined by the SCN neuronal network organization. *PLOS ONE* 4: e4976.
111. Sugino, T., Shimazoe, T., Ikeda, M., and Watanabe, S. (2006). Role of nociceptin and opioid receptor like 1 on entrainment function in the rat suprachiasmatic nucleus. *Neuroscience* 137: 537–544.
112. Mintz, E. M., Marvel, C. L., Gillespie, C. F., Price, K. M., and Albers, H. E. (1999). Activation of NMDA receptors in the suprachiasmatic nucleus produces light-like phase shifts of the circadian clock *in vivo*. *Journal of Neuroscience* 19: 5124–5130.

113. Mintz, E. M., Jasnow, A. M., Gillespie, C. F., Huhman, K. L., and Albers, H. E. (2002). GABA interacts with photic signaling in the suprachiasmatic nucleus to regulate circadian phase shifts. *Neuroscience* 109: 773–778.
114. Novak, C. M. and Albers, H. E. (2002). *N*-methyl-D-aspartate microinjected into the suprachiasmatic nucleus mimics the phase-shifting effects of light in the diurnal Nile grass rat (*Arvicanthis niloticus*). *Brain Research* 951: 255–263.
115. Gamble, K. L., Novak, C. M., and Albers, H. E. (2004). Neuropeptide Y and *N*-methyl-D-aspartic acid interact within the suprachiasmatic nuclei to alter circadian phase. *Neuroscience* 126: 559–565.
116. Colwell, C. S., Ralph, M. R., and Menaker, M. (1990). Do NMDA receptors mediate the effects of light on circadian behavior? *Brain Research* 523: 117–120.
117. Colwell, C. S. and Menaker, M. (1992). NMDA as well as non-NMDA receptor antagonists can prevent the phase-shifting effects of light on the circadian system of the golden hamster. *Journal of Biological Rhythms* 7: 125–136.
118. Hannibal, J., Ding, J. M., Chen, D., Fahrenkrug, J., Larsen, P. J., Gillette, M. U., and Mikkelsen, J. D. (1997). Pituitary adenylate cyclase-activating peptide (PACAP) in the retinohypothalamic tract: A potential daytime regulator of the biological clock. *Journal of Neuroscience* 17: 2637–2644.
119. Bergström, A. L., Hannibal, J., Hindersson, P., and Fahrenkrug, J. (2003). Light-induced phase shift in the Syrian hamster (*Mesocricetus auratus*) is attenuated by the PACAP receptor antagonist PACAP6-38 or PACAP immunoneutralization. *European Journal of Neuroscience* 18: 2552–2562.
120. Hannibal, J., Hindersson, P., Østergaard, J., Georg, B., Heegaard, S., Larsen, P. J., and Fahrenkrug, J. (2004). Melanopsin is expressed in PACAP-containing retinal ganglion cells of the human retinohypothalamic tract. *Investigative Ophthalmology and Visual Science* 45: 4202–4209.
121. Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Lelièvre, V., Hu, Z., and Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology* 287: R1194–R1201.
122. Minami, Y., Furuno, K., Akiyama, M., Moriya, T., and Shibata, S. (2002). Pituitary adenylate cyclase-activating polypeptide produces a phase shift associated with induction of *mPer* expression in the mouse suprachiasmatic nucleus. *Neuroscience* 113: 37–45.
123. Yannielli, P. C., Brewer, J. M., and Harrington, M. E. (2004). Blockade of the NPY Y5 receptor potentiates circadian responses to light: Complementary *in vivo* and *in vitro* studies. *European Journal of Neuroscience* 19: 891–897.
124. Lahmam, M., El M'rabet, A., Ouarour, A., Pévet, P., Challet, E., and Vuillez, P. (2008). Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent *Lemniscomys barbarus*. *Chronobiology International* 25: 882–904.
125. Huhman, K. L. and Albers, H. E. (1994). Neuropeptide Y microinjected into the suprachiasmatic region phase shifts circadian rhythms in constant darkness. *Peptides* 15: 1475–1478.
126. Biello, S. M. and Mrosovsky, N. (1996). Phase response curves to neuropeptide Y in wildtype and *tau* mutant hamsters. *Journal of Biological Rhythms* 11: 27–34.
127. Harrington, M. E. and Schak, K. M. (2000). Neuropeptide Y phase advances the in vitro hamster circadian clock during the subjective day with no effect on phase during the subjective night. *Canadian Journal of Physiology and Pharmacology* 78: 87–92.
128. Knoch, M. E., Gobes, S. M. H., Pavlovska, I., Su, C., Mistlberger, R. E., and Glass, J. D. (2004). Short-term exposure to constant light promotes strong circadian phase-resetting responses to nonphotic stimuli in Syrian hamsters. *European Journal of Neuroscience* 19: 2779–2790.
129. Soscia, S. J. and Harrington, M. E. (2005). Neuropeptide Y does not reset the circadian clock in NPY $Y2^{-/-}$ mice. *Neuroscience Letters* 373: 175–178.
130. Yannielli, P. C. and Harrington, M. E. (2000). Neuropeptide Y applied *in vitro* can block the phase shifts induced by light *in vivo*. *NeuroReport* 11: 1587–1591.
131. Van den Pol, A. N. (1986). Gamma-aminobutyrate, gastrin releasing peptide, serotonin, somatostatin, and vasopressin: Ultrastructural immunocytochemical localization in presynaptic axons in the suprachiasmatic nucleus. *Neuroscience* 17: 643–659.
132. Morin, L. P. (1992). Serotonergic reinnervation of the hamster suprachiasmatic nucleus and intergeniculate leaflet without functional circadian rhythm recovery. *Brain Research* 599: 98–104.
133. Prosser, R. A., Dean, R. R., Edgar, D. M., Heller, H. C., and Dement, W. C. (1993). Serotonin and the mammalian circadian system. I. *In vitro* phase shifts by serotonergic agonists and antagonists. *Journal of Biological Rhythms* 8: 1–16.
134. Pickard, G. E., Smith, B. N., Belenky, M., Rea, M. A., Dudek, F. E., and Sollars, P. J. (1999). 5-HT_{1B} receptor-mediated presynaptic inhibition of retinal input to the suprachiasmatic nucleus. *Journal of Neuroscience* 19: 4034–4045.
135. Manrique, C., Héry, F., Faudon, M., and François-Bellan, A. M. (1999). Indirect evidence for an association of 5-HT_{1B} binding sites with retinal and geniculate axon terminals in the rat suprachiasmatic nucleus. *Synapse* 33: 314–323.
136. Belenky, M. A. and Pickard, G. E. (2001). Subcellular distribution of 5-HT_{1B} and 5-HT_7 receptors in the mouse suprachiasmatic nucleus. *Journal of Comparative Neurology* 432: 371–388.
137. Sanggaard, K. M., Hannibal, J., and Fahrenkrug, J. (2003). Serotonin inhibits glutamate- but not PACAP-induced *per* gene expression in the rat suprachiasmatic nucleus at night. *European Journal of Neuroscience* 17: 1245–1252.
138. Moore, R. Y. and Speh, J. C. (2004). Serotonin innervation of the primate suprachiasmatic nucleus. *Brain Research* 1010: 169–173.
139. Glass, J. D., Grossman, G. H., Farnbauch, L., and DiNardo, L. (2003). Midbrain raphe modulation of nonphotic circadian clock resetting and 5-HT release in the mammalian suprachiasmatic nucleus. *Journal of Neuroscience* 23: 7451–7460.
140. Edgar, D. M., Miller, J. D., Prosser, R. A., Dean, R. R., and Dement, W. C. (1993). Serotonin and the mammalian circadian system. II. Phase-shifting rat behavioral rhythms with serotonergic agonists. *Journal of Biological Rhythms* 8: 17–31.
141. Cutrera, R. A., Saboureau, M., and Pévet, P. (1996). Phase-shifting effects of 8-OH-DPAT, a 5-HT_{1A}/5-HT_7 receptor agonist, on locomotor activity in golden hamster in constant darkness. *Neuroscience Letters* 210: 1–4.
142. Bobrzynska, K. J., Godfrey, M. H., and Mrosovsky, N. (1996). Serotonergic stimulation and nonphotic phase-shifting in hamsters. *Physiology and Behavior* 59: 221–230.
143. Challet, E., Scarbrough, K., Penev, P. D., and Turek, F. W. (1998). Roles of suprachiasmatic nuclei and intergeniculate leaflets in mediating the phase-shifting effects of a serotonergic agonist and their photic modulation during subjective day. *Journal of Biological Rhythms* 13: 410–421.

144. Horikawa, K. and Shibata, S. (2004). Phase-resetting response to (+)8-OH-DPAT, a serotonin 1A/7 receptor agonist, in the mouse in vivo. *Neuroscience Letters* 368: 130–134.
145. Basu, P., Singaravel, M., and Haldar, C. (2012). L-5-hydroxytryptophan resets the circadian locomotor activity rhythm of the nocturnal Indian pygmy field mouse, *Mus terricolor. Naturwissenschaften* 99: 233–239.
146. Prosser, R. A. (2003). Serotonin phase-shifts the mouse suprachiasmatic circadian clock in vitro. *Brain Research* 966: 110–115.
147. Antle, M. C., Marchant, E. G., Niel, L., and Mistlberger, R. E. (1998). Serotonin antagonists do not attenuate activity-induced phase shifts of circadian rhythms in the Syrian hamster. *Brain Research* 813: 139–149.
148. Challet, E., Turek, F. W., Laute, M. A., and Van Reeth, O. (2001). Sleep deprivation decreases phase-shift responses of circadian rhythms to light in the mouse: Role of serotonergic and metabolic signals. *Brain Research* 909: 81–91.
149. Antle, M. C., Ogilvie, M. D., Pickard, G. E., and Mistlberger, R. E. (2003). Response of the mouse circadian system to serotonin 1A/2/7 agonists in vivo: Surprisingly little. *Journal of Biological Rhythms* 18: 145–158.
150. Moriya, T., Yoshinobu, Y., Ikeda, M., Akiyama, M., and Shibata, S. (1998). Potentiating action of MKC-242, a selective 5-HT_{1A} receptor agonist, on the entrainment of the circadian activity rhythm in hamsters. *British Journal of Pharmacology* 125: 1281–1287.
151. Byku, M. and Gannon, R. L. (2000). Effects of the $5HT_{1A}$ agonist/antagonist BMY 7378 on light-induced phase advances in hamster circadian activity rhythms during aging. *Journal of Biological Rhythms* 15: 300–305.
152. Rea, M. A., Barrera, J., Glass, J. D., and Gannon, R. L. (1995). Serotonergic potentiation of photic phase shifts of the circadian activity rhythm. *NeuroReport* 6: 1289–1292.
153. Gannon, R. L. (2003). Serotonergic $serotonin_{1A}$ mixed agonists/antagonists elicit large-magnitude phase shifts in hamster circadian wheel-running rhythms. *Neuroscience* 119: 567–576.
154. Mistlberger, R. E. and Antle, M. C. (1998). Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. *Brain Research* 786: 31–38.
155. Challet, E., Pévet, P., and Malan, A. (1997). Lesion of the serotonergic terminals in the suprachiasmatic nuclei limits the phase advance of body temperature rhythm in food-restricted rats fed during daytime. *Journal of Biological Rhythms* 12: 235–244.
156. Mistlberger, R. E., Bossert, J. M., Holmes, M. M., and Marchant, E. G. (1998). Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. *Behavioural Brain Research* 96: 93–99.
157. Prosser, R. A. (2001). Glutamate blocks serotonergic phase advances of the mammalian circadian pacemaker through AMPA and NMDA receptors. *Journal of Neuroscience* 21: 7815–7822.
158. Sollars, P. J., Ogilvie, M. D., Rea, M. A., and Pickard, G. E. (2002). 5-HT_{1B} receptor knockout mice exhibit an enhanced response to constant light. *Journal of Biological Rhythms* 17: 428–437.
159. Folkard, S., Arendt, J., Aldhous, M., and Kennett, H. (1990). Melatonin stabilises sleep onset time in a blind man without entrainment of cortisol or temperature rhythms. *Neuroscience Letters* 113: 193–198.
160. Hastings, M. H., Mead, S. M., Vindlacheruvu, R. R., Ebling, F. J. P., Maywood, E. S., and Grosse, J. (1992). Non-photic phase shifting of the circadian activity rhythm of Syrian hamsters: The relative potency of arousal and melatonin. *Brain Research* 591: 20–26.
161. Grosse, J., Velickovic, A., and Davis, F. C. (1996). Entrainment of Syrian hamster circadian activity rhythms by neonatal melatonin injections. *American Journal of Physiology* 270: R533–R540.
162. Hao, H. and Rivkees, S. (2000). Melatonin does not shift circadian phase in baboons. *Journal of Clinical Endocrinology and Metabolism* 85: 3618–3622.
163. Chiba, A., Kikuchi, M., and Aoki, K. (1995). Entrainment of the circadian locomotor activity rhythm in the Japanese newt by melatonin injections. *Journal of Comparative Physiology A* 176: 473–477.
164. Oshima, I., Yamada, H., Goto, M., Sato, K., and Ebihara, S. (1989). Pineal and retinal melatonin is involved in the control of circadian locomotor activity and body temperature rhythms in the pigeon. *Journal of Comparative Physiology A* 166: 217–226.
165. Chabot, C. C. and Menaker, M. (1992). Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons. *Journal of Comparative Physiology A* 170: 615–622.
166. Heigl, S. and Gwinner, E. (1994). Periodic melatonin in the drinking water synchronizes circadian rhythms in sparrows. *Naturwissenschaften* 81: 83–85.
167. Lumineau, S., Guyomarc'h, C., Vivien-Roels, B., and Houdelier, C. (2002). Cyclic melatonin synchronises the circadian rhythm of feeding activity in Japanese quail, *Coturnix c. japonica*. *Comptes Rendus Biologies* 325: 205–212.
168. Houdelier, C., Guyomarc'h, C., Lumineau, S., and Richard, J. P. (2002). Circadian rhythms of oviposition and feeding activity in Japanese quail: Effects of cyclic administration of melatonin. *Chronobiology International* 19: 1107–1119.
169. Warren, W. S., Hodges, D. B., and Cassone, V. M. (1993). Pinealectomized rats entrain and phase-shift to melatonin injections in a dose-dependent manner. *Journal of Biological Rhythms* 8: 233–245.
170. Duffield, G. E., Hastings, M. H., and Ebling, F. J. P. (1998). Investigation into the regulation of the circadian system by dopamine and melatonin in the adult Siberian hamster (*Phodopus sungorus*). *Journal of Neuroendocrinology* 10: 871–884.
171. Kirsch, R., Belgnaoui, S., Gourmelen, S., and Pévet, P. (1993). Daily melatonin infusion entrains free-running activity in Syrian and Siberian hamsters. In: Wetterberg, L. (Ed.), *Light and Biological Rhythms in Man*. New York: Pergamon, pp. 107–120.
172. Schuhler, S., Pitrosky, B., Kirsch, R., and Pévet, P. (2002). Entrainment of locomotor activity rhythm in pinealectomized adult Syrian hamsters by daily melatonin infusion. *Behavioural Brain Research* 133: 343–350.
173. Sharma, V. K. and Chidambaram, R. (2003). Entrainment of circadian locomotor activity rhythm of the nocturnal field mouse *Mus booduga* using daily injections of melatonin. *Journal of Experimental Zoology* 296A: 30–37.
174. Redman, J. R. and Francis, A. J. P. (1998). Entrainment of rat circadian rhythms by the melatonin agonist S-20098 requires intact suprachiasmatic nuclei but not the pineal. *Journal of Biological Rhythms* 13: 39–51.
175. Slotten, H. A., Pitrosky, B., and Pévet, P. (1999). Influence of the mode of daily melatonin administration on entrainment of rat circadian rhythms. *Journal of Biological Rhythms* 14: 347–353.
176. Pitrosky, B., Kirsch, R., Malan, A., Mocaer, E., and Pévet, P. (1999). Organization of rat circadian rhythms during daily infusion of melatonin or S20098, a melatonin agonist. *American Journal of Physiology* 277: R812–R828.

177. Slotten, H. A., Pitrosky, B., and Pévet, P. (2000). Entrainment of rat circadian rhythms by melatonin does not depend on the serotonergic afferents to the suprachiasmatic nuclei. *Brain Research* 876: 10–16.
178. Slotten, H. A., Pitrosky, B., Krekling, S., and Pévet, P. (2002). Entrainment of circadian activity rhythms in rats to melatonin administered at T cycles different from 24 hours. *Neurosignals* 11: 73–80.
179. Slotten, H. A., Krekling, S., and Pévet, P. (2005). Photic and nonphotic effects on the circadian activity rhythm in the diurnal rodent *Arvicanthis ansorgei*. *Behavioural Brain Research* 165: 91–97.
180. Masuda, K. and Zhdanova, I. V. (2010). Intrinsic activity rhythms in *Macaca mulatta*: Their entrainment to light and melatonin. *Journal of Biological Rhythms* 25: 361–371.
181. Zhdanova, I. V., Masuda, K., Bozhokin, S. V., Rosene, D. L., González-Martínez, J., Schettler, S., and Samorodnitsky, E. (2012). Familial circadian rhythm disorder in the diurnal primate *Macaca mulatta*. *PLOS ONE* 7: e33327.
182. Sack, R. L., Lewy, A. J., Blood, M. L., Stevenson, J., and Keith, L. D. (1991). Melatonin administration to blind people: Phase advances and entrainment. *Journal of Biological Rhythms* 6: 249–261.
183. Middleton, B., Arendt, J., and Stone, B. M. (1997). Complex effects of melatonin on human circadian rhythms in constant dim light. *Journal of Biological Rhythms* 12: 467–477.
184. Lockley, S. W., Skene, D. J., James, K., Thapan, K., Wright, J., and Arendt, J. (2000). Melatonin administration can entrain the free-running circadian system of blind subjects. *Journal of Endocrinology* 164: R1–R6.
185. Sack, R. L., Brandes, R. W., Kendall, A. R., and Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
186. Hack, L. M., Lockley, S. W., Arendt, J., and Skene, D. J. (2003). The effects of low-dose 0.5-mg melatonin on the free-running circadian rhythms of blind subjects. *Journal of Biological Rhythms* 18: 420–429.
187. Lewy, A. J., Emens, J. S., Bernert, R. A., and Lefler, B. J. (2004). Eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation: Clinical implications. *Journal of Biological Rhythms* 19: 68–75.
188. Weaver, D. R., Rivkees, S. A., and Reppert, S. M. (1989). Localization and characterization of melatonin receptors in rodent brain by in vitro autoradiography. *Journal of Neuroscience* 9: 2581–2590.
189. Weaver, D. R. and Reppert, S. M. (1996). The Mel_{1a} melatonin receptor gene is expressed in human suprachiasmatic nuclei. *NeuroReport* 8: 109–112.
190. Gauer, F., Schuster, C., Poirel, V. J., Pévet, P., and Masson-Pévet, M. (1998). Cloning experiments and developmental expression of both melatonin receptor Mel_{1A} mRNA and melatonin binding sites in the Syrian hamster suprachiasmatic nuclei. *Molecular Brain Research* 60: 193–202.
191. Hunt, A. E., Al-Ghoul, W. M., Gillette, M. U., and Dubocovich, M. L. (2001). Activation of MT_2 melatonin receptors in rat suprachiasmatic nucleus phase advances the circadian clock. *American Journal of Physiology* 280: C110–C118.
192. Jin, X., von Gall, C., Pieschl, R. L., Gribkoff, V. K., Stehle, J. H., Reppert, S. M., and Weaver, D. R. (2003). Targeted disruption of the mouse Mel_{1b} melatonin receptor. *Molecular and Cellular Biology* 23: 1054–1060.
193. Benloucif, S. and Dubocovich, M. L. (1996). Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN mouse. *Journal of Biological Rhythms* 11: 113–125.
194. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M., and Subbaraj, R. (1999). In the field mouse *Mus booduga* melatonin phase response curves (PRCs) have a different time course and wave form relative to light PRC. *Journal of Pineal Research* 26: 153–157.
195. Sumová, A. and Illnerová, H. (1996). Melatonin instantaneously resets intrinsic circadian rhythmicity in the rat suprachiasmatic nucleus. *Neuroscience Letters* 218: 181–184.
196. Weaver, D. R., Liu, C., and Reppert, S. M. (1996). Nature's knockout: The Mel_{1b} receptor is not necessary for reproductive and circadian responses to melatonin in Siberian hamsters. *Molecular Endocrinology* 10: 1478–1487.
197. Lewy, A. J., Ahmed, S., Jackson, J. M. L., and Sack, R. L. (1992). Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiology International* 9: 380–392.
198. Deacon, S., English, J., and Arendt, J. (1994). Acute phase-shifting effects of melatonin associated with suppression of core body temperature in humans. *Neuroscience Letters* 178: 32–34.
199. Attenburrow, M. E. J., Dowling, B. A., Sargent, P. A., Sharpley, A. L., and Cowen, P. J. (1995). Melatonin phase advances circadian rhythm. *Psychopharmacology* 121: 503–505.
200. Rajaratnam, S. M. W., Dijk, D. J., Middleton, B., Stone, B. M., and Arendt, J. (2003). Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *Journal of Clinical Endocrinology and Metabolism* 88: 4303–4309.
201. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C., and Weber, J. M. (2004). Evening melatonin and bright light administration induce additive phase shifts in dim light melatonin onset. *Journal of Pineal Research* 36: 192–194.
202. Burke, T. M., Markwald, R. R., Chinoy, E. D., Snider, J. A., Bessman, S. C., Jung, C. M., and Wright, K. P. (2013). Combination of light and melatonin time cues for phase advancing the human circadian clock. *Sleep* 36: 1617–1624.
203. Morin, L. P., Fitzgerald, K. M., and Zucker, I. (1977). Estradiol shortens the period of hamster circadian rhythms. *Science* 196: 305–307.
204. Takahashi, J. S. and Menaker, M. (1980). Interaction of estradiol and progesterone: Effects on circadian locomotor rhythm of female golden hamsters. *American Journal of Physiology* 239: R497–R504.
205. Zucker, I., Fitzgerald, K. M., and Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.
206. Lumineau, S., Guyomarc'h, C., Boswell, T., Richard, J. P., and Leray, D. (1998). Induction of circadian rhythm of feeding activity by testosterone implantations in arrhythmic Japanese quail males. *Journal of Biological Rhythms* 13: 278–287.
207. Schull, J., McEachron, D. L., Adler, N. T., Fiedler, L., Horvitz, J., Noyes, A., Olson, M., and Shack, J. (1988). Effects of thyroidectomy, parathyroidectomy and lithium on circadian wheel running in rats. *Physiology and Behavior* 42: 33–39.
208. Bauer, M. S., Soloway, A., Dratman, M. B., and Kreider, M. (1992). Effects of hypothyroidism on rat circadian activity and temperature rhythms and their response to light. *Biological Psychiatry* 32: 411–425.

209. Duncan, W. C. and Schull, J. (1994). The interaction of thyroid state, MAOI drug treatment, and light on the level and circadian pattern of wheel-running in rats. *Biological Psychiatry* 35: 324–334.
210. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O., and Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
211. Prosser, R. A. and Bergeron, H. E. (2003). Leptin phase-advances the rat suprachiasmatic circadian clock in vitro. *Neuroscience Letters* 336: 139–142.
212. Byku, M. and Gannon, R. L. (2000). Opioid induced non-photic phase shifts of hamster circadian activity rhythms. *Brain Research* 873: 189–196.
213. Byku, M. and Gannon, R. L. (2000). SNC 80, a δ-opioid agonist, elicits phase advances in hamster circadian activity rhythms. *NeuroReport* 11: 1449–1452.
214. Tierno, A., Fiore, P., and Gannon, R. L. (2002). Delta opioid inhibition of light-induced phase advances in hamster circadian activity rhythms. *Brain Research* 937: 66–73.
215. Cote, N. K. and Harrington, M. E. (1993). Histamine phase shifts the circadian clock in a manner similar to light. *Brain Research* 613: 149–151.
216. Meyer, J. L., Hall, A. C., and Harrington, M. E. (1998). Histamine phase shifts the hamster circadian pacemaker via an NMDA dependent mechanism. *Journal of Biological Rhythms* 13: 288–295.
217. Elliott, K. J., Weber, E. T., and Rea, M. A. (2001). Adenosine A_1 receptors regulate the response of the hamster circadian clock to light. *European Journal of Pharmacology* 414: 45–53.
218. Antle, M. C., Steen, N. M., and Mistlberger, R. E. (2001). Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. *NeuroReport* 12: 2901–2905.
219. Cagampang, F. R. A., Okamura, H., and Inouye, S. T. (1994). Circadian rhythms of norepinephrine in the rat suprachiasmatic nucleus. *Neuroscience Letters* 173: 185–188.
220. Earnest, D. J. and Turek, F. W. (1985). Neurochemical basis for the photic control of circadian rhythms and seasonal reproductive cycles: Role for acetylcholine. *Proceedings of the National Academy of Sciences of the United States of America* 82: 4277–4281.
221. Meijer, J. H., van der Zee, E., and Dietz, M. (1988). The effects of intraventricular carbachol injections on the free-running activity rhythm of the hamster. *Journal of Biological Rhythms* 3: 333–348.
222. Wee, B. E. F., Anderson, K. D., Kouchis, N. S., and Turek, F. W. (1992). Administration of carbachol into the lateral ventricle and suprachiasmatic nucleus (SCN) produces dose-dependent phase shifts in the circadian rhythm of locomotor activity. *Neuroscience Letters* 137: 211–215.
223. Liu, C. and Gillette, M. U. (1996). Cholinergic regulation of the suprachiasmatic nucleus circadian rhythm via a muscarinic mechanism at night. *Journal of Neuroscience* 16: 744–751.
224. Bina, K. G. and Rusak, B. (1996). Muscarinic receptors mediate carbachol-induced phase shifts of circadian activity rhythms in Syrian hamsters. *Brain Research* 743: 202–211.
225. Erhardt, C., Galani, R., Jeltsch, H., Cassel, J. C., Klosen, P., Menet, J. S., Pévet, P., and Challet, E. (2004). Modulation of photic resetting in rats by lesions of projections to the suprachiasmatic nuclei expressing p75 neurotrophin receptor. *European Journal of Neuroscience* 19: 1773–1788.
226. Colwell, C. S., Kaufman, C. M., and Menaker, M. (1993). Phase-shifting mechanisms in the mammalian circadian system: New light on the carbachol paradox. *Journal of Neuroscience* 13: 1454–1459.
227. Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A., and Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740.
228. Lee, C., Parikh, V., Itsukaichi, T., Bae, K., and Edery, I. (1996). Resetting the Drosophila clock by photic regulation of PER and a PER-TIM complex. *Science* 271: 1740–1744.
229. Emery, P., So, W. V., Kaneko, M., Hall, J. C., and Rosbash, M. (1998). CRY, a *Drosophila* clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. *Cell* 95: 669–679.
230. Ceriani, M. F., Darlington, T. K., Staknis, D., Más, P., Petti, A. A., Weitz, C. J., and Kay, S. A. (1999). Light-dependent sequestration of TIMELESS by CRYPTOCHROME. *Science* 285: 553–556.
231. Ivanchenko, M., Stanewsky, R., and Giebultowicz, J. M. (2001). Circadian photoreception in *Drosophila*: Functions of cryptochrome in peripheral and central clocks. *Journal of Biological Rhythms* 16: 205–215.
232. Lin, F. J., Song, W., Meyer-Bernstein, E., Naidoo, N., and Sehgal, A. (2001). Photic signaling by cryptochrome in the *Drosophila* circadian system. *Molecular and Cellular Biology* 21: 7287–7294.
233. Koh, K., Zheng, X., and Sehgal, A. (2006). JETLAG resets the *Drosophila* circadian clock by promoting light-induced degradation of TIMELESS. *Science* 312: 1809–1812.
234. Klarsfeld, A., Malpel, S., Michard-Vanhée, C., Picot, M., Chélot, E., and Rouyer, F. (2004). Novel features of cryptochrome-mediated photoreception in the brain circadian clock of *Drosophila*. *Journal of Neuroscience* 24: 1468–1477.
235. Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J. C., and Stanewsky, R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30: 249–261.
236. Rieger, D., Stanewsky, R., and Helfrich-Förster, C. (2003). Cryptochrome, compound eyes, Hofbauer-Buchner eyelets, and ocelli play different roles in the entrainment and masking pathway of the locomotor activity rhythm in the fruit fly *Drosophila melanogaster*. *Journal of Biological Rhythms* 18: 377–391.
237. Rust, M. J., Golden, S. S., and O'Shea, E. K. (2011). Light-driven changes in energy metabolism directly entrain the cyanobacterial circadian oscillator. *Science* 331: 220–223.
238. Devlin, P. F. and Kay, S. A. (2001). Circadian photoperception. *Annual Review of Physiology* 63: 677–694.
239. Salomé, P. A. and McClung, C. R. (2004). The *Arabidopsis thaliana* clock. *Journal of Biological Rhythms* 19: 425–435.
240. Crosthwaite, S. K., Loros, J. J., and Dunlap, J. C. (1995). Light-induced resetting of a circadian clock is mediated by a rapid increase in *frequency* transcript. *Cell* 81: 1003–1012.
241. Crosthwaite, S. K., Dunlap, J. C., and Loros, J. J. (1997). *Neurospora wc-1* and *wc-2*: Transcription, photoresponses, and the origins of circadian rhythmicity. *Science* 276: 763–769.
242. Froehlich, A. C., Liu, Y., Loros, J. J., and Dunlap, J. C. (2002). White collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. *Science* 297: 815–819.
243. He, Q., Cheng, P., Yang, Y., Wang, L., Gardner, K. H., and Liu, Y. (2002). White collar-1, a DNA binding transcription factor and a light sensor. *Science* 297: 840–842.

244. Butcher, G. Q., Dziema, H., Collamore, M., Brugoon, P. W., and Obrietan, K. (2002). The p42/44 mitogen-activated protein kinase pathway couples photic input to circadian clock entrainment. *Journal of Biological Chemistry* 277: 29519–29525.
245. Shearman, L. P., Zylka, M. J., Weaver, D. R., Kolakowski, L. F., and Reppert, S. M. (1997). Two *period* homologs: Circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron* 19: 1261–1269.
246. Shigeyoshi, Y., Taguchi, K., Yamamoto, S., Takekida, S., Yan, L., Tei, H., Moriya, T. et al. (1997). Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the *mPer1* transcript. *Cell* 91: 1043–1053.
247. Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P. et al. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 282: 1490–1494.
248. Okamura, H., Miyake, S., Sumi, Y., Yamaguchi, S., Yasui, A., Muijtjens, M., Hoeijmakers, J. H. J., and van der Horst, G. T. J. (1999). Photic induction of *mPer1* and *mPer2* in *Cry*-deficient mice lacking a biological clock. *Science* 286: 2531–2534.
249. Wakamatsu, H., Takahashi, S. Moriya, T., Inouye, S. T., Okamura, H., Akiyama, M., and Shibata, S. (2001). Additive effect of *mPer1* and *mPer2* antisense oligonucleotides on light-induced phase shift. *NeuroReport* 12: 127–131.
250. Asai, M., Yamaguchi, S., Isejima, H., Janouchi, M., Moriya, T., Shibata, S., Kobayashi, M., and Okamura, H. (2001). Visualization of *mPer1* transcription in vitro: NMDA induces a rapid shift of *mPer1* gene in cultured SCN. *Current Biology* 11: 1524–1527.
251. Von Gall, C., Noton, E., Lee, C., and Weaver, D. R. (2003). Light does not degrade the constitutively expressed BMAL1 protein in the mouse suprachiasmatic nucleus. *European Journal of Neuroscience* 18: 125–133.
252. Paul, K. N., Fukuhara, C., Tosini, G., and Albers, H. E. (2003). Transduction of light in the suprachiasmatic nucleus: Evidence for two different neurochemical cascades regulating the levels of *per1* mRNA and pineal melatonin. *Neuroscience* 119: 137–144.
253. Butcher, G. Q., Lee, B., Cheng, H. M., and Obrietan, K. (2005). Light stimulates MSK1 activation in the suprachiasmatic nucleus via a PACAP-ERK/MAP kinase-dependent mechanism. *Journal of Neuroscience* 25: 5305–5313.
254. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U., and Nevo, E. (2002). Circadian genes in a blind subterranean mammal. II. Conservation and uniqueness of the three *Period* homologs in the blind subterranean mole rat, *Spalax ehrenbergi* superspecies. *Proceedings of the National Academy of Sciences of the United States of America* 99: 11718–11723.
255. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P., and Challet, E. (2003). Circadian profile and photic regulation of clock genes in the suprachiasmatic nucleus of a diurnal mammal, *Arvicanthis ansorgei*. *Neuroscience* 116: 583–591.
256. Beaulé, C., Houle, L. M., and Amir, S. (2003). Expression profiles of PER2 immunoreactivity within the shell and core regions of the rat suprachiasmatic nucleus. *Journal of Molecular Neuroscience* 21: 133–147.
257. Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H., and Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *Journal of Biological Rhythms* 18: 159–169.
258. Yan, L. and Silver, R. (2002). Differential induction and localization of mPer1 and mPer2 during advancing and delaying phase shifts. *European Journal of Neuroscience* 16: 1531–1540.
259. Yan, L. and Silver, R. (2004). Resetting the brain clock: Time course and localization of mPER1 and mPER2 protein expression in suprachiasmatic nuclei during phase shifts. *European Journal of Neuroscience* 19: 1105–1109.
260. Albrecht, U., Zheng, B., Larkin, D., Sun, Z. S., and Lee, C. C. (2001). *mPer1* and *mPer2* are essential for normal resetting of the circadian clock. *Journal of Biological Rhythms* 16: 100–104.
261. Olde Scheper, T., Klinkenberg, D., Pennartz, C., and van Pelt, J. (1999). A mathematical model for the intracellular circadian rhythm generator. *Journal of Neuroscience* 19: 40–47.
262. Leloup, J. C., and Goldbeter, A. (1998). A model for circadian rhythms in *Drosophila* incorporating the formation of a complex between the PER and TIM proteins. *Journal of Biological Rhythms* 13: 70–87.
263. Petri, B. and Stengl, M. (2001). Phase response curves of a molecular model oscillator: Implications for mutual coupling of paired oscillators. *Journal of Biological Rhythms* 16: 125–141.
264. Smolen, P., Baxter, D. A., and Byrne, J. H. (2001). Modeling circadian oscillations with interlocking positive and negative feedback loops. *Journal of Neuroscience* 21: 6644–6656.
265. Gonze, D., Halloy, J., and Gldbeter, A. (2002). Robustness of circadian rhythms with respect to molecular noise. *Proceedings of the National Academy of Sciences of the United States of America* 99: 673–678.
266. Geier, F., Becker-Weimann, S., Kramer, A., and Herzel, H. (2005). Entrainment in a model of the mammalian circadian oscillator. *Journal of Biological Rhythms* 20: 83–93.
267. Bagheri, N., Taylor, S. R., Meeker, K., Petzold, L. R., and Doyle, F. J. (2008). Synchrony and entrainment properties of robust circadian oscillators. *Journal of the Royal Society Interface* 5: S17–S28.
268. Akman, O. E., Watterson, S., Parton, A., Binns, N., Millar, A. J., and Ghazal, P. (2012). Digital clocks: Simple Boolean models can quantitatively describe circadian systems. *Journal of the Royal Society Interface* 9: 2365–2382.
269. Relógio, A., Westermark, P. O., Wallach, T., Schellenberg, K., Kramer, A., and Herzel, H. (2011). Tuning the mammalian circadian clock: Robust synergy of two loops. *PLoS Computational Biology* 7: e1002309.
270. Forger, D. B. and Peskin, C. S. (2003). A detailed predictive model of the mammalian circadian clock. *Proceedings of the National Academy of Sciences of the United States of America* 100: 14806–14811.
271. Teclemariam-Mesbah, R., Kalsbeek, A., Pévet, P., and Buijs, R. M. (1997). Direct vasoactive intestinal polypeptide-containing projection from the suprachiasmatic nucleus to spinal projecting hypothalamic paraventricular neurons. *Brain Research* 748: 71–76.
272. Teclemariam-Mesbah, R., Ter Horst, G. J., Postema, F., Wortel, J., and Buijs, R. M. (1999). Anatomical demonstration of the suprachiasmatic nucleus-pineal pathway. *Journal of Comparative Neurology* 406: 171–182.
273. Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J., and Buijs, R. M. (2000). Functional connections between the suprachiasmatic nucleus and the thyroid gland as revealed by lesioning and viral tracing techniques in the rat. *Endocrinology* 141: 3832–3841.

274. Scheer, F. A. J. L., Ter Horst, G. J., van der Vliet, J., and Buijs, R. M. (2001). Physiological and anatomic evidence for regulation of the heart by suprachiasmatic nucleus in rats. *American Journal of Physiology* 280: H1391–H1399.
275. Buijs, R. M., La Fleur, S. E., Wortel, J., van Heyningen, C., Zuiddam, L., Mettenleiter, T. C., Kalsbeek, A., Nagai, K., and Niijima, A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. *Journal of Comparative Neurology* 464: 36–48.
276. Leak, R. K. and Moore, R. Y. (2001). Topographic organization of suprachiasmatic nucleus projection neurons. *Journal of Comparative Neurology* 433: 312–334.
277. Leak, R. K., Card, J. P., and Moore, R. Y. (1999). Suprachiasmatic pacemaker organization analyzed by viral transynaptic transport. *Brain Research* 819: 23–32.
278. Deurveilher, S. and Semba, K. (2005). Indirect projections from the suprachiasmatic nucleus to major arousal-promoting cell groups in rat: Implications for the circadian control of behavioural state. *Neuroscience* 130: 165–183.
279. Vujovic, N., Gooley, J. J., Jhou, T. C., and Saper, C. B. (2015). Projections from the subparaventricular zone define four channels of output from the circadian timing system. *Journal of Comparative Neurology* 523: 2714–2737.
280. Kriegsfeld, L. J., Leak, R. K., Yackulic, C. B., LeSauter, J., and Silver, R. (2004). Organization of suprachiasmatic nucleus projections in Syrian hamsters (*Mesocricetus auratus*): An anterograde and retrograde analysis. *Journal of Comparative Neurology* 468: 361–379.
281. Munch, I. C., Møller, M., Larsen, P. J., and Vrang, N. (2002). Light-induced c-Fos expression in suprachiasmatic nuclei neurons targeting the paraventricular nucleus of the hamster hypothalamus: Phase dependence and immunochemical identification. *Journal of Comparative Neurology* 442: 48–62.
282. Smeraski, C. A., Sollars, P. J., Ogilvie, M. D., Enquist, L. W., and Pickard, G. E. (2004). Suprachiasmatic nucleus input to autonomic circuits identified by retrograde transsynaptic transport of pseudorabies virus from the eye. *Journal of Comparative Neurology* 471: 298–313.
283. Mistlberger, R. E. (2005). Circadian regulation of sleep in mammals: Role of the suprachiasmatic nucleus. *Brain Research Reviews* 49: 429–454.
284. Novak, C. M. and Nunez, A. A. (2000). A sparse projection from the suprachiasmatic nucleus to the sleep active ventrolateral preoptic area in the rat. *NeuroReport* 11: 93–96.
285. Deurveilher, S., Burns, J., and Semba, K. (2002). Indirect projections from the suprachiasmatic nucleus to the ventrolateral preoptic nucleus: A dual tract-tracing study in rat. *European Journal of Neuroscience* 16: 1195–1213.
286. Chou, T. C., Scammell, T. E., Gooley, J. J., Gaus, S. E., Saper, C. B., and Lu, J. (2003). Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *Journal of Neuroscience* 23: 10691–10702.
287. Aston-Jones, G., Chen, S., Zhu, Y., and Oshinsky, M. L. (2001). A neural circuit for circadian regulation of arousal. *Nature Neuroscience* 4: 732–738.
288. Noguchi, T., Watanabe, K., Ogura, A., and Yamaoka, S. (2004). The clock in the dorsal suprachiasmatic nucleus runs faster than that in the ventral. *European Journal of Neuroscience* 20: 3199–3202.
289. Novak, C. M. and Nunez, A. A. (1998). Daily rhythms in Fos activity in the rat ventrolateral preoptic area and midline thalamic nuclei. *American Journal of Physiology* 275: R1620–R1626.
290. Sun, X., Whitefield, S., Rusak, B., and Semba, K. (2001). Electrophysiological analysis of suprachiasmatic nucleus projections to the ventrolateral preoptic area in the rat. *European Journal of Neuroscience* 14: 1257–1274.
291. Stephan, F. K. and Nunez, A. A. (1977). Elimination of circadian rhythms in drinking, activity, sleep, and temperature by isolation of the suprachiasmatic nuclei. *Behavioral Biology* 20: 1–16.
292. Honma, S., Honma, K., and Hiroshige, T. (1984). Dissociation of circadian rhythms in rats with a hypothalamic island. *American Journal of Physiology* 246: R949–R954.
293. Hakim, H., DeBernardo, A. P., and Silver, R. (1991). Circadian locomotor rhythms, but not photoperiodic responses, survive surgical isolation of the SCN in hamsters. *Journal of Biological Rhythms* 6: 97–113.
294. Klein, D. C., Smoot, R., Weller, J. L., Higa, S., Markey, S. P., Creed, G. J., and Jacobowitz, D. M. (1983). Lesions of the paraventricular nucleus area of the hypothalamus disrupt the suprachiasmatic-spinal cord circuit in the melatonin generating rhythm. *Brain Research Bulletin* 10: 647–652.
295. Moore, R. Y. and Danchenko, R. L. (2002). Paraventricular-subparaventricular hypothalamic lesions selectively affect circadian function. *Chronobiology International* 19: 345–360.
296. Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., van der Vliet, J., van Heijningen, C., Simonneaux, V., Pévet, P., and Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: Inhibitory and stimulatory mechanisms. *European Journal of Neuroscience* 17: 221–228.
297. Lu, J., Zhang, Y. H., Chou, T. C., Gaus, S. E., Elmquist, J. K., Shiromani, P., and Saper, C. B. (2001). Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. *Journal of Neuroscience* 21: 4864–4874.
298. Abrahamson, E. E. and Moore, R. Y. (2006). Lesions of suprachiasmatic nucleus efferents selectively affect rest-activity rhythm. *Molecular and Cellular Endocrinology* 252: 46–56.
299. Nunez, A. A., Bult, A., McElhinny, T. L., and Smale, L. (1999). Daily rhythms of Fos expression in hypothalamic targets of the suprachiasmatic nucleus in diurnal and nocturnal rodents. *Journal of Biological Rhythms* 14: 300–306.
300. Hermes, M. L. H., Coderre, E. M., Buijs, R. M., and Renaud, L. P. (1996). GABA and glutamate mediate rapid neurotransmission from suprachiasmatic nucleus to hypothalamic paraventricular nucleus in rat. *Journal of Physiology* 496: 749–757.
301. Cui, L., Coderre, E., and Renaud, L. P. (2000). $GABA_B$ presynaptically modulates suprachiasmatic input to hypothalamic paraventricular magnocellular neurons. *American Journal of Physiology* 278: R1210–R1216.
302. Cui, L. N., Coderre, E., and Renaud, L. P. (2001). Glutamate and GABA mediate suprachiasmatic nucleus inputs to spinal-projecting paraventricular neurons. *American Journal of Physiology* 281: R1283–R1289.
303. Kalsbeek, A., Garidou, M. L., Palm, I. F., van der Vliet, J., Simonneauz, V., Pévet, P., and Buijs, R. M. (2000). Melatonin sees the light: Blocking GABA-ergic transmission in the paraventricular nucleus induces daytime secretion of melatonin. *European Journal of Neuroscience* 12: 3146–3154.
304. Lipton, J. M. (1968). Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiology and Behavior* 3: 165–169.
305. Lepkovsky, S., Snapir, N., and Furuta, F. (1968). Temperature regulation and appetitive behavior in chickens with hypothalamic lesions. *Physiology and Behavior* 3: 911–915.

306. Carlisle, H. J. (1969). Effect of preoptic and anterior hypothalamic lesions on behavioral thermoregulation in the cold. *Journal of Comparative and Physiological Psychology* 69: 391–402.
307. Satinoff, E. and Rutstein, J. (1970). Behavioral thermoregulation in rats with anterior hypothalamic lesions. *Journal of Comparative and Physiological Psychology* 71: 77–82.
308. Toth, D. M. (1973). Temperature regulation and salivation following preoptic lesions in the rat. *Journal of Comparative and Physiological Psychology* 82: 480–488.
309. Satinoff, E., Valentino, D., and Teitelbaum, P. (1976). Thermoregulatory cold-defense deficits in rats with preoptic/anterior hypothalamic lesions. *Brain Research Bulletin* 1: 553–565.
310. Van Zoeren, J. G. and Stricker, E. M. (1977). Effects of preoptic, lateral hypothalamic, or dopamine-depleting lesions on behavioral thermoregulation in rats exposed to the cold. *Journal of Comparative and Physiological Psychology* 91: 989–999.
311. Hagan, A. A. and Heath, J. E. (1980). Effects of preoptic lesions on thermoregulation in ducks. *Journal of Thermal Biology* 5: 141–150.
312. Morimoto, A. and Murakami, N. (1985). [^{14}C]deoxyglucose incorporation into rat brain regions during hypothalamic or peripheral thermal stimulation. *American Journal of Physiology* 248: R84–R92.
313. Joyce, M. P. and Barr, G. A. (1992). The appearance of Fos protein-like immunoreactivity in the hypothalamus of developing rats in response to cold ambient temperatures. *Neuroscience* 49: 163–173.
314. Bratincsák, A. and Palkovits, M. (2004). Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience* 127: 385–397.
315. Szymusiak, R., DeMory, A., Kittrell, E. M. W., and Satinoff, E. (1985). Diurnal changes in thermoregulatory behavior in rats with medial preoptic lesions. *American Journal of Physiology* 249: R219–R227.
316. Satinoff, E. and Shan, S. Y. Y. (1971). Loss of behavioral thermoregulation after lateral hypothalamic lesions in rats. *Journal of Comparative and Physiological Psychology* 77: 302–312.
317. Refinetti, R. and Carlisle, H. J. (1986). Effects of lateral hypothalamic lesions on thermoregulation in the rat. *Physiology and Behavior* 38: 219–228.
318. Refinetti, R., Kaufman, C. M., and Menaker, M. (1994). Complete suprachiasmatic lesions eliminate circadian rhythmicity of body temperature and locomotor activity in golden hamsters. *Journal of Comparative Physiology A* 175: 223–232.
319. Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.
320. Osborne, A. R. and Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.
321. Ruby, N. F., Ibuka, N., Barnes, B. M., and Zucker, I. (1989). Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *American Journal of Physiology* 257: R210–R215.
322. Ruby, N. F. and Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *American Journal of Physiology* 263: R353–R362.
323. Paul, M. J., Kauffman, A. S., and Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
324. Ruis, J. F., Rietveld, W. J., and Buys, P. J. (1987). Effects of suprachiasmatic nuclei lesions on circadian and ultradian rhythms in body temperature in ocular enucleated rats. *Journal of Interdisciplinary Cycle Research* 18: 259–273.
325. Eastman, C. I., Mistlberger, R. E., and Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology and Behavior* 32: 357–368.
326. Honma, S., Honma, K., Shirakawa, T., and Hiroshige, T. (1988). Rhythms in behavior, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. *Physiology and Behavior* 44: 247–255.
327. Warren, W. S., Champney, T. H., and Cassone, V. M. (1994). The suprachiasmatic nucleus controls the circadian rhythm of heart rate via the sympathetic nervous system. *Physiology and Behavior* 55: 1091–1099.
328. Wachulec, M., Li, H., Tanaka, H., Peloso, E., and Satinoff, E. (1997). Suprachiasmatic nuclei lesions do not eliminate homeostatic thermoregulatory responses in rats. *Journal of Biological Rhythms* 12: 226–234.
329. Liu, S., Chen, X. M., Yoda, T. Nagashima, K., Fukuda, Y., and Kanosue, K. (2002). Involvement of the suprachiasmatic nucleus in body temperature modulation by food deprivation in rats. *Brain Research* 929: 26–36.
330. Angeles-Castellanos, M., Salgado-Delgado, R., Rodriguez, K., Buijs, R. M., and Escobar, C. (2010). The suprachiasmatic nucleus participates in food entrainment: A lesion study. *Neuroscience* 165: 1115–1126.
331. Jansen, H. T., Sergeeva, A., Stark, G., and Sorg, B. A. (2012). Circadian discrimination of reward: Evidence for simultaneous yet separable food- and drug-entrained rhythms in the rat. *Chronobiology International* 29: 454–468.
332. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C., and Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
333. Satinoff, E., Liran, J., and Clapman, R. (1982). Aberrations of circadian body temperature rhythms in rats with medial preoptic lesions. *American Journal of Physiology* 242: R352–R357.
334. Briese, E. (1989). Do medial preoptic lesions interfere with the set-point of temperature regulation? *Brain Research Bulletin* 23: 137–144.
335. Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D., and Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus *in vivo*. *Journal of Neuroscience* 18: 10709–10723.
336. Ralph, M. R., Foster, R. G., Davis, F. C., and Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247: 975–978.
337. Saitoh, Y., Matsui, Y., Nihonmatsu, I., and Kawamura, H. (1991). Cross-species transplantation of the suprachiasmatic nuclei from rats to Siberian chipmunks (*Eutamias sibiricus*) with suprachiasmatic lesions. *Neuroscience Letters* 123: 77–81.
338. Romero, M. T., Lehman, M. N., and Silver, R. (1993). Age of donor influences ability of suprachiasmatic nucleus grafts to restore circadian rhythmicity. *Developmental Brain Research* 71: 45–52.
339. LeSauter, J. and Silver, R. (1994). Suprachiasmatic nucleus lesions abolish and fetal grafts restore circadian gnawing rhythms in hamsters. *Restorative Neurology and Neuroscience* 6: 135–143.

340. Viswanathan, N. and Davis, F. C. (1995). Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. *Brain Research* 686: 10–16.
341. Sollars, P. J., Kimble, D. P., and Pickard, G. E. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *Journal of Neuroscience* 15: 2109–2122.
342. Grosse, J. and Davis, F. C. (1998). Melatonin entrains the restored circadian activity rhythms of Syrian hamsters bearing fetal suprachiasmatic nucleus grafts. *Journal of Neuroscience* 18: 8032–8037.
343. Boer, G. J., van Esseveldt, K. E., van der Geest, B. A. M., Duindam, H., and Rietveld, W. J. (1999). Vasopressin-deficient suprachiasmatic nucleus grafts re-instate circadian rhythmicity in suprachiasmatic nucleus lesioned arrhythmic rats. *Neuroscience* 89: 375–385.
344. Earnest, D. J., Liang, F. Q., Ratcliff, M., and Cassone, V. M. (1999). Immortal time: Circadian clock properties of rat suprachiasmatic cell lines. *Science* 283: 693–695.
345. Zlomanczuk, P., Mrugala, M., de la Iglesia, H. O., Ourednik, V., Quesenberry, P. J., Snyder, E. Y., and Schwartz, W. J. (2002). Transplanted clonal neural stem-like cells respond to remote photic stimulation following incorporation within the suprachiasmatic nucleus. *Experimental Neurology* 174: 162–168.
346. Tousson, E. and Meissl, H. (2004). Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus. *Journal of Neuroscience* 24: 2983–2988.
347. Aguilar-Roblero, R., Morin, L. P., and Moore, R. Y. (1994). Morphological correlates of circadian rhythm restoration induced by transplantation of the suprachiasmatic nucleus in hamsters. *Experimental Neurology* 130: 250–260.
348. Lehman, M. N., LeSauter, J., and Silver, R. (1998). Fiber outgrowth from anterior hypothalamic and cortical xenografts in the third ventricle. *Journal of Comparative Neurology* 391: 133–145.
349. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N., and Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
350. Silver, R., LeSauter, J., Tresco, P. A., and Lehman, M. N. (1996). A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature* 382: 810–813.
351. Allen, G., Rappe, J., Earnest, D. J., and Cassone, V. M. (2001). Oscillating on borrowed time: Diffusible signals from immortalized suprachiasmatic nucleus cells regulate circadian rhythmicity in cultured fibroblasts. *Journal of Neuroscience* 21: 7937–7943.
352. Guo, H., Brewer, J. M., Champhekar, A., Harris, R. B. S., and Bittman, E. L. (2005). Differential control of peripheral circadian rhythms by suprachiasmatic-dependent neural signals. *Proceedings of the National Academy of Sciences of the United States of America* 102: 3111–3116.
353. Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schütz, G., and Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science* 289: 2344–2347.
354. Pezük, P., Mohawk, J. A., Wang, L. A., and Menaker, M. (2012). Glucocorticoids as entraining signals for peripheral circadian oscillators. *Endocrinology* 153: 4775–4783.
355. Liu, Y., Merrow, M., Loros, J. J., and Dunlap, J. C. (1998). How temperature changes reset a circadian oscillator. *Science* 281: 825–829.
356. Brown, S. A., Zumbrunn, G., Fleury-Olela, F., Preitner, N., and Schibler, U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Current Biology* 12: 1574–1583.
357. Ruoff, P. and Rensing, L. (2004). Temperature effects on circadian clocks. *Journal of Thermal Biology* 29: 445–456.
358. Buhr, E. D., Yoo, S. H., and Takahashi, J. S. (2010). Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* 330: 379–385.
359. Morf, J., Ray, G., Schneider, K., Stratmann, M., Fujita, J., Naef, F., and Schibler, U. (2012). Cold-inducible RNA-binding protein modulates circadian gene expression posttranscriptionally. *Science* 338: 379–383.
360. Morf, J. and Schibler, U. (2013). Body temperature cycles: Gatekeepers of circadian clocks. *Cell Cycle* 12: 539–540.
361. McDonald, M. J. and Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* 107: 567–578.
362. Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N., and Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32: 657–671.
363. Ceriani, M. F., Hogenesch, J. B., Yanovsky, M., Panda, S., Straume, M., and Kay, S. A. (2002). Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *Journal of Neuroscience* 22: 9305–9319.
364. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N., and Taghert, P. H. (2002). Influence of the *period*-dependent circadian clock on diurnal, circadian, and aperiodic gene expression in *Drosophila melanogaster. Proceedings of the National Academy of Sciences of the United States of America* 99: 9562–9567.
365. Kita, Y., Shiozawa, M., Jin, W., Majewski, R. R., Besharse, J. C., Greene, A. S., and Jacob, H. J. (2002). Implications of circadian gene expression in kidney, liver and the effects of fasting on pharmacogenomic studies. *Pharmacogenetics* 12: 55–65.
366. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H., and Kyriacou, C. P. (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Current Biology* 12: 540–550.
367. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H., and Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.
368. Oishi, K., Miyazaki, K., Kadota, K., Kikuno, R., Nagase, T., Atsumi, G., Ohkura, N. et al. (2003). Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. *Journal of Biological Chemistry* 278: 41519–41527.
369. Desai, V. G., Moland, C. L., Branham, W. S., Delongchamp, R. R., Fang, H., Duffy, P. H., Peterson, C. A., Beggs, M. L., and Fuscoe, J. C. (2004). Changes in expression level of genes as a function of time of day in the liver of rats. *Mutation Research* 549: 115–129.

370. Ptitsyn, A. A., Zvonic, S., Conrad, S. A., Scott, L. K., Mynatt, R. L., and Gimble, J. M (2006). Circadian clocks are resounding in peripheral tissues. *PLoS Computational Biology* 2(3): e16.
371. Hirao, J., Arakawa, S., Watanabe, K., Ito, K., and Furukawa, T. (2006). Effects of restricted feeding on daily fluctuations of hepatic functions including P450 monooxygenase activities in rats. *Journal of Biological Chemistry* 281: 3165–3171.
372. Cho, H., Zhao, X., Hatori, M., Yu, R. T., Barish, G. D., Lam, M. T., Chong, L. W. et al. (2012). Regulation of circadian behaviour and metabolism by *Rev-erbα* and *Rev-erbβ*. *Nature* 485: 123–127.
373. Marshall, E. (2004). Getting the noise out of gene arrays. *Science* 306: 630–631.

This page intentionally left blank

Section V

Applications



A patient undergoes surgical biopsy for breast cancer.
(*Photograph courtesy of the U.S. National Cancer Institute.*)

This page intentionally left blank

14 Optimal Timing on Earth and in Space

14.1 PATTERNS OF HUMAN MOBILITY

In Section I of this book, we discussed historical and methodological aspects of research in circadian physiology. We examined the phenomenology of circadian rhythms in Section II. Endogenous and environmental mechanisms that control circadian rhythms were discussed in Section III. In Section IV, we addressed the cellular and molecular substrates of circadian rhythms. It is now time to try to relate this vast knowledge of fundamental principles to the complexity of human life. The four chapters in Section V will deal with the optimal time of day for human performance (Chapter 14), the economic and medical implications of shift work and jet lag (Chapter 15), the application of circadian physiology to the diagnosis and treatment of human diseases (Chapter 16), and the application of circadian physiology to veterinary medicine (Chapter 17). In discussing the optimal timing for human performance in this chapter, we will start here on Earth (Sections 14.1 and 14.2) and will later consider the issue of space exploration (Section 14.3).

The first topic that we will address is that of the patterns of human mobility. Figure 14.1 shows my own mobility record on a nationwide scale during the years 2013 and 2014. It may help you to know that I used to live in South Carolina and later moved to Idaho, but it is clear that I traveled to other locations as well. If airlines had records such as these for every person in the country, they would be able to optimize flight routes for the convenience of travelers and for their own profit. Hotels, car rental companies, restaurants, and other businesses could also benefit from this information. This is, therefore, valuable information. Nonetheless, most people, most of the time, remain in their own cities. Knowing when people go to different places can help city administrators plan the construction of new roads, schedule bus routes, coordinate police schedules, arrange staffing of emergency rooms in hospitals, and so on. There is, therefore, great interest in mapping patterns of urban mobility.

One way to track the movement of people is to track the movement of their mobile phones (assuming that people bring their phones with them everywhere they go and that they don't lend their phones to friends and relatives). Figure 14.2 shows the mobility patterns of two individuals (denoted by A and B) over 10 days (numbered from bottom to top), as determined by the location of their mobile phones. Despite an occasional trip farther a way, both individuals exhibit a relatively regular mobility pattern, starting the day at home and coming back home at the end of the day (and traveling to the same workplace during the day).[1] Studies of human mobility have been consistent in finding that most people visit a few places at about the same time each day, day after day.[1–4] This has been the case in studies using location information from mobile phones,[1,5] public transportation usage,[4] or automobile GPS tracking.[3]

Our motion in space is important, of course, but the temporal pattern of our motion is also of great consequence. Arriving safely at a restaurant to meet someone special for dinner is important, but arriving 2 hours late has grave consequences. As a matter of fact, because most humans spend a lot of time at the same location, the plain temporal course of physical activity is a valuable variable. We saw in Chapter 2 that small data loggers containing accelerometers (actigraphs) can be worn on the wrist or the waist to record physical activity as people go about their normal daily routines. A sample 24-hour record of physical activity of a 39-year-old woman from Chicago living her regular life is shown in Figure 14.3. She seems to wake up several hours after sunrise, to become gradually more active, to be very active from 11 a.m. to 4 p.m., and to become much less active after sunset. With some variability, this daily pattern of activity resembles the patterns of activity of humans and various animal species that were described in Chapter 5. Although the activity rhythm has been extensively studied in laboratory animals, relatively few studies have been conducted with human subjects using laboratory or home sensors[6–11] or actigraphs worn by individuals carrying out their regular lives in the real world.[12–15]

Several million people around the world voluntarily edit articles in Wikipedia, the open-access encyclopedia. A study of the time of day when edits are made, with adjustment for local time, revealed a global pattern with a minimum of activity at 6 a.m. followed by a rapid rise, a high plateau from noon to 10 p.m., and a fall to the next trough at 6 a.m.[16]

In a study involving more than 2300 men and women from five different countries individually recorded with actigraphs, researchers found out that the daily distribution of activity is relatively similar in different countries, except that residents of different countries tend to start (and end) the day a few hours earlier or later.[15] Figure 14.4 shows the distribution of acrophases (peak times) of the activity rhythm in the five countries. The distributions are similar (except maybe for a flatter distribution in the United States), but the mode of the distribution is about 3 hours later in the United States than in Ghana, with the other countries being in between. The differences in acrophases between the countries were strongly correlated with geographical location: people living in countries farther from the Equator tended to be active later in the day—an observation similar to that previously made within large countries such as Brazil[17] and Russia.[18] Differences in acrophase related to latitude and photoperiod in the multicountry study may be partly confounded by the fact that people in many countries near the Equator tend to include a majority of persons who

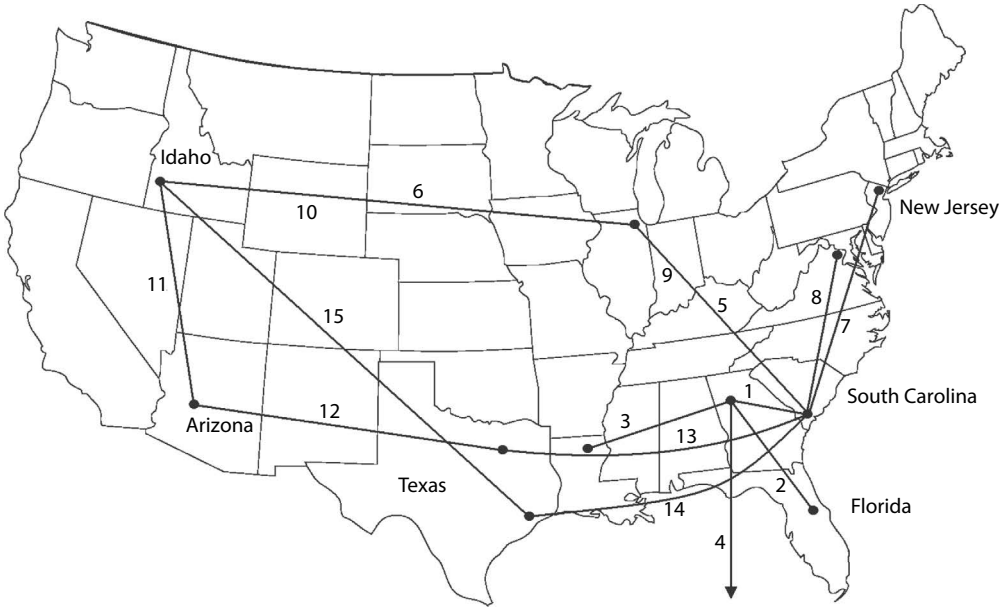


FIGURE 14.1 Traveling in America. This diagram summarizes the nationwide mobility pattern of a university professor in the years 2013 and 2014. Most trips were round trips, and the numbers indicate the order in which the trips occurred. Route 1 was a common first leg repeated for routes 2, 3, and 4. Route 4 was a round trip overseas.

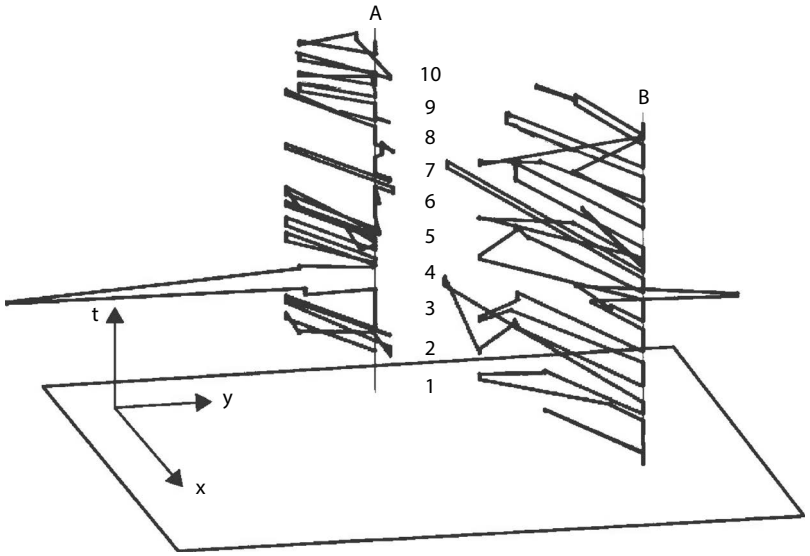


FIGURE 14.2 Tracking mobile phones. This diagram shows the mobility patterns of two individuals (A and B) over 10 days (numbered from bottom to top), as determined by the location of their mobile phones. (Adapted from Schneider, C.M. et al., *J. R. Soc. Interface*, 10, art. 20130246, 2013.)

time their daily work activities according to sunlight (such as farmers in Ghana), whereas people in countries farther from the Equator are often engaged in time-fixed office or industry occupations (such as urban dwellers in the United States).

When conducting further analysis of the data from the actigraphy study involving individuals from five different countries, the authors compared rhythmicity of physical activity on the daily, weekly, lunar, and seasonal time scales. They found robust daily rhythmicity in all countries (accounting for 23% of the total variance in the data), weaker weekly rhythmicity (accounting for 1% of the total variance), and no significant rhythmicity on the lunar or seasonal scales.[19] Thus, there is great variation in human physical activity between the day and the night, much less variation between days of the week, and only random variation across phases of the moon and months of the year. Whereas human rhythmicity according to the phases of the moon is probably only folklore,[19] the absence of rhythmicity according to the months of the year (seasons) is possibly due to the averaging of the activity patterns of many people. While it is true that office workers must report to work at the same time of day throughout the year (thus homogenizing the daily pattern of activity), it is also

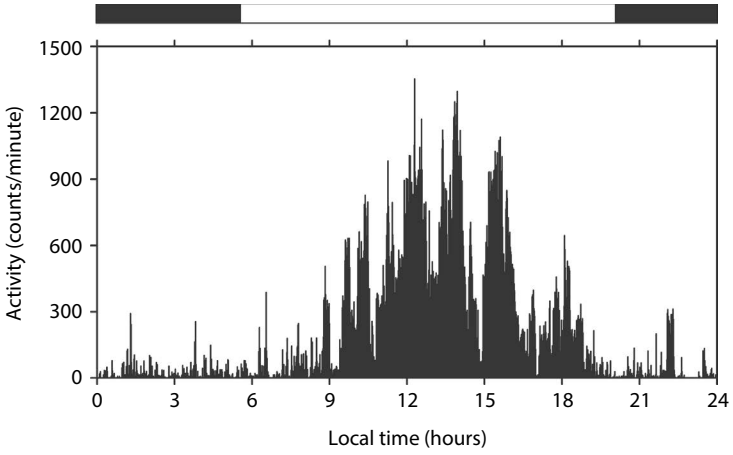


FIGURE 14.3 Daily activity pattern. The graph shows the physical activity (in 1-minute resolution averaged over 7 consecutive days) of a 39-year-old woman from Chicago living her regular life. The horizontal dark and white bars at the top of the graph indicate the duration of the dark and light phases of the prevailing light–dark cycle, respectively. (Data from Sani, M. et al., *Chronobiol. Int.*, 32, 650, 2015.)

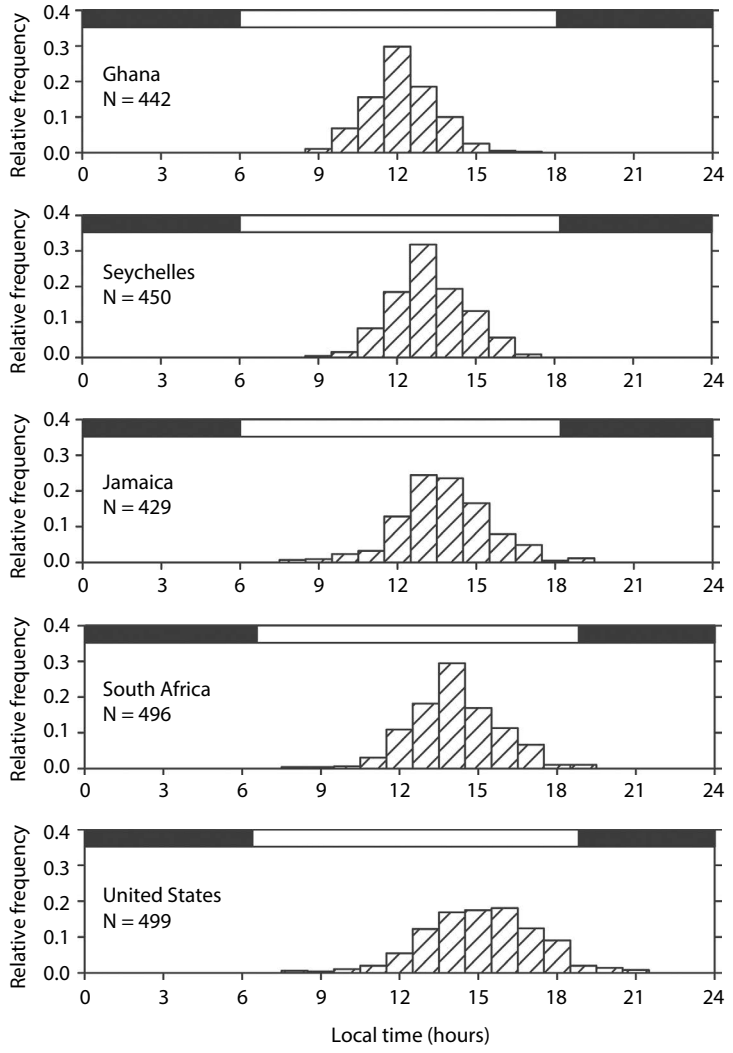


FIGURE 14.4 Different countries, different daily patterns. The graphs show the distributions of acrophases of the daily activity rhythms of residents of five different countries. The horizontal dark and white bars above the graphs indicate the duration of the dark and light phases of the prevailing light–dark cycles, respectively. Although the light–dark cycles are similar in the five countries, the mode of the distribution of acrophases varies by several hours from one country to another. (Data from Sani, M. et al., *Chronobiol. Int.*, 32, 650, 2015.)

true that most workers take vacations some time during the year, which should create a seasonal pattern of variation at the individual level. Alternatively, it is possible that the temporal pattern of activity remains the same during vacation time as during the rest of the year even though the specific types of activity (such as walking on the beach during vacation as opposed to walking through town during the rest of the year) may vary. Long-term longitudinal studies are needed to clarify this issue.

14.2 BEST TIME FOR SPORTS AND INTELLECTUAL ACTIVITIES

The knowledge acquired through research on basic mechanisms of circadian physiology can be expected to help us choose the best time of day to perform physical and intellectual activities. Most people overestimate the applicability of scientific knowledge to everyday life, but there is certainly valid advice that circadian physiologists can provide to the general public.

14.2.1 Physical Activities

Let us start with the issue of the best time of day for physical activities, such as sports (Figure 14.5). In previous chapters, we have seen over and over again that practically every variable in the body exhibits daily or circadian rhythmicity. Therefore, it is quite reasonable to expect that the ability to perform physical activities will exhibit daily rhythmicity. On the other hand, the previous chapters have shown us that quite a few "obvious" expectations are sometimes wrong. Rather than trust our intuition, we must examine the facts.

Laboratory studies on human subjects performing various forms of physical exercise have confirmed our expectation of daily rhythmicity. Whether one considers maximal isometric lifting strength,[20] endurance in concentric leg movements,[21] spontaneous variation in bicycle pedaling rate,[22,23] accuracy of badminton serve,[24] juggling performance in soccer,[25] anaerobic (strenuous) capacity,[26] or the subjective feeling of exertion,[27] the results are consistent in showing daily rhythmicity in performance. Performance is increased by about 10% in the late afternoon or early evening as compared to the early morning. As an example, Figure 14.6 shows the daily variation in knee-extension torque ("force") of trained bicyclists tested in the laboratory. Notice that torque is maximal at 19:00 hours (7 p.m.) and minimal in the early morning.[28]

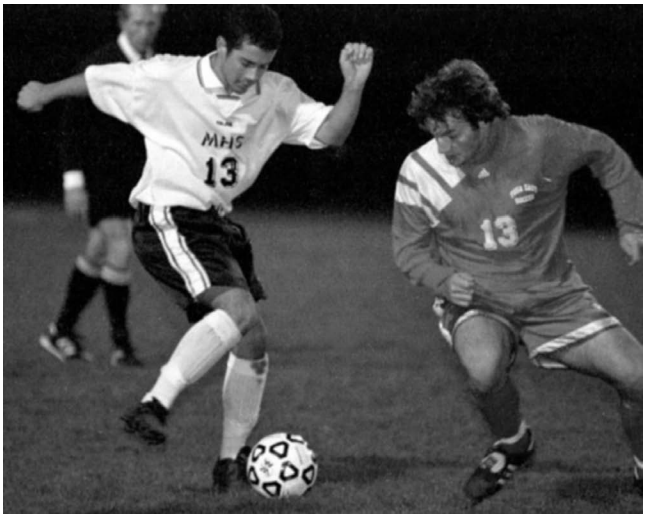


FIGURE 14.5 On the ball. Because many physiological variables exhibit circadian rhythmicity, performance in sports and other physical activities may depend on time of day. (Coyright ArtToday, Tucson, AZ.)

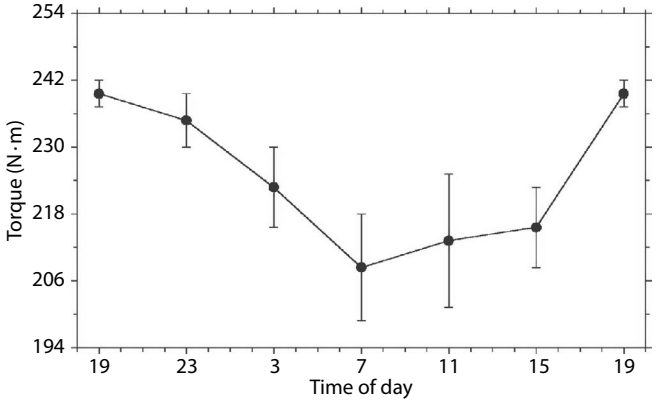


FIGURE 14.6 Daily variation in muscle torque. The graph shows the daily variation in torque of trained bicyclists. Torque was measured in the laboratory for the isometric contraction of the right knee extensor muscles at 65° knee flexion. The data points are the means (±SE) of 6 subjects. Notice that torque is lowest in the morning and early afternoon. (Data from Callard, D. et al., *Chronobiol. Int.*, 17, 693, 2000.)

Naturally, conditions in the laboratory are much more stable than in the real world. Variables under study in the laboratory are intentionally dissociated from other variables. Thus, the 10% variation in performance found in the laboratory may or may not be significant in the world of competitive sports. The real test here consists in looking at the records of performance of professional athletes. One group of researchers analyzed the performance of major league baseball teams during the years 1991 to 1993.[29] Of course, low-pay researchers cannot tell multimillion-dollar baseball players when to play. However, the regular schedule of games often involves travel from one coast of the United States to the other. Because games are played at the same local time on both coasts, the visiting team is forced to play 3 hours earlier (or later) when away than when at home. This provides the opportunity to compare the performance of the teams at different times of the biological day. Figure 14.7 shows the percentages of wins of the home teams. Teams from both coasts win 54% of home games played against local teams that do not have to travel. Eastward travel seems to impair the performance of the visiting teams, allowing the home teams on the East Coast to win 63% of the games played against teams coming from the West Coast. Curiously, westward travel does not seem to impair the performance of visiting teams, as West Coast teams win only 56% of the games played against teams coming from the East Coast (a winning rate that is comparable

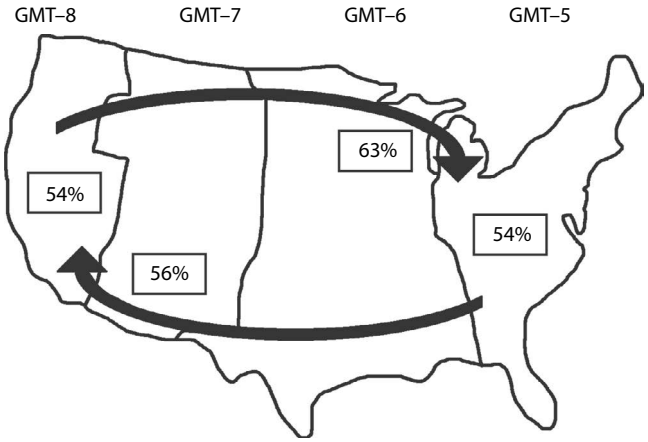


FIGURE 14.7 Home team advantage. The number of wins of American baseball teams depends on whether the visiting team travels westward or eastward. Percentages of wins over a 3-year period are shown for the home team according to the travel status of the visiting team (local, traveling eastward, or traveling westward). (Data from Recht, L.D. et al., *Nature*, 377, 583, 1995.)

to the winning rate in games played against local teams). The advantage of East Coast home teams, or, actually, the disadvantage of West Coast teams traveling eastward may be due to jet lag (which we will discuss in detail in Chapter 15) or may simply be an incidental occurrence in the 1991–1993 seasons. A separate group of researchers analyzed the performance of professional basketball teams (NBA) from 1987 to 1995.[30] Although there was a small difference in the percentages of games won by home teams in the two coasts, the difference was not statistically significant.

Considering that performance in a game is affected not only by circadian variation in strength and endurance but also by innate and acquired skills, fitness, motivation, concentration, and pregame rest (Figure 14.8), it makes sense that *not* playing at one's best time of day will *not* have a major

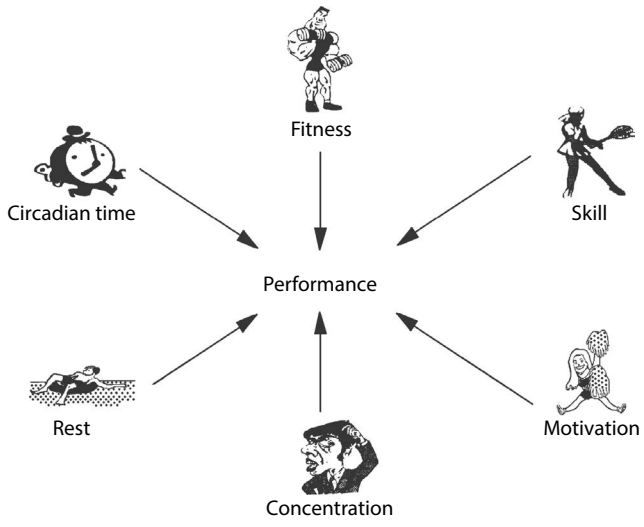


FIGURE 14.8 More than just hard work. This diagram indicates some factors that affect athletic performance.

effect on one's overall performance. Indeed, evaluation of the existing literature reveals no consistent evidence of daily rhythmicity in sport-specific performance,[31,32] even though athletes do seem to perform better in the late afternoon and early evening, possibly as the result of a combination of optimal circadian time, sleep status, and environmental factors.[33,34] Even in physically fit but nonprofessional athletes tested under seminatural conditions, time of day does not seem to affect overall performance.[35–37] An example is shown in Figure 14.9. Assiduous members of a fitness club (who work out an average of 4 days per week) were asked to do their workout at different times of the day. Body temperature, measured immediately before the workout, exhibited the expected daily variation with a peak in the midafternoon to late afternoon. However, physical vigor (measured as the number of repetitions of movements in the strength-training machine of the subject's choice) showed only a small, nonstatistically significant dip after lunch. So, although the program **Health**—which is part of the software package that accompanies this book—will help you determine your best time of day for physical performance (see Exercise 14.1), you should keep in mind that circadian variation in physical prowess is only one of many variables that determine your overall performance. Depending on your level of fitness, on your motivation, and on how much rest you have had, the effect of circadian time on performance may be negligible.

14.2.2 Intellectual Activities

The existence of daily and circadian rhythmicity in cognitive processes was briefly mentioned in Chapter 5. Such rhythmicity may or may not have significant implications for a variety of intellectual activities, including learning and exam taking (Figure 14.10). I have taught Introduction to Psychology (Psyc 101) to undergraduate students for many years. I have taught the exact same material, and given the exact same exams, in morning, afternoon, and evening classes. I cannot guarantee that the students taking classes at the three different times of the day are equally bright or equally motivated, but, assuming that they are, I can use the mean exam grades as a measure of student performance (in learning and exam taking) at three different times of the day. As shown in the upper panel of Figure 14.11, the morning classes attain, on average, higher grades than the afternoon classes, and the afternoon classes attain higher grades than the evening classes. You may notice that all grades are rather low (with a grand mean of 60 in a scale of 0–100), but this is another story to be discussed elsewhere. The important aspect of the data is the daily variation in performance. Actually, the main aspect is the *appearance* of daily variation. Because I did not plot the standard errors of the means, you have no way to know what the real data look like. The individual means of the 21 classes used to produce Panel A are shown in Panel B. As you can see now, the variability in grades from one class to another within each time of day is so large that one cannot reliably compare performance at the three times of the day. The mean differences apparent in Panel A can be easily explained by random

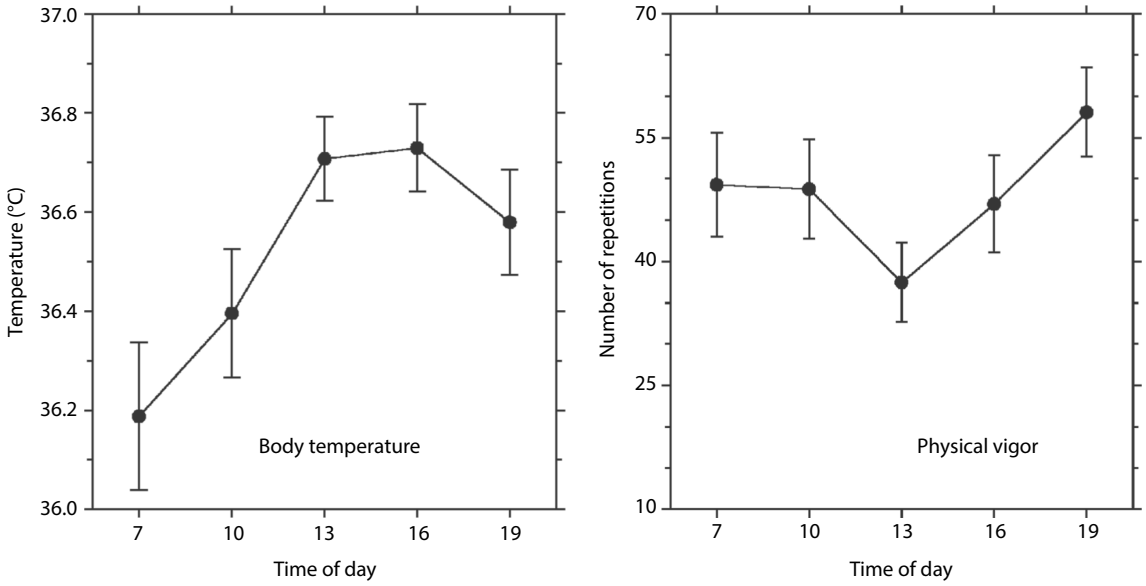


FIGURE 14.9 Best time to work out? The graphs show the daily variation in body core temperature and physical vigor of assiduous members of a fitness club (who work out on average 3.8 days per week). The data points correspond to the means (±SE) of 25 subjects. Physical vigor was measured as the number of repetitions of movements in the strength-training machine of the subject's choice (with the instruction to perform "as many repetitions as possible without hurting yourself"). Notice that body temperature shows the expected peak in the midafternoon to late afternoon, but physical vigor shows only a nonstatistically significant dip after lunch. (Data from Refinetti, R. and Laney, M.Y., *Biol. Rhythm Res.*, 34, 115, 2003.)



FIGURE 14.10 Exam time! Because many psychological variables exhibit circadian rhythmicity, performance in exams may depend on time of day. (Copyright ArtToday, Tucson, AZ.)

variability ($p > 0.30$). The reason why I bothered to show you Figure 14.11 is that I wanted to emphasize the importance of proper data analysis procedures in the interpretation of real-world data. Too often, educators and members of the lay media publicize differences in mean performance without accompanying indices of intragroup variability. This practice is very dangerous, and can be intentionally deceiving. You should be very careful when interpreting them.

Numerous laboratory studies have demonstrated daily (and, occasionally, circadian) rhythmicity in cognitive performance. Whether one considers general alertness,[38–44] reaction time,[39,41,45,46] memory,[39,41,47] the accuracy of estimates of time,[48,49] pianists' fine motor skills,[50] or visual-search speed,[51,52] performance has been shown to be worst in the early morning (around wake-up time) and best in the late afternoon or early evening (around the time of the peak of the body temperature rhythm), with a variation in performance of 20%–100% between peak and trough. There is even evidence that people may be more likely to buy a new brand of shampoo when they watch the advertisement in the evening than when they watch it in the morning or afternoon.[53] Regarding visual search, which is directly relevant to real-life applications such as air-traffic control and baggage screening, it seems that circadian phase and time awake (including chronic sleep deprivation) impact speed but not accuracy in tasks that require mental image transformation,[52] which is comforting to those of us who often need to travel by airplane and must trust the visual-search capabilities of others.

Somewhat frustrating is the fact that several studies found the rhythms of cognitive performance to peak at other times of the day than that associated with the peak of the body temperature rhythm.[54–59] Two alternative explanations for the disagreement are evident: either the low temporal resolution of data collection used in many studies leads to inconsistencies in the determination of peaks and troughs, or the rhythms of different cognitive processes actually peak at different times of the day.

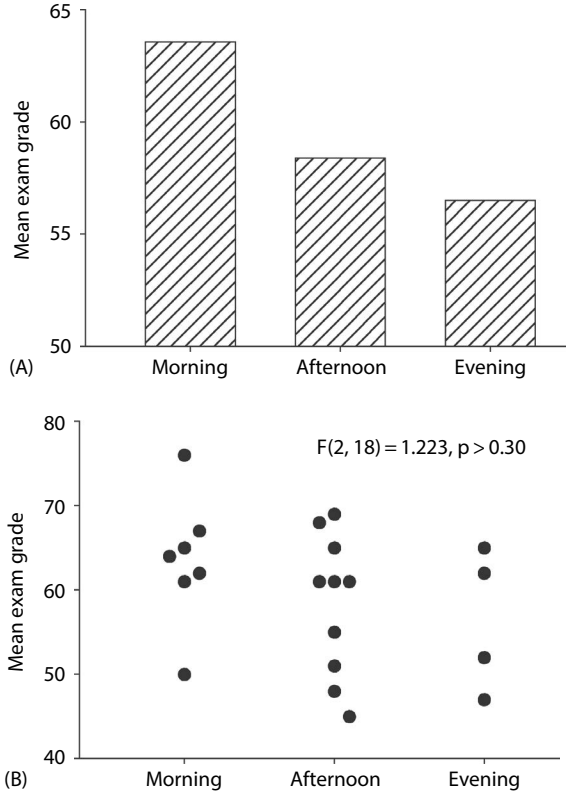


FIGURE 14.11 Comparing exam grades. The top graph (A) shows the mean grades attained by students in the first midterm exam in introductory psychology classes taught by the same professor—at the same university—in the morning, in the afternoon, or in the evening between 1999 and 2003. Although the mean grades are low in all classes, they are lower in the evening than in the afternoon and lower in the afternoon than in the morning. The actual distribution of class means shown in the lower graph (B) indicates that the differences between the three times of day are not statistically significant. (Data from R. Refinetti's archives of Psychology 101 grades.)

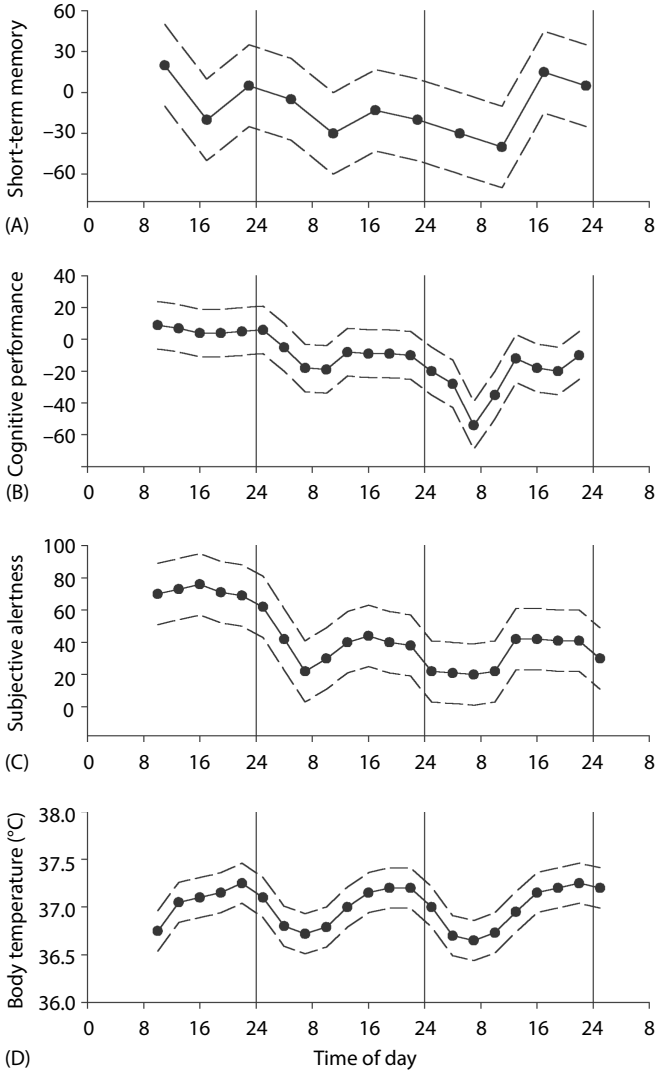


FIGURE 14.12 Daily variation in psychological functions. The graphs show the daily variation in three psychological functions (as well as body temperature) of human subjects studied under a constant routine protocol (that is, in continuous bed rest, receiving equally spaced meals, and not allowed to sleep) for 60 consecutive hours. The data points are the means (±SE) of 5 subjects. Notice that the rhythm of body temperature (Panel D) is very robust but that the rhythms of the psychological functions (Panels A through C) are rather sloppy. (Data from Johnson, M.P. et al., *J. Sleep Res.*, 1, 24, 1992.)

A series of pre-1990 studies seemed to support the hypothesis that the daily variation in cognitive processes depends on task demands, so that performance in some tasks is better early in the day, whereas performance in other tasks is better later in the day.[60] However, pre-1990 studies generally did not collect data with an adequate temporal resolution and did not use a longitudinal approach capable of ensuring the reproducibility of daily rhythms. In 1992, Charles Czeisler and his research team at Harvard Medical School conducted a very-well-controlled study in which subjects were kept in bed rest (but awake) for 60 consecutive hours. Body temperature and several cognitive variables were continuously recorded.[61] Some of the results are shown in Figure 14.12. At Panel D, notice that the body temperature rhythm exhibits the expected circadian rhythmicity with daily peaks in the early evening. The cognitive variables (short-term memory, cognitive performance, and subjective alertness) exhibit a more complex pattern that is very instructive. All three variables have elevated values during the first few hours of the first experimental day, which is probably a consequence of excitement (or boredom) of the subjects as they habituate to the experimental setup. This suggests that studies conducted over a single 24-hour interval cannot provide reliable results. During the second and third days, daily rhythmicity in short-term memory is still not very clear, but rhythmicity in cognitive performance and subjective alertness is quite robust. Notice that the cognitive rhythms peak in the early evening, as does body temperature. Because the subjects were not allowed to sleep during the experiment, it is evident that the circadian variation in cognitive performance and subjective alertness is not contingent on the increase of sleepiness during wake hours (as we discussed previously in Chapter 10). In a more recent study using the constant routine protocol,

a different research team showed that tasks that vary in difficulty are phased equally, so that performance is worst in the early morning and best in the early evening regardless of task complexity.[62]

In view of the foregoing, it would seem appropriate to conclude that the studies that found the rhythms of cognitive performance to peak at times other than that associated with the peak of the body temperature rhythm[54–59] suffered from methodological deficiencies that led to inconsistencies in the determination of peaks and troughs. I must add, however, that the phasing of circadian rhythms in real-world situations may very well differ from that observed in the laboratory. For instance, a study of subjective estimates of tiredness in women engaged in their normal workplace routines found that tiredness was lowest at noon and increased from that point until bedtime.[63] This implies that alertness (assuming that alertness is the opposite of tiredness) was highest at noon and not in the early evening. Quite possibly, the continuing demands of the workplace created a cumulative fatigue that is not observed in the relaxed environment of the laboratory. The effects of fatigue would then have overridden the intrinsic circadian variation in alertness.

Of particular interest to many readers may be the effect of daily variation in cognitive processes on the safety of automobile driving. A group of researchers in Sweden conducted a very interesting analysis of data on highway traffic accidents involving personal injury or death.[64] Panel A in Figure 14.13 shows the well-known fact that more traffic accidents occur at rush hour (8 a.m. and 5 p.m.) than at other times of the day. However, because rush hour is the time of the day when there is the largest number of vehicles on the road, the increase in the number of accidents may be a simple consequence of the increase in the number of vehicles on the road. An odds ratio of accidents can be easily calculated as the quotient of the number of accidents and the number of vehicles on the road at each time of the day. As shown in Panel B, the odds ratio peaks at 4 o'clock in the morning. Thus, although one's absolute chance of getting into an accident is higher at rush hour, one's relative chance of getting into an accident is much higher late at night. If you have to drive late at night, you should feel lucky that there are not a lot of other people driving at that time. As a matter of fact, you are likely to get into an accident at that time even if you are the only driver on the road!

As was the case for physical activities, it is quite possible that performance in most intellectual activities—such as playing the piano (Figure 14.14)—is affected by so many variables that the effect of circadian variation becomes negligible. This does not seem to be the case for traffic accidents, but no thorough study has been conducted about it. Anecdotal evidence suggests that most late-night traffic accidents are caused by drowsy drivers who fall asleep at the wheel. Quite possibly, driving competence is lower at this time because the driver has been awake for more than the usual 16 hours a day, and not because cognitive processes are at their circadian low point. A survey of drivers involved in traffic accidents revealed that, even for diurnal accidents, 12% of drivers

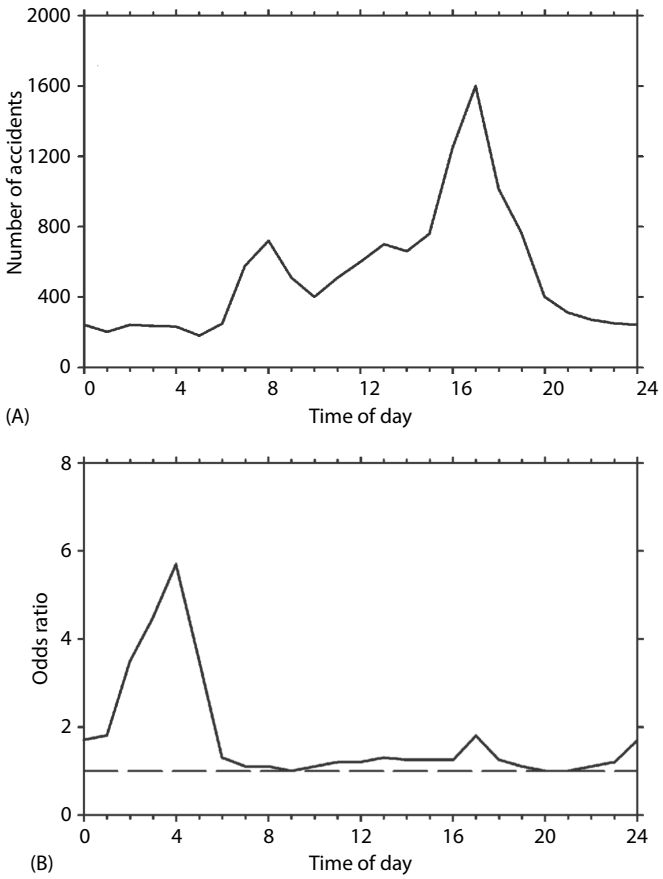


FIGURE 14.13 Daily variation in highway traffic accidents. The top graph (A) shows the hourly distribution of all highway traffic accidents involving personal injury or death in Sweden from 1987 to 1991. There are clearly more accidents around 8 a.m. and 5 p.m. Of course, there is also more traffic at these times of day, so the graph on the bottom (B) shows the hourly distribution of odds ratios (that is, number of accidents divided by the number of vehicles on the road). The large peak at 4 a.m. suggests that drivers are less competent at this time of day. (Data from Åkerstedt, T. et al., *Sleep*, 24, 401, 2001.)

involved in accidents had sleep-related risk factors.[65] In a study in which subjects drove 200 km (125 miles) at night, drinking coffee or taking a 1-hour nap before the drive significantly reduced driving impairment.[66] Similarly, when school start time at high schools in a Kentucky county were moved back 1 hour (thus starting at 8:30 a.m. instead of 7:30 a.m.), students increased the number of hours slept per night and there was a significant reduction in car crashes involving teenage drivers.[67]

The case of tiredness in the workplace mentioned earlier also suggested that the circadian signal may be overridden by other signals. Evidently, more research is needed on this issue. Everything else being equal, a pianist should perform better in the early evening; but "everything else" is rarely equal. Talent, practice, fatigue, mood, behavior of the audience, and numerous other variables contribute to the quality of the performance to such an extent that we cannot confidently say that circadian variation in cognitive processes plays a significant role in it. The importance of the circadian system in



FIGURE 14.14 A musical score for the piano. Music playing requires accurate temporal discrimination, detailed memory, precise coordination of muscle movements, and many other abilities. Some or all of these abilities may vary during the day. (Excerpt from *Anita* by R. Refinetti, Copyright 1983–2001.)

the determination of intellectual performance in real-world activities is yet to be adequately demonstrated. A study of the quality of emergency care provided by medical personnel to patients at two hospitals over 3 years concluded that the quality of care during the night shift was not significantly different from the quality of care provided during the day shift, even though the medical personnel worked at opposite phases of the circadian cycle.[68]

Because motivation is usually a strong modulator of intellectual performance, mention should be made of the normal daily variation in mood. It was briefly mentioned in Chapter 5 that a common distinction in mood assessment is the one between *positive affect* and *negative affect*.[69] Although many of us think of happiness as the opposite of sadness, psychologists have come to realize that happiness (positive affect) is actually a psychological dimension distinct from sadness (negative affect). Positive affect can vary from joviality/enthusiasm to drowsiness/dullness, whereas negative affect varies from distress/hostility to calm/rest. Research has consistently documented daily rhythmicity in positive affect, whereas rhythmicity in negative affect has been found in some studies,[70–72] but not in others.[56,58,73] A study using social media data from more than 2 million people around the world found that positive affect peaks at around midnight (with a second, smaller peak at wake-up time) and that negative affect also peaks at around midnight but with a smaller amplitude of daily oscillation.[72] The smaller amplitude of oscillation in negative affect may explain why no rhythmicity was found in some of the studies involving small numbers of subjects.

Finally, it has been shown that people engage in more unethical behavior (such as lying and cheating) in tasks performed in the afternoon than in tasks performed in the morning.[74] The time-of-day effect is, however, very dependent on people's temporal habits,[75] as we will discuss in the following section.

14.2.3 Morningness–Eveningness Typology

We have just seen that, all other conditions being equal, physical and intellectual capacities are higher in the evening for an *average* human being. However, many human beings are not *average*. Thus, if we are to advise people about "when to work and when to play,"[76] we need to take individual differences into consideration. In Chapters 7 and 8, we discussed the mechanisms that determine the phase relationship between the rhythms of an organism and the rhythms of environmental factors. We saw that the environmental alternation of light and darkness (the light–dark cycle) is usually the most important *zeitgeber* for circadian rhythms, and that the timing of circadian rhythms is determined primarily by the mechanism of *entrainment*—which, on its turn, is predicated on the free-running period of the organism, the period of the zeitgeber, and the properties of the *phase–response curve*. Although the characteristics of entrainment of an organism are relatively stable and permanent, different individuals of a species establish slightly different *phase angles of entrainment* with the zeitgeber because of individual differences in free-running period or in the phase–response curve. Figure 14.15 shows the distribution of phase angles of entrainment of a group of 50 Nile grass rats maintained under a light–dark cycle with 12 hours of light per day (LD 12:12). Most grass rats start running on their running wheels 40–60 minutes before lights on each day, but some start only 20 minutes before lights on, whereas others start as early as 80 minutes before lights on. Thus, some grass rats are *late risers* and some are *early risers*. This diversity of *chronotypes* has been noted in other animal species also, such as mice,[77] degus,[78] and rhesus monkeys.[79]

As mentioned in Chapter 2, a *morningness–eveningness inventory* for humans was developed by Horne and

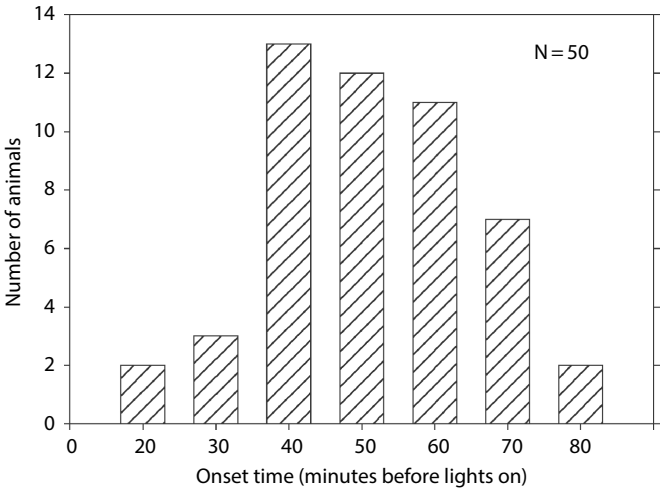


FIGURE 14.15 Interindividual variability in the daily start of activity. The graph shows the distribution of activity onset times of 50 Nile grass rats (*Arvicanthis niloticus*) housed individually with running wheels under a light–dark cycle (LD 12:12). Most animals start running on the wheels 40–60 minutes before the lights come on each day. However, there is a spread of a full hour from early risers to late risers. (Data from Refinetti, R., *Acta Sci. Veter.*, 32, 1, 2004.)

Östberg in 1976,[80] and similar inventories have been developed since then,[81–83] particularly the Munich Chronotype Questionnaire,[84,85] which has become popular in recent years.[18,50,86,87] The idea of classifying people along a continuum from *morning types* (people who usually wake up early and are more productive in the morning) to *evening types* (people who usually wake up late and are more productive later in the day) adds nothing to the understanding of fundamental principles of circadian physiology, but it has a great appeal to those of us humans who must live in a world structured around the 24-hour day (Figure 14.16). Many visitors to my website (www.circadian.org) write to me lamenting that, as extreme evening types, they are frequently chastised for being "lazy" or "disorganized." I sympathize with them. Not being normal can certainly be frustrating. However, I always point out to the frustrated writers that the norms about official work times are most likely wrong and, just as importantly, that being abnormal may many times be a good thing. I will explain these two arguments in detail in the following paragraphs.

I will start by providing more detail about the morningness–eveningness typology. Figure 14.17 shows the distribution of morningness–eveningness scores for 2135 university students from Italy and Spain. The distribution is unremarkable as far as distributions of biological and psychological functions go. It is bell shaped with a mean of 48 and a standard deviation of 10.[88] Lower scores indicate eveningness (*evening chronotype*), whereas higher scores indicate morningness (*morning chronotype*). The cutoff points for the typology are arbitrary.



FIGURE 14.16 Sunrise at the beach. Some people do not like to wake up early; some do. (Copyright ArtToday, Tucson, AZ.)

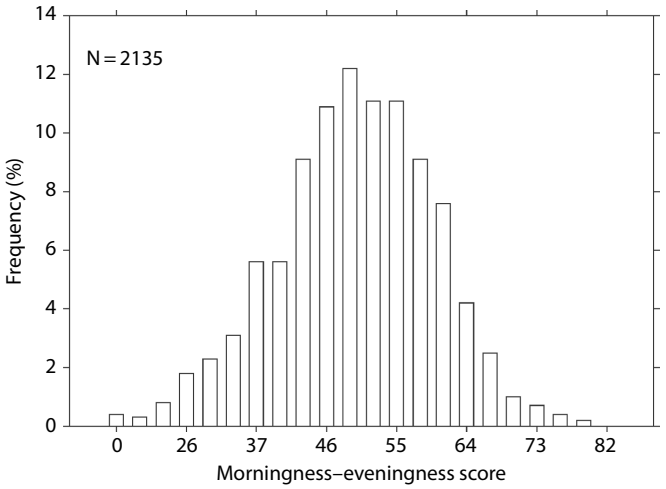


FIGURE 14.17 Morning types, evening types, and everyone in between. The graph shows the distribution of scores in the morningness–eveningness questionnaire of 2135 university students from Italy and Spain. The distribution is bell shaped with a mean of 48.2. Lower scores characterize evening types; higher scores characterize morning types. (Data from Adan, A. and Natale, V., *Chronobiol. Int.*, 19, 709, 2002.)

Horne and Östberg[80] considered evening types to have scores below 42 and morning types to have scores above 58. People with scores between 42 and 58 (the majority of people) were considered to be *intermediate* types (or *neither types*).

Originally developed in Sweden and formalized in England, the Horne–Östberg inventory has been adapted and used in numerous countries, including Australia,[89] Brazil,[90–92] Canada,[93,94] England,[95] Finland,[96] France,[97,98] Germany,[99] Italy,[59,88,100,101] Japan,[102,103] Korea,[102] Poland,[104] Spain,[88,105] and the United States.[47,106–110] Morning types usually wake up 2 hours earlier than evening types (for example, 7:20 a.m. and 9:20 a.m., respectively, in a study in England).[111] Consistently, the daily rhythms of body temperature,[80,107,112] heart rate,[97,113] and melatonin secretion[93,99,109,112] peak earlier in the day in morning types than in evening types. Morning types are more alert at wake-up time,[59,82] are better at recognition of sentences presented in the morning than in the afternoon,[100] are less stressed out by morning commute driving than evening types are,[89] and prefer having sex earlier in the day than evening types do.[114] Morning types also tend to be happier (displaying more positive affect) than evening types,[110,115,116] whereas evening types tend to be more impulsive than morning types.[104,117–119] Evening-type students like online classes more than morning-type students do, even though the two groups perform equally well in online classes.[120] A study on the social perseverance of chronotypes revealed a stable distribution of scores for two generations of university students tested 21 years apart.[81]

Figure 14.18 exemplifies how the morningness–eveningness typology affects the best time of day for cognitive performance (*alertness*). Although all three chronotypes exhibit daily variation in alertness, only intermediate types (who constitute the majority of people) exhibit the regular temporal pattern with a peak in the early evening. In morning types, the alertness

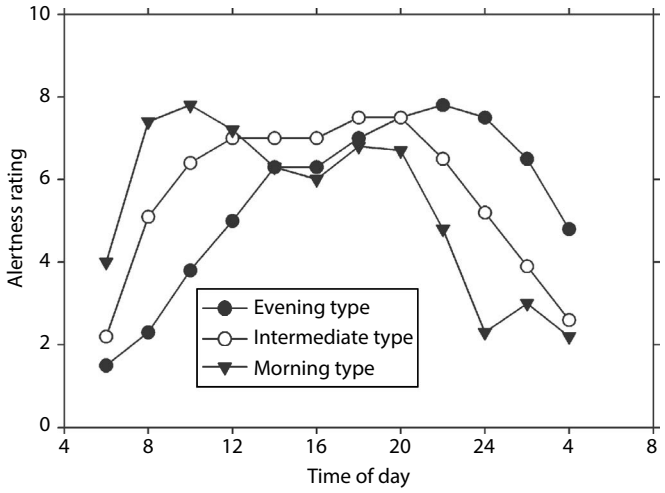


FIGURE 14.18 Morningness–eveningness typology and alertness. The graph shows the daily variation in alertness of morning types, intermediate types, and evening types. Subjective self-evaluation of alertness was conducted by 1800 university students from 6 countries. Mean values for each of the three types are shown. Notice that, for intermediate types, alertness reaches a daily maximum at noon and stays elevated until 8 p.m. For morning types and evening types, alertness rises a few hours earlier or later. (Data from Smith, C.S. et al., *Person. Indiv. Diff.*, 32, 949, 2002.)

rhythm peaks much earlier in the day, whereas the rhythm peaks much later in the day in evening types.[82]

Although some researchers have reported that women tend to be slightly more morning oriented than men,[84,88,94,95,117] others have found no sexual difference.[18,119,121–124] The discrepancy in findings may be related to an interaction between the effects of sex and age. Studies have been consistent in finding young children to be more morning oriented than young adults[18,103,121,122,125–127] and young adults to be more evening oriented than older adults.[14,47,85,92,98,105,106,115,117,124,128–130] Thus, morningness seems to decrease (by about 20%) from childhood to adult years and to return to the childhood level in old age. Women seem to reach the eveningness peak a few years earlier than men,[125,127] which creates a sex difference over the years.

People who live in rural areas seem to be more morning oriented than people who live in urban areas,[92] which suggests that the social environment may play a role in the determination of chronotype. This may be so, but studies with twins have documented also a sizable effect of genetic inheritance. Studies in England and Finland comparing identical twins with fraternal twins estimated chronotype heritability to be 50%.[95,129]

A negative correlation ($r = -0.60$) was found between morningness–eveningness scores and circadian periods in individuals studied in the laboratory under a forced desynchrony protocol,[108] which may provide a simple explanation for the morningness–eveningness typology. Because the phase angle of entrainment to a 24-hour light–dark cycle depends on circadian period (see Chapter 7), the shorter circadian period of morning types could easily explain why morning types have phase-advanced rhythms (and why evening types, who have longer circadian periods, have phase-delayed rhythms). Curiously, one study found an association between the morningness–eveningness typology and a polymorphism in the human *clock* gene.[131] However, follow-up studies by other research teams found no such association.[128,132]

Regarding my argument that "the norms about official work times are most likely wrong," it is based on the fact that the various studies of the morningness–eveningness typology have indicated that the *standard* work hours of 8 a.m. to 6 p.m. (or, in the United States, 9 a.m. to 5 p.m.) are not consistent with the preferred wake-up times of the majority of the population. As one group of researchers from Germany and Switzerland put it, "proverbs praising early chronotypes [such as 'the early bird catches the worm'] are abundant, but the results shown here indicate that worm catchers are rare birds in modern society."[84] Standard work schedules are tailored to a lark minority. They impose an unintended program of sleep deprivation on the majority of the work force and create an almost intolerable situation for evening types.

As for my argument that "being abnormal may many times be a good thing," it is based on two principles. The first principle is that abnormality per se should not have a negative connotation. Bill Gates, cofounder and chairman of Microsoft Corporation, is the richest man in the world.[133] He is, unquestionably, *abnormally rich*, and most people would be very happy to be so abnormally rich. Of course, the evening chronotype might actually have negative attributes. A survey of university students at Michigan State University identified small ($r < 0.30$) but significant correlations between morningness and both achievement tendency and aversion to time wasting.[134] So, it is possible that morning types are slightly more diligent and goal oriented than evening types. If true, the difference might be explained by the fact that society tends to concentrate sophisticated forms of entertainment (such as concerts, parties, and dinner celebrations) at night. Because evening types are more likely to be awake at night, they are also more likely to enjoy this type of entertainment, which necessarily takes time away from productive activities. However, the modest size of the correlations found in the study implies that only a minority of evening types would be in this situation. For the majority of evening types, being *abnormal* means simply being *different*.

The second principle is that some features that may be unfavorable in certain circumstances can be favorable in other circumstances. Being an extreme evening type may be unfavorable to a person who works as a cook at a breakfast parlor and has to arrive at work every day at 5 o'clock in the morning. Such a person may often arrive late for work and may be very unproductive for most of the workday. However, this same person would be an excellent employee as a cook at a fancy restaurant that opens only for dinner and stays open until 2 o'clock in the morning. As a matter of fact, a *normal* person, who prefers to work during light hours, would not be as good an employee at the fancy restaurant as the extreme-evening-type person would. Evidently, the issue of normality depends on the existing norms—and there are enough different norms in the current marketplace to

accommodate almost any chronotype. As is true in so many other aspects of life, finding the right match is the doorway to job satisfaction.

14.3 SPACE EXPLORATION

Cosmonaut Yuri Gagarin's orbital flight in 1961 and astronaut Neil Armstrong's landing on the moon in 1969 were rightfully heralded at the time as living proof of the possibility of space exploration. Later in the twentieth century, the Russian space station and the American space shuttle programs added support to the idea.[135] The International Space Station, which has been continuously occupied since November 2000 (Figure 14.19), was built to spearhead space-science research in the twenty-first century. The largest scientific and technological endeavor ever undertaken by humankind, the station was built by the United States in collaboration with Canada, Japan, Russia, Brazil, Belgium, Denmark, France, Germany, Italy, Holland, Norway, Spain, Sweden, Switzerland, and the United Kingdom. During construction, the program involved more than 100,000 people at space agencies and contractor companies around the world. The station provides a permanent laboratory for research and development in multiple fields, from materials science to human physiology.

The website of the National Aeronautics and Space Administration (www.nasa.gov) proudly states that "NASA is designing and building capabilities to send humans farther into the solar system than ever before, including to an asteroid and Mars." Colonization of other planets is a more remote but not unlikely possibility (Figure 14.20). Aside from the complexity of new developments in spacecraft design and operation—such as new propulsion systems[136] and onboard computers capable of assuming complete control of the spacecraft—[137] what type of challenges can we expect? How will humans adapt to life away from Earth?

The bulk of research on human physiology in space has been on the effects of reduced gravity. It is well established that microgravity leads to bone loss,[138,139] which is a major problem, but little is known about the effects of microgravity on circadian rhythms. It is not currently possible to distinguish the impact of microgravity on circadian rhythms from the impact of other environmental factors experienced in space; consequently, very little is known about negative effects of space travel on the circadian system.[140]



FIGURE 14.19 The International Space Station. The International Space Station, constructed in Earth's orbit, is the largest scientific and technological endeavor ever undertaken by humankind. (Image courtesy of NASA, Washington, DC.)

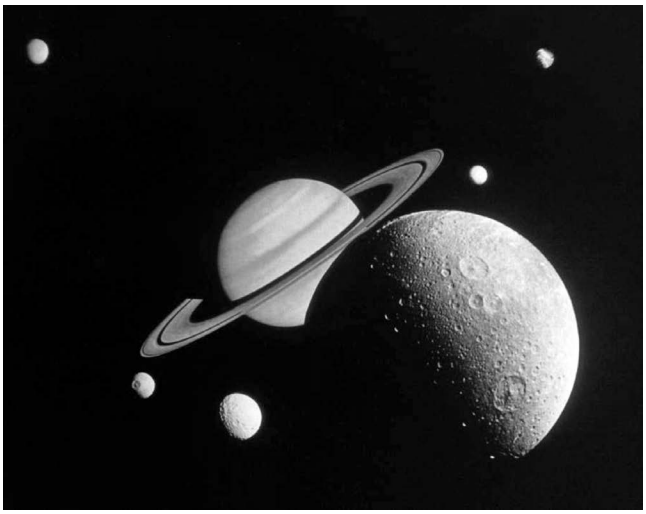


FIGURE 14.20 Out in the solar system. Saturn, the second largest planet in the solar system, is seen here with some of its many moons. (Copyright ArtToday, Tucson, AZ.)

Although reductions in sleep time and changes in sleep pattern (as compared to preflight baseline measurements) have been observed in orbiting astronauts,[141] fundamental parameters of circadian rhythmicity have not been properly investigated.[142] Poor sleep is a common complaint, but circadian disruption is less likely the cause of it than are uncomfortable living quarters. Indeed, the primary environmental nuisance responsible for sleep disturbance in space is spacecraft noise resulting from equipment and crewmember activity in the spacecraft.[143] Locomotor activity rhythms of beetles aboard the Russian space station Mir were found not to differ from rhythms recorded on Earth under light–dark cycles, constant darkness, or constant light.[144] Rhythms of heart rate, activity, and axillary temperature of monkeys aboard Cosmos missions exhibited changes in amplitude and phase,[145] but the changes were small and not consistent across the three variables.

Studies conducted on astronauts and cosmonauts in space also have failed to identify major alterations in circadian rhythmicity. In an astronaut who spent 8 days in space, a small delay (and no change in other parameters) in the rhythms of body temperature and alertness was observed, the delay not being clearly attributable either to circadian disruption or to a freerun in the absence of a strong environmental synchronizer.[146] In a study aboard Mir, only a 2-hour delay (and no change in other parameters) was noticed in the body temperature rhythm,[147] and the shift could have been due to a shift in the work schedule. In a study aboard the American space shuttle Columbia, where disruptions in the work schedule were minimized, no significant changes in the amplitude and

phase of the rhythms of body temperature, urinary melatonin, and cortisol were observed.[148] In neither of the studies (which lasted 30 and 17 days, respectively) were the rhythms found to freerun. In the Columbia study, the astronauts slept in light- and sound-shielded bunks with no light during the sleep period and spent the day performing their duties under artificial illumination, which was less bright than that experienced during the baseline period on Earth but, it would seem, was sufficient to maintain entrainment. In another study of astronauts aboard space shuttle missions, the robustness of the body temperature rhythm was found to be reduced during the flight,[149] but the awkward sleep schedule that was forced on the astronauts most likely masked the body temperature rhythm. In order to coordinate the times of launching and landing, NASA must advance the time-of-day of landing by 4–5 hours in relation to launching. Instead of effecting the phase shift on the day of landing, NASA spreads it out over the duration of the mission, so that the astronauts are placed on a sleep schedule with 23.7- or 23.4-hour days. Presumably, NASA has conducted its own classified research to determine that this procedure is less stressful to the astronauts than the simple implementation of a single shift on the landing day, but, regardless of NASA's motives, it is not surprising that the body temperature rhythm should be less robust during the flight because of the unusual sleep schedule. In a study of an astronaut who stayed aboard Mir for 5 months (living regular 24-hour days), the body temperature rhythm remained robust for the first 3 months, although it did lose robustness later on.[150]

If reduced gravity and the lack of other geophysical influences do not have a great impact on circadian rhythms, then the major concern about space exploration will be about rhythm entrainment, as Pittendrigh predicted back in 1967.[151] Apparently, the artificial living conditions maintained in spacecraft are sufficient to preserve entrainment. However, artificial conditions are not viable in large-scale enterprises. If we had to maintain a full light–dark cycle artificially in a whole planet, the costs would be exorbitant. So, if we are ever to colonize a planet whose day is longer or shorter than 24 hours, we had better learn to adapt our circadian systems to non-24-hour days. Our earthly biological clock can be entrained to light–dark cycles that do not differ much from 24 hours. However, even within our solar system, few planets have day lengths similar to Earth's (Table 14.1). What could we do if we wanted to colonize a planet that has, for instance, a 35-hour day? In some cases, frequency demultiplication (discussed in Chapter 7) could help us deal with unusual day lengths (say, 48-hour days), but this will not be the case for planets with 15- or 35-hour days—or 5830-hour days, such as in Venus! A better understanding of entrainment mechanisms might lead to the development of potent and inexpensive zeitgebers capable of entraining us to the unusual day length prevailing in other planets, although we would probably still face the problem of wanting to be awake for much of the long alien night. As we learn more about the mechanisms of action of circadian genes, biological engineering of a circadian mutant gene for humans (which could set our circadian period to that of our destination planet) may provide the optimal solution for planets whose day lengths differ from Earth's by several hours.

TABLE 14.1
Planets of the Solar System

Planet	Distance from Sun (10^6 km)	Diameter (10^3 km)	Duration of a Day (Hours)	Duration of a Year (Days)
Mercury	58	4.9	1410	88
Venus	108	12.1	5830	225
Earth	150	12.8	24	365
Mars	228	6.8	24	687
Jupiter	778	142.8	10	4,380
Saturn	1427	120.7	11	10,750
Uranus	2870	51.8	17	30,660
Neptune	4497	49.5	16	59,400
Pluto[a]	5900	2.3	144	90,520

Source: *TIME Almanac 2004*, TIME Books, Des Moines, IA, 2003.
[a] Pluto was divested of planet status in 2006.

Before we colonize an alien planet, we must get there. Humans have already been to the Moon (Figure 14.21), and there are immediate plans to return there.[152–154] The Chinese Space Agency landed a rover (Yutu) on the Moon in 2013,[155] and the European Space Agency is planning a mission for robotic exploration of the Moon.[156] Planets in faraway galaxies present a much greater challenge. As much as we may increase spacecraft speed, we will be limited to the speed of light, which means that voyages may take not weeks but generations. Assuming that cryopreservation is not an option, travelers will likely spend their whole lives in space. The costs of maintaining an appropriate light–dark cycle will probably be negligible in comparison with the costs of generating breathable air and providing food, neutral ambient temperature, and health care for all the travelers. Nevertheless, accurate knowledge of human circadian rhythms and the zeitgebers

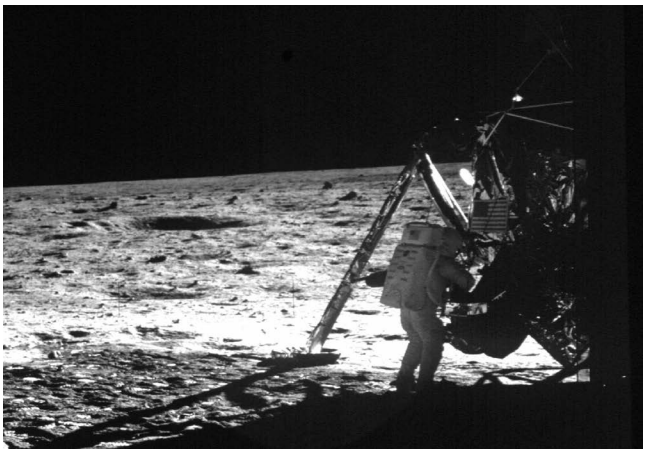


FIGURE 14.21 Man on the Moon. Neil Armstrong was the first man to set foot on the Moon on July 20, 1969. (Photo courtesy of NASA, Washington, DC.)

that affect them will be essential to provide the sojourners with an environment that relieves rather than accentuates the stress of the journey. In a simulated flight to Mars and back, lasting 520 days, the crew of six men exhibited an undesired reduction in activity level, sleep quantity, and sleep quality,[157] possibly because optimal environmental conditions for circadian entrainment were not provided.

Not only crewmembers, but also mission control workers require attention. For instance, while managing solar-powered exploration devices on the surface of Mars, Earth-based mission control personnel must follow the 24.65-hour Martian day (rather than the 24.00-hour Earthly day) for several months. Without proper training, many mission control workers failed to follow the protocol during the Mars Pathfinder mission. For the Phoenix Mars Lander mission, basic countermeasures were attempted with 20 volunteers,[158] although the countermeasures were insufficient from the perspective of circadian physiology and produced unremarkable results.

In the immediate future, colonization of the Moon is the most realistic goal. Even this short-distance migration would be a monumental enterprise, however.[159,160] Just the occupational and environmental health issues would be daunting. These issues include adequate food supply, medical care, mental health promotion, environmental hazard control, occupational injury prevention, preflight training, crew selection, and numerous aspects of environmental health, such as air quality, water, sanitation, and waste management.[160] Given the complexity of the preparations, a closed ecosystem would have to be developed and tested on Earth as a rehearsal for "the real thing." Developing a closed ecosystem was the main goal of the Biosphere 2 Center (Figure 14.22), which was privately constructed in the late 1980s and entrusted to Columbia University in 1996. Unfortunately, the project was not successful, and the university bailed out in 2003.[161] The project has since been taken over by the University of Arizona and serves as a center for research, teaching, and outreach.[162,163]



FIGURE 14.22 Biosphere. The Biosphere 2 Center, in Arizona, was built in the late 1980s to test whether humans could thrive in a closed environment akin to the one that would be required for the exploration of other planets. (Courtesy of Biosphere 2 Center, Columbia University, Oracle, AZ.)

Although Russia and the United States were the only spacefaring nations until a few decades ago, numerous countries now have organized space programs (Figure 14.23), particularly China.[164] In the beginnings of the US space program in the early 1960s, Pittendrigh pointed out that biologists had great interest in the exploration of Mars.[165] Evidently, not only biologists, and not only Americans, have interest in exploring Mars. As shown in Table 14.2, several unmanned missions to Mars have been conducted in recent years, and many more are planned for the near future.[166] Figure 14.24 is a self-portrait of NASA's Curiosity Mars rover taken next to Mount Sharp in January 2015. Manned missions to Mars have been proposed,[167,168] and then US President George W. Bush presented his own view on human exploration of Mars in 2004.[154,169,170] NASA is planning a Martian Sample Return mission for 2020 to collect rock samples from Mars.[171] There is also support in the US Congress for the idea of funding a multibillion-dollar mission to Jupiter's Europa moon, where some believe that life may be found in the oceans beneath the icy shell.[172]

Farther away, astronomers believe that they have discovered, depending on the research methods used, between 700 and 2600 extrasolar planets in the past 20 years.[173,174] It is likely that there are many Earth-sized planets close to their host stars, but planets of this type that also have surface conditions that can support life as we know it are yet to be located.[175] The prospect of exploring other planets is an exciting one, and circadian physiologists will certainly be on hand to provide their share of expertise to the advancement and ultimate success of the enterprise.

SUMMARY

1. Patterns of human mobility are similar in different parts of the world. Most people visit a few places at about the same time each day, day after day. There is great variation in human physical activity between the day and the night, much less variation between days of the week, and only random variation across phases of the moon and months of the year in urban settings.
2. In laboratory studies conducted under controlled conditions, performance in physical and intellectual activities is shown to exhibit daily rhythmicity. The best performance is achieved around the time of the peak of the body temperature rhythm, which usually takes place in the late afternoon. The exact timing varies from individual to individual, and this variability is often expressed in terms of the morningness–eveningness typology. In real-world situations, variables such as innate and acquired skills, motivation, concentration, and fatigue may overcome the effect of circadian variation in physical and cognitive capabilities.
3. Microgravity in space does not seem to have major effects on fundamental properties of circadian rhythms. Nonetheless, circadian physiology can provide significant contributions to the space exploration enterprise by assisting in the design of work and

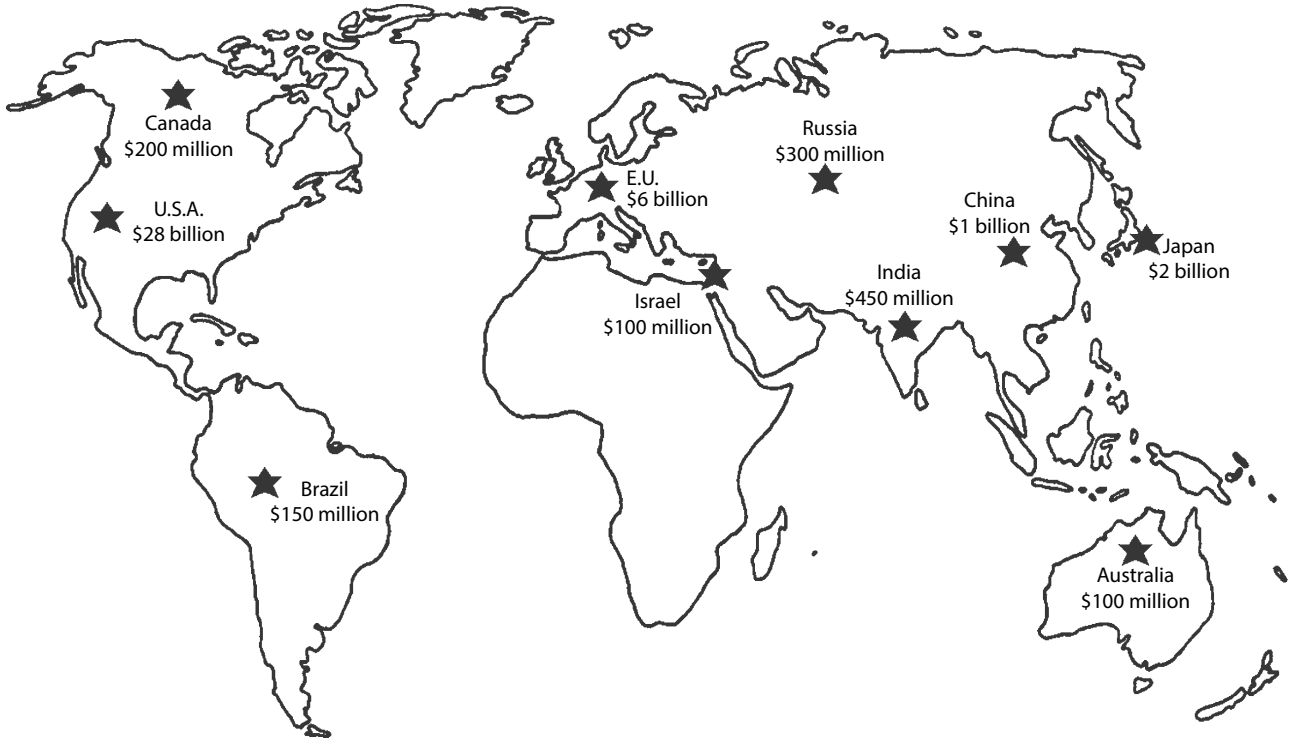


FIGURE 14.23 The space club. Ten nations currently have active space programs. The United States invests in space science and technology more than all other nations combined (28 billion dollars per year). (Data from Verger, F. et al., *Cambridge Encyclopedia of Space*, Cambridge University Press, New York, 2003.)

TABLE 14.2
Recent and Planned Expeditions to Mars

Year	Mission	Space Agency[a]
1996	Pathfinder lander	NASA
2001	Odyssey orbiter	NASA
2003	Exploration Spirit rover	NASA
2003	Exploration Opportunity rover	NASA
2003	Mars Express orbiter	ESA
2005	Reconnaissance orbiter	NASA
2007	Phoenix lander	NASA
2011	Curiosity rover	NASA
2013	Mars orbiter	ISRO
2013	MAVEN orbiter	NASA
2016	ExoMars lander and rover	ESA
2016	InSight lander	NASA
2020	Mars 2020 rover	NASA

Source: Courtesy of The Planetary Society, Pasadena, CA.

[a] *Abbreviations:* ESA, European Space Agency; ISRO, Indian Space Research Organization; NASA, U.S. National Aeronautics and Space Administration.

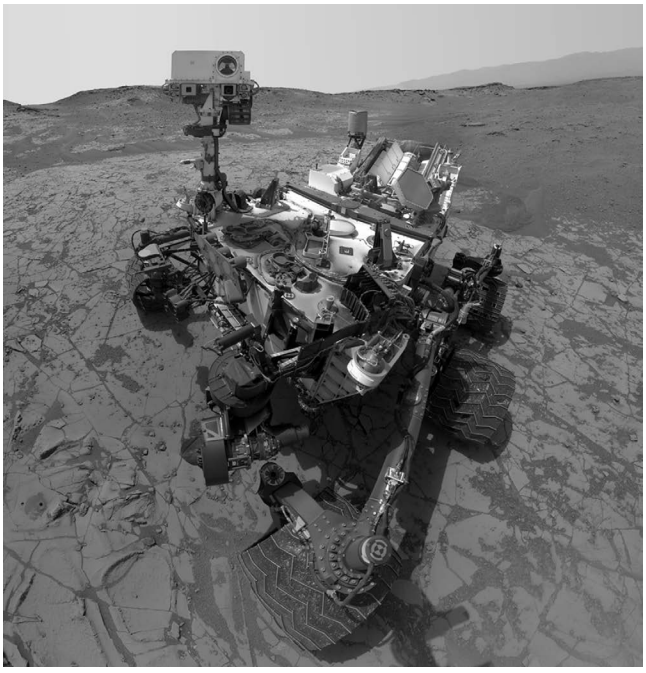


FIGURE 14.24 Selfie in Mars. This self-portrait of NASA's Curiosity Mars rover shows the vehicle in front of Mount Sharp in Mars. (Photo courtesy of NASA, Washington, DC.)

living schedules that optimize physical and cognitive performance in environments that lack the 24-hour cycle of light and darkness prevailing on Earth.

EXERCISES

14.1 Calculating your best time of day. In Section 14.1, we discussed how the knowledge of daily rhythmicity in the human body can help us estimate the best time of day for the performance of physical and intellectual activities. The program **Health** (the third icon from the right in the Circadian banner) contains a section that estimates the best time of day for you based on your usual wake time and bedtime. Because many people do not sleep as much as they would like to, their wake time and bedtime are unlikely to be good markers of the state of the circadian clock. Thus, the program makes adjustments based on the user's judgment of the adequacy of his or her sleeping pattern. The program is very simple and easy to use. Just start **Health**, select the first option from the bottom in the main menu ("I would like to know the time of day for my optimal physical or intellectual performance"), and answer the few questions that follow. You may want to share the program with friends and family members.

14.2 Daily variation in memory/attention. In this exercise, we will examine in greater detail one of the many intellectual functions that exhibit daily rhythmicity. Although the task involved is mostly a short-term memory task, variations in attention level may also influence it.

1. Ideally, you would use one of those handheld electronic games that require you to repeat a random sequence of stimuli. Two versions of the toy are shown in Figure 14.25. Most toy stores should have an equivalent version. If you cannot obtain the toy, you may use a computer program that comes on the CD that accompanies this book. Although the program does not appear in the Circadian banner, it should have been copied to your hard drive when the software package was installed. Look for it (**Game.exe**) in the folder where the other programs are ("\Program Files\Circadian") and check it out. It works very similarly to the handheld game, except, of course, that you must carry your computer with you when you go anywhere.
2. Whether you use the toy version or the computer version, you must practice using it several times before starting the study. This is necessary because your skills will improve as you become familiar with the game.
3. When you are ready to start, get a pencil and a sheet of paper to record the data. All you will need to do is to record how many times you can repeat the random pattern of lights (and/or sounds) at different times of the day. One trial per hour for 2 or more consecutive days would be ideal. Occasionally, you may also use an alarm clock to briefly wake you up in the middle of the night for nocturnal trials (but don't do this too often, otherwise you may disturb the body's clock).
4. If at all possible, you should recruit a friend to count the number of repetitions for you. If not, as you will soon find out, you may get distracted by the counting and end up making errors that you would not otherwise make.
5. You should be warned that the pattern of stimuli generated by the game is almost truly random, which means that some patterns may be extremely easy to remember (such as 5 consecutive times with the same color followed by 5 consecutive times with another color). You might discard patterns that are too easy and redo the trial, but you will have a hard time coming up with an objective definition of *too easy*. The best solution is probably to conduct two or three trials one after the other and then average the results.
6. When you have finished collecting the data, draw a graph showing number of repetitions attained (Y-axis) as a function of time (X-axis). You should be able to observe a daily rhythm, although probably not as clearly as in Exercises 1.2 and 5.1. If you find no daily rhythm in the raw data, try to average all days into a single day. This will limit your analysis to a single 24-hour cycle but will also average out random variations in performance, which may unmask the daily oscillation.

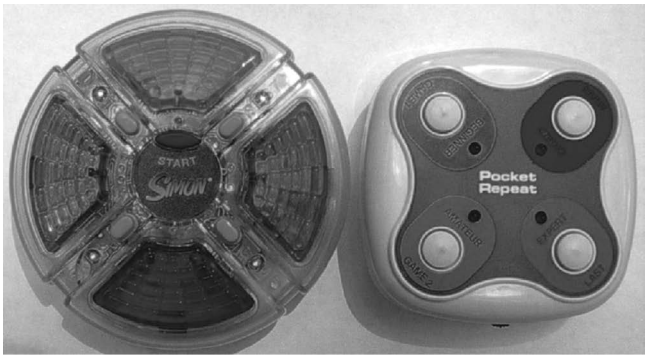


FIGURE 14.25 Simon says. Shown are two commercially available handheld electronic games that may be used for the memory/attention task in Exercise 14.2.

SUGGESTIONS FOR FURTHER READING

As in previous chapters, the source articles listed in the "References" section may be consulted for detailed information about topics covered in this chapter. For more general further reading, the following sources may be useful:

Edlund, M. (2003). *The Body Clock Advantage: Finding Your Best Time of Day to Succeed in Love, Work, Play, Exercise*. Avon, MA: Adams Media.

A general-audience book on the applications of circadian physiology to everyday life. This is a helpful book, with the caveats presented in this chapter.

Roenneberg, T. (2012). *Internal Time: Chronotypes, Social Jet Lag, and Why You're So Tired*. Cambridge, MA: Harvard University Press.

Written for an educated lay audience, this book deals extensively with the matter of chronotypes and how mismatches with the outside world cause "social jet lag."

Gardner, H., Kornhaber, M. L., and Wake, W. K. (1995). *Intelligence: Multiple Perspectives*. Belmont, CA: Wadsworth.

This book does not address the issue of circadian variation in intellectual ability, but it provides a good overview of modern theories of intelligence, which are quite different from those of 20 or more years ago.

Tenenbaum, G. and Eklund, R. C. (Eds.) (2007). *Handbook of Sport Psychology*, 3rd edn. New York: Wiley.

Written for sport psychologists, coaches, and athletes, this handbook covers basic and applied research in sport psychology.

Stern, S. A. (Ed.) (2003). *Worlds Beyond: The Thrill of Planetary Exploration as Told by Leading Experts*. New York: Cambridge University Press.

An edited book in which 10 planetary scientists describe their favorite planets in the solar system.

Verger, F., Sourbès-Verger, I., and Ghirardi, R. (2003). *The Cambridge Encyclopedia of Space*. New York: Cambridge University Press.

A thorough but accessible account of space activities (both commercial and scientific) conducted by all countries since the midtwentieth century.

WEBSITES TO EXPLORE

China National Space Administration: http://www.cnsa.gov.cn/n615709/cindex.html.
European Space Agency: http://www.esa.int/.
International Olympic Committee: http://www.olympic.org/.
NASA: http://www.nasa.gov/.
Science Career Network: http://recruit.sciencemag.org/.
U.S. National Science Foundation: http://www.nsf.gov/.
World University Rankings: http://www.timeshighereducation.co.uk/world-university-rankings/.

REFERENCES

1. Schneider, C. M., Belik, V., Couronné, T., Smoreda, Z., and González, M. C. (2013). Unravelling daily human mobility motifs. *Journal of the Royal Society Interface* 10: art. 20130246.
2. González, M. C., Hidalgo, C. A., and Barabási, A. L. (2008). Understanding individual human mobility patterns. *Nature* 453: 779–782.
3. Herder, E. and Siehndel, P. (2012). Daily and weekly patterns in human mobility. *CEUR Workshop Proceedings*, Vol. 872, art. 3.
4. Sun, L., Axhausen, K. W., Lee, D. H., and Huang, X. (2013). Understanding metropolitan patterns of daily encounters. *Proceedings of the National Academy of Sciences of the United States of America* 110: 13774–13779.
5. Song, C., Qu, Z., Blumm, N., and Barabási, A. L. (2010). Limits of predictability in human mobility. *Science* 327: 1018–1021.
6. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F., and Pauly, J. E. (Eds.), *Chronobiology*. Tokyo, Japan: Igaku Shoin, pp. 451–459.
7. Gander, P. H., Connell, L. J., and Graeber, R. C. (1986). Masking of the circadian rhythm of heart rate and core temperature by the rest-activity cycle in man. *Journal of Biological Rhythms* 1: 119–135.
8. Chandrashekaran, M. K., Marimuthu, G., and Geetha, L. (1997). Correlations between sleep and wake in internally synchronized and desynchronized circadian rhythms in humans under prolonged isolation. *Journal of Biological Rhythms* 12: 26–33.
9. Endo, T., Honma, S., Hashimoto, S., and Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
10. Natale, V. (2002). Circadian motor asymmetries in humans. *Neuroscience Letters* 320: 102–104.
11. Austin, D., Cross, R. M., Hayes, T., and Kaye, J. (2014). Regularity and predictability of human mobility in personal space. *PLOS ONE* 9: e90256.
12. Binkley, S., Tome, M. B., Crawford, D., and Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
13. Yoneyama, S., Hashimoto, S., and Honma, K. (1999). Seasonal changes of human circadian rhythms in Antarctica. *American Journal of Physiology* 277: R1091–R1097.
14. Evans, D. S., Snitker, S., Wu, S. H., Mody, A., Njajou, O. T., Perlis, M. L., Gehrman, P. R., Shuldiner, A. R., and Hsueh, W. C. (2011). Habitual sleep/wake patterns in the Old Order Amish: Heritability and association with non-genetic factors. *Sleep* 34: 661–669.
15. Sani, M., Refinetti, R., Jean-Louis, G., Pandi-Perumal, S. R., Durazo-Arvizu, R. A., Dugas, L. R., Kafensztok, R. et al. (2015). Daily activity patterns of 2316 men and women from five countries differing in socioeconomic development. *Chronobiology International* 32: 650–656.
16. Yasseri, T., Sumi, R., and Kertész, J. (2012). Circadian patterns of Wikipedia editorial activity: A demographic analysis. *PLOS ONE* 7: e30091.
17. Miguel, M., de Oliveira, V. C., Pereira, D., and Pedrazzoli, M. (2014). Detecting chronotype differences associated to latitude: A comparison between Horne-Östberg and Munich Chronotype questionnaires. *Annals of Human Biology* 41: 105–108.
18. Borisenkov, M. F. (2011). The pattern of entrainment of the human sleep-wake rhythm by the natural photoperiod in the North. *Chronobiology International* 28: 921–929.
19. Refinetti, R., Sani, M., Jean-Louis, G., Pandi-Perumal, S. R., Durazo-Arvizu, R. A., Dugas, L. R., Kafensztok, R. et al. (2015). Evidence for daily and weekly rhythmicity but not lunar or seasonal rhythmicity of physical activity in a large cohort of individuals from five different countries. *Annals of Medicine* 47: 530–537.

20. Birch, K. and Reilly, T. (2002). The diurnal rhythm in isometric muscular performance differs with eumenorrheic menstrual cycle phase. *Chronobiology International* 19: 731–742.
21. Deschenes, M. R., Kraemer, W. J., Bush, J. A., Doughty, T. A., Kim, D., Mullen, K. M., and Ramsey, K. (1998). Biorhythmic influences on functional capacity of human muscle and physiological responses. *Medicine and Science in Sports and Exercise* 30: 1399–1407.
22. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C., and Van Hoecke, J. (2001). Nycthemeral variations in core temperature and heart rate: Continuous cycling exercise versus continuous rest. *International Journal of Sports Medicine* 22: 553–557.
23. Moussay, S., Dosseville, F., Gauthier, A., Larue, J., Sesboüe, B., and Davenne, D. (2002). Circadian rhythms during cycling exercise and finger-tapping task. *Chronobiology International* 19: 1137–1149.
24. Edwards, B. J., Lindsay, K., and Waterhouse, J. (2005). Effect of time of day on the accuracy and consistency of the badminton serve. *Ergonomics* 48: 1488–1498.
25. Reilly, T., Atkinson, G., Edwards, B., Waterhouse, J., Farrelly, K., and Fairhurst, E. (2007). Diurnal variation in temperature, mental and physical performance, and tasks specifically related to football (soccer). *Chronobiology International* 24: 507–519.
26. Hill, D. W. and Smith, J. C. (1991). Circadian rhythm in anaerobic power and capacity. *Canadian Journal of Sport Science* 16: 30–32.
27. Waterhouse, J., Edwards, B., Bedford, P., Hughes, A., Robinson, K., Nevill, A., Weinert, D., and Reilly, T. (2004). Thermoregulation during mild exercise at different circadian times. *Chronobiology International* 21: 253–275.
28. Callard, D., Davenne, D., Gauthier, A., Lagarde, D., and Van Hoecke, J. (2000). Circadian rhythms in human muscular efficiency: Continuous physical exercise versus continuous rest, a crossover study. *Chronobiology International* 17: 693–704.
29. Recht, L. D., Lew, R. A., and Schwartz, W. J. (1995). Baseball teams beaten by jet lag. *Nature* 377: 583.
30. Steenland, K. and Deddens, J. A. (1997). Effect of travel and rest on performance of professional basketball players. *Sleep* 20: 366–369.
31. Youngstedt, S. D. and O'Connor, P. J. (1999). The influence of air travel on athletic performance. *Sports Medicine* 28: 197–207.
32. Youngstedt, S. D. and Buxton, O. M. (2003). Jet lag and athletic performance. *American Journal of Medicine in Sports* 5: 219–226.
33. Reilly, T. and Waterhouse, J. (2009). Sports performance: Is there evidence that the body clock plays a role? *European Journal of Applied Physiology* 106: 321–332.
34. Thun, E., Bjorvatn, B., Flo, E., Harris, A., and Pallesen, S. (2015). Sleep, circadian rhythms, and athletic performance. *Sleep Medicine Reviews* 23: 1–9.
35. O'Connor, P. J., Morgan, W. P., Koltyn, K. F., Raglin, J. S., Turner, J. G., and Kalin, N. H. (1991). Air travel across four time zones in college swimmers. *Journal of Applied Physiology* 70: 756–763.
36. Refinetti, R. and Laney, M. Y. (2003). Nycthemeral variation in intra-aural temperature and attention/memory but not in physical vigor of assiduous members of a fitness club. *Biological Rhythm Research* 34: 115–121.
37. Racinais, S., Hue, O., Hertogh, C., Damiani, M., and Blonc, S. (2004). Time-of-day effects in maximal anaerobic leg exercise in tropical environment: A first approach. *International Journal of Sports Medicine* 25: 186–190.
38. Van Dongen, H. P. A., Kerkhof, G. A., and Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
39. Wright, K. P., Hull, J. T., and Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *American Journal of Physiology* 283: R1370–R1377.
40. Hull, J. T., Wright, K. P., and Czeisler, C. A. (2003). The influence of subjective alertness and motivation on human performance independent of circadian and homeostatic regulation. *Journal of Biological Rhythms* 18: 329–338.
41. Varkevisser, M. and Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: Validating a self-constructed test battery. *Chronobiology International* 20: 109–121.
42. Horowitz, T. S., Cade, B. E., Wolfe, J. M., and Czeisler, C. A. (2003). Searching night and day: A dissociation of effects of circadian phase and time awake on visual selective attention and vigilance. *Psychological Science* 14: 549–557.
43. Cohen, D. A., Wang, W., Wyatt, J. K., Krinauer, R. E., Dijk, D. J., Czeisler, C. A., and Klerman, E. B. (2010). Uncovering residual effects of chronic sleep loss on human performance. *Science Translational Medicine* 2: 14ra3.
44. Valdez, P., Ramírez, C., García, A., Talamantes, J., and Cortez, J. (2010). Circadian and homeostatic variation in sustained attention. *Chronobiology International* 27: 393–416.
45. Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A., and Cajochen, C. (2004). Circadian and wake-dependent modulation of fastest and slowest reaction times during psychomotor vigilance task. *Physiology and Behavior* 80: 695–701.
46. Bratzke, D., Rolke, B., Ulrich, R., and Peters, M. (2007). Central slowing during the night. *Psychological Science* 18: 456–461.
47. May, C. P., Hasher, L., and Stoltzfus, E. R. (1993). Optimal time of day and the magnitude of age differences in memory. *Psychological Science* 4: 326–330.
48. Campbell, S. S., Murphy, P. J., and Boothroyd, C. E. (2001). Long-term time estimation is influenced by circadian phase. *Physiology and Behavior* 72: 589–593.
49. Kuriyama, K., Uchiyama, M., Suziki, H., Tagaya, H., Ozaki, A., Aritake, S., Kamei, Y., Nishikawa, T., and Takahashi, K. (2003). Circadian fluctuation of time perception in healthy human subjects. *Neuroscience Research* 46: 23–31.
50. Van Vugt, F. T., Treutler, K., Altenmüller, E., and Jabusch, H. C. (2013). The influence of chronotype on making music: Circadian fluctuations in pianists' fine motor skills. *Frontiers in Human Neuroscience* 7: art. 347.
51. De Gennaro, L., Ferrara, M., Curcio, G., and Bertini, M. (2001). Visual search performance across 40 h of continuous wakefulness: Measures of speed and accuracy and relation with oculomotor performance. *Physiology and Behavior* 74: 197–204.
52. Pomplun, M., Silva, E. J., Ronda, J. M., Cain, S. W., Münch, M. Y., Czeisler, C. A., and Duffy, J. F. (2012). The effects of circadian phase, time awake, and imposed sleep restriction on performing complex visual tasks: Evidence from comparative visual search. *Journal of Vision* 12: 1–19.
53. Pornpitakpan, C. (2004). The persuasive effect of circadian arousal, endorser expertise, and argument strength in advertising. *Journal of Global Marketing* 17: 141–172.
54. Gates, A. I. (1916). Diurnal variations in memory and association. *University of California Publications in Psychology* 1: 323–344.
55. De Castro, J. M. (1987). Circadian rhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology and Behavior* 40: 437–446.

56. Watson, D., Wiese, D., Vaidya, J., and Tellegen, A. (1999). The two general activation systems of affect: Structural findings, evolutionary considerations, and psychobiological evidence. *Journal of Personality and Social Psychology* 76: 820–838.
57. Kraemer, S., Danker-Hopfe, H., Dorn, H., Schmidt, A., Ehlert, I., and Herrmann, W. M. (2000). Time-of-day variations of indicators of attention: Performance, physiologic parameters, and self-assessment of sleepiness. *Biological Psychiatry* 48: 1069–1080.
58. Murray, G., Allen, N. B., and Trinder, J. (2002). Mood and the circadian system: Investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
59. Natale, V., Alzani, A., and Cicogna, P. (2003). Cognitive efficiency and circadian typologies: A diurnal study. *Personality and Individual Differences* 35: 1089–1105.
60. Folkard, S. (1990). Circadian performance rhythms: Some practical and theoretical implications. *Philosophical Transactions of the Royal Society of London B* 327: 543–553.
61. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M., and Czeisler, C. A. (1992). Short-term memory, alertness and performance: A reappraisal of their relationship to body temperature. *Journal of Sleep Research* 1: 24–29.
62. Van Eekelen, A. P. J. and Kerkhof, G. A. (2003). No interference of task complexity with circadian rhythmicity in a constant routine protocol. *Ergonomics* 46: 1578–1593.
63. Kahneman, D., Krueger, A. B., Schkade, D. A., Schwarz, N., and Stone, A. A. (2004). A survey method for characterizing daily life experience: The day reconstruction method. *Science* 306: 1776–1780.
64. Åkerstedt, T., Kecklund, G., and Hörte, L. G. (2001). Night driving, season, and the risk of highway accidents. *Sleep* 24: 401–406.
65. Lucidi, F., Mallia, L., Violani, C., Giustiniani, G., and Persia, L. (2013). The contributions of sleep-related risk factors to diurnal car accidents. *Accident Analysis and Prevention* 51: 135–140.
66. Philip, P., Taillard, J., Moore, N., Delord, S., Valtat, C., Sagaspe, P., and Bioulac, B. (2006). The effects of coffee and napping on nighttime highway driving: A randomized trial. *Annals of Internal Medicine* 144: 785–791.
67. Danner, F. and Phillips, B. (2008). Adolescent sleep, school start times, and teen motor vehicle crashes. *Journal of Clinical Sleep Medicine* 4: 533–535.
68. Silbergleit, R., Kronick, S. L., Philpott, S., Lowell, M. J., and Wagner, C. (2006). Quality of emergency care on the night shift. *Academic Emergency Medicine* 13: 325–330.
69. Watson, D. and Clark, L. A. (1997). Measurement and mismeasurement of mood: Recurrent and emergent issues. *Journal of Personality Assessment* 68: 267–297.
70. Mitsutake, G., Otsuka, K., Cornélissen, G., Herold, M., Günther, R., Dawes, C., Burch, J. B., Watson, D., and Halberg, F. (2001). Circadian and infradian rhythms in mood. *Biomedicine and Pharmacotherapy* 55: 94–100.
71. Mitsutake, G., Cornélissen, G., Otsuka, K., Dawes, C., Burch, J., Rawson, M. J., Siegelová, J. et al. (2002). Relationship between positive and negative moods and blood pressure in a clinically healthy man. *Scripta Medica* 75: 315–320.
72. Golder, S. A. and Macy, M. W. (2011). Diurnal and seasonal mood vary with work, sleep, and daylength across diverse cultures. *Science* 333: 1878–1881.
73. Murray, G., Nicholas, C. L., Kleiman, J., Dwyer, R., Carrington, M. J., Allen, N. B., and Trinder, J. (2009). Nature's clocks and human mood: The circadian system modulates reward motivation. *Emotion* 9: 705–716.
74. Kouchaki, M. and Smith, I. H. (2014). The morning morality effect: The influence of time of day on unethical behavior. *Psychological Science* 25: 95–102.
75. Gunia, B. C., Barnes, C. M., and Sah, S. (2014). The morality of larks and owls: Unethical behavior depends on chronotype as well as time of day. *Psychological Science* 25: 2272–2274.
76. Brody, J. E. (1981). Body's many rhythms send messages on when to work and when to play. *New York Times*, August 11, 1981, pp. C1–C2.
77. Low-Zeddies, S. S. and Takahashi, J. S. (2001). Chimera analysis of the *Clock* mutation in mice shows that complex cellular integration determines circadian behavior. *Cell* 105: 25–42.
78. Labyak, S. E., Lee, T. M., and Goel, N. (1997). Rhythm chronotypes in a diurnal rodent, *Octodon degus*. *American Journal of Physiology* 273: R1058–R1066.
79. Masuda, K. and Zhdanova, I. V. (2010). Intrinsic activity rhythms in *Macaca mulatta*: Their entrainment to light and melatonin. *Journal of Biological Rhythms* 25: 361–371.
80. Horne, J. A. and Östberg, O. (1976). A self-assessment questionnaire to determine morningness–eveningness in human circadian rhythms. *International Journal of Chronobiology* 4: 97–110.
81. Koscec, A., Radosevic-Vidacek, B., and Kostovic, M. (2001). Morningness–eveningness across two student generations: Would two decades make a difference? *Personality and Individual Differences* 31: 627–638.
82. Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M. and Tisak, J. (2002). Investigation of morning–evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.
83. Di Milia, L., Smith, P. A., and Folkard, S. (2004). Refining the psychometric properties of the circadian type inventory. *Personality and Individual Differences* 36: 1953–1964.
84. Roenneberg, T., Wirz-Justice, A., and Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
85. Zavada, A., Gordijn, M. C. M., Beersma, D. G. M., Daan, S., and Roenneberg, T. (2005). Comparison of the Munich chronotype questionnaire with the Horne-Östberg's morningness–eveningness score. *Chronobiology International* 22: 267–278.
86. Roenneberg, T., Allebrandt, K. V., Merrow, M., and Vetter, C. (2012). Social jet lag and obesity. *Current Biology* 22: 939–943.
87. Parsons, M. J., Moffitt, T. E., Gregory, A. M., Goldman-Mellor, S., Nolan, P. M., Poulton, R., and Caspi, A. (2015). Social jetlag, obesity and metabolic disorder: Investigation in a cohort study. *International Journal of Obesity* 39: 842–848.
88. Adan, A. and Natale, V. (2002). Gender differences in morningness–eveningness preference. *Chronobiology International* 19: 709–720.
89. Langford, C. and Glendon, A. I. (2002). Effects of neuroticism, extraversion, circadian type and age on reported driver stress. *Work and Stress* 16: 316–334.
90. Benedito-Silva, A. A., Menna-Barreto, L., Alam, M. F., Rotenberg, L., Moreira, L. F. S., Menezes, A. A. L., Pereira da Silva, H., and Marques, N. (1998). Latitude and social habits as determinants of the distribution of morning and evening types in Brazil. *Biological Rhythm Research* 29: 591–597.

91. Medeiros, A. L. D., Mendes, D. B. F., Lima, P. F., and Araujo, J. F. (2001). The relationships between sleep-wake cycle and academic performance in medical students. *Biological Rhythm Research* 32: 263–270.
92. Von Schantz, M., Taporoski, T. P., Horimoto, A. R. V., Duarte, N. E., Vallada, H., Krieger, J. E., Pedrazzoli, M., Negrão, A. B., and Pereira, A. C. (2015). Distribution and heritability of diurnal preference (chronotype) in a rural Brazilian family-based cohort, the Baependi study. *Scientific Reports* 5: 9214.
93. Laberge, L., Carrier, J., Lespérance, P., Lambert, C., Vitaro, F., Tremblay, R. E., and Montplaisir, J. (2000). Sleep and circadian phase characteristics of adolescent and young adult males in a naturalistic summertime condition. *Chronobiology International* 17: 489–501.
94. Mongrain, V., Paquet, J., and Dumont, M. (2006). Contribution of the photoperiod at birth to the association between season of birth and diurnal preference. *Neuroscience Letters* 406: 113–116.
95. Barclay, N. L., Eley, T. C., Buysse, D. J., Archer, S. N., and Gregory, A. M. (2010). Diurnal preference and sleep quality: Same genes? A study of young adult twins. *Chronobiology International* 27: 278–296.
96. Johansson, C., Willeit, M., Smedh, C., Ekholm, J., Paunio, T., Kieseppä, T., Lichtermann, D. et al. (2003). Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. *Neuropsychopharmacology* 28: 734–739.
97. Taillard, J., Sanchez, P., Lemoine, P., and Mouret, J. (1990). Heart rate circadian rhythm as a biological marker of desynchronization in major depression: A methodological and preliminary report. *Chronobiology International* 7: 305–316.
98. Taillard, J., Philip, P., Chastang, J. F., and Bioulac, B. (2004). Validation of Horne and Ostberg morningness–eveningness questionnaire in a middle-aged population of French workers. *Journal of Biological Rhythms* 19: 76–86.
99. Roemer, H. C., Griefahn, B., Kuenemund, C., Blaszkewicz, M., and Gerngroß, H. (2003). The reliability of melatonin synthesis as an indicator of the individual circadian phase position. *Military Medicine* 168: 674–678.
100. Natale, V. and Lorenzetti, R. (1997). Influences of morningness–eveningness and time of day on narrative comprehension. *Personality and Individual Differences* 23: 685–690.
101. Mecacci, L., Righi, S., and Rocchetti, G. (2004). Cognitive failures and circadian typology. *Personality and Individual Differences* 37: 107–113.
102. Park, Y. M., Matsumoto, K., Seo, Y. J., and Shinkoda, H. (1997). Scores on morningness–eveningness and sleep habits of Korean students, Japanese students, and Japanese workers. *Perceptual and Motor Skills* 85: 143–154.
103. Shinkoda, H., Matsumoto, K., Park, Y. M., and Nagashima, H. (2000). Sleep-wake habits of schoolchildren according to grade. *Psychiatry and Clinical Neurosciences* 54: 287–289.
104. Stolarski, M., Ledzinska, M., and Matthews, G. (2013). Morning is tomorrow, evening is today: Relationships between chronotype and time perspective. *Biological Rhythm Research* 44: 181–196.
105. Adan, A. and Almirall, H. (1991). Horne and Östberg morningness–eveningness questionnaire: A reduced scale. *Personality and Individual Differences* 12: 241–253.
106. May, C. P. and Hasher, L. (1998). Synchrony effects in inhibitory control over thought and action. *Journal of Experimental Psychology: Human Perception and Performance* 24: 363–379.
107. Baehr, E. K., Revelle, W., and Eastman, C. I. (2000). Individual differences in the phase and amplitude of the human circadian temperature rhythm with an emphasis on morningness–eveningness. *Journal of Sleep Research* 9: 117–127.
108. Duffy, J. F., Rimmer, D. W., and Czeisler, C. A. (2001). Association of intrinsic circadian period with morningness–eveningness, usual wake time, and circadian phase. *Behavioral Neuroscience* 115: 895–899.
109. Martin, S. K. and Eastman, C. I. (2002). Sleep logs of young adults with self-selected sleep times predict the dim light melatonin onset. *Chronobiology International* 19: 695–707.
110. Hasler, B. P., Allen, J. J. B., Sbarra, D. A., Bootzin, R. R., and Bernert, R. A. (2010). Morningness–eveningness and depression: Preliminary evidence for the role of BAS and positive effect. *Psychiatry Research* 176: 166–173.
111. Horne, J. A. and Östberg, O. (1977). Individual differences in human circadian rhythms. *Biological Psychology* 5: 179–190.
112. Lack, L., Bailey, M., Lovato, N., and Wright, H. (2009). Chronotype differences in circadian rhythms of temperature, melatonin, and sleepiness as measured in a modified constant routine protocol. *Nature and Science of Sleep* 1: 1–8.
113. Kerkhof, G. A., Van Dongen, H. P. A., and Bobbert, A. C. (1998). Absence of endogenous circadian rhythmicity in blood pressure? *American Journal of Hypertension* 11: 373–377.
114. Jankowski, K. S., Díaz-Morales, J. F., and Randler, C. (2014). Chronotype, gender, and time for sex. *Chronobiology International* 31: 911–916.
115. Biss, R. K. and Hasher, L. (2012). Happy as a lark: Morning-type younger and older adults are higher in positive affect. *Emotion* 12: 437–441.
116. Dagys, N., McGlinchey, E. L., Talbot, L. S., Kaplan, K. A., Dahl, R. E., and Harvey, A. G. (2012). Double trouble? The effects of sleep deprivation and chronotype on adolescent affect. *Journal of Child Psychology and Psychiatry* 53: 660–667.
117. Caci, H., Mattei, V., Baylé, F. J., Nadalet, L., Dossios, C., Robert, P., and Boyer, P. (2005). Impulsivity but not venturesomeness is related to morningness. *Psychiatry Research* 134: 259–265.
118. Digdon, N. K. and Howell, A. J. (2008). College students who have an eveningness preference report lower self-control and greater procrastination. *Chronobiology International* 25: 1029–1046.
119. Ponzi, D., Wilson, M. C., and Maestripieri, D. (2014). Eveningness is associated with higher risk-taking, independent of sex and personality. *Psychological Reports* 115: 932–947.
120. Jovanovski, D. and Bassili, J. N. (2007). The relationship between morningness–eveningness preference and online learning. *Biological Rhythm Research* 38: 355–365.
121. Kim, S., Dueker, G. L., Hasher, L., and Goldstein, D. (2002). Children's time of day preference: Age, gender and ethnic differences. *Personality and Individual Differences* 33: 1083–1090.
122. Giannotti, F., Cortesi, F., Sebastiani, T., and Ottaviano, S. (2002). Circadian preference, sleep and daytime behaviour in adolescence. *Journal of Sleep Research* 11: 191–199.
123. Caci, H., Adan, A., Bohle, P., Natale, V., Pornpitakpan, C., and Tilley, A. (2005). Transcultural properties of the composite scale of morningness: The relevance of the "morning affect" factor. *Chronobiology International* 22: 523–540.
124. Mansour, H. A., Wood, J., Chowdari, K. V., Dayal, M., Thase, M. E., Kupfer, D. J., Monk, T. H., Devlin, B., and Nimgaonkar, V. L. (2005). Circadian phase variation in Bipolar I disorder. *Chronobiology International* 22: 571–584.

125. Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., and Merrow, M. (2007). Epidemiology of the human circadian clock. *Sleep Medicine Reviews* 11: 429–438.
126. Schneider, A. M. and Randler, C. (2009). Daytime sleepiness during transition into daylight saving time in adolescents: Are owls higher at risk? *Sleep Medicine* 10: 1047–1050.
127. Nielsen, T. (2010). Nightmares associated with the evening chronotype. *Journal of Biological Rhythms* 25: 53–62.
128. Robilliard, D. L., Archer, S. N., Arendt, J., Lockley, S. W., Hack, L. M., English, J., Leger, D. et al. (2002). The 3111 *Clock* gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. *Journal of Sleep Research* 11: 305–312.
129. Koskenvuo, M., Hublin, C., Partinen, M., Heikkilä, K., and Kaprio, J. (2007). Heritability of diurnal type: A nationwide study of 8753 adult twin pairs. *Journal of Sleep Research* 16: 156–162.
130. Chung, J. K., Lee, K. Y., Kim, S. H., Kim, E. J., Jeong, S. H., Jung, H. Y., Choi, J. E., Ahn, Y. M., Kim, Y. S., and Joo, E. J. (2012). Circadian rhythm characteristics in mood disorders: Comparison among bipolar I disorder, bipolar II disorder and recurrent major depressive disorder. *Clinical Psychopharmacology and Neuroscience* 10: 110–116.
131. Katzenberg, D., Young, T., Finn, L., Lin, L., King, D. P., Takahashi, J. S., and Mignot, E. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep* 21: 569–576.
132. Chang, A. M., Buch, A. M., Bradstreet, D. S., Klements, D. J., and Duffy, J. F. (2011). Human diurnal preference and circadian rhythmicity are not associated with the CLOCK 3111C/T gene polymorphism. *Journal of Biological Rhythms* 26: 276–279.
133. Grossman, L. (2004). 10 Questions for Bill Gates. *Time* 163(10): 8.
134. Watts, B. L. (1982). Individual differences in circadian activity rhythms and their effects on roommate relationships. *Journal of Personality* 50: 374–384.
135. Verger, F., Sourbès-Verger, I., and Ghirardi, R. (2003). *The Cambridge Encyclopedia of Space* (Trans. by S. Lyle and P. Reilly). Cambridge, U.K.: Cambridge University Press.
136. Weed, W. S. (2003). Star trek. *Discover* 24(8): 34–41.
137. Normile, D. (1998). NASA craft to take the controls in flight. *Science* 282: 604–605.
138. Lang, T., LebLanc, A., Evans, H., Lu, Y., Genant, H., and Yu, A. (2004). Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. *Journal of Bone and Mineral Research* 19: 1006–1012.
139. Tavella, S., Ruggiu, A., Giuliani, A., Brun, F., Canciani, B., Manescu, A., Marozzi, K. et al. (2012). Bone turnover in wild type and pleiotrophin-transgenic mice housed for three months in the International Space Station. *PLOS ONE* 7: e33179.
140. Wang, D., Zhang, L., Wan, Y., Chen, X., Liang, X., Shen, H., and Guo, J. (2014). Space meets time: Impact of gravity on circadian timing system. In: Sanders, S. (Ed.), *Human Performance in Space: Advancing Astronautics Research in China*. Washington, DC: American Association for the Advancement of Science, pp. 15–17.
141. Izumi, R., Ishioka, N., Mizuno, K., and Goka, T. (2001). Space environment, electromagnetic fields, and circadian rhythm. *Biomedicine and Pharmacotherapy* 55: 25–31.
142. Cassone, V. M. and Stephan, F. K. (2002). Central and peripheral regulation of feeding and nutrition by the mammalian circadian clock: Implications for nutrition during manned space flight. *Nutrition* 18: 814–819.
143. Mallis, M. M. and DeRoshia, C. W. (2005). Circadian rhythms, sleep, and performance in space. *Aviation, Space, and Environmental Medicine* 76(S): B94–B107.
144. Hoban-Higgins, T. M., Alpatov, A. M., Wassmer, G. T., Rietveld, W. J., and Fuller, C. A. (2003). Gravity and light effects on the circadian clock of a desert beetle, *Trigonoscelis gigas*. *Journal of Insect Physiology* 49: 671–675.
145. Fuller, C. A., Hoban-Higgins, T. M., Klimovitsky, V. Y., Griffin, D. W., and Alpatov, A. M. (1996). Primate circadian rhythms during spaceflight: Results from Cosmos 2044 and 2229. *Journal of Applied Physiology* 81: 188–193.
146. Gundel, A., Nalishiti, V., Reucher, E., Vejvoda, M., and Zulley, J. (1993). Sleep and circadian rhythm during a short space mission. *Clinical Investigator* 71: 718–724.
147. Gundel, A., Polyakov, V. V., and Zulley, J. (1997). The alteration of human sleep and circadian rhythms during spaceflight. *Journal of Sleep Research* 6: 1–8.
148. Monk, T. H., Buysse, D. J., Billy, B. D., Kennedy, K. S., and Willrich, L. M. (1998). Sleep and circadian rhythms in four orbiting astronauts. *Journal of Biological Rhythms* 13: 188–201.
149. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J. et al. (2001). Sleep, performance, circadian rhythms, and light-dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
150. Monk, T. H., Kennedy, K. S., Rose, L. R., and Linenger, J. M. (2001). Decreased human circadian pacemaker influence after 100 days in space: A case study. *Psychosomatic Medicine* 63: 881–885.
151. Pittendrigh, C. S. (1967). Circadian rhythms, space research and manned space flight. *Life Sciences and Space Research* 5: 122–134.
152. Lawler, A. (2003). The new race to the Moon. *Science* 300: 724–727.
153. Spudis, P. D. (2003). The new moon. *Scientific American* 289(6): 86–93.
154. Kluger, J. (2004). Mission to Mars. *Time* 163(4): 42–50.
155. Xiao, L., Zhu, P., Fang, G., Xiao, Z., Zou, Y., Zhao, J., Zhao, N. et al. (2015). A young multilayered terrane of the northern Mare Imbrium revealed by Chang'E-3 mission. *Science* 347: 1226–1229.
156. Gibney, E. (2014). Europe plans Moon landing. *Nature* 516: 153.
157. Basner, M., Dinges, D. F., Mollicone, D., Ecker, A., Jones, C. W., Hyder, E. C., Di Antonio, A. et al. (2013). Mars 520-d mission simulation reveals protracted crew hypokinesis and alterations of sleep duration and timing. *Proceedings of the National Academy of Sciences of the United States of America* 110: 2635–2640.
158. Barger, L. K., Sullivan, J. P., Vincent, A. S., Fiedler, E. R., McKenna, L. M., Flynn-Evans, E. E., Gilliland, K. et al. (2012). Learning to live on a Mars day: Fatigue countermeasures during the Phoenix Mars Lander mission. *Sleep* 35: 1423–1435.
159. Bluth, B. J. (1988). Lunar settlements: A socio-economic outlook. *Acta Astronautica* 17: 659–667.
160. Litton, C. E. (1997). Lessons learned studying design issues for lunar and Mars settlements. *Life Support and Biosphere Science* 4: 127–144.
161. Mervis, J. (2003). Bye, bye Biosphere 2. *Science* 302: 2053.
162. Stokstad, E. (2011). Cash advance, new approach aim to relaunch Biosphere 2. *Science* 333: 146.
163. Stokstad, E. (2012). Experimental landscapes raise stakes at Biosphere 2. *Science* 338: 1417.
164. Stone, R. (2012). A new dawn for China's space scientists. *Science* 336: 1630–1637.

165. Pittendrigh, C. S. (1965). On the biological problems to be attacked with a series of U.S. satellites in 1966. *Life Sciences and Space Research* 3: 206–214.
166. Kunzig, R. (2003). Mars Express. *Discover* 24(5): 34–43.
167. Jaffe, S. (2003). Shuttle squeezes science in space program. *Scientist* 17(20): 46–47.
168. Stone, R. (2003). One nuclear leap to Mars? *Science* 301: 906–909.
169. Lawler, A. (2004). Scientists add up gains, losses in Bush's new vision for NASA. *Science* 303: 444–445.
170. Lawler, A. (2004). How much space for science? *Science* 303: 610–612.
171. Hand, E. (2014). NASA planners gear up for Martian sample return. *Science* 344: 787–788.
172. Mervis, J. and Malakoff, D. (2014). Science agencies make gains despite tight U.S. budget. *Science* 346: 1437–1438.
173. Bhattacharjee, Y. (2011). A distant glimpse of alien life? *Science* 333: 930–932.
174. Howard, A. W. (2013). Observed properties of extrasolar planets. *Science* 340: 572–576.
175. Gaidos, E., Haghighipour, N., Agol, E., Latham, D., Raymond, S., and Rayner, J. (2007). New worlds on the horizon: Earth-sized planets close to other stars. *Science* 318: 210–213.

15 Jet Lag and Shift Work

15.1 JET-LAG SYNDROME

Jet lag is a malaise often associated with transmeridian (i.e., long-distance) airplane travel (Figure 15.1). Anyone who has traveled by airplane across several time zones (for instance, from New York to London) probably has experienced jet lag. Some of the symptoms that are often reported are fatigue, irritability, and inability to concentrate during the day, difficulty in sleeping at night, and gastrointestinal discomfort.[1–3] The symptoms usually last only a few days (a little over a week, if many time zones are crossed), but they can be very distressing, especially for businessmen who must make important business decisions while mentally impaired by jet lag.

15.1.1 Diagnosis

There are three main elements involved in transmeridian flights: the traveling itself, the crossing of time zones, and the arrival at a different geographical area. The traveling itself—especially if it is long—may make you feel bored and tired. The aviation industry is well aware of travelers' complaints about air transportation (such as airport tumult, barometric pressure changes, immobility, noise, vibration, and radiation) and strives to improve passenger comfort.[4] However, the stress of an airplane trip is usually nothing that a good night of sleep cannot overcome. Jet lag is a much more serious condition. After all, if you walk across several time zones (which is far more exhausting than sitting in an airplane but allows you to slowly adapt to the successive zones), you do not experience jet lag. Even better, if you fly from New York to Lima in South America (which is a long trip but involves no crossing of time zones), you do not experience jet lag.

Arriving at a different geographical area may also make you feel distressed. A sudden change in temperature, humidity, eating habits, and social customs can certainly make your trip much less enjoyable than you anticipated. As a matter of fact, depending on one's predisposition, even everyday problems and the minor nuisances of life can be important forms of stress with significant impact on one's health.[5] However, changes in the environment are—for better or for worse—an essential part of traveling. Some people like them, some do not, and this does not seem to have a major effect on jet lag. Indeed, even though the environmental changes can be as radical in a north–south flight as in a west–east flight, jet lag is much stronger in the latter than in the former.

Thus, the major element seems to be the rapid crossing of time zones. The cause of jet lag is travel by *jet* (Figure 15.2). In terms of circadian rhythms, what does this mean? Let us start by reviewing the concept of time zone. By convention, the world is divided into 24 time zones, starting at the Greenwich meridian, in England (Figure 15.3). Greenwich mean time (GMT) is the reference point (GMT 0). When it is noon in London, it is still 4 a.m. in California (GMT −8) and it is already 9 p.m. in Japan (GMT +9). In principle, each time zone corresponds to a 15° section of the Earth's circumference, but many exceptions are made for political and practical reasons.

Next, let us consider an eastbound trip across 8 time zones, such as from New York to Moscow, Russia (Figure 15.4). At current commercial air travel speeds, the flight should take about 10 hours. If you leave at 9 p.m., you should arrive at 7 a.m. on the next day (according to New York time, which is also your internal time). After a taxicab drive to the hotel and a refreshing shower, you will be ready to do business at 9 a.m. However, your 9 a.m. is actually 5 p.m. in Moscow (±1 hour, depending on daylight-saving schedules). Particularly if you managed to get some sleep on the plane, you will be quite awake and ready to do business at a time when everyone is getting ready to go home. Worse, when the Moscow clock shows 12 midnight (time to go to sleep), your clock says it is only 4 o'clock in the afternoon. You obviously do not feel sleepy. By the time you do get sleepy (say, 1 a.m. in your internal clock, or 9 a.m. in the Moscow clock), you are in the middle of a business meeting. No wonder you cannot think clearly! Notice that local residents will not be jet lagged like you are. They have been in that time zone for quite a while and are fully adapted. So will you, if you can afford to "waste" more than a week sitting by the swimming pool and waiting for your internal clock to be ready to do business in Russia. This is why jet lag is a transient illness—it will go away if you do nothing about it. The problem is that usually you cannot wait (and you feel crummy during the whole process, anyway).

Still in Figure 15.4, notice that a flight from New York to Santiago (in Chile) is just as long as the flight to Moscow but involves a change of only 1 time zone, which is usually negligible. Similarly, a flight from Perth (in Australia) to Cape Town (in South Africa) is just as long as a flight to Tokyo (in Japan), but the flight to Cape Town involves a change of 6 time zones, whereas the flight to Tokyo involves a change of only 1 time zone. Small changes in time zones may be slightly disruptive, but large changes essentially cause you to want to be awake when it is time to sleep (and to be asleep when it is time to be awake), which is the heart of the problem.

So, the trouble with jet lag is that one is suddenly expected to be awake during one's night and asleep during one's day. Since the person cannot sleep during the day (local night), he or she will be very tired, irritable, and unable to concentrate at night (local day). This sounds very simple, but is it true? Have controlled studies actually been conducted? Yes. In animal studies, jet lag is often simulated by a shift in the light–dark cycle. Advances in the light–dark cycle correspond to eastbound travel (because the Sun rises earlier in the east



FIGURE 15.1 Touristic attractions. Places like New York, London, Rio, and Cairo attract visitors from all over the world. (Copyright ArtToday, Tucson, AZ.)



FIGURE 15.2 Flying overseas. As the best-selling jetliner in the world, the Boeing 737 takes millions of travelers in transmeridian flights. (Copyright Boeing, Chicago, IL. Used under license.)

and, consequently, the day starts earlier as one moves eastward instantly), while delays correspond to westbound travel. Figure 15.5 shows the records of running-wheel activity of two Nile grass rats that were subjected to phase shifts of the light–dark cycle, as indicated by the white and dark bars above the actograms. The animal whose records are shown in Panel A was subjected to an 8-hour phase advance of the zeitgeber, which corresponds to the flight from New York to Moscow.

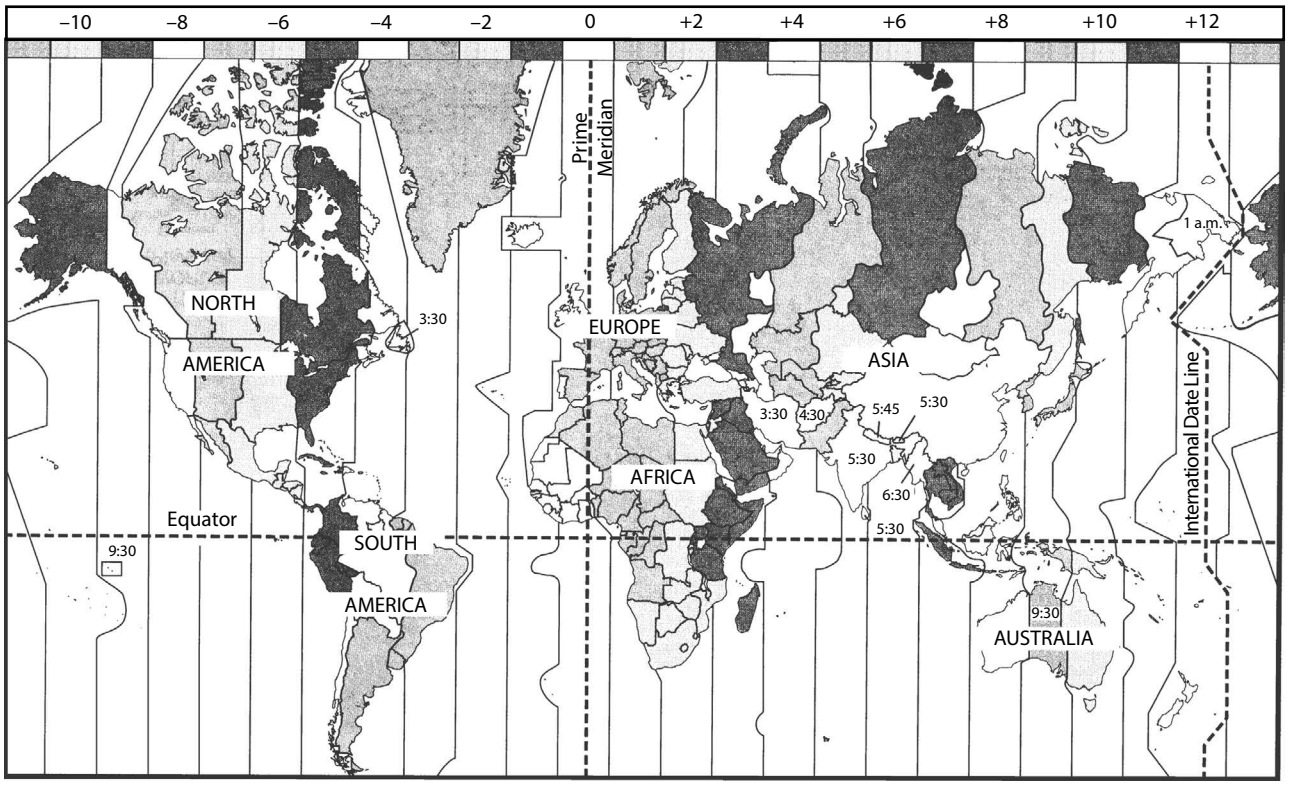


FIGURE 15.3 Time zones. By convention, the world is divided into 24 time zones, starting at the Greenwich meridian (England). When it is noon in London, it is still 4 a.m. in California (GMT −8) and it is already 9 p.m. in Japan (GMT +9). GMT, Greenwich mean time. (Adapted from *TIME Almanac*, TIME Books, Des Moines, IA, 2009.)

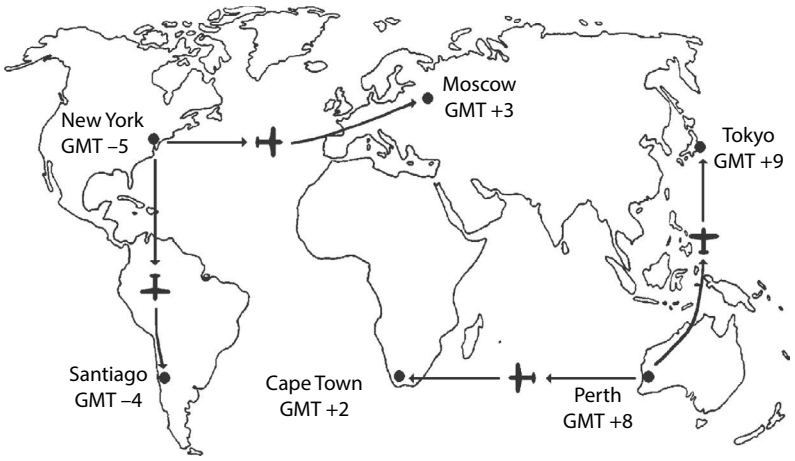


FIGURE 15.4 Traveling east–westward or north–southward. Although a flight from New York to Moscow (Russia) is about as long as a flight to Santiago (Chile), the trip to Moscow requires the crossing of 8 time zones, whereas the trip to Santiago requires the crossing of only 1 time zone. Similarly, a trip from Perth (Australia) to Cape Town (South Africa) requires the crossing of 6 time zones, whereas a trip to Tokyo (Japan) requires the crossing of only 1 time zone.

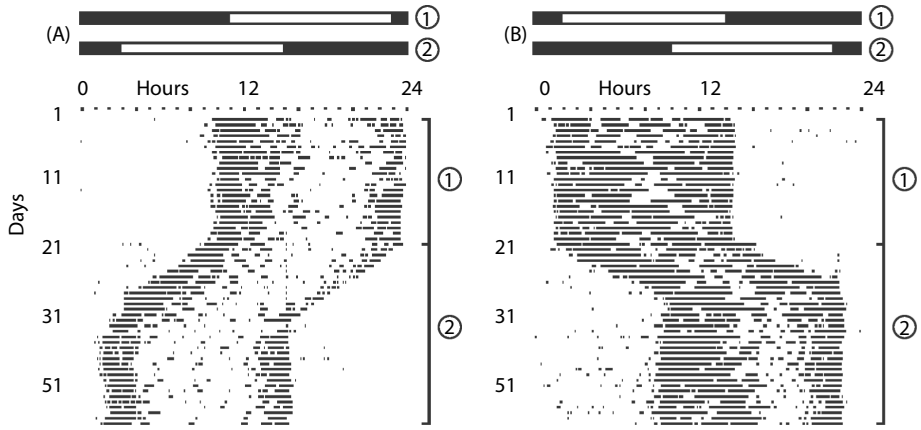


FIGURE 15.5 Simulating jet lag. The actograms show the rhythms of running-wheel activity of two Nile grass rats (*Arvicanthis niloticus*) subjected to phase shifts of the light–dark cycle as simulations of transmeridian flights. As indicated by the dark and white horizontal bars above the actograms, the condition depicted in Panel A is an 8-hour phase advance, as that experienced after a flight from New York to Moscow. The condition depicted in Panel B is an 8-hour phase delay, as that experienced after a flight from Tokyo to Rome. Reentrainment requires many days in both conditions, but synchronization is achieved more rapidly after a phase delay (westward flight) than after a phase advance (eastward flight). (Data from the archives of the Refinetti lab.)

The animal whose records are shown in Panel B was subjected to an 8-hour phase delay of the zeitgeber, which corresponds to a flight from Tokyo (Japan) to Rome (Italy). Notice that reentrainment takes several days in both cases but that the phase delay (Panel B) is accomplished in 10 days, whereas the phase advance (Panel A) requires 16 days. Based on the principles discussed in Chapter 7, we know that the rate of reentrainment depends on the circadian period of the organism, on the amplitude of the phase–response curve, and on various properties of the zeitgeber (such as the proportion of light and darkness and the magnitude of the shift).

This type of jet-lag simulation has been conducted in numerous species, including domestic mice,[6–17] laboratory rats,[18–20] other rodents,[21–27] cats,[28] birds,[29] and primates.[30–32] Although cases of instantaneous reentrainment have been observed,[7,19] they most likely reflected the additional influence of photic masking (which is much more common in nocturnal animals than in diurnal ones). In most studies, reentrainment was found to require approximately 1 day for each hour of the shift in the zeitgeber. For instance, in one of my own studies, I recorded the body temperature and activity rhythms of golden hamsters before and after a 6-hour phase advance in the light–dark cycle, which corresponds to a flight from New York to Paris (France). The rhythms of body temperature and activity gradually advanced during the following days, and full reentrainment was attained in 6 days (that is, 1 day for each hour of the full shift).[24] In a similar study in a different laboratory, it took rats 6–7 days to reentrain after an 8-hour advance in the light–dark cycle.[18]

Studies in which many physiological variables were simultaneously recorded indicated that different variables may reentrain at different rates. For instance, in rats subjected to

a 12-hour shift of the light–dark cycle, the body temperature rhythm reentrained in 9 days, while the rhythms of drinking and feeding reentrained in 7 days.[33] In rhesus monkeys subjected to a 6-hour phase advance, the activity rhythm reentrained in 4 days, whereas the body temperature rhythm reentrained in 7 days.[34] In squirrel monkeys subjected to an 8-hour phase delay, the rhythms of drinking, feeding, activity, urination, and body temperature all reentrained at different rates.[35] As discussed in Chapter 9, differences in phase-shift rates of various rhythms are probably due to differences in the output pathways from the central clock. The clock itself shifts at a rate that depends on the timing of photic exposure.

Snapshots of the phase shift that occurs within the clock can be seen in Figure 15.6. Expression of the *per1* gene was measured in the suprachiasmatic nucleus (SCN) of rats that had been subjected to a 6-hour advance of the light–dark cycle. Groups of animals were euthanized at various time points before and after the shift, and the number of cells expressing *per1* was measured both in the ventrolateral and dorsomedial regions of the SCN.[36] As you remember from Chapter 12, the ventrolateral SCN receives most of the photic afferent input, whereas the dorsomedial SCN contains most cells that are intrinsically rhythmic. Notice in the figure that, before the shift of the light–dark cycle, the rhythms of *per1* expression peaked during the day in both regions of the SCN. After the shift of the light–dark cycle, the ventrolateral SCN shifted almost immediately, thus reflecting the shifted photic input. In contrast, it took 5–9 days for the dorsomedial SCN to complete the shift, which is consistent with the lag time of the locomotor activity rhythm. A different research team, conducting research on mice, noticed that the rhythms of *per1* and *per2* shifted rapidly in the SCN (they did not look at the two subdivisions separately), but that the rhythm of *cry1* shifted rapidly during phase delays and slowly during phase advances.[37] This is an interesting finding because, as we saw in Figure 15.5, behavioral phase delays are usually attained more rapidly than phase advances.

A number of studies have been conducted with human subjects in actual and simulated jet-lag conditions.[38–46] The time needed for reentrainment was found to be a little less than 1 day per time zone—say, about 6 days for an 8-hour shift—and differences were found between eastbound and westbound conditions. Although complaints of discomfort during the day, and of difficulty sleeping at night, are common, it is not clear whether the performance of well-trained athletes is actually impaired by jet lag,[47,48] as was mentioned in Chapter 14. A recent study involving the German Olympic gymnastics team noticed disturbance in various physiological and behavioral parameters (including subjective ratings of jet-lag intensity) after westward flights across 6 time zones and eastward flights across 8 time zones, but it is not evident that athletic performance was actually impaired.[49] Whether athletes or sedentary subjects are studied, subjective ratings of jet lag are very closely related to subjective ratings of fatigue,[50] so that it is difficult to ascertain whether impairments in intellectual or physical performance are due to disturbed circadian phase or merely to lack of sleep. Of course, lack of sleep is itself a consequence of disturbed circadian phase caused by the rapid crossing of time zones.

Most people do not travel long distance on a regular basis, but some (such as airplane pilots and aircraft crew) do. It is relevant, therefore, to investigate whether repeated phase shifts of the circadian clock can be harmful. Deleterious effects of phase shifts on people's long-term health are not well known, but, at least in golden hamsters, repeated shifts can be deadly. In cardiomyopathic hamsters, animals subjected to 12-hour phase shifts of the light–dark cycle on a weekly basis experienced an 11% reduction in life span in reference to animals maintained under a constant light–dark cycle.[51] In humans, this would correspond to an 8-year reduction in life span (from 76 to 68 years). In a similar study in aged mice, mortality rate was 33% higher in animals subjected to a weekly 6-hour phase advance as compared to animals maintained under a constant light–dark cycle.[52] In another study, young mice didn't die because of frequent phase shifts of the light–dark cycle, but they ate more and moved around less than mice kept under a constant light–dark cycle—and became obese.[53] Female mice subjected to frequent phase advances of the light–dark cycle right after becoming pregnant had a full-term pregnancy success rate of 22%, whereas females kept under a constant light–dark cycle had a pregnancy success rate of 90%.[54] Presumably the negative effects of frequent phase shifts of the light–dark cycle demonstrated in these studies are the result of chronic desynchronization, possibly leading to disruption of the organism's internal temporal order. When circadian disruption was induced by very frequent phase shifts of the light–dark cycle (8 hours every 2 days), mice exhibited weak rhythms of activity and body temperature and, when inoculated with transplantable osteosarcoma fragments, developed larger tumors than control mice did.[55]

15.1.2 Treatment

As we look for ways to avoid or reduce jet lag, we must, of course, consider all that we learned about circadian rhythms in the preceding chapters. The most effective way to prevent jet lag is *not* to travel by plane. Travel by car, by train, or by boat is so slow that our bodies have enough time to gradually adapt to the time-zone changes. I realize, however, that this solution to the problem of jet lag is akin to the prevention of unwanted pregnancy by the avoidance of sex altogether. Thus, let us move on to a more realistic solution. The next possible solution is to be lucky enough to have to do business on the west arm of the trip, rather than on the east arm. Many frequent travelers have found out on their own that jet lag is much less of a problem in westbound flights because they can simply go to sleep a little later (according to their internal clock) on the first night at the destination and will wake up ready for business the following morning. On the other hand, after an eastbound flight, they have to try unsuccessfully to go to bed several hours before their usual bedtime—as discussed earlier regarding a flight from New York to Moscow (Figure 15.4). Let us say that you are flying back home from Moscow to New York. You leave at 4 p.m., local time (which by now is also your internal time). You fly 10 hours and arrive in New York on the next day at 2 a.m., Moscow time.

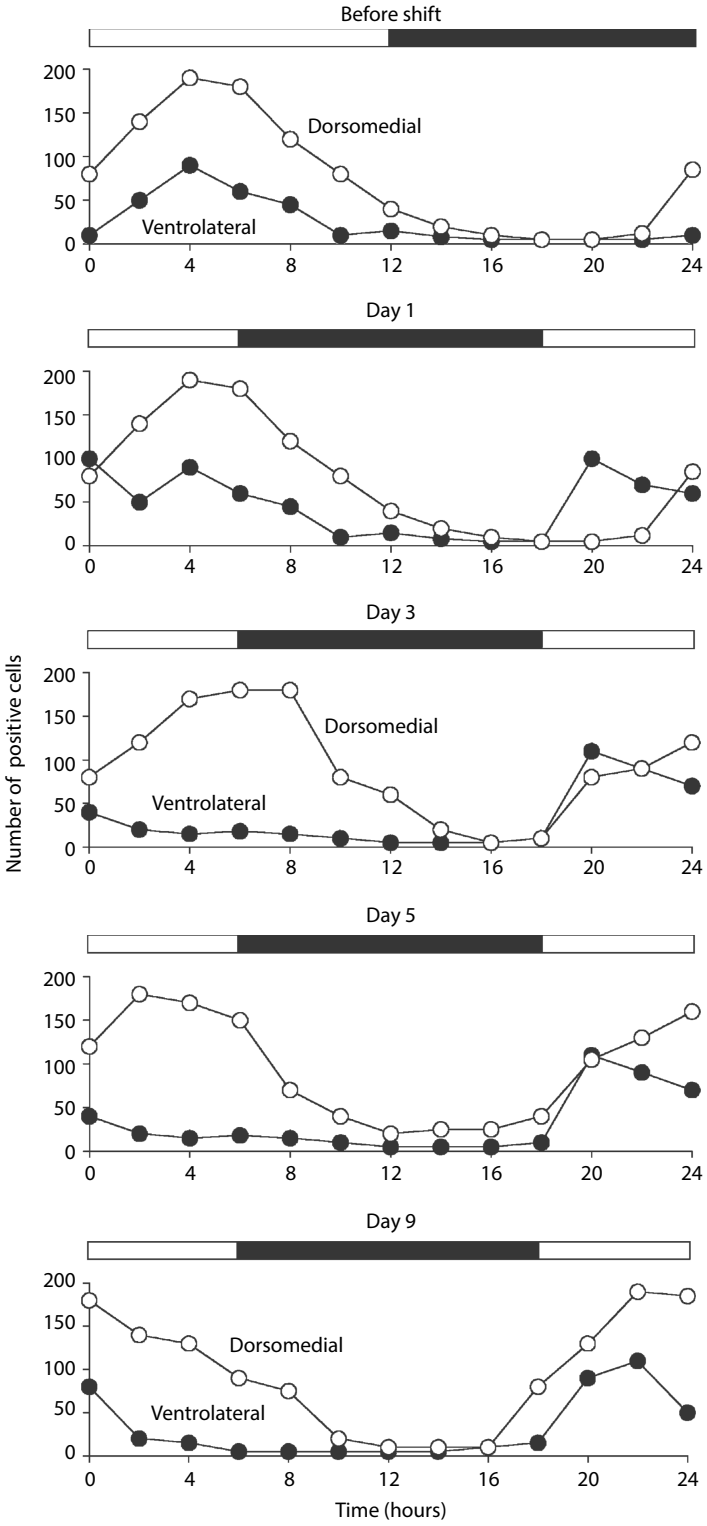


FIGURE 15.6 Looking for the molecular substrate of jet lag. The graphs show the daily variation in the expression of the *per1* gene in the SCN of rats (*Rattus norvegicus*) subjected to a 6-hour advance of the light–dark cycle. Notice that the *per1*-expression rhythm in the ventrolateral section of the SCN shifts almost immediately after the shift of the light–dark cycle, whereas the rhythm in the dorsomedial section takes more than 5 days to shift. The 5-day duration of the desynchronization between the two sections of the SCN matches the 5-day duration of jet lag expected after a 6-hour shift of the light–dark cycle. (Data from Nagano, M. et al., *J. Neurosci.*, 23, 6141, 2003.)

Because you crossed 8 time zones, 2 a.m. is actually 6 p.m. in New York (on the previous calendar day). So, it is past your internal bedtime, but you surely can manage to stay awake for a couple of extra hours and go to bed "early," say, at 8 p.m. local time (which is 4 a.m. your time). Having gone to bed very late (in terms of your internal clock), you manage to sleep 10 hours and wake up at 6 a.m. (local time). A jog in the park, a long shower, extra commuting time, and you are not too early for the 8:30 a.m. meeting. You may be a little tired at the end of the business day (5 p.m. local time, 1 a.m. your time), but overall, it was not a bad day. Of course, if it will take you 6–8 days to adapt to the 8-hour change in time zones, you are bound to run into synchronization problems later in the week. However, if you are scheduled to fly back east soon, you may be able to avoid jet lag entirely. Indeed, if you can keep your origination schedule at your destination, you will not experience jet lag at all. The entertainment industry in Las Vegas is very aware of this and keeps the casinos open 24 hours a day, so that travelers from all over the world can gamble at their own origination schedule and can get back home without ever experiencing jet lag.

In some instances, a traveler can avoid jet lag by selecting the best time of the year to travel. When traveling between two countries that are on different time zones, but that are also on opposite sides of the Equator, one rarely encounters the standard difference in time zones if the two countries adopt daylight-saving time (which we will discuss in greater detail later on). Because the seasons are inverted in the two hemispheres, the 1-hour delay of daylight-saving time is applied at the opposite time of the year in the two hemispheres. Consequently, the standard time-zone difference is encountered only a few weeks each year, as indicated by the stippled bars in Figure 15.7. Most of the year, the time-zone difference is either increased or decreased by daylight-saving time. For example, when flying from Chicago (Illinois) to Rio de Janeiro (Brazil) in December, the traveler will experience a 4-hour time change, which can be disruptive for business activities (Figure 15.8). In July, however, only a 2-hour change will be experienced. This is due to the fact that the standard time difference between Chicago and Rio is 3 hours, but in July, Chicagoans have their clocks advanced by 1 hour (their summer), whereas *cariocas* are

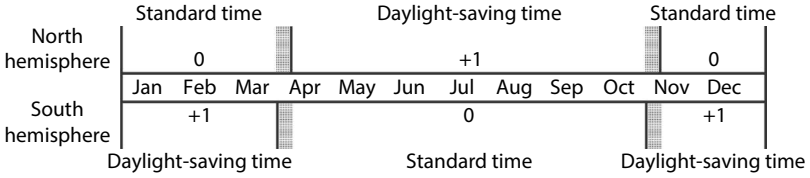


FIGURE 15.7 Hemispheric differences in daylight-saving time. When traveling between two countries that are on different time zones, but that are on opposite sides of the Equator and adopt daylight-saving time, one rarely encounters the standard difference in time zones. Because the 1-hour delay of daylight-saving time is applied at the opposite time of the year in the two hemispheres, the standard time-zone difference is encountered only a few weeks a year (denoted by the stippled bars). Most of the year, the standard time-zone difference is either increased or decreased by daylight-saving time.

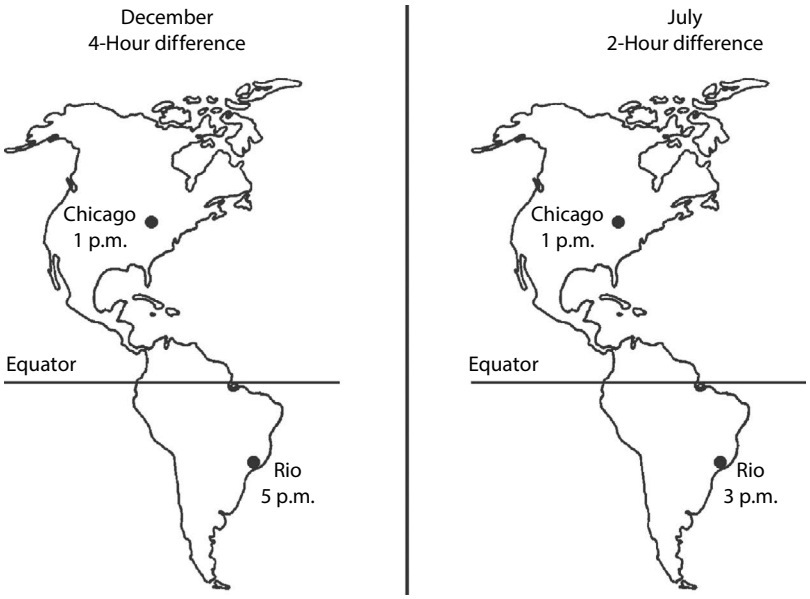


FIGURE 15.8 Two-, three-, or four-hour difference? The standard time difference between Chicago and Rio is 3 hours. However, during most of the year, daylight-saving time causes the difference to be either 4 hours (northern winter) or 2 hours (northern summer).

back on standard time (their winter)—thus, 3 – 1 = 2-hour difference. In December, Chicagoans are back on standard time (their winter), but *cariocas* have their clocks advanced by 1 hour (their summer)—thus, 3 + 1 = 4 hours difference. If you cannot benefit from this hemispheric discrepancy, you may still be able to reduce jet lag by planning your trip with full awareness of the number of time zones involved. The program **Jet-lag**, which is part of the software package that accompanies this book, can help you do it (see Exercise 15.2).

If you cannot prevent the collision between your internal clock and your destination clock, you may try to convince your hosts to live by your clock (so that they—rather than you—are jet lagged), although it is unlikely that you will succeed. It would be more realistic to try to make your clock shift faster than it normally would. To accomplish this, you must use one of the various procedures known to phase-shift circadian rhythms. None of the procedures studied in the laboratory (such as light pulses, melatonin, and exercise) have been thoroughly tested in real-life jet-lag situations, but many studies have produced encouraging results. Before we discuss them, let us review the rationale. Figure 15.9 will serve as a guide.

In Panel A of Figure 15.9, let us assume that, at home (the *origin*), both the passenger's internal clock and the external clock indicate 1 a.m. After an instantaneous trip eastward across 4 time zones, the internal clock will still indicate 1 a.m., but the external clock at the *destination* will indicate 5 a.m. Thus, the *treatment* for jet lag will require a 4-hour advance of the internal clock to match the time of the external clock. We saw in Chapter 7 that the human circadian system can be phase-shifted by discrete pulses of light presented at the appropriate circadian time (CT).[56–77] Panel B gives the details about how this knowledge can be used in the treatment of jet lag. All three diagrams refer to the traveler's internal clock. Let us assume that sunlight at the origin lasted from 5 a.m. to 7 p.m. (of the *previous day*). Then, sunlight at the destination that is 4 time zones ahead will last (according to the internal clock) from 1 a.m. to 3 p.m. (of the *next day*). Hence, to avoid jet lag, we need to advance the internal clock by 4 hours. According to the human phase–response curve for photic stimuli (Panel C), a 4-hour phase advance can be obtained by a light pulse presented at CT 2. Thus, on the travel day (middle diagram in Panel B), the traveler must spend the whole day in darkness except for a few hours—say, 3 hours—starting at (or centered around) CT 2. Because CT 2 is 2 hours after the traveler's wake-up time (presumably 5 a.m.), the pulse must be presented from 7 a.m. to 10 a.m. Of course, the traveler cannot travel in actual darkness. However, he or she can *experience* darkness by wearing dark goggles that allow the passage of only enough light to allow him or her to move around but not enough to stimulate his or her circadian system (which, as we saw in Chapter 7, is less sensitive to light than the visual system is). This would provide the appropriate conditions for the required 4-hour shift.

A problem in using a single light pulse as the phase-shifting agent is that the amplitude of the human phase–response curve is relatively modest. A phase shift of 4 hours is about the largest phase shift that can be attained. Still, 4 hours are better than nothing (and would be plenty for a trip that involves the crossing of only 4 time zones). Thus, several researchers put this protocol to the test.[66,78–81] In one study, human volunteers were kept in isolation in the laboratory for 2 weeks and were subjected to an 8-hour eastbound travel simulation. The body temperature rhythm of subjects who received a 3-hour bright light pulse in the morning advanced more than 7 hours during the first 5 days after the shift; the rhythm of subjects who did not receive the light-pulse treatment advanced only 5 hours during the same time.[66] The properly timed light pulse supposedly caused an instantaneous phase shift of the pacemaker to supplement the gradual phase shift caused by the displacement of the light–dark cycle.

Results from a study in which the subjects actually traveled from Zurich (Switzerland) to New York are shown in Figure 15.10. New York is 6 hours behind Zurich, so that a 6-hour phase delay of the internal clock was required. Subjects in the experimental group wore a special visor that delivered a 3-hour pulse of bright white light (3000 lux) at about CT 18 (the point in the phase–response curve associated with the largest phase delay) upon arrival in New York. Subjects in the control group wore a visor that delivered a 3-hour pulse of dim red light (10 lux) at CT 18, which is not expected to affect the circadian system. Phase shifts of the rhythm of melatonin secretion were measured 2 days after arrival. As shown in the figure, the mean phase shift in the control group was 1.6 hours, which is what one would expect 2 days after arrival. In contrast, the mean phase shift in the experimental group was 2.6 hours. Thus, the bright light treatment speeded up reentrainment. Of course, the treatment added only 1 hour to the shift, whereas a light pulse at CT 18 would be expected to add 3 or 4 hours to the shift. Also, the authors reported that the faster reentrainment of the melatonin rhythm was *not* accompanied by improvement in sleep, performance, or subjective assessment of jet-lag symptoms.[79] Evidently, it is not this study that will cause newspapers to print the headline "Cure for Jet Lag Found!"

Another inconclusive study involved female soccer players traveling eastward across 5 time zones. The experimenters intended to use properly timed supplemental light (2500 lux) to phase-advance the players' clocks and thus prevent jet lag. Symptoms of jet lag were not reduced by the treatment, but the study provided no control of natural light exposure,[82] so that it is likely that the treatment was obfuscated by natural light—and, not surprisingly, produced no effects.

A very popular remedy for jet lag these days is the hormone melatonin, which—as discussed in Chapter 13—has the ability to shift the mammalian clock. Since melatonin is not a regulated drug, it can be purchased at natural-food stores and be used by anyone for self-medication. *Theoretically*, melatonin can be almost as effective as light pulses,[74,83–87] but it does not require the awkward use of light boxes or dark goggles. *In practice*, its effectiveness remains to be rigorously demonstrated. At least two best-selling popular books on this topic provide plenty of advice on how to obtain and use melatonin.[88,89] Articles in the medical literature have claimed both that melatonin can relieve symptoms of jet lag[90] and that it cannot.[91] In a laboratory study, human subjects under a

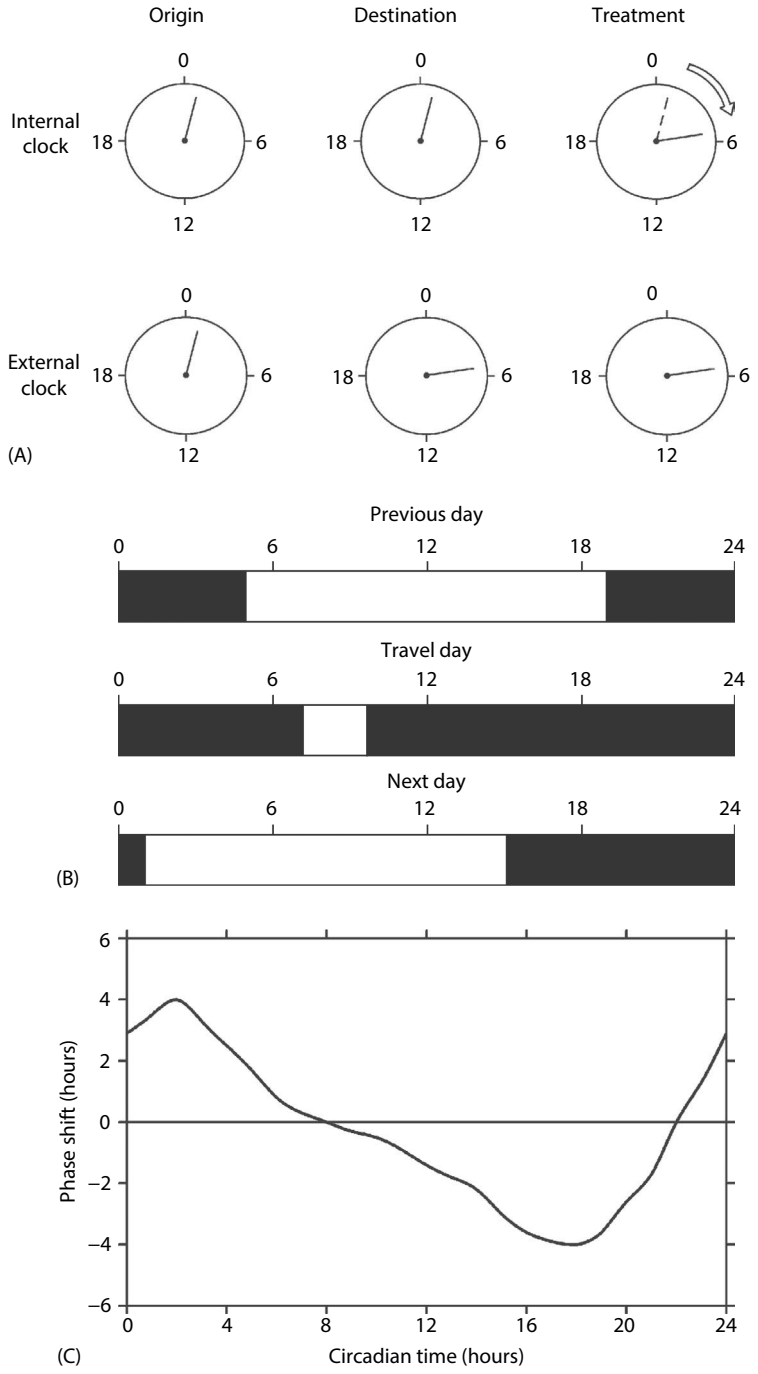


FIGURE 15.9 Treatment of jet lag: theory. The diagrams in Panel A illustrate the fact that after a flight eastward across 4 time zones, the internal clock is 4 hours behind the external clock at the destination and that, therefore, treatment must produce a 4-hour advance of the internal clock. The diagrams in Panel B illustrate the fact that a 4-hour advance of the internal clock may be achieved by appropriately timed exposure to light on the travel day. Panel C shows the human photic phase–response curve (values averaged from four independently determined phase–response curves). (Data for the phase–response curve: Honma, K. and Honma, S., *Jpn. J. Psych. Neurol.*, 42, 167, 1988; Dawson, D. et al., *Chronobiol. Int.*, 10, 94, 1993; Jewett, M.E. et al., *J. Biol. Rhythms*, 9, 295, 1994; Khalsa, S.B.S. et al., *J. Physiol.*, 549, 945, 2003.)

constant routine protocol were either exposed to a light pulse or given melatonin—or received both treatments. Subjects receiving both treatments showed a greater phase shift of the endogenous melatonin rhythm than subjects receiving either treatment alone, and subjects receiving either treatment alone phase-shifted more than subjects receiving no treatment.[87] These were encouraging results, but, even in the condition with the two treatments combined, the total phase shift was only 1 hour, which is rather disappointing.

Other potential treatments for jet lag include the use of exercise "pulses" to shift the clock after transmeridian flight[92–94] and the administration of drugs known to have phase-shifting properties,[95–97] although additional studies are certainly needed to verify their safety and effectiveness.

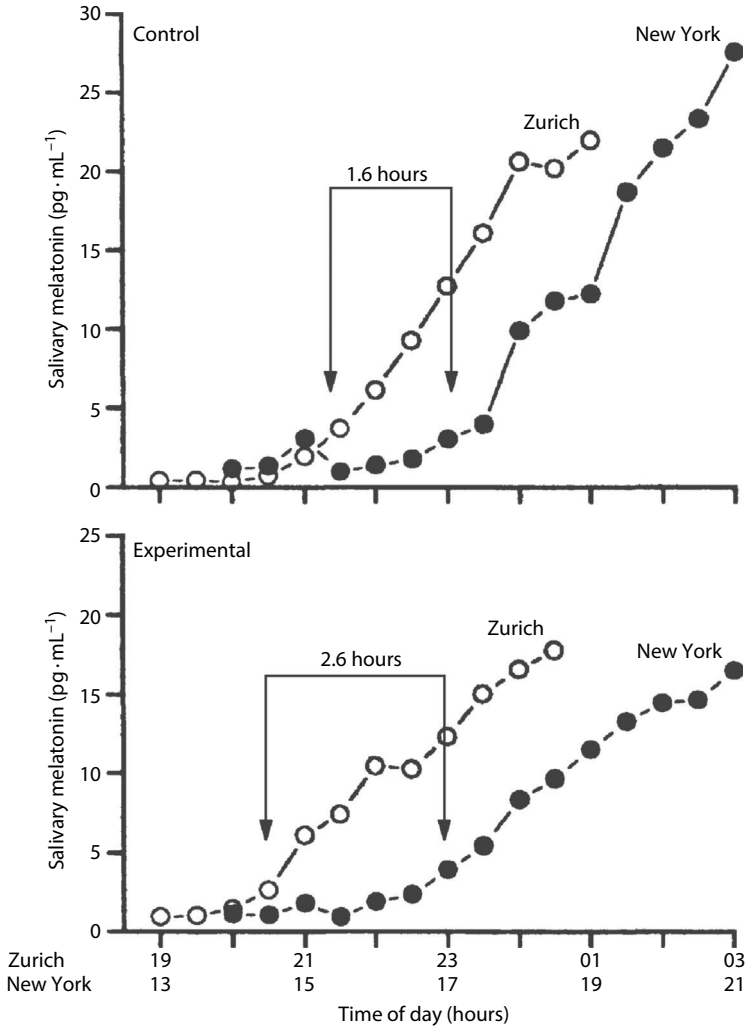


FIGURE 15.10 Treatment of jet lag: real case. The graphs show the results of a study that investigated the use of light visors as a remedy for jet lag after a flight from Zurich (Switzerland) to New York. As indicated in the abscissa, New York is 6 hours behind Zurich, which means that the internal clock must be delayed by 6 hours. The state of the internal clock is inferred from the phase of the daily rhythm of melatonin secretion (dim light melatonin onset) 2 days after arrival in New York. The data points are the means of 8 experimental subjects (who wore a visor emitting white light at 3000 lux for 3 hours starting at circadian time 18 after arrival in New York) and 8 control subjects (who wore a visor emitting dim red light at 10 lux). Notice that the subjects of the experimental group exhibited a significantly greater delay than the members of the control group. (Data from Boulos, Z. et al., *Aviat. Space Environ. Med.*, 73, 953, 2002.)

A recent animal study involving degus showed that inhibition of cortisol synthesis can accelerate reentrainment after a 6-hour shift of the light–dark cycle.[98] Because cortisol secretion is associated with stress, and because transmeridian flights are often stressful (due to crowded airports, uncomfortable airplane seats, unpalatable meals, lost luggage, and so on), efforts to reduce stress might lead to shorter reentrainment times and, consequently, to faster recovery from jet lag. In a study involving mice, the phase shift of the rhythms of locomotor activity, body temperature, and clock gene expression in the brain following a phase shift of the light–dark cycle was greatly accelerated when receptors of the neurotransmitter vasopressin were blocked either pharmacologically or by genetic engineering (i.e., knockout of the genes that code for the V1a and V1b receptors).[14] Unfortunately, there is no clear indication of how the blockade of synapses that use vasopressin produces the acceleration in reentrainment.

Finally, when inability to sleep at night is the major complaint, sleeping pills can be prescribed as treatment for jet lag.[99] As a matter of fact, melatonin has soporific effects,[100] and its action as a medicine for the treatment of jet lag—if it does have an action—may derive from its sleep-inducing property.[101] Although chemically-induced sleep takes care only of the homeostatic component of sleep regulation (leaving the *real circadian cause* of jet lag untreated), it can relieve one of the major symptoms of the disorder. Likewise, airlines often schedule long eastward transmeridian flights to arrive at the destination early in the day, so that, by the end of the local day, the traveler has been awake for some many hours that he or she has no problem falling asleep at night. Of course, jet lag will be experienced in the following days, but the airlines hope that by then the traveler will have forgotten that the flight was the cause of the whole problem!

15.2 SHIFT-WORK MALAISE

Shift work refers to work performed in shifts (i.e., changes in configuration), although the term is most often used in connection with *night* shifts or *rotating* shifts. Truck drivers (Figure 15.11) and emergency medical personnel (Figure 15.12) are just two examples of workers who often must labor on nonstandard shifts. Aside from the inconvenience of having to work when most people are enjoying free time, shift work often produces a malaise characterized both by sleepiness and inability to concentrate during work hours and by difficulty in sleeping at the scheduled sleep time.[102] Complaints of gastrointestinal disturbances are also common.[103] Considering that two out of five American workers are employed in nonstandard shifts,[104] the malaise associated with shift work has the potential to be a major public health issue.

In addition to immediate health hazards, the impairment in performance during nocturnal work hours can create further health problems due to accidents. For instance, researchers conducting a study of accidental exposure to blood-borne pathogens in medical staff found that, when adjusted for the number of people working on the night shift, accidental exposure is 50% greater at night than during the day.[105] Similarly, a study of the incidence of injury at work among police officers revealed that the incidence of injury on the midnight shift was 72% larger than on the day shift.[106] This procedure controlled for variations in the number of crimes, but you may wonder whether there are more crimes, and consequently more opportunity for injury, on the midnight shift. That this is not the case is evident in Figure 15.13, which shows that both crimes against people (assault, homicide, kidnapping, sex offenses, etc.) and crimes against property (arson, burglary, vandalism, larceny, etc.) are less frequent during the late night and early morning.



FIGURE 15.11 Truck stop. Truck drivers often have to drive throughout the night and then sleep during the day, which creates a conflict between the internal clock and the external clock. (Photo by R. Refinetti.)

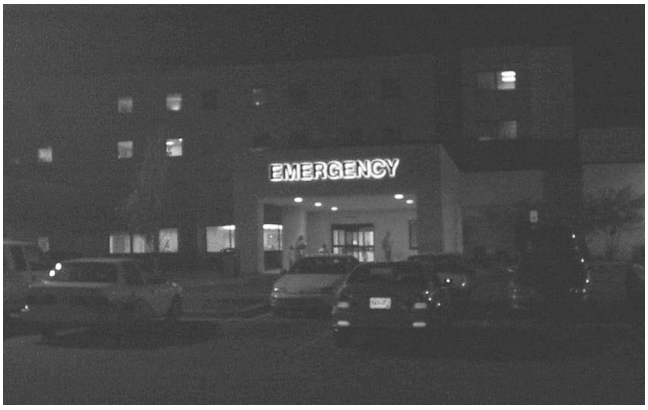


FIGURE 15.12 Emergency room. Night-shift work is a common job requirement for personnel in the emergency room of a hospital. (Photo by R. Refinetti.)

15.2.1 Diagnosis

We saw in the previous section that the central problem in jet lag is that the person is suddenly expected to be awake during his or her night and to be asleep during his or her day. Now, except for the fact that there is no travel involved, this is the same situation faced by shift workers—but there is a major difference between the two situations, as diagrammed in Figure 15.14. After a transmeridian flight, the traveler's internal clock (*person*) is out of phase with the external clock (*clock*) and with the cycle of sunlight and darkness (*light*), but the internal clock can be resynchronized in a few days. In shift work, the person's clock cannot synchronize to the new environment, for the simple reason that the cycle of sunlight and darkness has not actually changed. That is, the worker's work schedule has changed (*clock*), but the Sun still rises and sets at the same time (and so do all other people in the community, except for other shift workers). Even worse, shift workers are usually kept on a given shift for only a few days, so that, even if the worker managed to synchronize to the new schedule, he or she would be desynchronized again very soon!

One might argue that rotating shifts were the brainstorm of someone who had no knowledge whatsoever of circadian rhythms. After all, why would someone subject shift workers to the endless torture of repeated phase shifts just when the workers are resynchronizing to the new schedule? This criticism would be unfair, however. First, it is unlikely that shift workers actually resynchronize to the new schedule (remember: the light–dark cycle and the behavior of the rest of the population do not follow the change in schedule of the shift worker). Second, many people will not accept the idea that they have become "night rats." That is, workers themselves demand having days off from the night shift so that, say, they can go to the park with their families on a Sunday afternoon. Thus, not only are shift workers unable to truly adapt to shift work, but they would not want to do it even if they were given the option. Does this mean that shift workers are doomed to have a miserable existence? Not necessarily. Let us have a look at what research in this field has revealed.

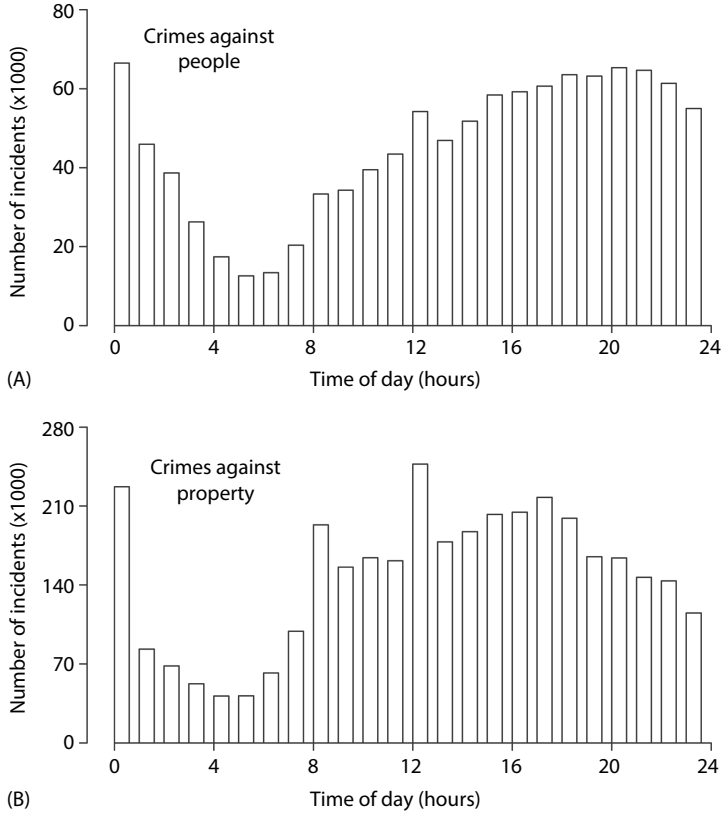


FIGURE 15.13 Time for crime. There is a clear daily oscillation in the number of crimes against people (A) and crimes against property (B). The noticeable peaks at 12 noon and 12 midnight are likely due to rounding of reported times when the exact times were not known. (Data from U.S. National Incident-Based Reporting System, Federal Bureau of Investigation, Washington, DC.)

Many businesses have three basic types of work shift: day (7 a.m. to 3 p.m. or 8 a.m. to 5 p.m.), swing (3 p.m. to 11 p.m. or 4 p.m. to 1 a.m.), and graveyard (11 p.m. to 7 a.m. or midnight to 9 a.m.). Workers in the swing and graveyard shifts complain of health problems much more often than workers in the day shift do.[107] As early as 1904, Francis Benedict, at Wesleyan University (in Connecticut), conducted a series of careful studies of the body temperature rhythm of shift workers.[108] Being dissatisfied with the use of mercury thermometers in long-term studies, he was one of the first physiologists to develop what is today a standard piece of equipment: the thermistor (a temperature-sensitive resistor that allows continuous recording of body temperature based on the variations in electrical resistance). By studying the body temperature rhythm of shift workers and day workers, he found out that even long-term graveyard workers do not synchronize to the night schedule (that is, their body temperature still peaks in the late afternoon, as does that of day workers). Various studies conducted more recently have concurred with Benedict's conclusion that circadian rhythms tend not to adapt properly to the reversed work schedule.[109–115] Data from one of these studies are shown in Figure 15.15. As a marker of the phase of the circadian pacemaker, serum melatonin concentration was measured in regular day workers and night workers during a 24-hour stay in the laboratory. The records from three representative members of each of the two groups show that, despite the opposite phases of work, the melatonin rhythm peaks in the middle of the night in both groups.[109] Subject E exhibits an earlier peak, but not earlier enough to characterize entrainment to the nocturnal work schedule. The study involved 6 day workers and 9 night workers, and subject E was the only subject out of the 15 subjects who did not exhibit a peak in the middle of the night.

Alain Reinberg's team, at the Rothschild Foundation (in France), looked into the body temperature rhythms of shift workers in great detail. By comparing the temperature rhythms of individual workers with the gravity of their health-related complaints, Reinberg's team found that the temperature rhythm of people who are less tolerant of shift work has a smaller amplitude (that is, it has lower peaks and higher troughs).[116] They also found out that rhythms with smaller amplitude are more easily shifted, as assessed on the first night of shift work.[117] Consequently, people who are less tolerant to shift work synchronize to the new schedule more rapidly.[118] This suggests that the malaise associated with shift work may be due to an abrupt shift in the bodily rhythms, and not to the imposition of a shift per se. The conclusion has intuitive appeal if one considers that very slow shifts (say, half an hour per day) are unlikely to result in any distress to the individual. In principle, then, the problem with shift work would not be the fact that one has to work during the night but that one has

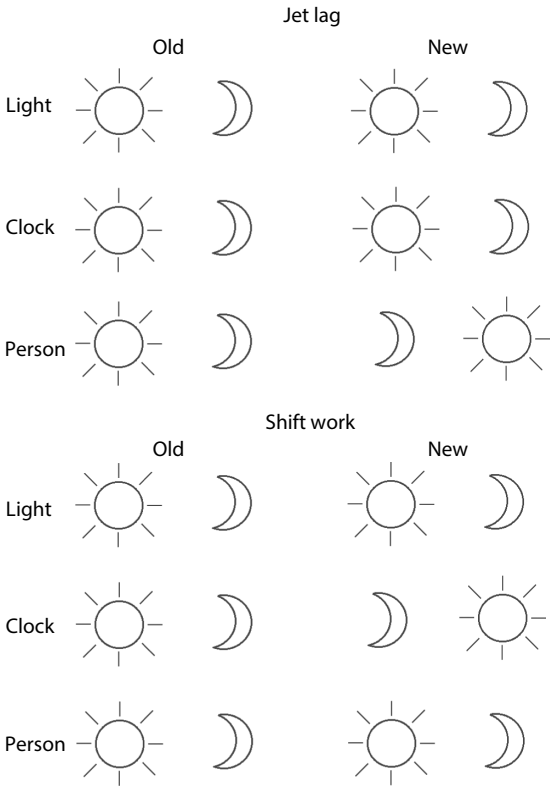


FIGURE 15.14 Comparing jet lag and shift work. The diagram illustrates the fact that although both jet lag and shift work involve a mismatch between the timing of a person's internal clock and the timing of the external clock, shift work is complicated by the mismatch between sunlight and the external clock. See text for details.

to make the transition from day to night work. Thus, shift work would not be different from jet lag after all. However, Reinberg's team did not follow up the workers' rhythms long enough to ascertain that they actually synchronize to the night schedule. As a matter of fact, even the initial finding of smaller amplitude of the temperature rhythm in people less tolerant to shift work was obtained in cross-sectional studies and may, therefore, result from individual characteristics developed during the course of shift work. Stjepan Vidacek's team, at the University of Zagreb (in Croatia), conducted a longitudinal study (that is, examining the body temperature rhythms before the individuals ever started shift work and comparing them with health-related complaints after shift work was started) and found out that the body temperature rhythm of people who are less tolerant of shift work has a larger amplitude than that of people who are more tolerant[119] (that is, the opposite of Reinberg's finding). Therefore, further studies—especially by different researchers—are necessary to elucidate the phenomenon.

The misalignment of body rhythms with environmental cycles that is usually found in night-shift workers characterizes a condition of chronic desynchronization similar to that produced by persistent jet lag, as described earlier, and is just as likely to engender disruption of the organism's internal temporal order, thus producing the usual symptoms of poor quality of sleep, fatigue, and irritability.[120,121] Whether more serious health problems also result is not well established. For example, a large study of lung cancer risk among female textile workers in China (Figure 15.16), which involved a cohort of 267,400 workers in Shanghai, did not

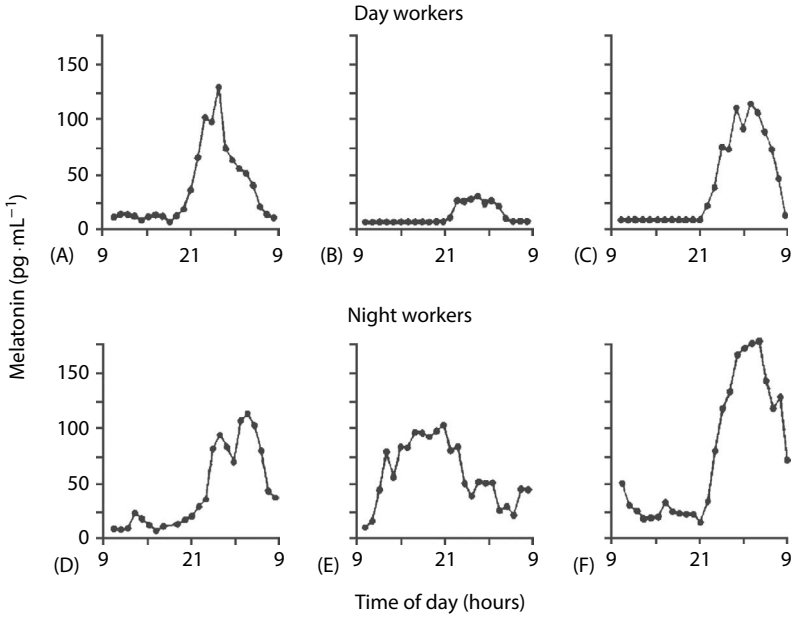


FIGURE 15.15 Shift workers do not shift. The graphs show the daily rhythm of melatonin secretion (as determined by serum melatonin concentration) of 3 day workers (A–C) and 3 night workers (D–F). Day workers were active from 09:00 to 18:00 daily, whereas night workers had been working 5 to 6 night shifts per week (19:00 to 07:00) for at least a year. Notice that the melatonin rhythm peaks in the middle of the night for both groups. The rhythm of subject E peaks earlier than that of the other subjects, but not as early as needed to match the shift in work schedule. Of 6 day workers and 9 night workers, subject (E) was the only one not to exhibit a peak around 04:00. (Data from Roden, M. et al., *Am. J. Physiol.*, 265, R261, 1993.)



FIGURE 15.16 Textile plant in China. The textile industry is a major component of the Chinese economy. (Copyright Dreamstime, Brentwood, TN.)

reveal a consistent increase in lung cancer among night-shift workers,[122] but a study in Seattle (Washington) found that women who work in the night shift have a 60% increased risk of breast cancer.[123]

15.2.2 Treatment

The cure for the shift-work malaise is as nebulous as that for jet lag. Behaviorally, the best way to avoid the malaise is to avoid shift work altogether. Obviously, this is not an option for most shift workers. In some cases, the schedule of work hours is so bad that there is probably nothing that can be done about it. For example, some health-care workers are placed on 48-hour shifts with 12 hours of continuous work followed by 36 hours of time off. By the end of the 12-hour work shift, the workers are so exhausted that the quality of their performance is clearly compromised.[124] Even more absurd is the work schedule of American submarine crew. Sailors are required to live under an 18-hour schedule (6 hours on duty and 12 hours off duty), which is incompatible with entrainment of human circadian rhythms.[125]

For those who must work nonstandard but reasonable shifts, one possible advice is to reduce the number of shift changes and allow the internal clock to adapt to whatever schedule that is required. It is generally believed that, when rotating shifts are necessary, the rotation should move clockwise (that is, day shift first, then swing shift, then graveyard shift, then back to day shift, and so on).[126] However, recent studies revealed no benefit of clockwise rotation as compared to counterclockwise rotation.[127,128] Theoretically, the best approach to avoid the malaise associated with shift work is not to rotate shifts and to use artifices to phase-shift the circadian clock. Of course, one cannot attain a stable phase shift of the circadian clock if the light–dark cycle remains unchanged. However, a change in the light–dark cycle can be accomplished by exposure to artificial bright light during the night and the use of dark goggles during the day (to avoid light exposure during sunlight hours). The rationale is described in Figure 15.17. Standard work hours in the United States (*day shift*) are 9 a.m. to 5 p.m., an interval that is approximately in the middle of sunlight hours. A night shift from 9 p.m. to 5 a.m. (*night shift*) will cause a full 12-hour desynchrony with the light–dark cycle and consequent sleepiness during work hours and restlessness during the rest hours. To treat this condition, one must make the Sun join the night shift—which, of course, is not feasible. However, one can simulate a reversal of the light–dark cycle (*treatment*) by using artificial lighting at night and dark goggles during the day. The reversal of the light–dark cycle will allow reentrainment of the circadian clock after a week or so.

In actual studies, treatment with light exposure at night and use of dark goggles during the day produced encouraging results.[129–131] An example is shown in Figure 15.18. Nurses with many years of experience in night work were studied under laboratory conditions. Tests were conducted during usual night work and during a temporary interval of day work immediately after return from vacation. Control subjects followed their usual routine, whereas experimental subjects were exposed to bright light during the first 6 hours of the 8-hour work shift and were instructed to wear dark goggles when outdoors during the day. Estimates of circadian phase were obtained by measurements of body temperature and melatonin secretion. To facilitate inspection, the figure shows the time and duration of sleep as hatched rectangles. Notice that control subjects exhibited only a 5-hour delay in the rhythms of body temperature and melatonin secretion in response to the 10-hour delay in work (and sleep) schedule. The experimental subjects exhibited a full 10-hour delay of the rhythms.[130] In principle, control subjects should not have exhibited any shift, but the experimental subjects clearly exhibited much

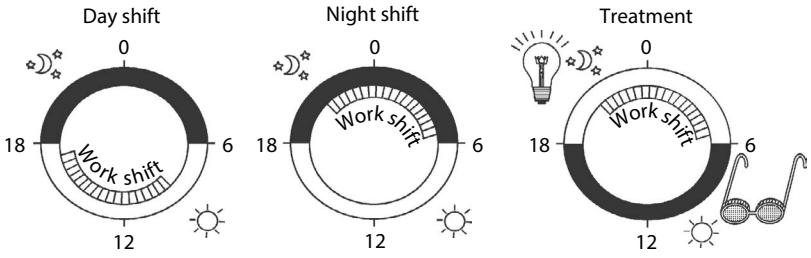


FIGURE 15.17 Treatment of the shift-work malaise. The ideal treatment for the malaise associated with shift work is to simulate a shift in the daily cycle of light and darkness that matches the shift in work schedule. This can be achieved by the use of artificial light during the night and dark goggles during the day. The night shift must be permanent (that is, nonrotating).

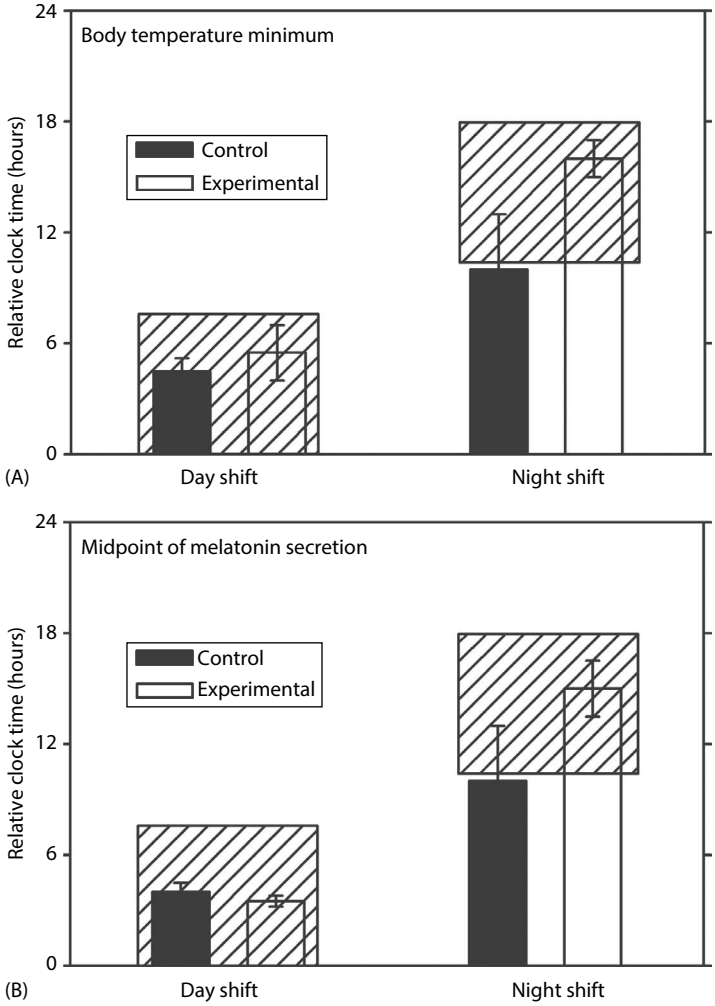


FIGURE 15.18 Testing the treatment. The graphs show the mean times of the trough of the body temperature rhythm (Panel A) and the midpoint of the rhythm of melatonin secretion (Panel B) of workers on day and night shifts under normal conditions (control) or after treatment with bright light during the first 6 hours of the work shift and use of dark goggles during off-work hours (experimental). The hatched rectangles indicate time in bed (*sleep time*). Measurements were conducted under a 36-hour constant routine protocol. Notice that night workers in the experimental group, but not in the control group, exhibited a 10-hour delay of the temperature and melatonin rhythms in accordance with the 10-hour delay in work schedule. (Data from Boivin, D.B. and James, F.O., *J. Biol. Rhythms*, 17, 556, 2002.)

greater shifts, thus indicating that the treatment was effective. Some researchers used light pulses at night without control of photic exposure during the day,[132–135] and the results were not encouraging. Other researchers used physical exercise,[136,137] melatonin,[138,139] or mere enforcement of diurnal sleep time,[140–142] without great success.

You may wonder why shift work is used at all if it is so bad. Even if employers did not care about the health of their employees, they should at least care about the employees' performance. They certainly do. However, mediocre performance is still better than no performance at all (that is, no work at night). In the services sector of the economy, some customers, for a variety of reasons, desire around-the-clock access to goods such as gasoline, groceries, and medicine. In the industrial sector, specialized machinery is so expensive and becomes obsolete so rapidly that it has to be operated 24 hours a day, 7 days a week, in order to be profitable.[126] Thus, as long as the night worker's performance is not so bad as to compromise the quality of the product, the reduction in productivity is not a major concern. Even poor performance is infinitely better than no performance (and no financial gain). It is probably no coincidence that most night workers have jobs that are low in the occupational hierarchy.[104]

15.3 DAYLIGHT-SAVING TIME

The regimen of daylight-saving time (*summer time*) resembles the condition of shift work albeit in a very small scale. The regimen consists of advancing the clocks by 1 hour in the spring and returning them back in the fall (Figure 15.19). In the United States, daylight-saving time was introduced by an act of Congress in 1918, the same year in which time zones were introduced.[143] The rationale for daylight-saving time was mainly the conservation of fuel in a time of war (World War I). As we saw in Chapter 4, there are many more hours of sunlight in a day in the summer than in the winter. Because many more

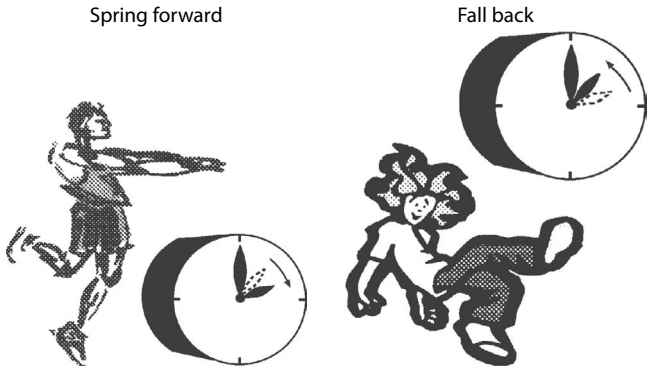


FIGURE 15.19 Daylight-saving time. In regions that observe daylight-saving time, the clocks are advanced by 1 hour in the spring and delayed by 1 hour in the fall.

people are active at dusk than at dawn, the 1-hour advance of clocks in the summer provides an additional hour of sunlight at the end of each day without a noticeable subtraction in the early morning. Interestingly, because more people go for walks in the evening than in the morning, and because the extra hour of light in the evening during daylight-saving time provides enhanced visibility for motor-vehicle drivers, there is an estimated reduction of 13% in pedestrian fatalities during daylight-saving time as compared to standard time.[144,145]

In terms of entrainment of circadian rhythms, daylight-saving time has a small effect that is inconsequential for most people. Direct your attention to Figure 15.20. The figure shows the annual variation in day length. The magnitude of the variation depends on latitude, so we need to pick an arbitrary latitude. In the northern part of the United States, day length varies from 16 hours in the summer to 8 hours in the winter. In comparison to this variation, the 1-hour change of daylight-saving time is very small. The savings in electricity that result from the extra hour of sunlight at the end of the day, however, are substantial. Thus, another act of Congress in 1966 instituted daylight-saving time as a permanent fixture in the lives of American residents.[143] Many countries around the world observe daylight-saving time in some form.[146]

Although daylight-saving time is not a major nuisance for the human circadian system, some people do not like it for other reasons. The US Congress allowed each of the states to choose whether it wanted to adopt daylight-saving time or not. Hawaii and Arizona chose not to adopt it. Until 2005, Indiana adopted it in some counties but not in others. Idaho is considering no longer to adopt it. In states that adopt it, daylight-saving time starts on the second Sunday in March and ends on the first Sunday in November.[146] Many people do not realize that daylight-saving time lasts most of the year (8 out of 12 months) and is not just a "summer time" change.

One of the complaints that early risers have about daylight-saving time is that, for several weeks after the clocks are advanced in the spring, it is still dark when they wake up in the morning. Ironically, late risers complain that it gets dark too early when daylight-saving time ends in the fall. People who are not early risers or late risers often complain about having

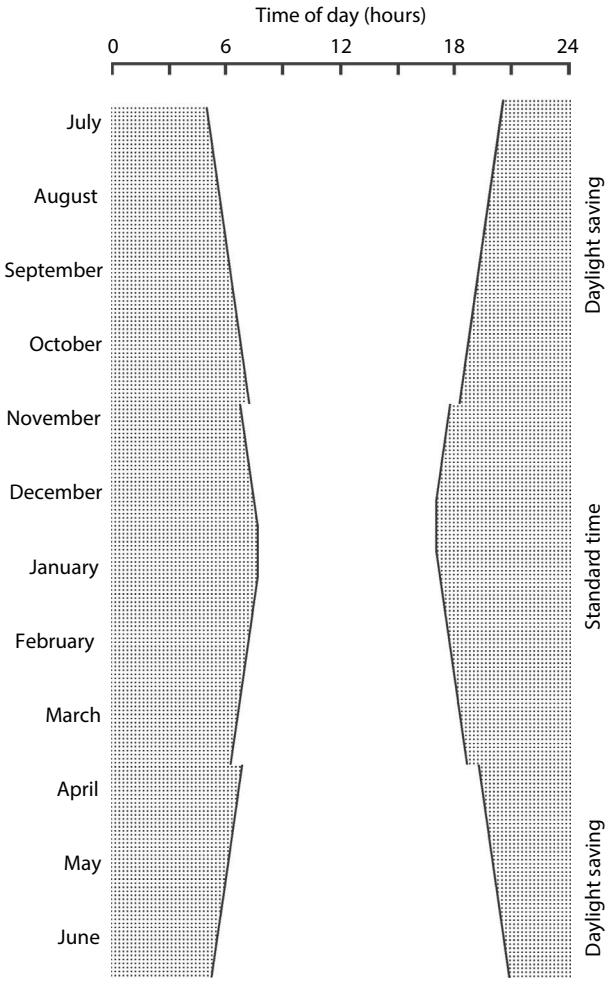


FIGURE 15.20 Photoperiod and daylight-saving time. The graph shows the annual variation in photoperiod at a latitude slightly north of the Tropic of Cancer with the adjustment for daylight-saving time. Because of the large seasonal effect, the 1-hour change of daylight-saving time is barely noticeable.

to go through the house adjusting the clocks twice a year every year. Some complain about losing an hour on the day when the clocks are advanced in the spring. Whether these various complaints are serious enough to justify the abolishment of daylight-saving time depends on whom you ask.

There have been occasional reports of negative consequences of daylight-saving time. A small study in Finland suggested that the transition to daylight-saving time causes a reduction in sleep duration and sleep efficiency, as determined a week after the transition in comparison with measurements made a week before the transition.[147] A larger study in Germany, involving 469 teenagers, noted a slight trend toward daytime sleepiness for the first 3 weeks after the transition to daylight-saving time.[148] In the United States, analysis of national accident and labor statistics indicated that the frequency of mining injuries has a peak on the day after the transition to daylight-saving time.[149] Again, the magnitude of the effects is small, and the number of studies is also very small, so that no authoritative statements about negative consequences of daylight-saving time can be made at this time.

SUMMARY

1. Jet lag is a malaise often associated with airplane travel across multiple time zones. It results from a transient misalignment of the circadian clock with the external clock. The symptoms are fatigue, irritability, and inability to concentrate during the day; difficulty in sleeping at night; and gastrointestinal discomfort. If untreated, the condition undergoes spontaneous remission in 1–10 days, depending on the number of time zones crossed and on the direction of travel. Jet lag can sometimes be prevented by careful planning of the time of travel, but no effective posttravel treatment has been rigorously documented.
2. The symptoms of the malaise associated with shift work are similar to those of jet lag. Although the two disorders share a common etiology, the shift-work malaise does not abate spontaneously because the external clock is not in phase with the alternation of day and night. Treatment involves the artificial reversal of day and night, which can be attained by presentation of bright artificial light during work time (at night) and prevention of photic stimulation during sunlight hours (by the use of dark goggles). Alternative treatments have been proposed but not adequately validated.
3. By advancing the clocks by 1 hour in the spring and returning them back in the fall, the regimen of daylight-saving time resembles the condition of shift work albeit in a very small scale. Although the regimen is not a major nuisance for the human circadian system, some people do not like it for other reasons.

EXERCISES

15.1 Dealing with shift work and jet lag. In this chapter, we discussed the problems associated with shift work and jet lag. Generally, these problems result from a lack of synchronization between the body's clock and the external clock. The program **Health** (the third icon from the right in the Circadian banner) contains two sections dealing with this issue.

1. Start the program **Health**.
2. Select the third option from the top in the main menu ("I can't get myself on a schedule"). Then answer the questions to obtain advice on how to solve your particular problem. The program will judge whether you actually have a problem, and if you do, it will advise you on how to solve it. The advice is based on the basic principle of synchronization of the body's clock by the natural cycle of daylight.
3. The other section of **Health** pertinent to this chapter is the fourth option from the top ("I traveled recently and can't get over my jet lag"). This is a very brief section intended mainly to remind you that jet lag is, by its very own nature, a transient illness. Exercise 15.2 deals with the prevention of jet lag.

15.2 Planning a transcontinental trip. This exercise is meant for entertainment just as much as for education. You may want to share it with family members and friends. It provides simple guidelines for the minimization of jet lag after transmeridian flights.

1. Start the program **Jet-lag** (fourth icon from the right in the Circadian banner).
2. Click at the button under *Origin*, and then click on Florida in the map (which should be bright blue in most monitors).
3. Click at the button under *Destination*, and then click on western Europe in the map (dark blue).
4. Because this trip takes you out of North America, a message is displayed to remind you that the beginning and end of daylight-saving time are not identical in other parts of the world. Thus, if you are traveling around Easter or Halloween, the time-zone differential may be an hour off. Click on the button under *Travel*.
5. A message is displayed telling you that you will cross 6 time zones eastward. The best way to avoid jet lag under this condition is to arrive late at night, go to bed late, and wake up late the next day. This will help you to sleep on your first night in Europe and not to be sleepy the following day. Essentially, you will behave as if the external clock had not been phase-advanced by 6 hours.
6. Now, let us change the origin of the trip. Click at the button under *Origin*, then click on California (purple), and then click on the button under *Travel*.
7. The program will tell you that you will cross 9 time zones to go from California to western Europe. This will require a large shift of your internal clock. Although you could try the same approach as in the previous trip (item 5), most airlines give up on attempting to attain synchronization and simply try to optimize your ability to sleep on the first night at the destination. They have you travel overnight (and get very little sleep) and arrive early in the morning (local time). By the time the locals are going to bed, you will have been awake for so long that you will be likely to sleep reasonably well even though your internal clock says that it is not time to sleep.
8. Repeat the procedure with any origin and destination that you wish. If your travel requires you to cross the Equator, a message will be displayed to remind you that daylight-saving times, if applicable, are effective at opposite times of the year in the two hemispheres. Thus, the time-zone differential may be off by up to 2 hours. You should consult the consulate of the particular country to determine if and when daylight-saving time begins and ends. All calculations provided by this program are based on standard time at both origin and destination.

SUGGESTIONS FOR FURTHER READING

Ehret, C. F. and Scanlon, L W. (1997). *Overcoming Jet Lag*. New York: Berkley.

A "cookbook" for preventing jet lag after transmeridian travel. As is the case for any book aimed at popular audiences, this book should be read with a skeptical attitude, even though most of the advice given by the authors is based on rigorous scientific research.

Monk, T. H. and Folkard, S. (1992). *Making Shiftwork Tolerable*. New York: Taylor & Francis.

Expert advice on how to reduce or prevent the malaise associated with shift work. As for the book on jet lag, a skeptical attitude is recommended.

Moore-Ede, M. (1997). *Working Nights Health and Safety Guide*. Cambridge, MA: Circadian Information.

An unpretentious booklet (32 pages) providing basic information on how to reduce or prevent the malaise associated with shift work.

WEBSITES TO EXPLORE

Circadian Technologies Inc.: http://www.circadian.com/.
Jet Lag Optimizer: http://www.lumie.com/pages/jet-lag-optimiser/.
Official U.S. Time: http://www.time.gov/.
Shiftwork Solutions LLC: http://www.shift-work.com/.
Time Zone Information: http://www.timeanddate.com/time/.
Travel Online: http://www.travel.com/.
Traveler's Health (U.S. Centers for Disease Control): http://www.cdc.gov/travel/.
World Clock: http://www.timeanddate.com/worldclock/.

REFERENCES

1. Siegel, P. V., Gerathewohl, S. J., and Mohler, S. R. (1969). Time-zone effects. *Science* 164: 1249–1255.
2. McFarland, R. A. (1974). Influence of changing time zones on air crews and passengers. *Aerospace Medicine* 45: 648–658.
3. Katz, G., Durst, R., Zislin, Y., Barel, Y., and Knobler, H. Y. (2001). Psychiatric aspects of jet lag: Review and hypothesis. *Medical Hypotheses* 56: 20–23.
4. Rayman, R. B. (1997). Passenger safety, health and comfort: A review. *Aviation, Space, and Environmental Medicine* 68: 432–440.
5. Kohn, P. M., Lafreniere, K., and Gurevich, M. (1991). Hassles, health, and personality. *Journal of Personality and Social Psychology* 61: 478–482.
6. Halberg, F., Barnum, C. P., Silber, R. H., and Bittner, J. J. (1958). 24-Hour rhythms at several levels of integration in mice on different lighting regimens. *Proceedings of the Society for Experimental Biology* 97: 897–900.
7. Kilduff, T. S., Vugrinic, C., Lee, S. L., Milbrandt, J. D., Mikkelsen, J. D., O'Hara, B. F., and Heller, H. C. (1998). Characterization of the circadian system of *NGFI-A* and *NGFI-A/NGFI-B* deficient mice. *Journal of Biological Rhythms* 13: 347–357.
8. Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Lelièvre, V., Hu, Z., and Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology* 287: R1194–R1201.
9. Kopp, C., Ressel, V., Wigger, E., and Tobler, I. (2006). Influence of estrous cycle and ageing on activity patterns in two inbred mouse strains. *Behavioural Brain Research* 167: 165–174.
10. Mendoza, J., Pévet, P., and Challet, E. (2008). High-fat feeding alters the clock synchronization to light. *Journal of Physiology* 586: 5901–5910.
11. Minami, Y., Kasukawa, T., Kakazu, Y., Iigo, M., Sugimoto, M., Ikeda, S., Yasui, A., van der Horst, G. T., Soga, T., and Ueda, H. R. (2009). Measurement of internal body time by blood metabolomics. *Proceedings of the National Academy of Sciences of the United States of America* 106: 9890–9895.
12. van der Veen, D. R. and Archer, S. N. (2010). Light-dependent behavioral phenotypes in PER3-deficient mice. *Journal of Biological Rhythms* 25: 3–8.
13. Yang, Y., Duguay, D., Bédard, N., Rachalski, A., Baquiran, G., Na, C. H., Fahrenkrug, J. et al. (2012). Regulation of behavioral circadian rhythms and clock protein PER1 by the deubiquitinating enzyme USP2. *Biology Open* 1: 789–801.
14. Yamaguchi, Y., Suzuki, T., Mizoro, Y., Kori, H., Okada, K., Chen, Y., Fustin, J. M. et al. (2013). Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. *Science* 342: 85–90.
15. An, S., Harang, R., Meeker, K., Granados-Fuentes, D., Tsai, C. A., Mazuski, C., Kim, J., Doyle, F. J., Petzold, L. R., and Herzog, E. D. (2013). A neuropeptide speeds circadian entrainment by reducing intercellular synchrony. *Proceedings of the National Academy of Sciences of the United States of America* 110: E4355–E4361.
16. Wolff, G., Duncan, M. J., and Esser, K. A. (2013). Chronic phase advance alters circadian physiological rhythms and peripheral molecular clocks. *Journal of Applied Physiology* 115: 373–382.
17. Husse, J., Leviavski, A., Tsang, A. H., Oster, H., and Eichele, G. (2014). The light-dark cycle controls peripheral rhythmicity in mice with a genetically ablated suprachiasmatic nucleus clock. *FASEB Journal* 28: 4950–4960.
18. Takamure, M., Murakami, N., Takahashi, K., Huroda, H., and Etoh, T. (1991). Rapid reentrainment of the circadian clock itself, but not the measurable activity rhythms, to a new light-dark cycle in the rat. *Physiology and Behavior* 50: 443–449.
19. Marumoto, N., Murakami, N., Kuroda, H., and Murakami, T. (1996). Melatonin accelerates reentrainment of circadian locomotor activity rhythms to new light-dark cycles in the rat. *Japanese Journal of Physiology* 46: 347–351.
20. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O., and Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
21. Weber, E. T. and Hohn, V. M. (2005). Circadian activity rhythms in the spiny mouse, *Acomys cahirinus*. *Physiology and Behavior* 86: 427–433.
22. Cohen, R. and Kronfeld-Schor, N. (2006). Individual variability and photic entrainment of circadian rhythms in golden spiny mice. *Physiology and Behavior* 87: 563–574.
23. Honrado, G. I. and Mrosovsky, N. (1989). Arousal by sexual stimuli accelerates the re-entrainment of hamsters to phase advanced light-dark cycles. *Behavioral Ecology and Sociobiology* 25: 57–63.
24. Refinetti, R. and Menaker, M. (1993). Effects of imipramine on circadian rhythms in the golden hamster. *Pharmacology, Biochemistry and Behavior* 45: 27–33.

25. Schöttner, K., Oosthuizen, M. K., Broekman, M., and Bennett, N. C. (2006). Circadian rhythms of locomotor activity in the Lesotho mole-rat, *Cryptomys hottentotus* subspecies from Sani Pass, South Africa. *Physiology and Behavior* 89: 205–212.
26. Lahmam, M., El M'rabet, A., Ouarour, A., Pévet, P., Challet, E., and Vuillez, P. (2008). Daily behavioral rhythmicity and organization of the suprachiasmatic nuclei in the diurnal rodent *Lemniscomys barbarus*. *Chronobiology International* 25: 882–904.
27. Basu, P. and Singaravel, M. (2013). Accurate and precise circadian locomotor activity rhythms in male and female Indian pygmy field mice, *Mus terricolor. Biological Rhythm Research* 44: 531–539.
28. Johnson, R. F. and Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
29. Kumar, V. and Gwinner, E. (2005). Pinealectomy shortens resynchronisation times of house sparrow (*Passer domesticus*) circadian rhythms. *Naturwissenschaften* 92: 419–422.
30. Schilling, A., Richard, J. P., and Servière, J. (1999). Duration of activity and period of circadian activity-rest rhythm in a photoperiod-dependent primate, *Microcebus murinus*. *Comptes Rendus de l'Académie des Sciences* 322: 759–770.
31. Perret, M., Aujard, F., Séguy, M., and Schilling, A. (2003). Olfactory bulbectomy modifies photic entrainment and circadian rhythms of body temperature and locomotor activity in a nocturnal primate. *Journal of Biological Rhythms* 18: 392–401.
32. Rappold, I. and Erkert, H. G. (1994). Re-entrainment, phase-response and range of entrainment of circadian rhythms in owl monkeys (*Aotus lemurinus g.*) of different age. *Biological Rhythm Research* 25: 133–152.
33. Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D., and Lalouette, A. (1994). Dissociation in body temperature, drinking and feeding rhythms following a light-dark cycle inversion in the rat. *Medical Science Research* 22: 53–55.
34. Tapp, W. N. and Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
35. Moore-Ede, M. C., Kass, D. A., and Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
36. Nagano, M., Adachi, A., Nakahama, K., Nakamura, T., Tamada, M., Meyer-Bernstein, E., Sehgal, A., and Shigeyoshi, Y. (2003). An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *Journal of Neuroscience* 23: 6141–6151.
37. Reddy, A. B., Field, M. D., Maywood, E. S., and Hastings, M. H. (2002). Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. *Journal of Neuroscience* 22: 7326–7330.
38. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.
39. Elliott, A. L., Mills, J. N., Minors, D. S., and Waterhouse, J. M. (1972). The effect of real and simulated time-zone shifts upon the circadian rhythms of body temperature, plasma 11-hydroxycorticosteroids, and renal excretion in human subjects. *Journal of Physiology* 221: 227–257.
40. Mills, J. N., Minors, D. S., and Waterhouse, J. M. (1978). Adaptation to abrupt time shifts of the oscillator(s) controlling human circadian rhythms. *Journal of Physiology* 285: 455–470.
41. Wever, R. A. (1980). Phase shifts of human circadian rhythms due to shifts of artificial zeitgebers. *Chronobiologia* 7: 303–327.
42. Monk, T. H., Moline, M. L., and Graeber, R. C. (1988). Inducing jet lag in the laboratory: Patterns of adjustment to an acute shift in routine. *Aviation, Space, and Environmental Medicine* 59: 703–710.
43. Folkard, S., Minors, D. S., and Waterhouse, J. M. (1991). "Demasking" the temperature rhythm after simulated time zone transitions. *Journal of Biological Rhythms* 6: 81–91.
44. Lowden, A. and Åkerstedt, T. (1999). Eastward long distance flights, sleep and wake patterns in air crews in connection with a two-day layover. *Journal of Sleep Research* 8: 15–24.
45. Waterhouse, J., Edwards, B., Nevill, A., Carvalho, S., Atkinson, G., Buckley, P., Reilly, T., Godfrey, R., and Ramsay, R. (2002). Identifying some determinants of "jet lag" and its symptoms: A study of athletes and other travellers. *British Journal of Sports Medicine* 36: 54–60.
46. Waterhouse, J., Kao, S., Edwards, B., Weinert, D., Atkinson, G., and Reilly, T. (2005). Transient changes in the pattern of food intake following a simulated time-zone transition to the East across eight time zones. *Chronobiology International* 22: 299–319.
47. Youngstedt, S. D. and O'Connor, P. J. (1999). The influence of air travel on athletic performance. *Sports Medicine* 28: 197–207.
48. Youngstedt, S. D. and Buxton, O. M. (2003). Jet lag and athletic performance. *American Journal of Medicine in Sports* 5: 219–226.
49. Lemmer, B., Kern, R. I., Nold, G., and Lohrer, H. (2002). Jet lag in athletes after eastward and westward time-zone transition. *Chronobiology International* 19: 743–764.
50. Waterhouse, J., Nevill, A., Edwards, B., Godfrey, R., and Reilly, T. (2003). The relationship between assessments of jet lag and some of its symptoms. *Chronobiology International* 20: 1061–1073.
51. Penev, P. D., Kolker, D. E., Zee, P. C., and Turek, F. W. (1998). Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. *American Journal of Physiology* 275: H2334–H2337.
52. Davidson, A. J., Sellix, M. T., Daniel, J., Yamazaki, S., Menaker, M., and Block, G. D. (2006). Chronic jet-lag increases mortality in aged mice. *Current Biology* 16: R914–R916.
53. Tsai, L., Tsai, Y., Huang, K., Huang, Y., and Tzeng, J. (2005). Repeated light-dark shifts speed up body weight gain in male F344 rats. *American Journal of Physiology* 289: E212–E217.
54. Summa, K. C., Vitaterna, M. H., and Turek, F. W. (2012). Environmental perturbation of the circadian clock disrupts pregnancy in the mouse. *PLOS ONE* 7: e37668.
55. Filipski, E., Delaunay, F., King, V. M., Wu, M. W., Claustrat, B., Gréchez-Cassiau, A., Guettier, C., Hastings, M. H., and Lévi, F. (2004). Effects of chronic jet lag on tumor progression in mice. *Cancer Research* 64: 7879–7885.
56. Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sánchez, R., Ríos, C. D., Freitag, W. O., Richardson, G. S., and Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* 233: 667–671.
57. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N., and Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.

58. Honma, K., Honma, S., and Wada, T. (1987). Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. *Experientia* 43: 1205–1207.
59. Honma, K. and Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167–168.
60. Dijk, D. J., Beersma, G. M., Daan, S., and Lewy, A. J. (1989). Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *American Journal of Physiology* 256: R106–R111.
61. Frennan, M., Kripke, D. F., and Gillin, J. C. (1989). Bright light can delay human temperature rhythm independent of sleep. *American Journal of Physiology* 257: R136–R141.
62. Shanahan, T. L. and Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *Journal of Clinical Endocrinology and Metabolism* 73: 227–235.
63. Buresová, M., Dvoráková, M., Zvolsky, P., and Illnerová, H. (1991). Early morning bright light phase advances the human circadian pacemaker within one day. *Neuroscience Letters* 121: 47–50.
64. Dawson, D., Lack, L., and Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102.
65. Boivin, D. B., Duffy, J. F., Kronauer, R. E., and Czeisler, C. A. (1996). Dose-response relationships for resetting of human circadian clock by light. *Nature* 379: 540–542.
66. Honma, K., Honma, S., Nakamura, K., Sasaki, M., Endo, T., and Takahashi, T. (1995). Differential effects of bright light and social cues on reentrainment of human circadian rhythms. *American Journal of Physiology* 268: R528–R535.
67. Tzischinsky, O. and Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.
68. Shanahan, T. L., Kronauer, R. E., Duffy, J. F., Williams, G. H., and Czeisler, C. A. (1999). Melatonin rhythm observed throughout a three-cycle bright-light stimulus designed to reset the human circadian pacemaker. *Journal of Biological Rhythms* 14: 237–253.
69. Zeitzer, J. M., Dijk, D. J., Kronauer, R. E., Brown, E. N., and Czeisler, C. A. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: Melatonin phase resetting and suppression. *Journal of Physiology* 526: 695–702.
70. Khalsa, S. B. S., Jewett, M. E., Cajochen, C., and Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.
71. Lockley, S. W., Brainard, G. C., and Czeisler, C. A. (2003). High sensitivity of the human circadian melatonin rhythm to resetting by short wavelength light. *Journal of Clinical Endocrinology and Metabolism* 88: 4502–4505.
72. Warman, V. L., Dijk, D. J., Warman, G. R., Arendt, J., and Skene, D. J. (2003). Phase advancing human circadian rhythms with short wavelength light. *Neuroscience Letters* 342: 37–40.
73. Burgess, H. J., Fogg, L. F., Young, M. A., and Eastman, C. I. (2004). Bright light therapy for winter depression: Is phase advancing beneficial? *Chronobiology International* 21: 759–775.
74. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C., and Weber, J. M. (2004). Evening melatonin and bright light administration induce additive phase shifts in dim light melatonin onset. *Journal of Pineal Research* 36: 192–194.
75. St. Hilaire, M. A., Gooley, J. J., Khalsa, S. B. S., Kronauer, R. E., Czeisler, C. A., and Lockley, S. W. (2012). Human phase response curve to a 1 h pulse of bright white light. *Journal of Physiology* 590: 3035–3045.
76. Rüger, M., St. Hilaire, M. A., Brainard, G. C., Khalsa, S. S., Kronauer, R. E., Czeisler, C. A., and Lockley, S. W. (2013). Human phase response curve to a single 6.5 h pulse of short-wavelength light. *Journal of Physiology* 591: 353–363.
77. Mien, I. H., Chua, E. C., Lau, P., Tan, L. C., Lee, I. T., Yeo, S. C., Tan, S. S., and Gooley, J. J. (2014). Effects of exposure to intermittent versus continuous red light on human circadian rhythms, melatonin suppression, and pupillary constriction. *PLOS ONE* 9: e96532.
78. Whitson, P. A., Putcha, L., Chen, Y. M., and Baker, E. (1995). Melatonin and cortisol assessment of circadian shifts in astronauts before flight. *Journal of Pineal Research* 18: 141–147.
79. Boulos, Z., Macchi, M. M., Stürchler, M. P., Stewart, K. T., Brainard, G. C., Suhner, A., Wallace, G., and Steffen, R. (2002). Light visor treatment for jet lag after westward travel across six time zones. *Aviation, Space, and Environmental Medicine* 73: 953–963.
80. Boivin, D. B. and James, F. O. (2002). Phase-dependent effect of room light exposure in a 5-h advance of the sleep-wake cycle: Implications for jet lag. *Journal of Biological Rhythms* 17: 266–276.
81. Burgess, H. J., Crowley, S. J., Gazda, C. J., Fogg, L. F., and Eastman, C. I. (2003). Preflight adjustment to eastward travel: 3 days of advancing sleep with and without morning bright light. *Journal of Biological Rhythms* 18: 318–328.
82. Thompson, A., Batterham, A. M., Jones, H., Gregson, W., Scott, D., and Atkinson, G. (2013). The practicality and effectiveness of supplementary bright light for reducing jet-lag in elite female athletes. *International Journal of Sports Medicine* 34: 582–589.
83. Lewy, A. J., Ahmed, S., Jackson, J. M. L., and Sack, R. L. (1992). Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiology International* 9: 380–392.
84. Lewy, A. J., Ahmed, S., and Sack, R. L. (1996). Phase shifting the human circadian clock using melatonin. *Behavioural Brain Research* 73: 131–134.
85. Matsumoto, M., Sack, R. L., Blood, M. L., and Lewy, A. J. (1997). The amplitude of endogenous melatonin production is not affected by melatonin treatment in humans. *Journal of Pineal Research* 22: 42–44.
86. Rajaratnam, S. M. W., Dijk, D. J., Middleton, B., Stone, B. M., and Arendt, J. (2003). Melatonin phase-shifts human circadian rhythms with no evidence of changes in the duration of endogenous melatonin secretion or the 24-hour production of reproductive hormones. *Journal of Clinical Endocrinology and Metabolism* 88: 4303–4309.
87. Burke, T. M., Markwald, R. R., Chinoy, E. D., Snider, J. A., Bessman, S. C., Jung, C. M., and Wright, K. P. (2013). Combination of light and melatonin time cues for phase advancing the human circadian clock. *Sleep* 36: 1617–1624.
88. Reiter, R. J. and Robinson, J. (1996). *Melatonin: Breakthrough Discoveries That Can Help You*. New York: Bantam Books.
89. Pierpaoli, W., Davies, O., and Regelson, W. (1995). *The Melatonin Miracle: Nature's Age-Reversing, Disease-Fighting, Sex-Enhancing Hormone*. New York: Simon & Schuster.
90. Sack, R. L., Lewy, A. J., and Hughes, R. J. (1998). Use of melatonin for sleep and circadian rhythm disorder. *Annals of Medicine* 30: 115–121.

91. Edwards, B. J., Atkinson, G., Waterhouse, J., Reilly, T., Godfrey, R., and Budgett, R. (2000). Use of melatonin in recovery from jet-lag following an eastward flight across 10 time-zones. *Ergonomics* 43: 1501–1513.
92. Shiota, M., Sudou, M., and Ohshima, M. (1996). Using outdoor exercise to decrease jet lag in airline crewmembers. *Aviation, Space, and Environmental Medicine* 67: 1155–1160.
93. Buxton, O. M., Lee, C. W., L'Hermite-Balériaux, M., Turek, F. W., and Van Cauter, E. (2003). Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase. *American Journal of Physiology* 284: R714–R724.
94. Barger, L. K., Wright, K. P., Hughes, R. J., and Czeisler, C. A. (2004). Daily exercise facilitates phase delays of circadian melatonin rhythm in very dim light. *American Journal of Physiology* 286: R1077–R1084.
95. Van Reeth, O. and Turek, F. W. (1987). Adaptation of circadian rhythmicity to shift in light-dark cycle accelerated by a benzodiazepine. *American Journal of Physiology* 253: R204–R207.
96. Van Reeth, O. and Turek, F. W. (1992). Changes in the phase response curve of the circadian clock to a phase-shifting stimulus. *Journal of Biological Rhythms* 7: 137–147.
97. Buxton, O. M., Copinschi, G., van Onderbergen, A., Karrison, T. G., and van Cauter, E. (2000). A benzodiazepine hypnotic facilitates adaptation of circadian rhythms and sleep-wake homeostasis to an eight hour delay shift simulating westward jet lag. *Sleep* 23: 915–927.
98. Mohawk, J. A., Cashen, K., and Lee, T. M. (2005). Inhibiting cortisol response accelerates recovery from a photic phase shift. *American Journal of Physiology* 288: R221–R228.
99. Nicholson, A. N., Spencer, M. B., Pascoe, P. A., Stone, B. M., Roehrs, T., and Roth, T. (1986). Sleep after transmeridian flights. *Lancet* 8517: 1205–1208.
100. Zhdanova, I. V. and Wurtman, R. J. (1997). Efficacy of melatonin as a sleep-promoting agent. *Journal of Biological Rhythms* 12: 644–650.
101. Paul, M. A., Gray, G., Sardana, T. M., and Pigeau, R. A. (2004). Melatonin and zopiclone as facilitators of early circadian sleep in operational air transport crews. *Aviation, Space, and Environmental Medicine* 75: 439–443.
102. Rajaratnam, S. M. W. and Arendt, J. (2001). Health in a 24-h society. *Lancet* 358: 999–1005.
103. Caruso, C. C., Lusk, S. L., and Gillespie, B. W. (2004). Relationship of work schedules to gastrointestinal diagnoses, symptoms, and medication use in auto factory workers. *American Journal of Industrial Medicine* 46: 586–598.
104. Presser, H. B. (1999). Toward a 24-hour economy. *Science* 284: 1778–1779.
105. Parks, D. K., Yetman, R. J., McNeese, M. C., Burau, K., and Smolensky, M. H. (2000). Day-night pattern in accidental exposures to blood-borne pathogens among medical students and residents. *Chronobiology International* 17: 61–70.
106. Violanti, J. M., Fekedulegn, D., Andrew, M. E., Charles, L. E., Hartley, T. A., Vila, B., and Burchfiel, C. M. (2012). Shift work and the incidence of injury among police officers. *American Journal of Industrial Medicine* 55: 217–227.
107. Singer, B., Terborg, J., and Mayer, S. (1994). Attitudinal, circadian, circumstantial, and subject selection explanations of shiftwork effects on health. *Journal of Occupational Medicine* 36: 66–69.
108. Benedict, F. G. (1904). Studies in body temperature. I. Influence of the inversion of the daily routine; the temperature of night-workers. *American Journal of Physiology* 11: 145–169.
109. Roden, M., Koller, M., Pirich, K., Vierhapper, H., and Waldhauser, F. (1993). The circadian melatonin and cortisol secretion pattern in permanent night shift workers. *American Journal of Physiology* 265: R261–R267.
110. Pati, A. K. and Gupta, S. (1994). Time estimation circadian rhythm in shift workers and diurnally active humans. *Journal of Biosciences* 19: 325–330.
111. Weibel, L. and Brandenberger, G. (1998). Disturbances in hormonal profiles of night workers during their usual sleep and work times. *Journal of Biological Rhythms* 13: 202–208.
112. Dumont, M., Benhaberou-Brun, D., and Paquet, J. (2001). Profile of 24-h light exposure and circadian phase of melatonin secretion in night workers. *Journal of Biological Rhythms* 16: 502–511.
113. Waterhouse, J., Buckley, P., Edwards, B., and Reilly, T. (2003). Measurement of, and some reasons for, differences in eating habits between night and day workers. *Chronobiology International* 20: 1075–1092.
114. Arendt, J., Middleton, B., Williams, P., Francis, G., and Luke, C. (2006). Sleep and circadian phase in a ship's crew. *Journal of Biological Rhythms* 21: 214–221.
115. Boudreau, P., Dumont, G. A., and Boivin, D. B. (2013). Circadian adaptation to night shift work influences sleep, performance, mood and the autonomic modulation of the heart. *PLOS ONE* 8: e70813.
116. Andlauer, P., Reinberg, A., Fourré, L., Battle, W., and Duverneuil, G. (1979). Amplitude of the oral temperature circadian rhythm and the tolerance to shift-work. *Journal de Physiologie* 75: 507–512.
117. Reinberg, A., Vieux, N., Ghata, J., Chaumont, A. J., and Laporte, A. (1978). Is the rhythm amplitude related to the ability to phase-shift circadian rhythms of shift-workers? *Journal de Physiologie* 74: 405–409.
118. Reinberg, A., Andlauer, P., Guillet, P., Nicolai, A., Vieux, N., and Laporte, A. (1980). Oral temperature, circadian rhythm amplitude, ageing and tolerance to shift-work. *Ergonomics* 23: 55–64.
119. Vidacek, S., Radosevic-Vidacek, B., Kaliterna, L., and Prizmic, Z. (1993). Individual differences in circadian rhythm parameters and short-term tolerance to shiftwork: A follow-up study. *Ergonomics* 36: 117–123.
120. Di Milia, L., Waage, S., Pallesen, S., and Bjorvatn, B. (2013). Shift work disorder in a random population sample: Prevalence and comorbidities. *PLOS ONE* 8: e55306.
121. Smith-Coggins, R., Broderick, K. B., and Marco, C. A. (2014). Night shifts in emergency medicine: The American Board of Emergency Medicine longitudinal study of emergency physicians. *Journal of Emergency Medicine* 47: 372–378.
122. Kwon, P., Lundin, J., Li, W., Ray, R., Littell, C., Gao, D., Thomas, D. B., and Checkoway, H. (2015). Night shift work and lung cancer risk among female textile workers in Shanghai, China. *Journal of Occupational and Environmental Hygiene* 12: 334–341.
123. Davis, S., Mirick, D. K., and Stevens, R. G. (2001). Night shift work, light at night, and risk of breast cancer. *Journal of the National Cancer Institute* 93: 1557–1562.
124. Borges, F. N. S. and Fischer, F. M. (2003). Twelve-hour night shifts of healthcare workers: A risk to the patients? *Chronobiology International* 20: 351–360.
125. Kelly, T. L., Neri, D. F., Grill, J. T., Ryman, D., Hunt, P. D., Dijk, D. J., Shanahan, T. L., and Czeisler, C. A. (1999). Nonentrained circadian rhythms of melatonin in submariners scheduled to an 18-hour day. *Journal of Biological Rhythms* 14: 190–196.

126. Monk, T. H. (2000). What can the chronobiologist do to help the shift worker? *Journal of Biological Rhythms* 15: 86–94.
127. Cruz, C., Detwiler, C., Nesthus, T., and Boquet, A. (2003). Clockwise and counterclockwise rotating shifts: Effects on sleep duration, timing, and quality. *Aviation, Space, and Environmental Medicine* 74: 597–605.
128. Cruz, C., Boquet, A., Detwiller, C., and Nesthus, T. (2003). Clockwise and counterclockwise rotating shifts: Effects on vigilance and performance. *Aviation, Space, and Environmental Medicine* 74: 606–614.
129. Baehr, E. K., Fogg, L. F., and Eastman, C. I. (1999). Intermittent bright light and exercise to entrain human circadian rhythms to night work. *American Journal of Physiology* 277: R1598–R1604.
130. Boivin, D. B. and James, F. O. (2002). Circadian adaptation to night-shift work by judicious light and darkness exposure. *Journal of Biological Rhythms* 17: 556–567.
131. Crowley, S. J., Lee, C., Tseng, C. Y., Fogg, L. F., and Eastman, C. I. (2003). Combinations of bright light, scheduled dark, sunglasses, and melatonin to facilitate circadian entrainment to night shift work. *Journal of Biological Rhythms* 18: 513–523.
132. Gallo, L. C. and Eastman, C. I. (1993). Circadian rhythms during gradually delaying and advancing sleep and light schedules. *Physiology and Behavior* 53: 119–126.
133. Eastman, C. I., Liu, L., and Fogg, L. F. (1995). Circadian rhythm adaptation to simulated night shift work: Effect of nocturnal bright-light duration. *Sleep* 18: 399–407.
134. Leppämäki, S., Partonen, T., Piiroinen, P., Haukka, J., and Lönnqvist, J. (2003). Timed bright-light exposure and complaints related to shift work among women. *Scandinavian Journal of Work and Environmental Health* 29: 22–26.
135. Lowden, A., Åkerstedt, T., and Wibom, R. (2004). Suppression of sleepiness and melatonin by bright light exposure during breaks in night work. *Journal of Sleep Research* 13: 37–43.
136. Härmä, M. I., Ilmarinen, J., Knauth, P., Rutenfranz, J., and Hänninen, O. (1988). Physical training intervention in female shift workers: II. The effects of intervention on the circadian rhythms of alertness, short-term memory, and body temperature. *Ergonomics* 31: 51–63.
137. Eastman, C. I., Hoese, E. K., Youngstedt, S. D., and Liu, L. (1995). Phase-shifting human circadian rhythms with exercise during the night shift. *Physiology and Behavior* 58: 1287–1291.
138. James, M., Tremea, M. O., Jones, J. S., and Krohmer, J. R. (1998). Can melatonin improve adaptation to night shift? *American Journal of Emergency Medicine* 16: 367–370.
139. Sharkey, K. M., Fogg, L. F., and Eastman, C. I. (2001). Effects of melatonin administration on daytime sleep after simulated night shift work. *Journal of Sleep Research* 10: 181–192.
140. Jelínková-Vondrasová, D., Hájek, I., and Illnerová, H. (1999). Adjustment of the human circadian system to changes of the sleep schedule under dim light at home. *Neuroscience Letters* 265: 111–114.
141. Horowitz, T. S., Cade, B. E., Wolfe, J. M., and Czeisler, C. A. (2001). Efficacy of bright light and sleep/darkness scheduling in alleviating circadian maladaptation to night work. *American Journal of Physiology* 281: E384–E391.
142. Burgess, H. J. and Eastman, C. I. (2004). Early versus late bedtimes phase shift the human dim light melatonin rhythm despite a fixed morning lights on time. *Neuroscience Letters* 356: 115–118.
143. Jespersen, J. and Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency*, 2nd edn. Mineola, NY: Dover.
144. Ferguson, S. A., Preusser, D. F., Lund, A. K., Zador, P. L., and Ulmer, R. G. (1995). Daylight saving time and motor vehicle crashes: The reduction in pedestrian and vehicle occupant fatalities. *American Journal of Public Health* 85: 92–95.
145. Coate, D. and Markowitz, S. (2004). The effects of daylight and daylight saving time on US pedestrian fatalities and motor vehicle occupant fatalities. *Accident Analysis and Prevention* 36: 351–357.
146. Janssen, S. (Ed.) (2014). *The World Almanac and Book of Facts*. New York: Simon & Schuster.
147. Lahti, T. A., Leppämäki, S., Lönnqvist, J., and Partonen, T. (2006). Transition to daylight saving time reduces sleep duration plus sleep efficiency of the deprived sleep. *Neuroscience Letters* 406: 174–177.
148. Schneider, A. M. and Randler, C. (2009). Daytime sleepiness during transition into daylight saving time in adolescents: Are owls higher at risk? *Sleep Medicine* 10: 1047–1050.
149. Barnes, C. M. and Wagner, D. T. (2009). Changing to daylight saving time cuts into sleep and increases workplace injuries. *Journal of Applied Psychology* 94: 1305–1317.

This page intentionally left blank

16 Human Medicine

16.1 CHRONOTHERAPEUTICS

The prevention and treatment of disease informed by knowledge of circadian rhythms is called "chronotherapeutics." In Chapter 5, and throughout this book, we saw that circadian rhythmicity affects numerous physiological and behavioral processes. Therefore, it is not surprising to find out that the symptoms of chronic diseases—such as allergic rhinitis, angina, arthritis, asthma, epilepsy, hypertension, and ulcer disease—exhibit daily rhythmicity.[1] Awareness of these rhythmic patterns is essential for proper diagnosis of the diseases, as had been suggested by Arthur Jores in the 1930s[2] and Franz Halberg in the 1950s.[3]

The implications of circadian rhythmicity to human medicine start at the most basic level of physiological processes. For instance, we have discussed the rhythm of body temperature numerous times in previous chapters. Like other animals, humans exhibit robust rhythmicity of body temperature.[4–41] It does not take much thinking to realize that rhythmicity in body temperature can affect the identification of febrile conditions (*fever*). Figure 16.1 shows my own body temperature rhythm, as determined by multiple sublingual measurements at different times of the day over a 6-day interval many years ago. To obtain nocturnal measurements, I woke myself up with an alarm clock and went back to sleep immediately afterward. Notice the robust rhythmicity in these data obtained without the controlled conditions of a laboratory and without sophisticated equipment. The mean level of the rhythm is a little below the "normal" human temperature of 37.0°C (98.6°F).[37] More importantly, notice that the temperature oscillates below and above the normal temperature. If a nurse at a physician's office took my temperature with disregard to the time of day, he or she would conclude that I am hypothermic from midnight to noon and that I have a fever from noon to 9 p.m. Evidently, the normal threshold for a fever varies along the day. In my case, only a sublingual temperature above 37.6°C (99.7°F) could be considered a fever at any time of the day.

As early as 1951, Halberg documented daily rhythmicity in blood eosinophil count.[42] As shown in Figure 16.2, cell count is lowest in the morning and afternoon and highest at night. Eosinophils (or *eosinophilic leukocytes*) are white blood cells with distinctive antiparasitic functions that are especially abundant in the mucosa of the gastrointestinal, respiratory, and urinary tracts.[43] The daily oscillation in eosinophil count implies that the body's resistance to parasitic infection is dependent on the time of day, with a peak at night.

The basic vital signs that are routinely monitored in medical visits are body temperature, heart rate, and blood pressure. Because all three of these variables exhibit daily rhythmicity, disregard for the rhythmicity may lead to erroneous interpretation of the measurements—as we just saw regarding body temperature. Table 16.1 shows the normative ranges for oral temperature, heart rate, systolic blood pressure, and diastolic blood pressure in healthy human adults. The designations "morning," "afternoon," and "night" apply to "average" individuals who wake up around 8 a.m. and go to sleep around midnight. Notice that the ranges for blood pressure and heart rate are the same in the morning and afternoon. This explains why physicians can disregard daily rhythmicity when measurements are taken during regular office hours. However, emergency-room staff attending to patients at night should not disregard the daily variation in blood pressure and heart rate. A systolic pressure of 125 mm Hg is quite normal during the day but is suggestive of hypertension at night. Notice that interpretation of oral temperature measurements must consider the daily rhythmicity regardless of whether the measurements are made in the office or in the emergency room. A temperature of 37.1°C is indicative of fever at night, may indicate fever in the morning, and most likely does not indicate fever in the afternoon.

The ranges given in Table 16.1 are relatively wide because they incorporate the normal interindividual variability observed among healthy individuals. For long-term health care, normative ranges should be empirically determined for each patient. Hourly measurements conducted for a week, while the patient is healthy, could generate baseline curves to facilitate the interpretation of pathological deviations later on. Devices for ambulatory monitoring of body temperature, heart rate, and blood pressure are now easily available, so that patients may carry on their usual lives while the baseline data are acquired. Ambulatory monitors, priced between $1000 and $5000, are manufactured or marketed by several companies in the United States, such as Spacelabs Healthcare (Snoqualmie, WA), Tiba Medical (Portland, OR), and Welch Allyn (Skaneateles Falls, NY). Ambulatory monitoring of basic vital signs is a central component of *personalized health planning*, which has been recommended as a means of anticipating and minimizing a patient's risk for the onset and progression of disease.[44] In the particular case of blood pressure, ambulatory monitoring can not only provide the normative baseline data but also prevent the occurrence of *white-coat hypertension* (i.e., the spurious elevation of blood pressure caused by a patient's anxiety in the physician's office).[45–47]

Several medical conditions exhibit daily or circadian rhythmicity. People who suffer from migraines are more likely to be in pain in the morning than in the afternoon or night.[48] Morning stiffness is a common symptom of rheumatoid arthritis.[49] Suicide attempts by self-poisoning are more common in the late afternoon and evening.[50] Oral malodor ("bad breath") also oscillates daily, mostly because of reduced malodor after meals.[51] Saliva secretion is less intense at night,

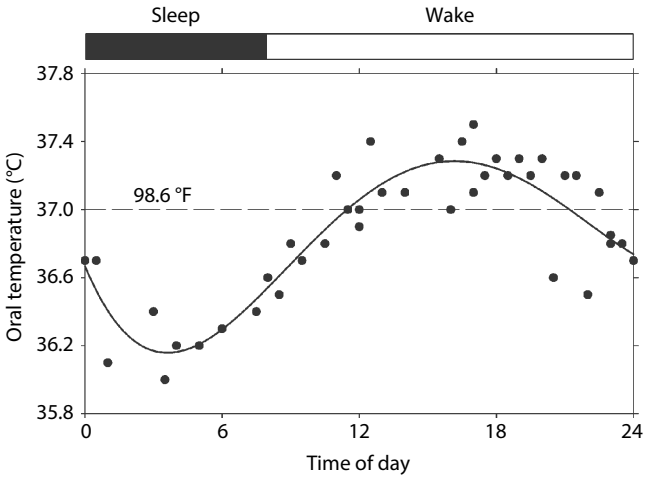


FIGURE 16.1 Do you have a fever? The graph shows the daily rhythm of oral temperature of a normal healthy human subject. Because the temperature oscillates from 36.0°C to 37.5°C, it makes no sense to use the traditional value of 37.0°C (98.6°F) as a criterion for ascertaining the presence of a fever. (Data from Refinetti, R., *Behav. Res. Methods Instrum. Comput.*, 24, 28, 1992.)

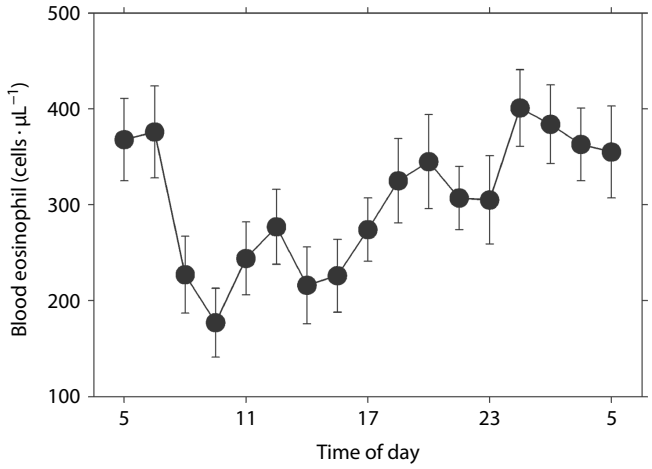


FIGURE 16.2 Daily rhythmicity in white blood cell count. The graph shows the daily oscillation of blood eosinophil (leukocytes with distinctive antiparasitic functions) in healthy human subjects. The data points correspond to the means (±SE) of 17 young men. Notice that cell count is higher during sleep time (between midnight and 7 a.m.). (Data from Halberg, F. et al., *J. Lancet*, 71, 312, 1951.)

so that "the most important time to carry out oral hygiene procedures [is] before going to bed."[52]

A condition called *night eating syndrome* is characterized by ingestion of one-third or more of daily calories after dinner and by night awakening for more eating. It affects approximately 2% of the population.[53–55] *Social jet lag* refers to an inadequate relationship between the internal clock and the external environment, as measured by the time difference between the midpoint of sleep during work days and the midpoint of sleep during free days. Greater social jet lag is associated with obesity,[56,57] although it is not known whether circadian disruption causes obesity or obesity causes circadian disruption.

TABLE 16.1
Normative Ranges for Human Vital Signs[a]

	Morning		Afternoon		Night	
	Low	**High**	**Low**	**High**	**Low**	**High**
Oral temperature (°F)	97.2	99.1	98.1	99.9	96.4	98.2
Oral temperature (°C)	36.2	37.3	36.7	37.7	35.8	36.8
Heart rate (bpm)	55	95	55	95	45	75
Systolic BP (mm Hg)	100	135	100	135	85	120
Diastolic BP (mm Hg)	60	85	60	85	45	70

[a] The designations "morning," "afternoon," and "night" apply to the patient's internal clock, not to the actual time of day, although for most patients, the internal clock will be synchronized to the external clock. It is assumed that the vital signs are measured at rest. The ranges given for each time of day incorporate the normal interindividual variability for healthy individuals.

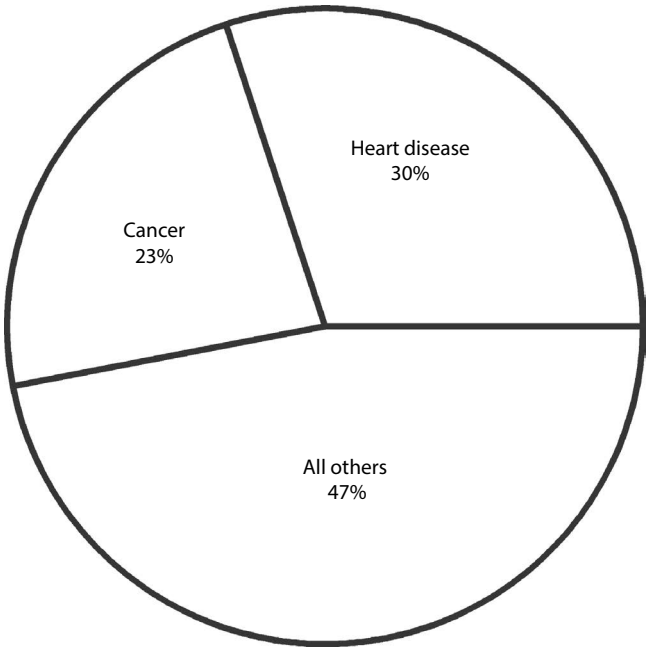


FIGURE 16.3 Leading causes of death in the United States. Two illnesses—heart disease and cancer—are responsible for more than half of all deaths. (Data from *The World Almanac and Book of Facts*, Simon & Schuster, New York, 2014.)

Although chronotherapeutics may be relevant for every human disease, space limitations force me to go into detail only about a few selected diseases. Two diseases—heart disease (or, more generally, cardiovascular disease) and cancer—are responsible for more than half of all deaths in the United States (Figure 16.3) and certainly deserve to be discussed here. It is true that every human being will die one day, so that reductions in mortality by one disease will necessarily lead to increases in mortality by other diseases; however, improvements in the treatment of cardiovascular disease and cancer can surely improve the patient's quality of life, even if it only postpones his or her death by other causes. Asthma is the third disease that I will cover.

16.1.1 Cardiovascular Disease

One of the leading causes of cardiovascular disease is hypertension (high blood pressure). Regular monitoring of blood pressure (Figure 16.4) is a simple, noninvasive way to ensure early diagnosis of hypertension. However, humans—like other animals—exhibit robust daily rhythmicity in cardiovascular parameters.[7,15,24,26,29,30,45–47,58–68] As mentioned earlier, this means that measurements of blood pressure must be interpreted in reference to the time of day when they are taken. It also means that treatment must be dispensed with consideration of the daily rhythm. Blood pressure has a definite and reproducible daily pattern: it is highest during the day, lowest during sleep, and increases rapidly from the very early morning to noon, as exemplified in Figure 16.5. Consequently, antihypertensive control for patients who need it should be concentrated in the early morning. In order to attain peak plasma level of the medication during the early morning period, controlled-onset, extended-release delivery systems have been developed and implemented. Such systems provide effective control of hypertension by supplying the antihypertensive drug at the time when blood pressure is highest, thus ensuring adequate dosage at the time when the drug is actually needed and avoiding toxic overdosage at other times.[67]

Halberg obtained preliminary data indicating an enormous effect of abnormalities in rhythm amplitude on morbidity from vascular disease. He conducted ambulatory measurements of blood pressure and heart rate in various individuals and then counted how many of them developed cerebral ischemia, myocardial infarction, nephropathy, or retinopathy during the following 6 years. As shown in Figure 16.6, very few people (8%) who had normal rhythms of blood pressure and heart rate became sick during the following 6 years. In contrast, 35% of people who had blood pressure rhythms with high amplitude (which in itself is not a morbid state and does not imply current hypertension) eventually developed vascular morbidity.[60,69,70]



FIGURE 16.4 Office monitoring of blood pressure. Frequent monitoring of blood pressure can facilitate the early detection of elevated blood pressure and the prevention of deadly cardiovascular diseases. (Copyright ArtToday, Tucson, AZ.)

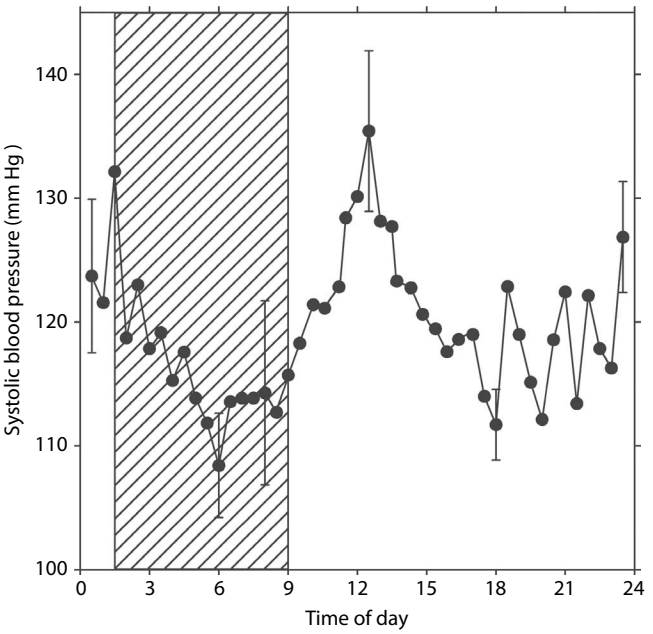


FIGURE 16.5 Ambulatory monitoring of blood pressure. Ambulatory blood pressure monitors can be used for around-the-clock monitoring of blood pressure. This graph shows the systolic blood pressure of the author of this book as measured every half hour and averaged over 7 consecutive days. The error bars are standard errors of the means shown at selected intervals to avoid clutter. The hatched area denotes the daily sleep time (from 01:30 to 09:00). (Data from the archives of the Refinetti lab.)

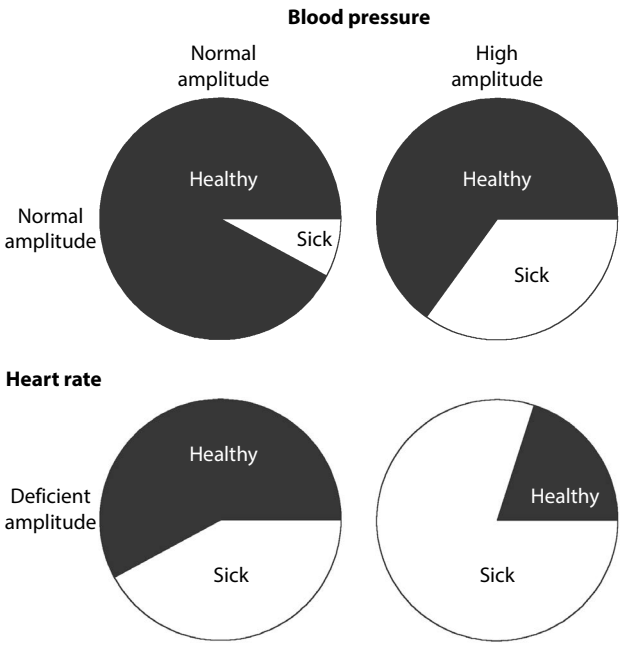


FIGURE 16.6 Prediction of morbidity by rhythm amplitude. Franz Halberg conducted ambulatory measurements of blood pressure and heart rate in a number of individuals and then counted how many of them developed cardiovascular morbidity during the following 6 years. The graphs show that large amplitude of the rhythm of blood pressure and small amplitude of the rhythm of heart rate (without abnormal mean levels) are strong predictors of morbidity. (Data courtesy of Franz Halberg.)

The situation was similar for people who had heart rate rhythms with unusually *low* amplitude (42% of them became sick). Amazingly, of those who had both types of rhythm abnormality, 80% developed vascular morbidity within 6 years.[71] Thus, the predictive power of amplitude abnormalities (i.e., high amplitude of the blood pressure rhythm and low amplitude of the heart rate rhythm) seems to be very high, and use of this predictive power could provide dramatic improvement in prophylactic health care. Great uncertainty derives, however, from the fact that the sample sizes in Halberg's exploratory studies were rather small, with about 20 patients in the high-amplitude blood pressure and low-amplitude heart rate groups, and only 5 patients in the group with both abnormalities. Evidently, a full-fledged clinical trial is urgently needed to evaluate the merits of the preliminary observations. Other investigators, using a larger number of subjects, noticed that absence of the daily rhythm of blood pressure (that is, blood pressure measurements with a *nondipping* nocturnal pattern) is a predictor of cardiovascular disorders.[72–74]

Regarding actual heart attacks, we saw in Chapter 5 that they tend to occur in the morning or early afternoon.[75–82] Ruptures of aortic aneurysms and cerebral aneurysms (stroke) also tend to occur in the morning.[83–86] Of course, these are populational rhythms, but the populational rhythm would not exist if individuals were not more likely to have cardiovascular problems early in the day. Thus, at-risk individuals may be advised to make a special effort to avoid threatening conditions early in the day. In addition, it has been shown that lack of daily rhythmicity in high-frequency power of the electrocardiogram is associated with ventricular arrhythmia, which means that analysis of ambulatory electrocardiogram data can help the early diagnosis of cardiac arrhythmia.[87]

16.1.2 Cancer

Recognition of the existence of circadian rhythms is important not only for diagnostic purposes but also for therapeutic purposes. Indeed, if physiological functions oscillate daily, it is to be expected that the sensitivity of the patient to therapeutic agents will also oscillate daily. Studies in laboratory animals have identified daily rhythmicity in the body's response to numerous drugs, including anesthetics,[88–90] immunosuppressants,[91] antibiotics,[92] and alcohol.[93] Likewise, clinical research on human patients has demonstrated that the pharmacodynamics and pharmacokinetics of H2-blockers, antiasthmatics (theophylline, β-agonists, anticholinergics, glucocorticoids), cardiovascular active drugs (β-blockers, organic nitrates, calcium channel blockers), and anticancer drugs, to name a few, display daily rhythmicity.[94,95]

Cancer treatment is an excellent example of the importance of chronopharmacology (i.e., the administration of drugs at specific times of the day when the drugs are most effective and have least side effects). Consider Figure 16.7. It shows daily variation in plasma concentration of fluorouracil (one of the oldest anticancer drugs, still widely used today) in patients with bladder carcinoma. The drug, which has a half-life in the blood of 10–20 minutes, was infused intravenously at a constant rate. Notice the clear daily variation in plasma concentration despite the constant rate of infusion.[96] Evidently, breakdown is much faster during the day than at night, which results in higher plasma concentration at night. This means that lower doses (with potentially lower toxicity) could be used at night.

Let me rush to point out that the story is a bit more complex. A separate research team showed that the daily variation in fluorouracil breakdown is rather small in comparison with other factors affecting blood concentration of the drug.[97] Parallel research conducted by Francis Lévi's team (in France) showed that fluorouracil is more effective and less toxic at night than during the day.[98] This means that the variation in drug breakdown can be ignored, and *higher* doses should be used at night. As a matter of fact, many oncologists administer fluorouracil only at night (with the assistance of time-controlled infusion pumps).[99] A comparison of the results of chemotherapy of colorectal cancer by constant infusion (*constant rate*) and by time-controlled infusion (*chronomodulated*) is shown in Figure 16.8. The results derive from two multicenter randomized trials involving a total of 278 patients. Fluorouracil was infused along with leucovorin and oxaliplatin. Toxicity is expressed as the percentage of patients hospitalized for toxic effects, and tumor response is expressed as the percentage of patients exhibiting greater than 50% reduction in tumor size. Notice that chronomodulated infusion resulted in both lower toxicity and higher tumor response.[99] As early as 1985, William Hrushesky (then at the Department of Veteran Affairs Medical Center, in Albany, NY) demonstrated greater efficacy and lower toxicity of chronomodulated administration of adriamycin and cisplatin in the treatment of ovarian cancer.[100]

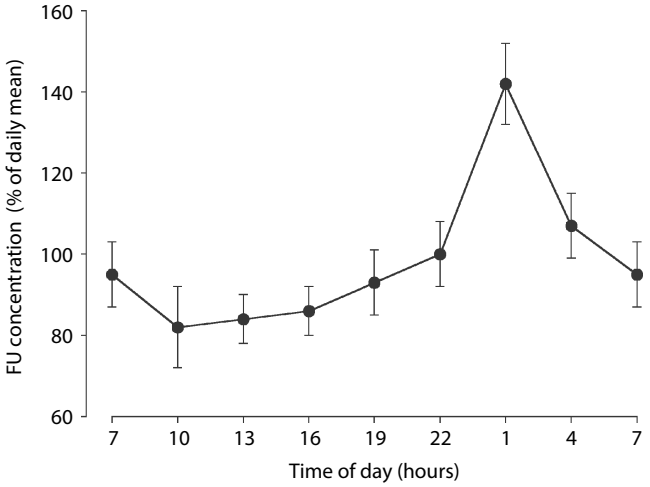


FIGURE 16.7 Daily rhythmicity in chemical breakdown of anticancer drug. The graph shows the daily oscillation in plasma concentration of fluorouracil (a drug used in cancer treatment) administered by continuous intravenous infusion at constant rate in human subjects. The data points correspond to the means (±SE) of 7 patients with bladder carcinoma. Notice the great daily variation in plasma concentration of the drug despite the continuous infusion. Plasma concentration is higher around midnight because breakdown is slower at this time of day. (Data from Milano, G. and Chamorey, A.L., *Chronobiol. Int.*, 19, 177, 2002.)

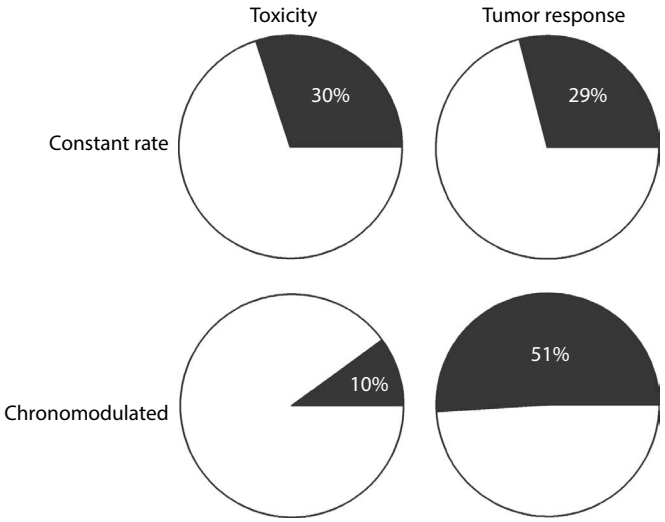


FIGURE 16.8 Chemotherapy of colorectal cancer. The graphs show the percentages of patients hospitalized for toxicity (*toxicity*) and the percentages of patients exhibiting greater than 50% reduction of tumor size (*tumor response*) associated with constant-rate or chronomodulated chemotherapy (fluorouracil, leucovorin, and oxaliplatin). The percentages are derived from two multicenter randomized trials involving a total of 278 patients with metastatic colorectal cancer. Notice that chronomodulated chemotherapy is less toxic and more effective than traditional chemotherapy. (Data from Lévi, F. et al., *Hepato-Gastroenterology*, 48, 320, 2001.)

At the molecular level, a study of 1457 patients with prostate cancer and 1352 controls provided information on single gene polymorphisms in 10 circadian clock genes. The results provided support for the hypothesis that there is a link between genetic variation in clock genes (particularly *npas2*) and prostate cancer.[101] In another study, polymorphisms in the *clk* gene were associated with breast cancer risk.[102]

It has been speculated that the development of breast cancer and prostate cancer may be more likely in individuals who lack robust circadian rhythms.[103,104] The speculation is somewhat supported by findings that patients with colorectal cancer who have robust rhythms of locomotor activity survive longer than patients who have weak rhythms.[105] Also, there seems to be a strong association between decreased expression of circadian clock genes and shorter metastasis-free survival in breast cancer patients.[106] Furthermore, an actigraphy study of 1734 patients found that low stability and high fragmentation of the activity rhythm is a predictor of all-cause mortality.[107] A major problem in interpreting the findings from these various studies is that the studies were observational, not experimental, so that one cannot know whether weak rhythmicity is a contributing factor to illness (and death) or is a consequence of the illness. As discussed in Chapter 6, illness is known to weaken circadian rhythms. If reverse causation can be documented, then strategies to strengthen circadian rhythmicity should be effective in preventing the illnesses. An experimental study in mice showed that surgical lesions of the master circadian pacemaker not only eliminate rhythmicity in locomotor activity, body temperature, and corticosterone secretion but also accelerate the growth of tumors induced by injection of a carcinogen.[108] Further studies would be very welcome.

16.1.3 Asthma

Asthma is *not* one of the major killers in our time, but it is a debilitating disease that affects 20 million people in the United Sates alone.[109] It is particularly worthy of mention here because its symptoms exhibit clear daily rhythmicity.[110,111] Asthma (or *bronchial asthma*) is a condition of the lungs (Figure 16.9) in which there is widespread narrowing of airways, due in varying degrees to spasm of smooth muscle, edema of the mucosa, and mucus in the lumen of the bronchi and bronchioles. The obvious symptom is difficulty in breathing.

The first large-scale investigation of daily rhythmicity in asthma was that of Margaret Turner-Warwick (in England) involving 7729 asthmatic outpatients.[112] Turner-Warwick confirmed previous anecdotal evidence of a worsening of symptoms during the late night and early morning. The "morning dip" in expiratory flow is truly an organic process and not a mere consequence of the lack of effective medication during the night.[113] However, controlled laboratory studies have not been conducted to exclude the role of environmental factors (such as changes in quantity and quality of allergens) in the determination of the daily oscillation. Thus, in strict terms, one can say that asthmatic symptoms exhibit *daily* rhythmicity but not necessarily *circadian* rhythmicity. As a matter of fact, one group of researchers has found that the rhythmicity in asthmatic symptoms disappears if the data are corrected for daily changes in psychosocial factors that contribute to asthma, such as activities, locations, social contacts, mood, and stressors.[114]

Two indices of expiratory flow have been used as objective measures to facilitate the management of asthma: peak expiratory flow rate[115–117] and forced expiratory volume in one

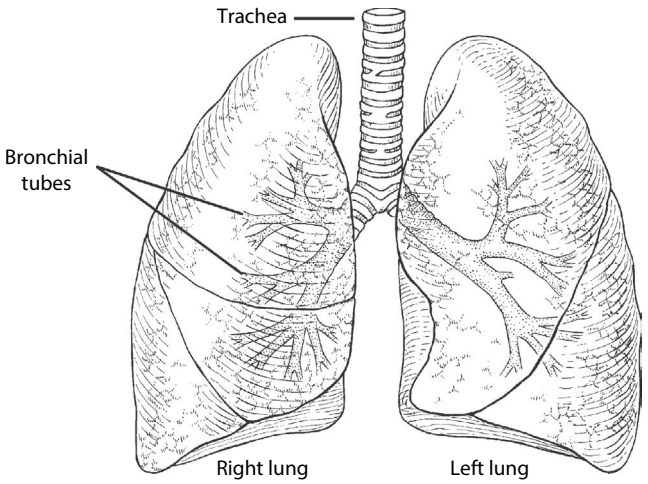


FIGURE 16.9 In the lungs. Asthma is a pulmonary disorder resulting from spasmodic contraction of the muscles of the bronchioles, from a swelling of the tissues of the bronchial tubes, and from the retention of the secretions of the bronchial glands. (Adapted from *Medical Illustration Library*, Williams & Wilkins, Baltimore, MD, 1994.)

second (FEV1).[118–120] The two measures correlate well with each other and with the caliber of the airway.[121] Measurements conducted at several time points during a day revealed clear rhythmicity in asthmatic patients and in normal subjects. For both groups, peak lung function occurs at approximately 4 p.m. and lowest lung function at 4 a.m. The two groups differ in the mean level and amplitude of the oscillation: asthmatic patients display lower lung function than normal subjects overall, and their "morning dip" is more accentuated than that of normal subjects.

In two related studies, asthmatic patients who presented nocturnally to the emergency department did not differ from other asthmatic patients in disease severity.[122,123] The authors interpreted this finding as an indication that daily rhythmicity in asthma severity is not clinically relevant for purposes of emergency management. This interpretation is correct in strict sense, but one should not infer from this that daily rhythmicity in asthma severity is not clinically relevant in general. Since a large number of uncontrolled variables determine a patient's decision to leave home in the middle of the night and go to a hospital emergency department, the studies have a natural sampling bias that prevents any generalization to the whole population of asthmatic patients.

Chronobiological treatment of asthma involves the same drugs used in traditional approaches (corticosteroids, theophylline, anticholinergics, or β-adrenergic agonists) but makes use of chronobiological information to maximize pharmacological effects and minimize side effects.[110,111] Since the greatest extent of bronchial inflammation is observed in the early morning, one would expect the best timing for drug administration to be the early evening (to allow enough time for drug action). Indeed, drug administration in the early evening has been found to be optimal for corticosteroids,[124] theophylline,[125] anticholinergics,[126] and β-adrenergic agonists.[127,128] While this approach does not provide new clues regarding an eventual cure for asthma, it does improve the patient's condition and offers a more effective way to prevent asthmatic episodes.

16.2 SLEEP DISORDERS

In Chapter 10, we discussed the regulation of sleep (Figure 16.10). We saw that sleep is a restorative process gated by the circadian system.[129,130] The longer you stay awake, the greater is your potential to sleep; however, the potential is converted into actual sleep propensity only when the circadian gate is open. Conversely, if the gate is open (if it is that time of the circadian cycle when your body temperature is falling) but you have just woken up, there will be little sleep propensity. To avoid a common misunderstanding, I should emphasize that the fall in body temperature with which sleep is associated is only a marker of the phase of the circadian pacemaker. Low body temperature per se is not sleep inducing. Large reductions in ambient temperature (which can cause a fall in body temperature) do not induce sleep; on the contrary, they impair sleep.[131–136] Humans fall asleep more easily when their skin temperature is higher, not lower than usual.[137,138] As a matter of fact, if there is a causal link between body temperature and sleep, it is in the opposite direction: falling asleep causes a small decrease in body temperature superimposed on the larger decrease determined by the circadian system.[23,139] Also, during the deeper stages of sleep, the homeostatic control of body temperature is relaxed,[131,135,140–151] which can lead to a further fall in body temperature if ambient temperature is low.



FIGURE 16.10 Sleeping beauty. Most healthy humans allowed to sleep to their heart's content spend approximately 8 hours sleeping each day. (Copyright ArtToday, Tucson, AZ.)

Because most humans are awake during the day and asleep at night, day length (photoperiod) can impact on sleep. Also, as we saw in Chapter 7, the phase angle of entrainment of circadian rhythms is dependent on photoperiod. Thus, it is important to examine how the photoperiod varies in different regions of the world. Because the Earth rotates around its axis, different parts of the world experience different times of day at any given instant (Figure 16.11). I pointed this out in Chapter 15 when we discussed the issue of time zones. However, I did not emphasize that at any given *latitude* the photoperiod is the same, even though the local time is different at different *longitudes*. Photoperiod is indicated by the small bars on the left margin of Figure 16.11. In the month of July, locations above the Arctic Circle experience 24 hours of sunlight each day, whereas locations below the Antarctic Circle experience total darkness. The exact proportions of light and darkness at a given latitude vary with the seasons, but—except for 2 days each year (the vernal and autumnal equinoxes)—the duration of daylight depends on latitude. We saw in Chapter 7, and also in Chapter 9, that animals expand and contract their daily intervals of activity in response to changes in photoperiod. Therefore, we can expect the activity interval of a given species to vary with latitude. This is generally not true for civilized human societies, however. As shown in Figure 16.12, there are geographical differences in the times when humans go to bed at night and get up in

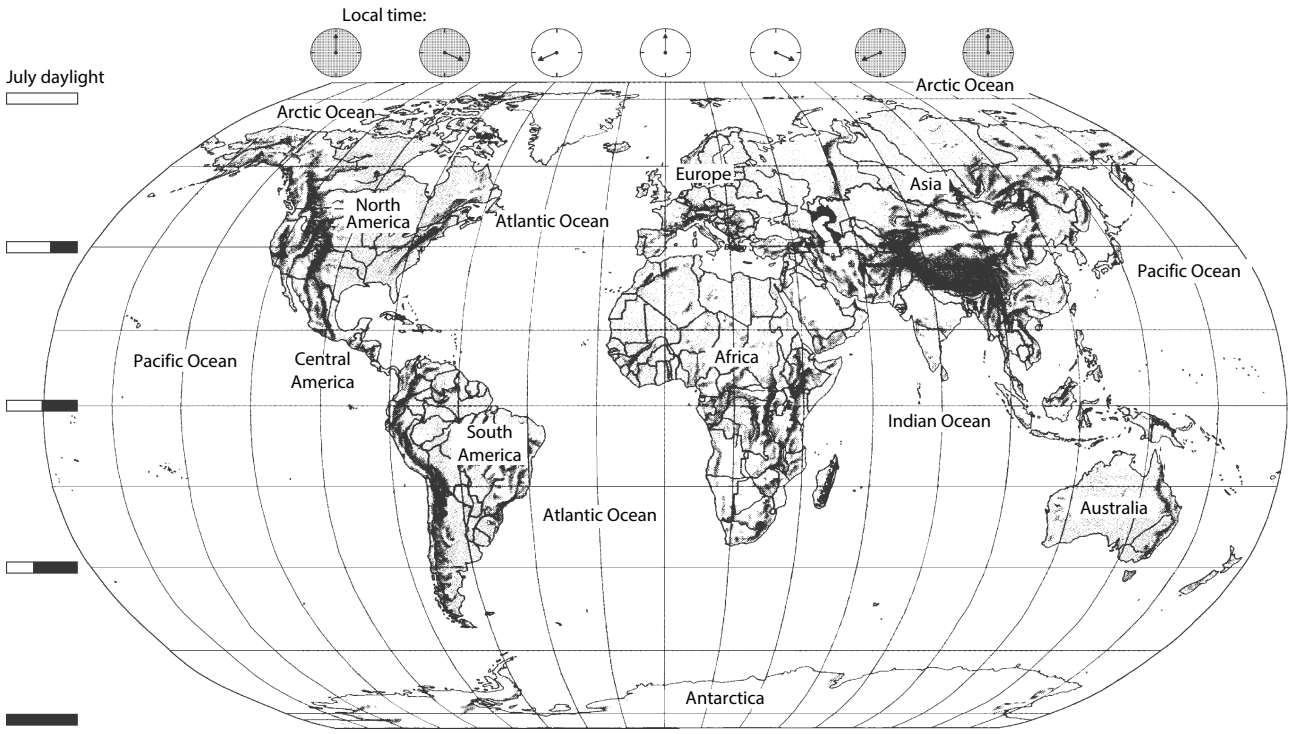


FIGURE 16.11 Latitude and longitude. Latitude and longitude affect the time of day differently. Longitude affects time of day according to the time zones, but the days are similar regarding local time. Latitude does not affect time of day, but it has a drastic effect on day length. As indicated by the horizontal bars on the left margin of the figure, the duration of daylight in a day may vary from 24 hours to 0 hours depending on latitude. (Data from *TIME Almanac*, TIME Books, Des Moines, IA, 2004.)

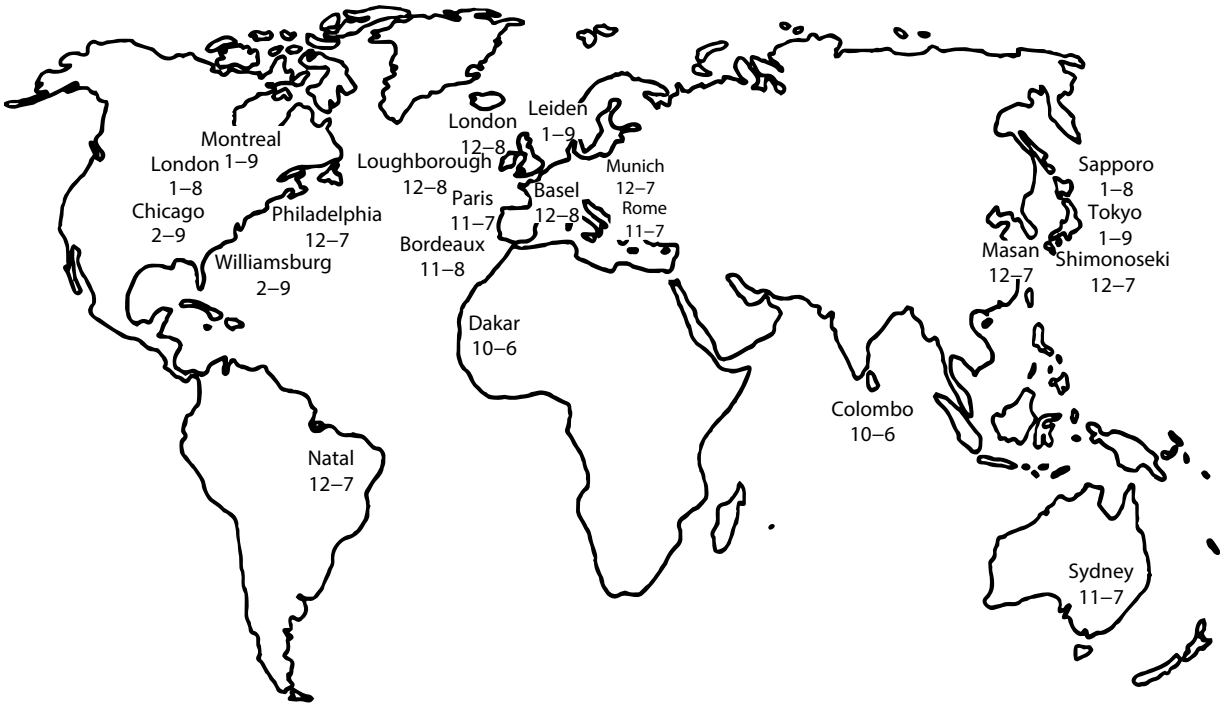


FIGURE 16.12 Sleep time. This diagram of the world indicates the habitual bedtimes and risetimes of young residents of various locations. To improve readability, values were rounded to full hours. All times are a.m. local time, except bedtimes 10 and 11, which are p.m. (From References 19, 22, 26, 32, 157, and 312–325.)

the morning, but these differences do not conform to a latitudinal gradient. Regardless of geographical location, *average* humans go to sleep sometime between 10 p.m. and 2 a.m. and wake up sometime between 6 a.m. and 9 a.m., most of them obtaining 7–8 hours of sleep each night. As discussed in Chapter 14, a small latitudinal gradient in activity times was described in a few studies within large countries[152,153] or across different countries,[154] although the differences are more likely due to sociocultural factors than to climatic factors. Interestingly, because time zones are set in 1-hour intervals (and are wider in many regions for political reasons), there is a *longitudinal* gradient in sunlight time within each time zone. Researchers have found that, at least in Germany, people living further east within a time zone tend to wake up and go to sleep earlier than people living further west in terms of official clock time.[155]

A survey of university students conducted in California in 1916 indicated that average bedtime and waketime were 11 p.m. and 7 a.m., respectively,[156] while a survey conducted in Virginia in 1995 indicated that average bedtime and waketime were 1:30 a.m. and 9 a.m.,[157] and a survey conducted in Rhode Island in 2013 indicated that the average bedtime and waketime were 2 a.m. and 9:30 a.m.[158] Thus, although college students are often said to be drastically sleep deprived, most of them incur less than a 1-hour sleep debt per night. As expected, however, waketimes are strongly determined by class schedules, so that a weekly pattern of waketimes (with delayed waketimes on weekends) can be observed in the records of many students.[157,159]

Because most of the studies whose data are shown in Figure 16.12 were conducted with university students, the bedtimes and waketimes are a little later than the averages for the whole population. Figure 16.13 shows the distribution of bedtimes and waketimes of 200 nontraditional university students (that is, individuals ranging in age from 18 to 65 years, many of them having other occupations and attending classes on a part-time basis) in rural South Carolina. The mean bedtime is 23:21, and the mean waketime is 07:35. Notice that some people go to sleep as late as 3 o'clock in the morning, and some people (not the same ones!) wake up as early as 5 o'clock in the morning. More extensive surveys involving 17,000 people in 24 countries[160] or 55,000 people in 4 countries[161] also found that most people go to sleep around midnight and wake up between 7 and 8 a.m.

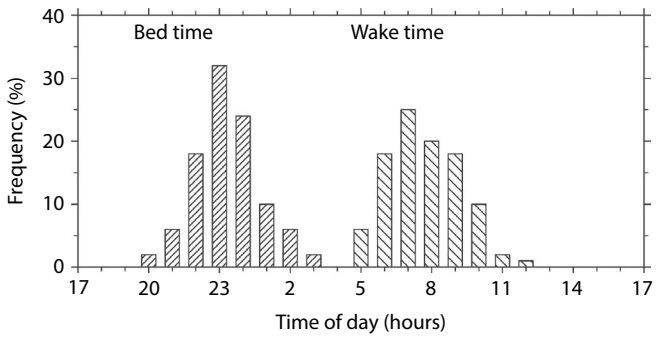


FIGURE 16.13 Distribution of sleep times. The graph shows the distributions of habitual bedtimes and waketimes of 200 adults in rural South Carolina. (Data from the archives of the Refinetti lab.)

When allowed to sleep as much as desired, most humans sleep 7–9 hours per night,[161–163] and the US National Sleep Foundation recommends that adults sleep 7–9 hours each day.[164] Sleep is not a luxury; it is needed for proper body function.[165,166] Correlational studies involving more than 1,300,000 people showed that mortality is higher for people who sleep much less or much more than 8 hours per night.[167]

Considering that most humans need 8 hours of sleep each day, the fact that most of us sleep fewer than 8 hours indicates that we are chronically sleep deprived. An informal survey reported in *TIME Magazine* indicated that 75% of American adults do not feel fully refreshed when waking up in the morning (Figure 16.14). Interestingly, it also indicated that 64% of the respondents hit the circadian gate on weekend mornings. In any event, most of us can get a good night of sleep if our work schedules allow us. Many people, however, face chronic sleep problems. Sleep disorders include insomnia (inability to sleep during the usual sleep time), hypersomnia (excessive sleepiness), narcolepsy (intrusion of sleep episodes into waketime), somnambulism (sleepwalking), and night terrors.[163] The prevalence of insomnia in the general population is reported to be quite high (10%–35%

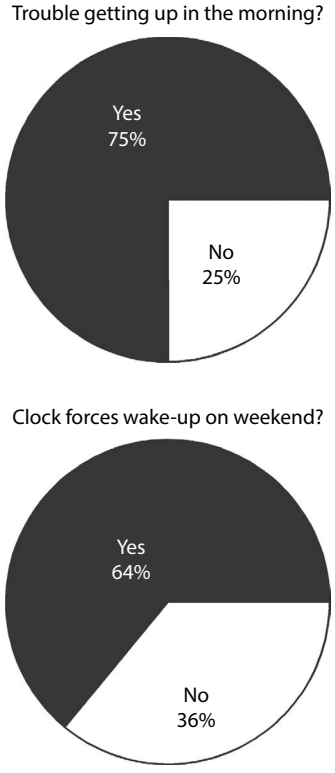


FIGURE 16.14 Common complaints about sleep. In a large—though not strictly scientific—survey conducted in the United States, randomly selected members of the general population were asked whether they regularly had difficulty getting up in the morning during week days and whether their body clocks forced them to wake up earlier than desired on weekends. (Data from *TIME*, New York, Vol. 161(21), p. 24, 2003.)

of all adults),[163] although it has been pointed out that many people who complain of inability to sleep actually misjudge their real needs and stay in bed longer than they need to.[168,169] As a matter of fact, in a study of 121 people who complained of insomnia and 56 who did not, electroencephalographic records over two nights in the laboratory showed no difference in sleep parameters between the complainers and the noncomplainers. Psychological tests conducted on these individuals indicated that the alleged insomniacs had more pathological personality profiles than the control subjects.[170]

Some instances of insomnia, such as those caused by sleep apnea, have no obvious relationship with circadian rhythms. Other instances, however, could potentially be due to disorders of the circadian system; among them are the so-called delayed sleep phase syndrome (DSPS) and advanced sleep phase syndrome (ASPS). DSPS and ASPS seem to be simple cases of an abnormal phase angle of entrainment.[171] ASPS patients feel very sleepy abnormally early in the evening and then wake up very early in the morning (say, 2 or 3 a.m.). Conversely, DSPS patients have difficulty falling asleep before 2 or 3 a.m. and, consequently, will not wake up spontaneously until late in the morning. DSPS is more common than ASPS, afflicting between 1 and 4 people out of 1000.[172] Neither syndrome involves actual lack of sleep if the patients can afford to set their work schedules according to their sleep habits, and studies of DSPS patients using electroencephalographic recording revealed no abnormalities in sleep structure.[173,174] However, most people do not have the luxury of determining their own work schedule. Consequently, DSPS and ASPS regularly result in chronic lack of sleep, which is experienced as *sleep-onset insomnia* in DSPS patients and as *sleep-termination insomnia* in ASPS patients.

As we saw in Chapter 14, some people are extreme "morning types" and some people are extreme "evening types." These chronotypes exhibit corresponding sleep times,[175] which could account for ASPS and DSPS. This is not the case for familial ASPS (FASPS), an ASPS condition that clearly runs in families and cannot be explained simply by an early chronotype.[176] FASPS has been traced to an abnormality in expression of the *per* gene[177] as a consequence of a mutation in the *ck1*δ gene.[178] DSPS may be related to a mutation in the *ck1*ε gene.[179] A mutation in the *dec2* gene is not related to either ASPS or DSPS but seems to affect the duration of sleep.[180]

Figure 16.15 shows diagrams depicting the presumptive abnormalities of phase angle of entrainment in ASPS and DSPS. The body temperature rhythm is used as an index of the circadian pacemaker. In normal people who wake up at about 7 a.m. and go to sleep at 11 p.m., the temperature rhythm peaks at 5 p.m. (indicated by the dashed line), or 6 hours before bedtime. In a person with ASPS, the clock is advanced by several hours. In Figure 16.15, an advance of 4 hours is depicted, which means that body temperature peaks at 1 p.m. and sleep is expected to start at 7 p.m. The person can usually stay awake past the expected bedtime (probably watching television) and not be troubled by the advanced rhythm. However, because the rhythm is advanced, his or her expected

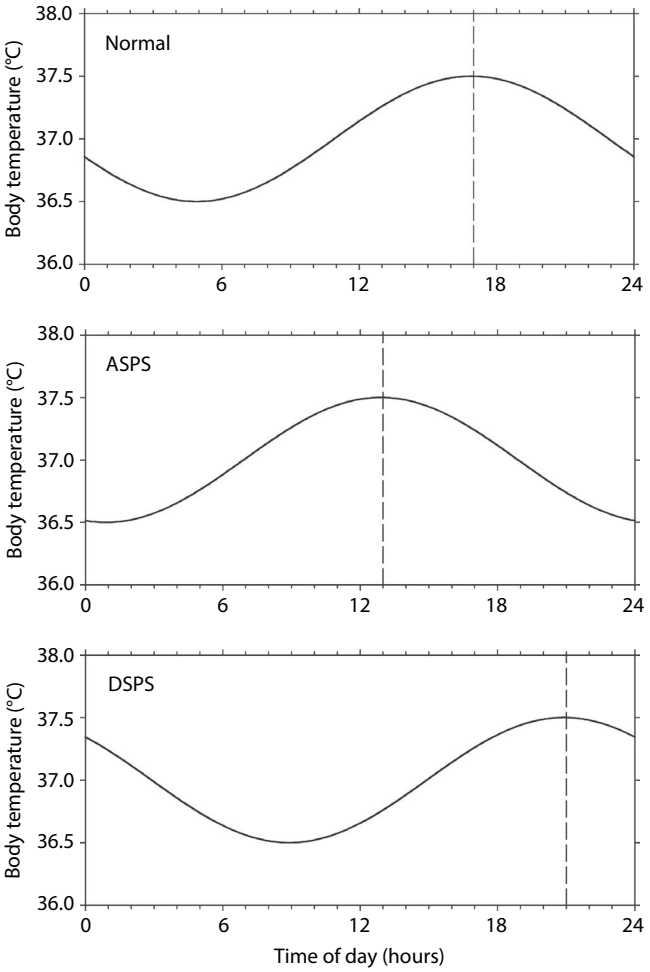


FIGURE 16.15 Sleep phase syndromes. These diagrams indicate that the phase of the circadian clock, as inferred from the phase of the human body temperature rhythm, is advanced in patients suffering from ASPS and is delayed in patients suffering from DSPS. The vertical dashed lines indicate the acrophase (time of peak) of the temperature rhythm.

wake-up time is 3 a.m. instead of 7 a.m. Thus, this person has no problem falling asleep at 11 p.m., but he or she will wake up too early, resulting in sleep-termination insomnia. In contrast, the clock is delayed by several hours in a person with DSPS. If this person's temperature rhythm peaks at 9 p.m., he or she will not feel sleepy until 3 a.m. Thus, this person will not be able to sleep at 11 p.m. (or midnight, or 1 a.m.) and will suffer from sleep-onset insomnia (which is what most people call simply *insomnia*). The alarm clock will wake him or her up at 7 a.m., but he or she will not feel refreshed.

If DSPS and ASPS actually derive from disturbances in the phase angle of entrainment (rather than from some other abnormality that only secondarily affects the phase angle), then our knowledge of circadian rhythms should allow us to understand the etiology of the disease. We saw in Chapter 7 that entrainment depends on three variables: the period of the pacemaker (τ), the period of the zeitgeber (T), and the phase–response curve of the pacemaker. The period of the zeitgeber is presumably 24 hours for all patients living in the real world,

so that we need only worry about the other two variables. If τ is shorter than 24 hours, the activity rhythm will be phase advanced in reference to the zeitgeber (that is, the person will want to wake up earlier); therefore, ASPS could result from τ being, say, 20 hours. Conversely, if τ is longer than 24 hours, the activity rhythm will be phase delayed; this is the case for most people (because the human τ is usually longer than 24 hours) but could be exaggerated in DSPS patients (say, τ = 28 hours). Just like the activity of the nocturnal mouse with a short τ was displaced into the daylight (Figure 7.25), the activity of the diurnal human with a long τ will be displaced into the nighttime. Alternatively (or in combination with an abnormal τ), the phase–response curve could be affected. Thus, the relative phase delay of DSPS patients could be due to reduced amplitude of the phase-advance region of the phase–response curve. A reduced amplitude would require that a wider section of the phase-advance region be photically stimulated, which could only be accomplished by starting the stimulation earlier during subjective night and terminating earlier during subjective day, thus extending the duration between lights off and bedtime. The combination of a small reduction in the amplitude of the phase–response curve and a small lengthening of τ would produce equivalent results.

Of course, all of this is hypothetical. It depends on whether DSPS and ASPS actually derive from disturbances in the phase angle of entrainment. Do they? The bedtime and wake-time data suggest that they do. However, we saw in Chapter 6 that the wake–sleep rhythm in humans can be very labile and not reflect the state of the circadian pacemaker. Have other rhythms, such as those of body temperature and melatonin secretion, been recorded in patients with sleep phase disorders? Yes, although only in a few studies.[181–184] Have the circadian period and the amplitude of the phase–response curve been determined in patients with sleep phase disorders? No,[185] except for one patient with FASPS, who did have a shorter circadian period consistent with the advanced phase angle of entrainment.[176]

Despite the scarcity of data about the etiology of sleep phase disorders, several forms of treatment have been employed. These have included chronotherapy, treatment with bright light exposure, and treatment with melatonin administration. All three of these forms of treatment have the peculiarity of not involving controlled drugs and, therefore, of not requiring a prescribing physician. *Sleeping pills*, which are traditionally used in the treatment of sleep disorders, do require a prescription from a physician. Sleeping pills (sedatives and hypnotics) are barbiturates, benzodiazepines, or other substances (such as zaleplon and eszopiclone).[186] The most common sleeping pills are benzodiazepines, such as diazepam (Valium) and estazolam (Prosom),[163] although they are being rapidly replaced by zaleplon (Sonata) and eszopiclone (Lunesta).[187] The structural formulas of diazepam and estazolam, which are very similar, are shown in Figure 16.16. Although sleeping pills are effective in inducing sleep, they do not necessarily affect the circadian system (and, even if they did, one would have to administer them not at bedtime but in accordance with the phase–response curve for each specific drug).

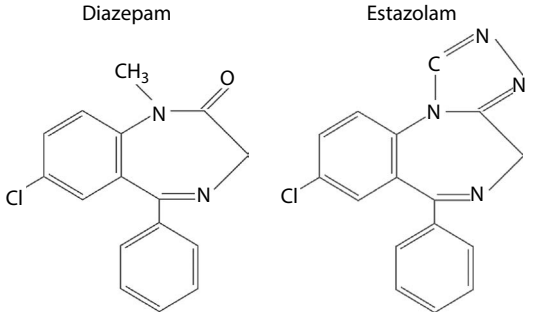


FIGURE 16.16 Sleeping pills. Benzodiazepines are often prescribed for the treatment of insomnia. The structural formulas of two benzodiazepines are shown: diazepam (Valium) and estazolam (Prosom). Diazepam is also widely prescribed for the treatment of anxiety. (From Sifton, D.W., ed., *Physician's Desk Reference*, 57th edn., Thomson PDR, Montvale, NJ, 2003.)

Therefore, sleeping pills correct only the homeostatic component of sleep, leaving the circadian component unaffected. Like most prescription drugs, they can also induce tolerance and dependence.

Chronotherapy is closer to a businesslike time-management initiative than to a medical treatment. It involves the realization that the problem at hand is simply one of doing the right thing at the right time. If the person is going to bed too late (and losing sleep only because he or she must wake up early in the morning to go to work), then the solution consists in moving the bedtime to an earlier time. Of course, the person cannot come up with a solution by himself/herself—otherwise, the problem would not exist to start with. However, considering that the usual human free-running period is longer than 24 hours, the adjustment in bedtime can be made if its direction is reversed: rather than trying to go to bed earlier, the person should delay the bedtime a few hours each day until he or she goes around the clock and reaches the desired bedtime.[188] The new phase angle of entrainment is unlikely to persist if the old phase angle was determined by abnormalities in τ or the phase–response curve, but it may very well persist if the true etiology is different. Except for the requirement of a week off from work to implement the transition, the procedure is very little disruptive and can be performed by the patient himself/herself without the consultation of a physician (or of a business adviser, or any other person at all). Therefore, it provides the convenience of a self-help method. As a matter of fact, the therapeutic action of chronotherapy may result simply from the establishment of a structured lifestyle, as it has been shown that high regularity in lifestyle is associated with high subjective sleep quality.[189] An attempt to obtain a 6-hour phase advance of the sleep–wake schedule of healthy subjects by successive 2-hour delays was unsuccessful, being plagued by disruptions in sleep, alertness, and circadian rhythms.[190]

Bright light treatment is based on the concept of light-induced phase shifting of the circadian pacemaker, as discussed in Chapter 7. Thus, if a patient is entrained to the 24-hour world with a chronic phase delay (DSPS), properly timed light pulses presented during the phase-advance region of the phase–response curve should cause a corrective

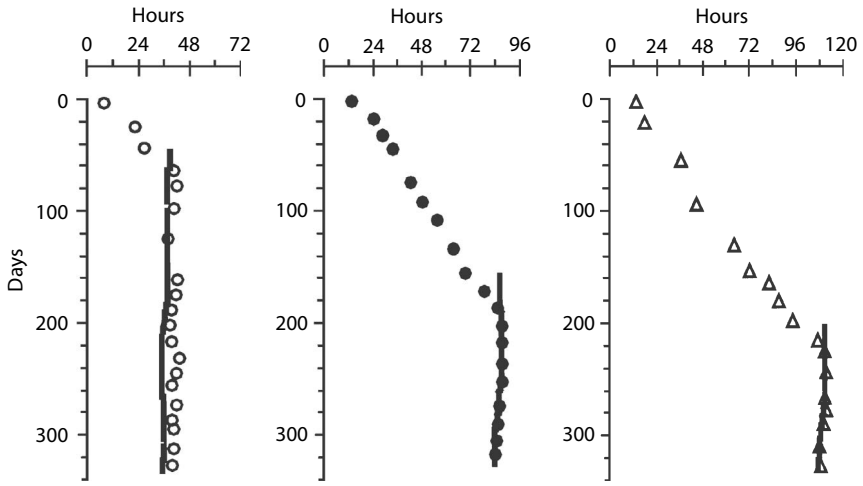


FIGURE 16.17 Entraining the blind. These pseudoactograms (single plots on multiple-day axes) show the onset times of the circadian rhythms of melatonin secretion of totally blind human subjects before and during daily administration of exogenous melatonin as a zeitgeber (0.5 mg orally). Before treatment, all three subjects exhibited free-running rhythms with periods longer than 24.0 hours. During treatment (vertical heavy lines), entrainment is apparent in all three cases. (Adapted from Lewy, A.J. et al., *J. Biol. Rhythms*, 19, 68, 2004.)

phase advance. Successful treatment of DSPS patients with phototherapy has been reported.[191–194] The program **Health**, which is part of the software package that accompanies this book, can help you work out the procedure on your own (see Exercise 16.2). I should note that this is one of those situations where the long-term success of a procedure contradicts the rationale on which it is based. If the circadian pacemaker was abnormally entrained because of its abnormal properties, then the phase shift caused by phototherapy should be very short-lived and, consequently, should be of very little clinical use. In other words, if the rationale for phototherapy is correct, then patients should require daily light pulses throughout their lives. The same is true about the apparent success in the use of *melatonin administration* in the treatment of sleep phase disorders.[195,196] It is possible that the therapeutic action of melatonin is dependent not on a nonparametric effect on the circadian pacemaker but rather on a hypnotic (sleep inducing) masking effect.[197,198] As shown in Figure 16.17, daily administration of melatonin seems to entrain human circadian rhythms, at least in totally blind persons who are not exposed to a conflicting cycle of light and darkness.[199] However, the rhythms were not allowed to freerun after the treatment, which means that true entrainment cannot be distinguished from masking. The distinction between masking and entrainment may be irrelevant for the patients. It is very relevant, however, that melatonin cannot be used simply as a corrective measure. In order to maintain entrainment (or masking), the patient would need to remain under melatonin treatment for the rest of his or her life.

Needless to say, the fact that physicians employ some form of treatment (and seem to obtain positive results) does not imply that the treatment is effective. As will be elaborated further in Section 16.3, physicians have the obligation to try to help their patients the best they can. They cannot afford to wait until all scientific doubts are eliminated. From a scientific perspective, the circadian etiology of ASPS and DSPS is largely hypothetical, and the efficacy of procedures used to treat these disorders may or may not exceed that of placebo. From a clinical perspective, however, potential solutions to the patients' predicaments are being offered—and oftentimes this is as much as physicians can hope for.

16.3 DEPRESSION

Mental disorders of one type or another affect the lives of one-third of all people. As shown in Figure 16.18, 35% of the US population either suffers from or is treated for mental illness. Notice that 8% of the population (or about 23 million people) is diagnosed and treated for mental illness. Almost the same percentage is treated even though not diagnosed (presumably because these people enjoy the experience of being treated). A full 20% (or one-fifth of the population) is diagnosed but not treated.[200] Although biomedical researchers have identified many genes and gene polymorphisms associated with mental disorders, the causes of mental illness remain mostly unknown.[201]

Affective disorders (which include depression, mania, and bipolar disorders) are a major component of mental illness. Figure 16.19 shows the percentages of US residents who are diagnosed with an affective disorder (whether treatment is rendered or not) each year or over their whole lifetimes. Each year, 14% of all women (and 9% of all men) is diagnosed with an affective disorder. Over the lifetime, the percentages rise to 24% for women and 15% for men.[202]

Although affective disorders usually do not involve the breakdown in logical thinking that is characteristic of other forms of mental illness popularly associated with the concept of *madness*, they can nevertheless be very incapacitating and may even lead to suicide. It is true that everyone is subject to occasional alterations in mood elicited by both internal and external factors (such as losing one's job and going through a divorce), and usually, there is nothing pathological in it.

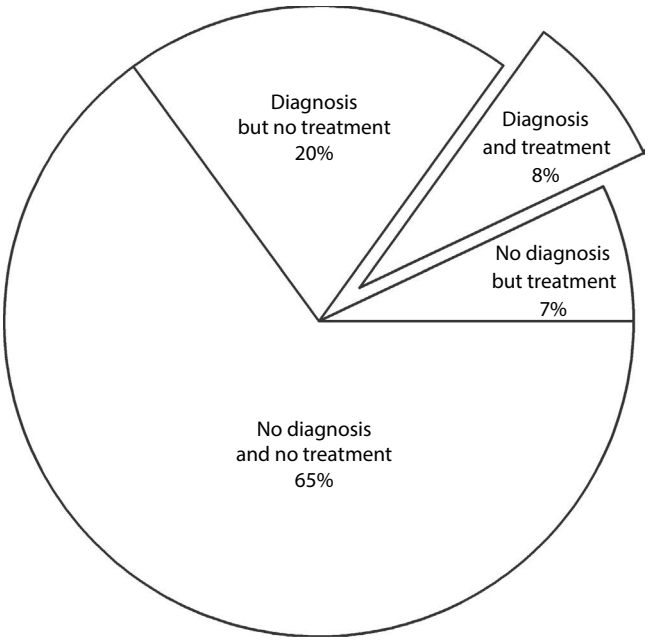


FIGURE 16.18 Prevalence of mental disorders and treatment. The graph shows the percentages of the US population that are diagnosed and treated for mental disorders each year. Notice that 8% of the population is diagnosed and treated each year and that 7% of the population is treated even though not diagnosed as having a mental disorder. Thus, 15% of the population (or about 43 million people) receives treatment for a mental disorder each year. (Data from U.S. Department of Health and Human Services, *Mental Health: A Report of the Surgeon General*, National Institute of Mental Health, Rockville, MD, 1999.)

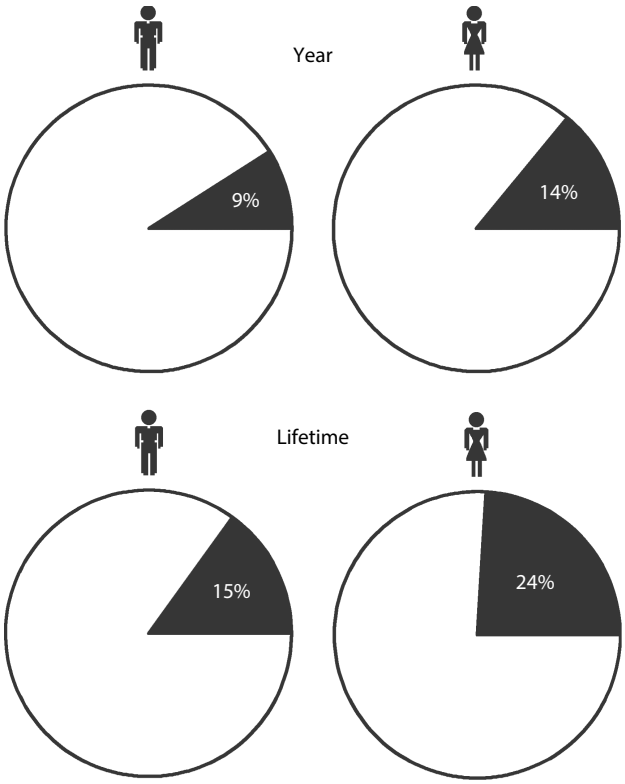


FIGURE 16.19 Prevalence of affective disorders. The diagrams show the prevalence of affective disorders (the most common of which is depression) in the US population. The values shown are the percentages of the population that were diagnosed with the disorder during the past 12 months (*year*) or during their lifetime (*lifetime*). (Data from Kessler, R.C. and Zhao, S., The prevalence of mental illness, in: Horvitz, A.V. and Scheid, T.L., eds., *A Handbook for the Study of Mental Health*, Cambridge University Press, New York, pp. 58–78, 1999.)

Overall happiness seems to be related much more to one's constitution than to events in one's life,[203] but small mood alterations are very common. Unfortunately, alterations in mood are sometimes strong enough to be diagnosed as depression or mania and, therefore, require medical attention. Depression is the most common affective disorder.[201] Table 16.2 shows the diagnostic criteria for depression (*major depressive disorder*) according to the American Psychiatric Association.[204] Five conditions must be met to justify the diagnosis of major depressive disorder, although the main defining feature is, of course, a state of profound sadness and lack of pleasure that persists for at least 2 weeks. In case you wonder whether you or a friend of yours are suffering from depression, the program **Health** (see Exercise 16.1) can help you conduct a brief self-diagnosis. Of course, the program is not a licensed therapist, and you should consult a psychiatrist or a psychologist if you believe that you need a professional diagnosis.

16.3.1 Traditional Treatment of Affective Disorders

Professionals involved in the treatment of mental disorders are generally either psychiatrists or psychologists. As medical doctors, psychiatrists can prescribe *psychotropic drugs*, whereas psychologists must rely on *psychotherapy* (the "talking cure"). Both psychiatrists and psychologists use scientific knowledge to guide their diagnostic and therapeutic procedures. However, as we try to relate the scientific knowledge of circadian rhythms discussed in previous chapters to its application in psychiatry and psychology, it is important to emphasize from the beginning that there is much more in medical and psychological practice than just the application of scientific knowledge to real-world situations. Although graduate school programs in clinical psychology often proclaim the goal of training *scientist practitioners*,[205] such expression is an oxymoron. The central mission of medicine (or psychological practice) is not to advance basic scientific knowledge but to improve the patient's health, to the same extent that the central mission of scientific research is to seek the truth regardless of its immediate application (Figure 16.20). When no scientific knowledge about a given condition is available, the practitioner is expected to do his or her best to alleviate the patient's ailment, whereas the scientist is expected to patiently chart the new territory at the pace dictated by empirical research. Only by understanding this fundamental difference between scientists and practitioners can we appreciate the efforts of both scientists and practitioners. As a matter of fact, lack of this understanding was likely the main cause of the division of circadian physiology into two antagonistic factions

TABLE 16.2
Diagnostic Criteria for Depression (Major Depressive Disorder)

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning: at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.
   1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feeling sad, empty, or hopeless) or observation made by others (e.g., appearing tearful).
   2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation).
   3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
   4. Insomnia or hypersomnia nearly every day.
   5. Psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down).
   6. Fatigue or loss of energy nearly every day.
   7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
   8. Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
   9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

C. The episode is not attributable to the physiological effects of a substance or to another medical condition. *Note:* Responses to a significant loss (e.g., bereavement, financial ruin, losses from a natural disaster, a serious medical illness or disability) may include the feelings of intense sadness, rumination about the loss, insomnia, poor appetite, and weight loss noted in Criterion A, which may resemble a depressive episode. Although such symptoms may be understandable or considered appropriate to the loss, the presence of a major depressive episode in addition to the normal response to a significant loss should also be carefully considered. This decision inevitably requires the exercise of clinical judgment based on the individual's history and the cultural norms for the expression of distress in the context of loss.

D. The occurrence of the major depressive episode is not better explained by schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, or other specified and unspecified schizophrenia spectrum and other psychotic disorders.

E. There has never been a manic episode or a hypomanic episode.

Source: Adapted from American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders*, 5th edn., American Psychiatric Publishing, Washington, DC, 2013.

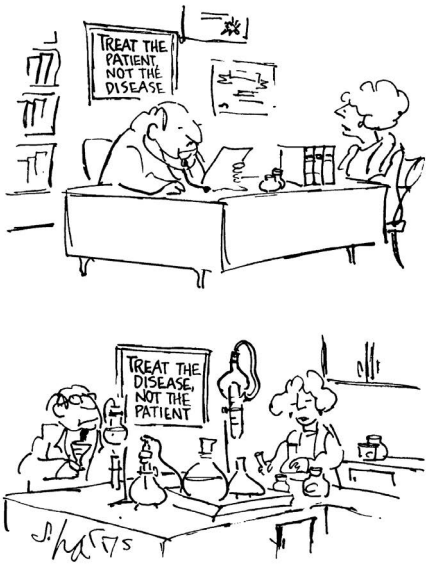


FIGURE 16.20 Physicians and researchers. This cartoon poignantly identifies the fundamental difference in the problem-solving approach of physicians and researchers. (Copyright 1991 Sidney Harris. Reprinted with permission.)

during most of its recent history, as we saw in Chapter 1. From the perspective of the scientist, practitioners misapply fundamental principles of circadian physiology in a manner that only a careless person would do. From the perspective of the practitioner, hardheaded adherence to fundamental principles deters applications that might relieve a patient's suffering. Thus, as we discuss the application of circadian physiology in psychiatric practice, it will be sensible to think of it not as a case of basic principles applied to practice but as a case of practice inspired by basic principles.

Traditional treatments of depression do not incorporate principles of circadian physiology. They rely either on psychotropic drugs or on psychotherapy (or both). Antidepressant drugs are effective in the treatment of depression, at least for some people.[206] Unfortunately, we know very little about how they work. A major roadblock in the understanding of the action of antidepressants (and other psychotropic drugs) is that, despite great progress in recent years, we know very little about how the brain processes mental activity. Because the antidepressant imipramine (Tofranil)—the first widely used antidepressant—was found to increase activity at synapses that utilize norepinephrine and dopamine as neurotransmitters, it was initially conjectured that depression might be the result of a deficiency in noradrenergic and dopaminergic

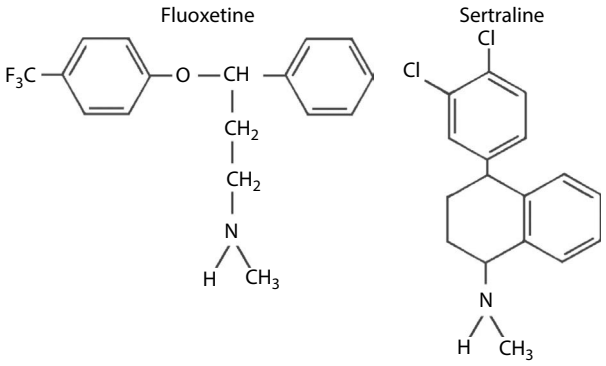


FIGURE 16.21 Antidepressant drugs. Selective serotonin reuptake inhibitors (SSRIs) are often prescribed for the treatment of depression. The structural formulas of two SSRIs are shown: fluoxetine (Prozac) and sertraline (Zoloft). (From Sifton, D.W., ed., *Physician's Desk Reference*, 57th edn., Thomson PDR, Montvale, NJ, 2003.)

neurotransmission.[207] Later on, fluoxetine (Prozac) and other *selective serotonin reuptake inhibitors (SSRIs)* (Figure 16.21) were introduced in the market. Because these potent antidepressants inhibit the synaptic reuptake of serotonin (thus increasing activity in serotonergic synapses), it was conjectured that depression might be the result of a deficiency in serotonergic neurotransmission.[208,209] In the late 1990s, Merck & Co. developed a new antidepressant (MK-869) that blocks synaptic receptors for the neurotransmitter *substance P* and does not seem to affect receptors for either serotonin or norepinephrine.[210] MK-869 turned out not to be better than placebo, though.[211] In 2008, Servier Laboratories released a new antidepressant called agomelatine (Valdoxan), which is a melatonergic agonist as well as a serotonergic antagonist and which performed well in clinical trials.[212,213]

The existence, and apparent efficacy, of all these antidepressant drugs indicates that we can produce drugs to treat depression—and that at least some of them work well. However, we can hardly say that we know how they really work. Even if we were confident about the neurotransmitter systems that are involved in depression, we would still need to identify the precise areas of the brain where they are located. A study on mice has raised the possibility that SSRIs act by stimulating neurogenesis in the hippocampus,[214] but, of course, further studies on humans are necessary to determine the significance of this finding. Recent studies suggest that depression may be caused by loss of synaptic connections in mood and emotion circuits in the brain, and that antidepressants can cure depression by increasing the number and function of synaptic connections.[215]

Evaluation of the efficacy of *psychotherapy* in the treatment of depression has a much more turbulent past. To start with, from a pharmacological perspective, all psychotherapy is placebo medication—for the simple fact that no drug is actually administered (although, of course, something must happen in the brain, otherwise there would be no behavioral change). As a matter of fact, even the behavioral action of psychotherapy is not well understood. Evaluation of the efficacy of psychotherapy is complicated by the fact that traditionally only a small fraction of people affected by mental disorders go through psychotherapy[200,202,216] (Figure 16.22). Thus, most cases of *antisocial personality* (such as psychotics, criminals, and juvenile delinquents) are isolated in special institutions and do not undergo psychotherapy (i.e., they receive other types of treatment or no treatment at all). Thirty to sixty percent of those who consult a psychotherapist and are accepted for treatment stop coming back before the therapist thinks they should do so. Of those who stay, about two-thirds are considered to show improvement. Thus, out of 100 people with mental disorders, only 40 undergo psychotherapy, 20 remain under treatment long enough, and 14 show improvement. Most of us would not call a 14:100 ratio a success. But we would not call it a failure either. After all, it is not the therapist's fault that patients are not sent in or do not wish to stay. What about the 2/3 improvement ratio? Well, this ratio may not be a success either. Since many people who are institutionalized do not undergo therapy, there is a natural control group against which to evaluate the efficacy of psychotherapeutic treatment. In 1952, H. J. Eysenck compared a group of adult

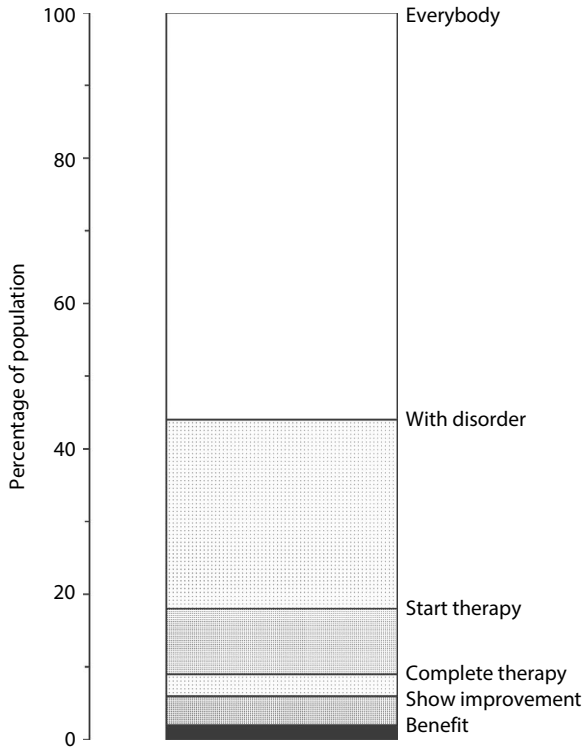


FIGURE 16.22 Benefit of psychotherapy. Less than half of the general population has a mental disorder. Less than half of those with a mental disorder undergo psychotherapy. Less than half of those who undergo psychotherapy show improvement. Less than half of those who show improvement actually benefit from psychotherapy. Still, about 3 million people in the United States benefit from psychotherapy each year. (Data from Bandura, A., *Principles of Behavior Modification*, Holt, Rinehart and Winston, New York, 1969; Shapiro, S. et al., *Arch. Gen. Psych.*, 41, 971, 1984; Kessler, R.C. and Zhao, S., The prevalence of mental illness, in: Horvitz, A.V. and Scheid, T.L., eds., *A Handbook for the Study of Mental Health*, Cambridge University Press, New York, 1999, pp. 58–78.)

neurotic patients undergoing psychotherapy with a similar group of patients not exposed to psychotherapy. His conclusion was straightforward: 2/3 of a group of adult neurotic patients will show some improvement within 2 years after the beginning of the illness irrespective of whether they underwent psychotherapy or not.[217] A few years later, a similar study on children reached a similar conclusion.[218]

The implications of the findings in the 1950s were very serious: 2/3 of the people who undergo therapy (and do not drop out) show improvement, but 2/3 of people who do *not* undergo therapy also show improvement. This means that psychotherapy provides no benefit at all! As the years went by, either psychotherapists improved their techniques or more sensitive measures of outcome were developed because, by the 1980s, a consensus developed that psychotherapy does have a small but real benefit.[219,220] There was a major problem, however: although there are many different types of psychotherapy—many of them based on principles totally different from, and often antithetical to, the others—the consensus reached by the experts was that all therapies are equally effective.[220,221] That is, in contrast to the therapist's belief (and the belief of most patients), the therapeutic value of psychotherapy resides simply in talking about one's problems to someone else—and not in learning how to *handle repressed feelings*, or how to *establish effective contingencies of reinforcement*, or how to *manage motivational needs*, or how to *develop optimal cognitive strategies*. Thus, the therapist's role is not that of a *scientist practitioner* but that of a *personal consultant* who advises a client the same way that a business consultant advises a business client. All the elaborate theories of psychopathology are just fancy costumes. This *demystification* of psychotherapy is consistent with the idea of *social construction* of the concept of mental illness.[222–224] As a matter of fact, it has been shown that even the participation of a therapist or consultant may not be necessary. Several health benefits can apparently be obtained by simply writing about one's own deep thoughts and feelings without any feedback from a therapist.[225] As recently as 2009, reputable clinicians lamented that "despite compelling research support for the merits of specific interventions for specific problems, clinical psychology, as a field, has failed to embrace these treatments, to standardize their use through formal practice guidelines, to promote and disseminate them widely through training, or to ensure that they are available to the patients who need them."[226] It has also been argued that some modalities of psychotherapy are not only ineffective but actually harmful.[227]

Figure 16.23 compares the response rates of various treatments of depression. "Response rate" refers to the percentage of treated patients who respond to the treatment (where *to respond* means to show some form of improvement, not necessarily to be cured). The best rate barely exceeds 50%, but notice that the rate for pill-placebo treatment is only 30%, which means that 20% (50% – 30% = 20%) of people who are treated and do not drop out actually benefit from the treatment. Interpersonal psychotherapy and cognitive behavioral psychotherapy have response rates comparable to that of pharmacotherapy (i.e., therapy based on antidepressant drugs).[206]

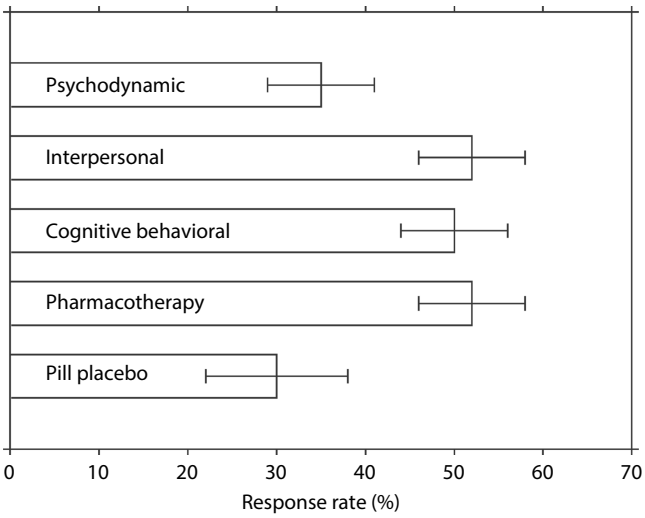


FIGURE 16.23 Efficacy of psychotherapy. The graph shows the mean (±SD) response rate of depressed patients who undergo different forms of psychotherapy as compared to that of patients receiving psychiatric medication or a pill placebo. Even for the best treatments, response rate is only 50%, whereas the response rate for placebo is up to 30%. (Means and approximate SDs computed from data provided by Hollon, S.D. et al., *Psychol. Sci. Public Interest*, 3, 39, 2002.)

A recent report suggests that the combination of the antidepressant venlafaxine (Effexor) with phototherapy may produce complete remission of depression in 76% of treated patients,[228] which is an unusually good outcome. We will talk more about phototherapy in Section 16.3.3, but it is clear that more research is needed to verify whether the combination of venlafaxine with phototherapy is indeed as effective as suggested by this study involving only 25 patients (and other 25 patients treated with venlafaxine without phototherapy).

16.3.2 Circadian Rhythmicity and Affective Disorders

Clinically depressed patients exhibit various abnormalities in circadian rhythms.[59,229–243] The most common abnormality reported in the literature is a small reduction in the amplitude of various daily rhythms, as exemplified in Figure 16.24. Daily rhythms of plasma cortisol were recorded in 14 patients suffering from major depressive disorder and 15 healthy controls. Both groups exhibited daily rhythmicity (with lower concentrations of cortisol around midnight) and responded to a meal (served at 19:00) with a transient elevation in cortisol secretion. Notice, however, that the nocturnal fall in cortisol concentration is slightly smaller in depressed patients than in normal subjects,[235] which results in a reduction in the amplitude of the rhythm. Another abnormality often reported is a change in the phase of the rhythms, although the findings are inconsistent. For instance, out of two studies conducted in the early 2000s, depressed patients were reported to have delayed rhythms in one study[237] but advanced rhythms in the other study.[239] It is possible that different rhythms behave differently (one group

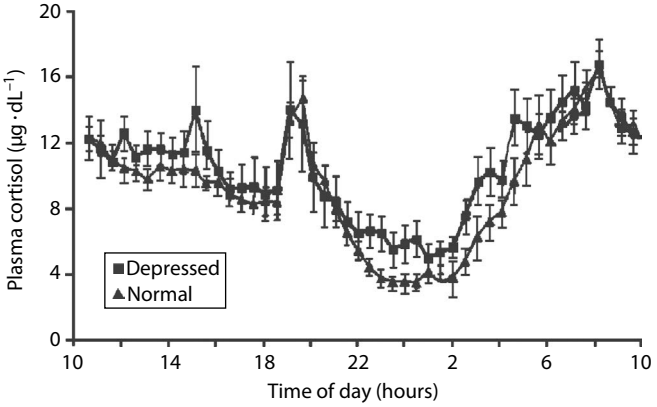


FIGURE 16.24 Depression and circadian rhythms. The graph shows the mean daily oscillation in plasma cortisol concentration (±SD) of 15 normal human subjects and 14 patients with major depression. Notice that the nocturnal trough of the rhythm is less accentuated in depressed patients than in healthy controls. (Adapted from Yehuda, R. et al., *Biol. Psych.*, 40, 79, 1996.)

studied the melatonin rhythm, whereas the other studied the cortisol rhythm), but, if so, the phase change is evidently not a characteristic of the central pacemaker. More importantly, all of these studies are of the correlational type, so that the question always remains whether the circadian abnormalities are the cause or the result of depression. Knowing that depression causes circadian dysfunction would be meaningful for clinical practice but would provide no breakthrough in the understanding of the disease. On the other hand, if circadian dysfunction were the *cause* of depression, then one might be on his or her way to a Nobel Prize in medicine.

Despite the lack of supporting evidence, several authors have suggested that disruption in circadian organization is an etiological (causal) factor in mental illness—particularly affective disorders.[213,244–250] One suggestion was that bipolar (manic depressive) disorder results from an abnormally phase-advanced clock.[231] The authors were renowned medical researchers with expertise in circadian physiology. Their effort to find a circadian physiological explanation for bipolar disorder is commendable. Should the causal link be true, one might be able to treat bipolar disorder with conveniently timed light pulses aimed at resetting the circadian clock. This would eliminate the undesirable side effects that often accompany psychotropic medication, would provide a *natural* alternative to pharmaceutical products, and would greatly reduce the financial burden of drug treatment (at the time of the writing of this book, a year's supply of Prozac, for example, cost over $6000). Unfortunately, the data on which the suggestion was based were very noisy and unconvincing. I averaged the various data sets and plotted the means in Figure 16.25. Daily rhythmicity is evident, but the cosine waves fitted to the means of the control group and to the means of the bipolar patients have identical acrophases. That is, the estimated phase of the circadian clock is the same for controls and patients. The data provide no evidence of an association between circadian phase and mental health.

Another team of physicians suggested that unipolar depression (major depressive disorder) is associated with a phase

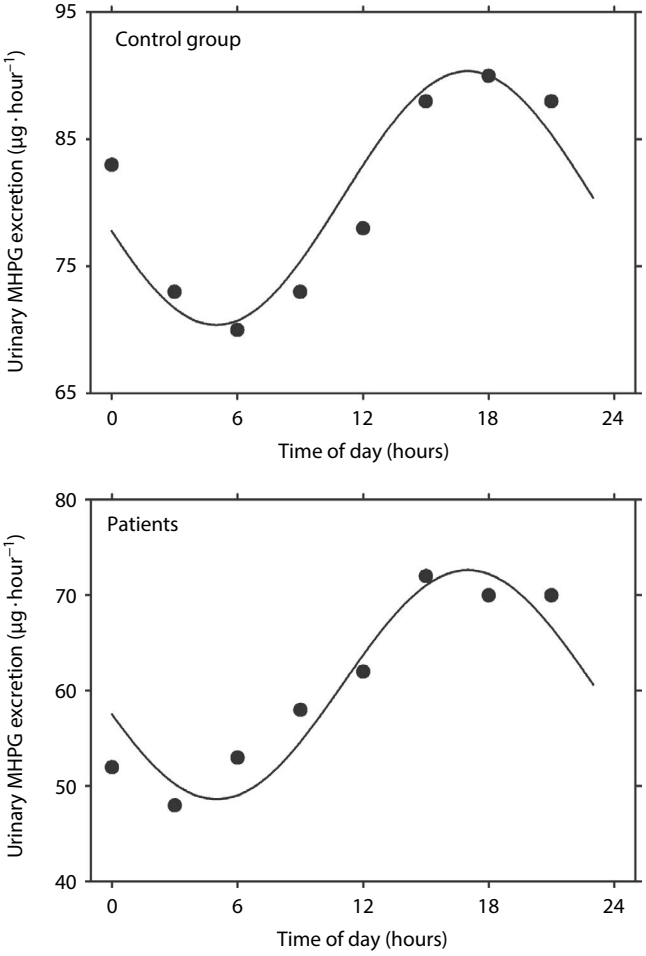


FIGURE 16.25 Are depressed patients phase advanced? It has been suggested that the circadian clock of bipolar patients (manic depressives) is 2–4 hours advanced in relation to that of healthy controls. The graphs show the data purported to support the phase-advance hypothesis. The data points are the means of 2 separate studies, one with 10 patients and 14 controls, the other with 8 patients and 15 controls. The drug 3-methoxy, 4-hydroxy-phenylglycol (MHPG) is a metabolite from brain norepinephrine whose concentration in urine was used as a phase marker of the circadian clock. The curves are single cosine waves interpolated to the data. There is absolutely no difference between the acrophases of the control group and of the patients. (Data from Wehr, T.A. and Wirz-Justice, A., *Pharmacopsychiatry*, 15, 31, 1982.)

delay of the circadian clock. Their data are shown in Figure 16.26. They correctly pointed out that the peak value of serum melatonin concentration is over an hour later in the depressed patients than in the controls (as indicated by the arrows).[237] However, the waveforms of the rhythms were not identical in the two groups, so that peak time is not a good criterion for comparing the groups. A better estimate of circadian phase can be obtained by first fitting a smooth cosine wave to the data. As you can see, the best-fitting cosine waves for the two groups have peaks at exactly the same time of day (3 a.m., as indicated by the dashed vertical line). Thus, in this case again, there is no difference in acrophase between the rhythms of patients and controls.

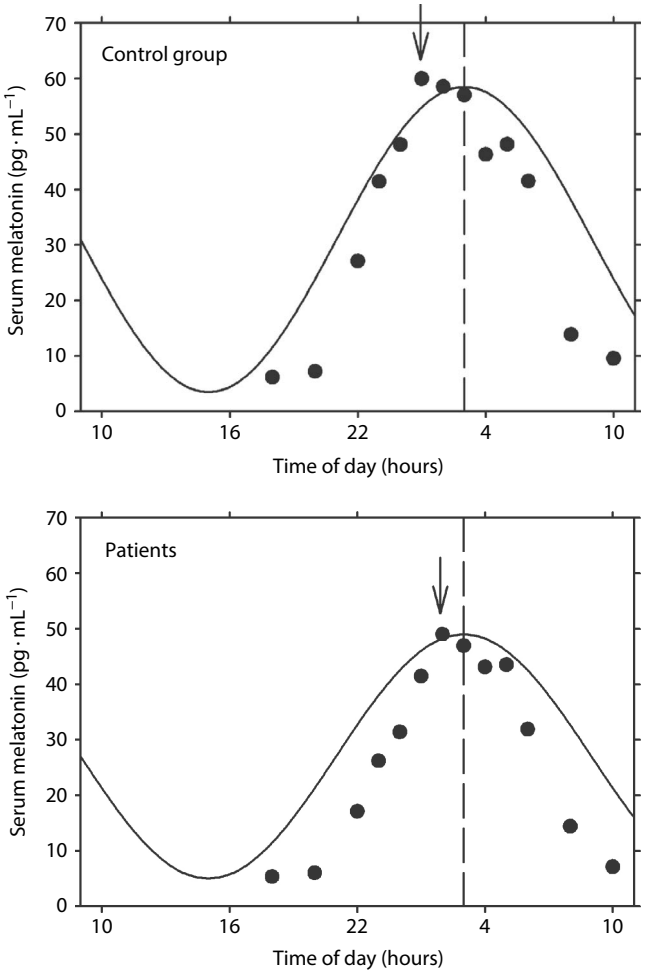


FIGURE 16.26 Are depressed patients phase delayed? The graphs show the mean concentrations of serum melatonin of 14 patients with major depression and 14 healthy controls, as measured in bed rest from 6 p.m. to 10 a.m. Because the highest mean value in patients occurred over an hour after the mean value in controls (arrows), the authors of the study suggested that depressed patients are phase delayed. However, the waveforms of the rhythms are different, so that fitting of a cosine wave is required for an appropriate comparison. The acrophases of the two cosine waves are identical (dashed vertical lines). (Data from Crasson, M. et al., *Psychoneuroendocrinology*, 29, 1, 2004.)

Because of the claims of correlation between circadian phase and depression, many basic researchers conducted experimental research in laboratory animals to determine whether the correlation implied causation. *Animal models* of depression admittedly have several limitations.[251] However, if one assumes that depression is the result of circadian disorganization, and it is known that antidepressant drugs relieve depression, then one must expect that antidepressants will affect circadian organization. Although imipramine (Tofranil) was shown not to affect circadian rhythms in laboratory animals,[252–255] other drugs such as lithium (Eskalith, Lithobid),[256–260] desipramine (Norpramin),[261] fluoxetine (Prozac),[262] clorgyline (an experimental antidepressant),[263,264] and rubidium (also an experimental antidepressant)[265] have been found to have small but significant effects. Using a different approach, researchers have shown that long exposure of nocturnal rodents to constant light (or moderate light at night under a light–dark cycle) can disrupt circadian rhythmicity at the same time as it produces depression-like behaviors.[266–268] One research team showed that exposure of mice to an ultradian light–dark cycle with 3.5 hours of light followed by 3.5 hours of darkness produces depression-like behavior and that this behavior is alleviated by administration of fluoxetine (Prozac).[269] Thus, the hypothesis that circadian disruption causes depression has not been refuted. It can hardly be said, however, that causality has been demonstrated. Real proof would consist of inducing depression in human subjects by experimental disruption of circadian rhythmicity and relieving the symptoms by restoring circadian organization. Of course, such an experiment would be unethical, if not criminal. More and better-conducted correlational studies in human patients and controls cannot answer the question of causation but could provide solid evidence in support of the correlational association.

Some promising studies have been conducted by Daniel Kripke (Figure 16.27) at the University of California, San Diego. He and his collaborators initially searched for single nucleotide polymorphisms in genes of patients suffering from affective disorders and identified a few candidate polymorphisms.[270] Later, they found a significant association of depressed mood with three single nucleotide polymorphisms of the *fmr1* (fragile X mental retardation) gene[271] as well as a significant association of bipolar disorder with a polymorphism of the *nf1a* (nuclear factor 1/A) gene.[272] Other research groups have found significant associations of bipolar disorder with single nucleotide polymorphisms of various circadian clock genes, such as *bmal1*,[273] *npas2*,[274] *cry2*,[275], *clk*,[276,277] and

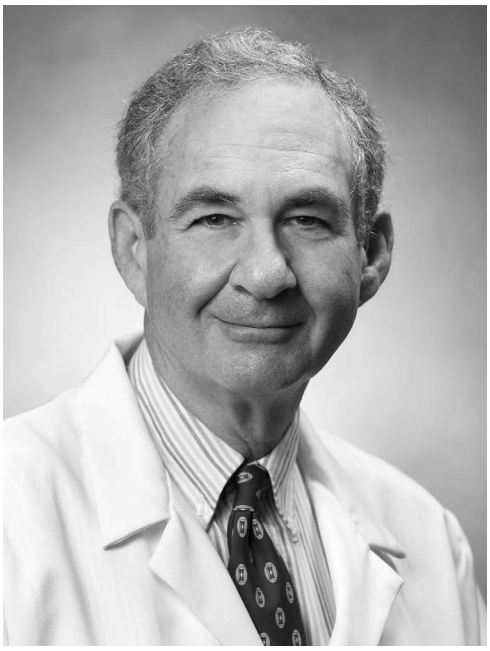


FIGURE 16.27 Daniel Kripke. This psychiatric researcher has conducted numerous studies relating psychiatric disorders to idiosyncrasies of circadian clock genes. (Photo courtesy of Daniel Kripke.)

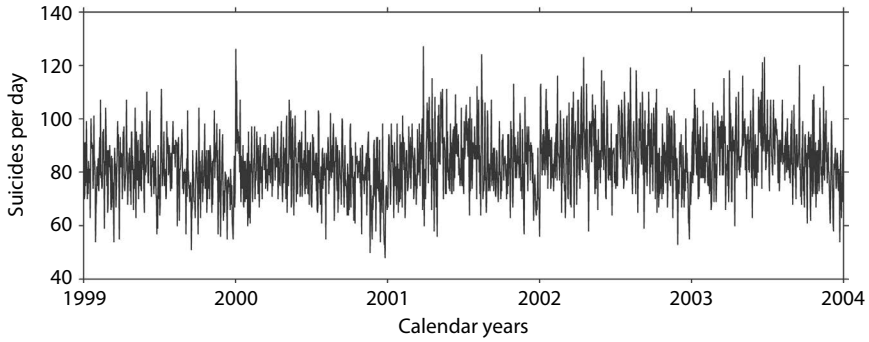


FIGURE 16.28 The season to die. The graph shows the daily number of suicides in the United States from the beginning of January 1999 to the end of December 2003. Looking past the day-to-day variability, one can see a seasonal oscillatory pattern. (Data from National Center for Health Statistics, U.S. Department of Health and Human Services.)

other genes.[278,279] As more is learned about circadian genes associated with affective disorders, a greater understanding of the relationship between the circadian system and affective disorders should be gained.

16.3.3 Seasonal Affective Disorder

A particular type of affective disorder, seasonal affective disorder (SAD), deserves special attention because of its rhythmic nature. SAD is a cyclic illness characterized by recurrent episodes of fall and winter depression alternating with periods of remission during the spring and summer.[280,281] We saw in Chapter 4 that many organismal variables, including mood, exhibit annual rhythmicity. Figure 16.28 shows national suicide data for the United States over an interval of 5 years. Despite significant day-to-day variability, a seasonal pattern can be seen, with the number of suicides per day tending to be higher than 80 around summer (June, July, and August) and lower than 80 around winter (December, January, and February). Making a loose assumption that people commit suicide when they are unhappy or annoyed, one could infer that more people feel unhappy or annoyed in the summer.

That the seasonal oscillation in SAD symptoms is not simply an expression of seasonal rhythmicity in human mood is demonstrated by the data shown in Figure 16.29, which depicts actual mood rather than suicides. Normal people (dark bars) feel best mostly in the spring and fall.[282,283] The distribution for SAD patients (white bars) is much sharper: no patient feels best during the winter months (December–February), and most of them feel best during the late spring and summer months (May–August).[283] It should be noted that patients with bipolar disorder sometimes exhibit seasonal variation in symptoms (and, thus, seasonal variation in depression), but most bipolar patients display no or only a mild seasonal pattern.[284]

Because of the strong seasonality in depressive symptoms of SAD patients, it was suggested early on that SAD might be under the influence of day length, as many annual rhythms in animals are (see Chapter 4). Indeed, although weather conditions were found to affect patients' self-rating of energy level, this effect was smaller than those of seasonal changes, which suggests that light exposure is the important variable.[285]

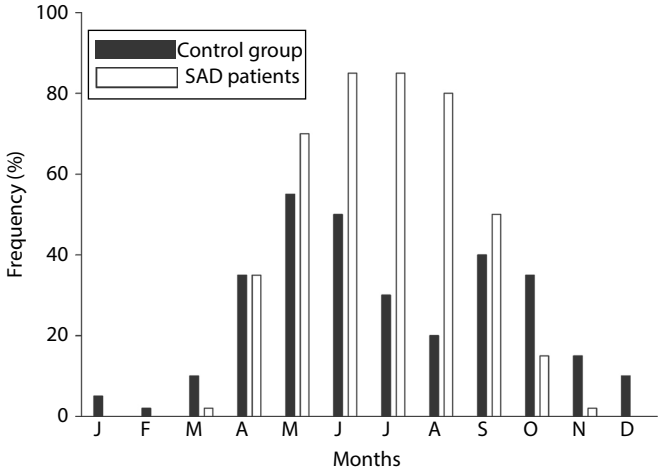


FIGURE 16.29 Seasonal depression. The graph shows the frequency distribution of answers to the question "When do you feel best?" asked to patients diagnosed with SAD and to a random sample of the general population (control group). Although people in the control group feel best mostly in the spring and fall, SAD patients show a much sharper seasonal variation in mood. (Data from Terman, M., *J. Biol. Rhythms*, 3, 155, 1988.)

SAD patients and healthy controls expose themselves to light similarly, but SAD patients could be more sensitive than controls to the seasonal variation in day length.[286] Accordingly, attempts were made to treat SAD by artificially extending the duration of daylight during the winter, typically by providing bright light stimulation for several hours in the early morning and early evening. A diagram of the rationale is shown in Figure 16.30. During the summer, the days are long (say, sunlight from 5 a.m. to 8 p.m.), whereas the days are short in the winter (say, from 7 a.m. to 4 p.m.). Thus, a summer day could be simulated in the winter by the provision of artificial light from 5 to 7 a.m. and from 4 to 8 p.m.

Various authors reported mood improvements in SAD patients subjected to this form of treatment,[287–290] and light boxes like the one pictured in Figure 16.31 gained popularity. However, it soon became clear that the effects of light therapy (or *phototherapy*) were not due to the extension of day length.[291–298] For instance, it was found that a single interval of light stimulation in the morning was just as effective as the morning–evening

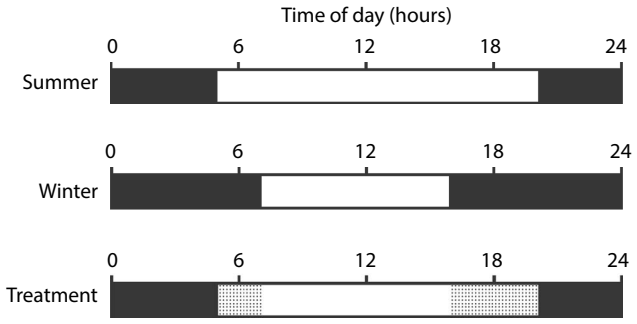


FIGURE 16.30 Rationale for phototherapy. Because SAD is more common in the winter than in the summer, phototherapy was designed to simulate a summer photoperiod during the winter. Artificial light (hatched area) was considered to be necessary in the early morning and in the evening. Later studies revealed that artificial light only once a day may be sufficient.



FIGURE 16.31 Phototherapy. Timed exposure to artificial light is a common treatment for SAD. (Image courtesy of BioLight Group, Santa Barbara, CA.)

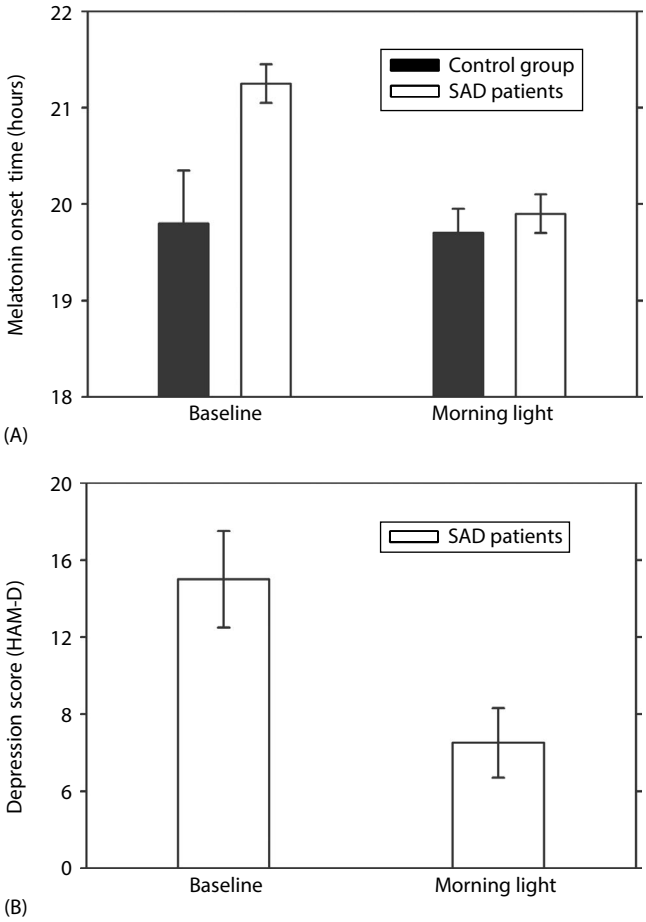


FIGURE 16.32 SAD and phase delay. The graphs show the onset time of melatonin secretion (as a phase marker of the circadian clock) and the depression score (in the Hamilton depression scale, HAM-D) of SAD patients and healthy controls before and after phototherapy. Phototherapy consisted of exposure to artificial light in the morning (6 a.m. to 8 a.m., 2500 lux) for a week. The bars correspond to the means (±SE) of 8 SAD patients and 7 controls. Notice that the SAD patients were phase delayed in relation to controls before the treatment. Phototherapy not only corrected the phase disorder (A) but also caused a significant reduction in the depression score (B). (Data from Lewy, A.J. et al., *Science*, 235, 352, 1987.)

combination and, as a matter of fact, that a single interval of stimulation in the early afternoon (which, of course, does not extend the day length) was also effective.[292] Attention was then focused on the potential phase-shifting effects of phototherapy. As discussed in Chapter 7, appropriately timed light pulses can shift the phase of circadian rhythms in humans and other animals. If, for instance, SAD were associated with an intrinsic phase delay of the circadian clock, then light pulses presented in the early morning could evoke a compensatory phase advance. The results of a study that tested this possibility are summarized in Figure 16.32. The circadian phase marker used in the study was the onset time of nocturnal melatonin secretion. Clearly, SAD patients had delayed rhythms, as compared to controls, during the pretreatment baseline (Panel A). After the treatment (daily morning light exposure for a week), the rhythms of the two groups had equivalent phases. Importantly, the depression scores of SAD patients were also reduced by the treatment (Panel B).[293]

A problem with this interpretation of the therapeutic effect of phototherapy is that different researchers have obtained very different results. While some found that morning light was more effective than afternoon or evening light in relieving depressive symptoms,[297,298] others did not.[299,300] Likewise, while some found untreated SAD patients to have phase-delayed rhythms,[297,298] others found them to have normally phased rhythms.[289,301–303] The results of a careful study conducted on SAD patients and healthy individuals under controlled laboratory conditions are summarized in Figure 16.33. Patients were tested both when they were depressed and when they were not. Mean values of circadian

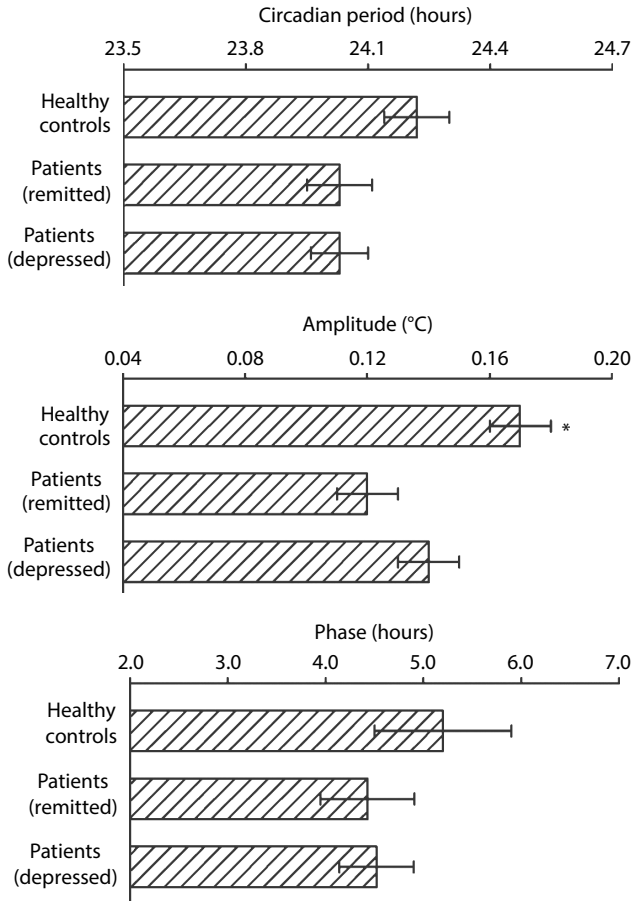


FIGURE 16.33 SAD and circadian rhythms. The graphs compare healthy controls with depressed and remitted SAD patients in terms of circadian period, amplitude, and phase of the rhythm of melatonin secretion. The bars correspond to the means (±SE) of 7 patients and 7 controls. Healthy controls have slightly higher means in the three variables, but significantly so only in amplitude (asterisk). Notice that depressed and remitted patients do not differ in any of the three variables. (Data from Koorengevel, K.M. et al., *J. Biol. Rhythms*, 17, 463, 2002.)

period, amplitude, and phase did not differ between the depressed and the remitted states. Also, the only significant difference between patients and controls was in the amplitude of the body temperature rhythm.[302] Similar to what we saw earlier concerning patients with major depressive disorder, SAD patients had rhythms with smaller amplitude. Notice, however, that the amplitude is smaller than that of controls both when the patients are depressed and when they have remitted. This implies that reduced amplitude is a constitutive characteristic of people predisposed to depression and not a functional property of the depressive state.

We saw in Chapter 11 that the circadian system senses light not only with retinal rods and cones but also with retinal ganglion cells that use the photopigment melanopsin. Because melanopsin is most sensitive to blue light, some practitioners proposed the use of blue light for phototherapy.[304] However, the fact remains that the circadian system uses rods and cones, so that a broader spectrum of stimulation (such as white light) is likely to be much more effective than monochromatic blue light.[305]

It would seem justified to conclude that the mechanism by which phototherapy relieves SAD symptoms is not yet known.[306] However, whether or not the therapeutic action of bright light stimulation is known, the treatment itself may be effective. The authors of a literature review of clinical studies concluded that phototherapy is as effective as pharmacotherapy in the treatment of depression and SAD,[307] but a major problem involving efficacy evaluation of treatment is that well-controlled, double-blind studies (i.e., studies in which neither the patient nor the investigator can subjectively bias the results) are not feasible when light treatment is involved, as the patient can always tell whether light is administered or not.[308] The average treatment efficacy of phototherapy is reportedly about 50%, which is only slightly higher than the high end of the placebo effect for antidepressant drugs (20%–40%).[283] A follow-up of SAD patients for almost 9 years revealed persistence of symptoms in 86% of the patients, which means that only 14% of the patients overcame the disease, even though 41% of them continued to use phototherapy (most of them in combination with antidepressant drugs).[309] Clearly, the outcome of treatment for SAD is not impressive. Attempts to conduct placebo-controlled studies by utilizing sounds and dim lights as the placebo condition produced minimal or null evidence that phototherapy is more effective than placebo alone.[310,311] Much more research is still needed to settle the issue. Because light at moderate intensities causes no harm, phototherapists can at least rest assured that they are not breaking their Hippocratic Oath, "Do no harm."

SUMMARY

1. Chronotherapeutics is the prevention and treatment of disease informed by knowledge of circadian rhythms. It refers to improved diagnosis attained by monitoring of daily oscillations in vital signs and by knowledge of rhythmic properties of pathological processes, as well as to improved treatment attained by knowledge of daily oscillations in the body's response to parasites and therapeutic drugs. Chronopharmacological treatments of hypertension, cancer, and asthma exemplify the use of chronobiological information to maximize pharmacological effects and minimize side effects.
2. Sleep disorders include insomnia, hypersomnia, narcolepsy, somnambulism, and night terrors. Some instances of insomnia seem to be due to disorders of the circadian system. People suffering from delayed sleep phase syndrome (DSPS) experience sleep-onset insomnia, whereas people suffering from advanced sleep phase syndrome (ASPS) experience sleep-termination insomnia. These sleep disorders are often treated by chronotherapy, by timed bright light exposure, or by melatonin administration. The treatments are safe, but their efficacy has not been rigorously demonstrated.

3. Depression (a state of profound sadness and lack of pleasure that persists for at least 2 weeks) is a serious psychiatric disorder that affects one out of five people at least once in their lifetimes. Traditional treatments of depression involve either psychotropic drugs or psychotherapy (or both). Although there is evidence that depression is associated with dysfunction of circadian rhythms, the direction of the causal link (if there is one) has not been adequately determined. This uncertainty applies to major depressive disorder as well as to bipolar disorder and seasonal affective disorder.

EXERCISES

16.1 Are you depressed? In Section 16.3, we saw that psychiatric depression may be related to circadian dysfunction. Whether or not the relation is significant, there is no doubt that depression is a debilitating disorder that afflicts a considerable number of people at least once in their lives. Therefore, a simple, informal diagnostic tool should be easily available. The program **Health** (the third icon from the right in the Circadian banner) contains such a tool. Just start the program and select the first item in the main menu ("I am depressed"). After you answer several questions, a diagnosis will be provided. Needless to say, the diagnosis will be unreliable if you are not honest or accurate in your answers. Also, although the questions are based on valid questionnaires developed by practicing psychiatrists, ***Health*** *is not a validated diagnostic instrument*. The diagnosis provided by the program is suggestive only. Use of the program should not, under any circumstances, deter you from seeking medical help if you feel sick or direct you to incur medical expenses if you do not feel sick.

16.2 Can you sleep well at night? In Section 16.2, we discussed sleep disorders, particularly sleep disorders due to circadian dysfunction. The same program **Health** that you used in the previous exercise has a section on sleep disorders. Just start the program and select the second item in the main menu ("I can't sleep well"). After you answer several questions, a tentative diagnosis will be provided. Depending on the diagnosis, a corrective course of action may also be provided. Of course, it is assumed that your sleep problems are only moderately annoying to you and that, consequently, you are willing to try a few simple corrective measures. If you have severe sleep problems, you should consult a sleep therapist right away. Most hospitals nowadays have a sleep clinic or are associated with one.

16.3 Crosswords. As we approach the end of the book, now is a good time to review some of the material covered in past chapters in a light manner through a popular pastime. About half of all words in these crosswords are related to circadian physiology. The crossword puzzle is in Figure 16.34. The key appears in Figure 16.35.

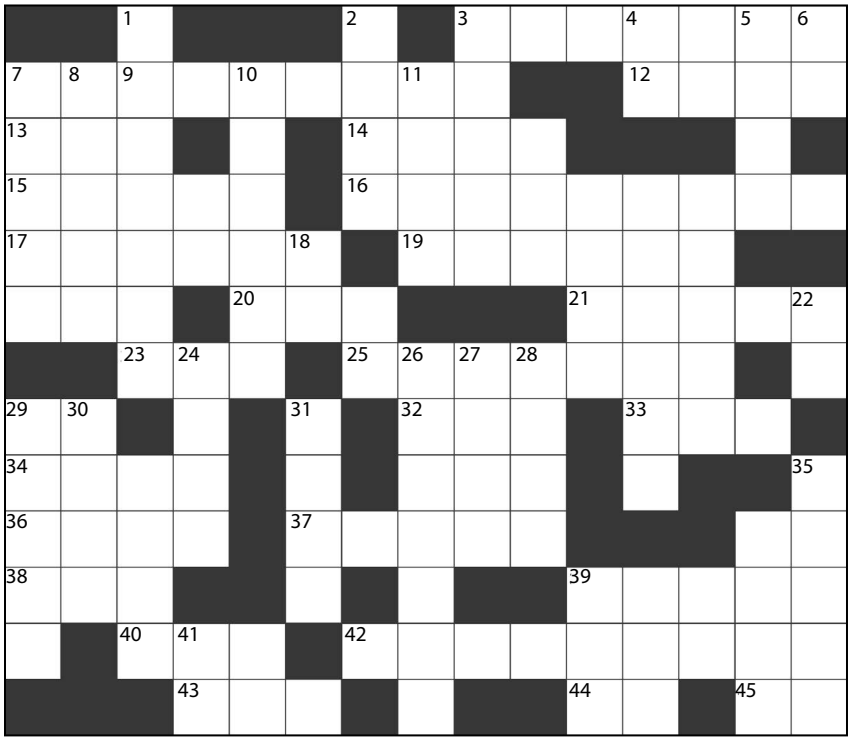


FIGURE 16.34 Crosswords. See Exercise 16.3.

Across

3 Disruption in the expression of overt rhythms without changes in phase or period of the pacemaker
7 The time at which the peak of a rhythm occurs
12 A woodwind instrument
13 Laughing out loud
14 The direction opposite to east
15 Uncomplicated; simple
16 The newer portion of the cerebral cortex that serves as the center of higher mental functions for humans
17 One who honks
19 One who drives
20 A common Boolean operator
21 A musical composition for developing a specific technique
23 A nocturnal bird
25 The state of a rhythm in the absence of effective zeitgebers
29 Anno Domini
32 The evening preceding a holiday
33 One of the Big Five studios of Hollywood's Golden Age
34 A nonspatial continuum in which events occur in apparently irreversible order
36 A disclosure of confidential information
37 With a spiral or coiled shape
38 The utmost possible
39 To clean one's body with water
40 A standard instrument in the study of sleep
42 Adjective describing a biological process that occurs in approximately 24-hour intervals
43 One of the three entities of Freud's theory of personality
44 Teaching assistant
45 North Dakota

Down

1 The city to which one flies to go to Disney World
2 The time each morning at which daylight begins
3 An estimate of central tendency of the distribution of values of an oscillating variable
4 Knockout
5 Informal "no"
6 A major company that produces electrical appliances
7 The segment of a circadian cycle during which the organism is active
8 Property of visual experience associated with electromagnetic wavelength
10 An endocrine gland that secretes melatonin
11 A ripened plant ovule containing an embryo
18 Registered nurse
22 "And" in Latin
24 A time interval consisting of 7 days
26 A newly engaged member of a military force
27 Morally bad or wrong
28 Evoking inexplicable fear or uneasiness
29 A book of maps
30 Same as nycthemeral
31 The part of the vertebrate body nearest to the spine
35 To make a uniform mixture
41 Electrical engineering

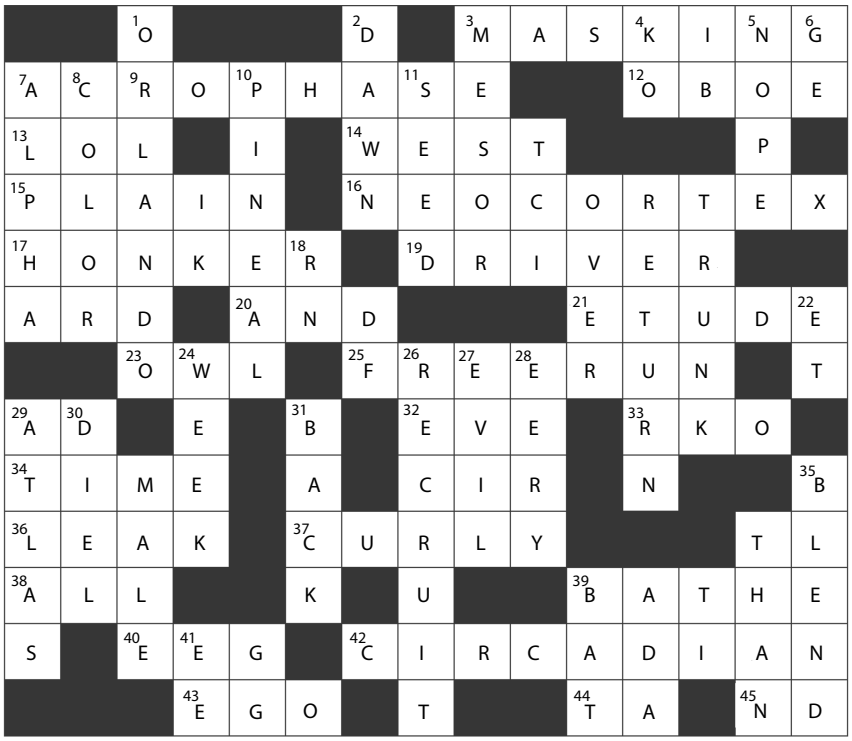


FIGURE 16.35 Solution to crosswords. This is the key for the crossword puzzle in Figure 16.34.

SUGGESTIONS FOR FURTHER READING

Halberg, F. and Watanabe, H. (Eds.) (1992). *Chronobiology and Chronomedicine*. Tokyo, Japan: Medical Review.

Proceedings of a Symposium on Chronomedicine held in Tokyo in 1990. Special emphasis is placed on the monitoring of blood pressure in human patients.

Youan, B. C. (Ed.) (2009). *Chronopharmaceutics: Science and Technology for Biological Rhythm Guided Therapy and Prevention of Diseases*. New York: Wiley.

An edited book with contributions from diverse authors covering various aspects of the timed administration of medical drugs.

Smolensky, M. and Lamberg, L. *The Body Clock Guide to Better Health: How to Use Your Body's Natural Clock to Fight Illness and Achieve Maximum Health*. New York: Henry Holt & Co., 2001.

A self-help book for general audiences written by an expert in chronotherapeutics.

Lévi, F. (Ed.) (2002). Cancer chronotherapeutics. *Chronobiology International* 19(1).

This 323-page special issue of the journal *Chronobiology International* is filled with review articles by specialists in a variety of fields of cancer therapy using chronobiological approaches.

Latta, F. and Van Cauter, E. (2002). Sleep and biological clocks. In: Weiner, I. B. (Ed.), *Handbook of Psychology* (*Biological Psychology*, Vol. 3). New York: Wiley, pp. 355–377.

A very readable review article that discusses the interaction of sleep and circadian rhythmicity in the control of the 24-hour profiles of behavior and physiological functions, particularly in humans.

Chokroverty, S. (Ed.) (2009). *Sleep Disorders Medicine: Basic Science, Technical Considerations, and Clinical Aspects*, 3rd edn. Philadelphia, PA: W.B. Saunders.

A handbook of sleep medicine for medical practitioners.

Ayers, S., Baum, A., McManus, C., Newman, S., Wallston, K., Weinman, J., and West, R. (Eds.) (2007). *Cambridge Handbook of Psychology, Health and Medicine*, 2nd edn. Cambridge, U.K.: Cambridge University Press.

A valuable reference tool for medical practitioners, this handbook of health psychology provides detailed information about the use of psychology for the improvement of physical health. Almost 200 contributors provide advice on topics ranging from abortion to the XYY syndrome.

Rosenthal, N. E. (2012). *Winter Blues: Seasonal Affective Disorder*, 4th edn. New York: Guilford Press.

A book on SAD written for general audiences by a renowned medical researcher from the National Institute of Mental Health.

Wirz-Justice, A., Benedetti, F., and Terman, M. (2013). *Chronotherapeutics for Affective Disorders*, 2nd edn. Basel, Switzerland: Karger.

A manual for nonpharmacological, nonpsychotherapeutical treatment of bipolar and unipolar affective disorders.

WEBSITES TO EXPLORE

American Psychiatric Association: http://www.psych.org/.
American Psychological Association: http://www.apa.org/.
American Circadian Lighting Association: http://www.americanhealthlights.org/.
Feeling Good (David D. Burns, MD): http://www.FeelingGood.com/.
International Circadian Lighting Association: http://www.circadianlighting.org/.
Lighting Research Center at Rensselaer Polytechnic Institute: http://www.lrc.rpi.edu/programs/lighthealth/.
National Center on Sleep Disorders Research: http://www.nhlbi.nih.gov/about/org/ncsdr/.
Sleep Disorders Guide: http://www.sleepdisordersguide.com/.
Sleep Network: http://www.sleepnet.com/.
Stanford Center for Sleep Sciences and Medicine: http://sleep.stanford.edu/.
U.S. National Institute of Mental Health: http://www.nimh.nih.gov/.

REFERENCES

1. Smolensky, M. H. and D'Alonzo, G. E. (1993). Medical chronobiology: Concepts and applications. *American Review of Respiratory Diseases* 147(6): S2–S19.
2. Jores, A. (1936). Rhythmikphysiologie und -pathologie des Menschen. *Naturwissenschaften* 24: 408–412.
3. Halberg, F. and Howard, R. B. (1958). 24 Hour periodicity and experimental medicine: Examples and interpretations. *Postgraduate Medicine* 24: 349–358.
4. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
5. Ogle, W. (1866). On the diurnal variations in the temperature of the human body in health. *St. George's Hospital Reports* 1: 221–245.
6. Benedict, F. G. (1904). Studies in body temperature. I. Influence of the inversion of the daily routine; the temperature of night-workers. *American Journal of Physiology* 11: 145–169.
7. Kleitman, N. and Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
8. Mellette, H. C., Hutt, B. K., Askovitz, S. I., and Horvath, S. M. (1951). Diurnal variations in body temperature. *Journal of Applied Physiology* 3: 665–675.
9. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.
10. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.
11. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
12. Aschoff, J., Gerecke, U., and Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.

13. Marotte, H. and Timbal, J. (1981). Circadian rhythm of temperature in man: Comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
14. Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
15. Gander, P. H., Connell, L. J., and Graeber, R. C. (1986). Masking of the circadian rhythm of heart rate and core temperature by the rest-activity cycle in man. *Journal of Biological Rhythms* 1: 119–135.
16. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
17. Scales, W. E., Vander, A. J., Brown, M. B., and Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
18. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K., and Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
19. Cisse, F., Martineaud, R., and Martineaud, J. P. (1991). Circadian cycles of central temperature in hot climate in man. *Archives Internationales de Physiologie, de Biochimie et de Biophysique* 99: 155–159.
20. Shanahan, T. L. and Czeisler, C. A. (1991). Light exposure induces equivalent phase shifts of the endogenous circadian rhythms of circulating plasma melatonin and core body temperature in men. *Journal of Clinical Endocrinology and Metabolism* 73: 227–235.
21. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T., and Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
22. Honma, K., Honma, S., Kohsaka, M., and Fukuda, N. (1992). Seasonal variation in the human circadian rhythm: Dissociation between sleep and temperature rhythm. *American Journal of Physiology* 262: R885–R891.
23. Barrett, J., Lack, L., and Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
24. Lee, Y. H. and Tokura, H. (1993). Circadian rhythm of human rectal and skin temperatures under the influences of three different kinds of clothing. *Journal of Interdisciplinary Cycle Research* 24: 33–42.
25. Pollak, C. P. and Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
26. Kräuchi, K. and Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *American Journal of Physiology* 267: R819–R829.
27. Honma, K., Honma, S., Nakamura, K., Sasaki, M., Endo, T., and Takahashi, T. (1995). Differential effects of bright light and social cues on reentrainment of human circadian rhythms. *American Journal of Physiology* 268: R528–R535.
28. Van Dongen, H. P. A., Kerkhof, G. A., and Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
29. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C., and Van Hoecke, J. (2001). Nycthemeral variations in core temperature and heart rate: Continuous cycling exercise versus continuous rest. *International Journal of Sports Medicine* 22: 553–557.
30. Moussay, S., Dosseville, F., Gauthier, A., Larue, J., Sesboüe, B., and Davenne, D. (2002). Circadian rhythms during cycling exercise and finger-tapping task. *Chronobiology International* 19: 1137–1149.
31. Murray, G., Allen, N. B., and Trinder, J. (2002). Mood and the circadian system: Investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
32. Varkevisser, M. and Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: Validating a self-constructed test battery. *Chronobiology International* 20: 109–121.
33. Gradisar, M. and Lack, L. (2004). Relationships between the circadian rhythms of finger temperature, core temperature, sleep latency, and subjective sleepiness. *Journal of Biological Rhythms* 19: 157–163.
34. Heikens, M. J., Gorbach, A. M., Eden, H. S., Savastano, D. M., Chen, K. Y., Skarulis, M. C., and Yanovski, J. A. (2011). Core body temperature in obesity. *American Journal of Clinical Nutrition* 93: 963–967.
35. Grimaldi, D., Provini, F., Pierangeli, G., Mazzella, N., Zamboni, G., Marchesini, G., and Cortelli, P. (2015). Evidence of a diurnal thermogenic handicap in obesity. *Chronobiology International* 32: 299–302.
36. Baker, F. C., Waner, J. I., Vieira, E. F., Taylor, S. R., Driver, H. S., and Mitchell, D. (2001). Sleep and 24 hour body temperatures: A comparison in young men, naturally cycling women and women taking hormonal contraceptives. *Journal of Physiology* 530: 565–574.
37. Mackowiak, P. A., Wasserman, S. S., and Levine, M. M. (1992). A critical appraisal of 98.6°F, the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich. *Journal of the American Medical Association* 268: 1578–1580.
38. Edwards, B., Waterhouse, J., Reilly, T., and Atkinson, G. (2002). A comparison of the suitabilities of rectal, gut, and insulated axilla temperatures for measurement of the circadian rhythm of core temperature in field studies. *Chronobiology International* 19: 579–597.
39. Shechter, A., Boudreau, P., Varin, F., and Boivin, D. B. (2011). Predominance of distal skin temperature changes at sleep onset across menstrual and circadian phases. *Journal of Biological Rhythms* 26: 260–270.
40. Bratzke, D., Rolke, B., Ulrich, R., and Peters, M. (2007). Central slowing during the night. *Psychological Science* 18: 456–461.
41. Kolodyazhniy, V., Späti, J., Frey, S., Götz, T., Wirz-Justice, A., Kräuchi, K., Cajochen, C., and Wilhelm, F. H. (2011). Estimation of human circadian phase via a multi-channel ambulatory monitoring system and a multiple regression model. *Journal of Biological Rhythms* 26: 55–67.
42. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., and Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *Journal-Lancet* 71: 312–319.
43. Barrett, K. E., Barman, S. M., Boitano, S., and Brooks, H. L. (2012). *Ganong's Review of Medical Physiology*, 24th edn. New York: McGraw-Hill.
44. Williams, R. S., Willard, H. F., and Snyderman, R. (2003). Personalized health planning. *Science* 300: 549.
45. Hermida, R. C., Ayala, D. E., Fernández, J. R., Mojón, A., Alonso, I., and Calvo, C. (2002). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiology International* 19: 461–481.

46. Leary, A. C., Struthers, A. D., Donnan, P. T., MacDonald, T. M., and Murphy, M. B. (2002). The morning surge in blood pressure and heart rate is dependent on levels of physical activity after waking. *Journal of Hypertension* 20: 865–870.
47. Hermida, R. C., Fernández, J. R., Ayala, D. E., Mojón, A., Alonso, I., and Calvo, C. (2004). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiology International* 21: 147–160.
48. Solomon, G. D. (1992). Circadian rhythms and migraine. *Cleveland Clinic Journal of Medicine* 59: 326–329.
49. Cutolo, M. and Masi, A. T. (2005). Circadian rhythms and arthritis. *Rheumatic Disease Clinics of North America* 31: 115–129.
50. Carroll, R., Metcalfe, C., Gunnell, D., Mohamed, F., and Eddleston, M. (2012). Diurnal variation in probability of death following self-poisoning in Sri Lanka: Evidence for chronotoxicity in humans. *International Journal of Epidemiology* 41: 1821–1828.
51. Fukui, Y., Yaegaki, K., Murata, T., Sato, T., Tanaka, T., Imai, T., Kamoda, T., and Herai, M. (2008). Diurnal changes in oral malodour among dental-office workers. *International Dental Journal* 58: 159–166.
52. Dawes, C. (1972). Circadian rhythms in human salivary flow rate and composition. *Journal of Physiology* 220: 529–545.
53. Rand, C. S. W., Macgregor, A. M. C., and Stunkard, A. J. (1997). The night eating syndrome in the general population and among postoperative obesity patients. *International Journal of Eating Disorders* 22: 65–69.
54. Lundgren, J. D., Allison, K. C., Crow, S., O'Reardon, J. P., Berg, K. C., Galbraith, J., Martino, N. S., and Stunkard, A. J. (2006). Prevalence of the night eating syndrome in a psychiatric population. *American Journal of Psychiatry* 163: 156–158.
55. Runfola, C. D., Allison, K. C., Hardy. K. K., Lock, J., and Peebles, R. (2014). Prevalence and clinical significance of night eating syndrome in university students. *Journal of Adolescent Health* 55: 41–48.
56. Roenneberg, T., Allebrandt, K. V., Merrow, M., and Vetter, C. (2012). Social jet lag and obesity. *Current Biology* 22: 939–943.
57. Parsons, M. J., Moffitt, T. E., Gregory, A. M., Goldman-Mellor, S., Nolan, P. M., Poulton, R., and Caspi, A. (2015). Social jetlag, obesity and metabolic disorder: Investigation in a cohort study. *International Journal of Obesity* 39: 842–848.
58. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F., and Pauly, J. E. (Eds.), *Chronobiology*. Tokyo, Japan: Igaku Shoin, pp. 451–459.
59. Taillard, J., Sanchez, P., Lemoine, P., and Mouret, J. (1990). Heart rate circadian rhythm as a biological marker of desynchronization in major depression: A methodological and preliminary report. *Chronobiology International* 7: 305–316.
60. Kumagai, Y., Shiga, T., Sunaga, K., Cornélissen, G., Ebihara, A., and Halberg, F. (1992). Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 19: 43–58.
61. Scheer, F. A. J. L., van Doornen, L. J. P., and Buijs, R. M. (1999). Light and diurnal cycle affect human heart rate: Possible role for the circadian pacemaker. *Journal of Biological Rhythms* 14: 202–212.
62. Viola, A. U., Simon, C., Ehrhart, J., Geny, B., Piquard, F., Muzet, A., and Brandenberger, G. (2002). Sleep processes exert a predominant influence on the 24-h profile of heart rate variability. *Journal of Biological Rhythms* 17: 539–547.
63. Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K., and Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s–44s.
64. O'Sullivan, C., Duggan, J., Atkins, N., and O'Brien, E. (2003). Twenty-four-hour ambulatory blood pressure in community-dwelling elderly men and women aged 60–102 years. *Journal of Hypertension* 21: 1641–1647.
65. Van Eekelen, A. P. J., Houtveen, J. H., and Kerkhof, G. A. (2004). Circadian variation in cardiac autonomic activity: Reactivity measurements to different types of stressors. *Chronobiology International* 21: 107–129.
66. Hadtstein, C., Wühl, E., Soergel, M., Witte, K., and Schaefer, F. (2004). Normative values for circadian and ultradian cardiovascular rhythms in childhood. *Hypertension* 43: 547–554.
67. Hermida, R. C., Calvo, C., Ayala, D. E., Domínguez, M. J., Covelo, M., Fernández, J. R., Fontao, M. J., and López, J. E. (2004). Administration-time-dependent effects of doxazosin GITS on ambulatory blood pressure of hypertensive subjects. *Chronobiology International* 21: 277–296.
68. Thomas, C., Wood, G. C., Langer, R. D., and Stewart, W. F. (2008). Elevated blood pressure in primary care varies in relation to circadian and seasonal changes. *Journal of Human Hypertension* 22: 755–760.
69. Otsuka, K., Cornélissen, G., and Halberg, F. (1996). Predictive value of blood pressure dipping and swinging with regard to vascular disease risk. *Clinical Drug Investigation* 11: 20–31.
70. Watanabe, Y., Cornélissen, G., Halberg, F., Bingham, C., Siegelová, J., Otsuka, K., and Kikuchi, T. (1997). Incidence pattern and treatment of a clinical entity: Overswinging or circadian hyperamplitudetension (CHAT). *Scripta Medica* 70: 245–261.
71. Halberg, F., Cornélissen, G., Katinas, G., Syutkina, E. V., Sothern, R. B., Zaslavskaya, R., Halberg, F. et al. (2003). Transdisciplinary unifying implications of circadian findings in the 1950s. *Journal of Circadian Rhythms* 1: art. 2.
72. Bilo, G., Giglio, A., Styczkiewicz, K., Caldara, G., Kawecka-Jaszcz, K., Mancia, G., and Parati, G. (2005). How to improve the assessment of 24-h blood pressure variability. *Blood Pressure Monitoring* 10: 321–323.
73. Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., McClory, S., Hond, E. D., McCormack, P., Staessen, J. A., and O'Brien, E. (2005). Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: The Dublin Outcome Study. *Hypertension* 46: 156–161.
74. Bastos, J. M., Bertoquini, S., Silva, J. A., and Polónia, J. (2006). Relação entre valores da pressurometria ambulatória e desenvolvimento futuro de eventos isquémicos cerebrovasculares e coronários em doentes hipertensos. *Revista Portuguesa de Cardiologia* 25: 305–316.
75. Fromm, R. E., Levine, R. L., and Pepe, P. E. (1992). Circadian variation in the time of request for helicopter transport of cardiac patients. *Annals of Emergency Medicine* 21: 1196–1199.
76. Spielberg, C., Falkenhahn, D., Willich, S. N., Wegscheider, K., and Völler, H. (1996). Circadian, day-of-week, and seasonal variability in myocardial infarction: Comparison between working and retired patients. *American Heart Journal* 132: 579–585.
77. Kentsch, M., Rodemerk, U., Ittel, T. H., Müller-Esch, G., and Mitusch, R. (2002). Tag-Nacht-Variabilität der Prähospital-Phase des akuten Myokardinfarktes. *Zeitschrift für Kardiologie* 91: 637–641.
78. Manfredini, R., La Cecilia, O., Boari, B., Steliu, J., Michelini, V., Carli, P., Zanotti, C., Bigoni, M., and Gallerani, M. (2002). Circadian pattern of emergency calls: Implications for ED organization. *American Journal of Emergency Medicine* 20: 282–286.

79. López-Messa, J. B., Garmendia-Leiza, J. R., Aguilar-García, M. D., Andrés-de-Llano, J. M., Alberola-López, C., and Ardura-Fernández, J. (2004). Factores de riesgo cardiovascular en el ritmo circadiano del infarto agudo de miocardio. *Revista Española de Cardiología* 57: 850–858.
80. Manfredini, R., Boari, B., Bressan, S., Gallerani, M., Salmi, R., Portaluppi, F., and Mehta, R. H. (2004). Influence of circadian rhythm on mortality after myocardial infarction: Data from a prospective cohort of emergency calls. *American Journal of Emergency Medicine* 22: 555–559.
81. Savopoulos, C., Ziakas, A., Hatzitolios, A., Delivoria, C., Kounanis, A., Mylonas, S., Tsougas, M., and Psaroulis, D. (2006). Circadian rhythm in sudden cardiac death: A retrospective study of 2,665 cases. *Angiology* 57: 197–204.
82. Reavey, M., Saner, H., Paccaud, F., and Marques-Vidal, P. (2013). Exploring the periodicity of cardiovascular events in Switzerland: Variation in deaths and hospitalizations across seasons, day of the week and hour of the day. *International Journal of Cardiology* 168: 2195–2200.
83. Mehta, R. H., Manfredini, R., Bossone, E., Hutchison, S., Evangelista, A., Boari, B., Cooper, J. V. et al. (2005). Does circadian and seasonal variation in occurrence of acute aortic dissection influence in-hospital outcomes? *Chronobiology International* 22: 343–351.
84. Kleinpeter, G., Schatzer, R., and Böck, F. (1995). Is blood pressure really a trigger for the circadian rhythm of subarachnoid hemorrhage? *Stroke* 26: 1805–1810.
85. Casetta, I., Granieri, E., Fallica, E., la Cecilia, O., Paolino, E., and Manfredini, R. (2002). Patient demographic and clinical features and circadian variation in onset of ischemic stroke. *Archives of Neurology* 59: 48–53.
86. Gupta, A. and Shetty, H. (2005). Circadian variation in stroke: A prospective hospital-based study. *International Journal of Clinical Practice* 59: 1272–1275.
87. Tükek, T., Yildiz, P., Atilgan, D., Tuzcu, V., Eren, M., Erk, O., Demirel, S., Akkaya, V., Dilmener, M., and Korkut, F. (2003). Effect of diurnal variability of heart rate on development of arrhythmia in patients with chronic obstructive pulmonary disease. *International Journal of Cardiology* 88: 199–206.
88. Scheving, L. E., Vedral, D. F., and Pauly, J. E. (1968). A circadian susceptibility rhythm in rats to pentobarbital sodium. *Anatomical Record* 160: 741–750.
89. Rebuelto, M., Ambros, L., Montoya, L., and Bonafine, R. (2002). Treatment-time-dependent difference of ketamine pharmacological response and toxicity in rats. *Chronobiology International* 19: 937–945.
90. Rebuelto, M., Ambros, L., Waxman, S., and Montoya, L. (2004). Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. *Chronobiology International* 21: 591–600.
91. Yamauchi, A., Oishi, R., and Kataoka, Y. (2004). Tacrolimus-induced neurotoxicity and nephrotoxicity is ameliorated by administration in the dark phase in rats. *Cellular and Molecular Neurobiology* 24: 695–704.
92. Soulban, G., Smolensky, M. H., and Yonovitz, A. (1990). Gentamicin-induced chronotoxicity: Use of body temperature as a circadian marker. *Chronobiology International* 7: 393–402.
93. Williams, R. L., Soliman, K. F. A., and Mizinga, K. M. (1993). Circadian variation in tolerance to the hypothermic action of CNS drugs. *Pharmacology, Biochemistry and Behavior* 46: 283–288.
94. Reinberg, A. E. (1996). Rythmes circadiens de la sensibilité des systèmes cibles aux médicaments: un phénomène sous-estimé. *Bulletin de l'Académie Nationale de Médecine* 180: 533–547.
95. Lemmer, B. (2000). Relevance for chronopharmacology in practical medicine. *Seminars in Perinatology* 24: 280–290.
96. Milano, G. and Chamorey, A. L. (2002). Clinical pharmacokinetics of 5-fluorouracil with consideration of chronopharmacokinetics. *Chronobiology International* 19: 177–189.
97. Fleming, G. F., Schumm, P., Friberg, G., Ratain, M. J., Njiaju, U. O., and Schilsky, R. L. (2015). Circadian variation in plasma 5-fluorouracil concentrations during a 24-hour constant-rate infusion. *BMC Cancer* 15: art. 69.
98. Lévi, F., Zidani, R., and Misset, J. L. (1997). Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. *Lancet* 350: 681–686.
99. Lévi, F., Giacchetti, S., Zidani, R., Brezault-Bonnet, C., Tigaud, J. M., Goldwasser, F., and Misset, J. L. (2001). Chronotherapy of colorectal cancer metastases. *Hepato-Gastroenterology* 48: 320–322.
100. Hrushesky, W. J. (1985). Circadian timing of cancer chemotherapy. *Science* 228: 73–75.
101. Zhu, Y., Stevens, R. G., Hoffman, A. E., FitzGerald, L. M., Kwon, E. M., Ostrander, E. A., Davis, S., Zheng, T., and Stanford, J. L. (2009). Testing the circadian gene hypothesis in prostate cancer: A population-based case-control study. *Cancer Research* 69: 9315–9322.
102. Hoffman, A. E., Yi, C. H., Zheng, T., Stevens, R. G., Leaderer, D., Zhang, Y., Holford, T. R., Hansen, J., Paulson, J., and Zhu, Y. (2010). CLOCK in breast tumorigenesis: Evidence from genetic, epigenetic, and transcriptional profiling analyses. *Cancer Research* 70: 1459–1468.
103. Keith, L. G., Oleszczuk, J. J., and Laguens, M. (2001). Circadian rhythm chaos: A new breast cancer marker. *International Journal of Fertility* 46: 238–247.
104. Sigurdardottir, L. G., Valdimarsdottir, U. A., Fall, K., Rider, J. R., Lockley, S. W., Schernhammer, E. S., and Mucci, L. A. (2012). Circadian disruption, sleep loss and prostate cancer risk: A systematic review of epidemiological studies. *Cancer Epidemiology, Biomarkers and Prevention* 21: 1002–1011.
105. Mormont, M. C., Waterhouse, J., Bleuzen, P., Giacchetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset, J. L., Touitou, Y., and Lévi, F. (2000). Marked 24-h rest/activity rhythms are associated with better quality of life, better response, and longer survival in patients with metastatic colorectal cancer and good performance status. *Clinical Cancer Research* 6: 3038–3045.
106. Cadenas, C., van de Sandt, L., Edlund, K., Lohr, M., Hellwig, B., Marchan, R., Schmidt, M., Rahnenführer, J., Oster, H., and Hengstler, J. G. (2014). Loss of circadian clock gene expression is associated with tumor progression in breast cancer. *Cell Cycle* 13: 3282–3291.
107. Zuurbier, L. A., Luik, A. I., Hofman, A., Franco, O. H., Van Someren, E. J. W., and Tiemeier, H. (2015). Fragmentation and stability of circadian activity rhythms predict mortality: The Rotterdam study. *American Journal of Epidemiology* 181: 54–63.
108. Filipski, E., King, V. M., Li, X. M., Granda, T. G., Mormont, M. C., Liu, X. H., Claustrat, B., Hastings, M. H., and Lévi, F. (2002). Host circadian clock as a control point in tumor progression. *Journal of the National Cancer Institute* 94: 690–697.
109. National Center for Environmental Health (2003). *Behavioral Risk Factor Surveillance System: Asthma*. Atlanta, GA: Centers for Disease Control and Prevention.
110. Pincus, D. J., Beam, W. R., and Martin, R. J. (1995). Chronobiology and chronotherapy of asthma. *Clinics in Chest Medicine* 16: 699–713.

111. Jindal, S. K., Aggarwal, A. N., and Gupta, D. (2002). Diurnal variability of peak expiratory flow. *Journal of Asthma* 39: 363–373.
112. Turner-Warwick, M. (1988). Epidemiology of nocturnal asthma. *American Journal of Medicine* 85(1B): 6–8.
113. Dethlefsen, U. and Repgas, R. (1985). Ein neues Therapieprinzip bei nachtlichen Asthma. *Klinische Medizin* 80: 44–47.
114. Smyth, J. M., Soefer, M. H., Hurewitz, A., Kliment, A., and Stone, A. A. (1999). Daily psychosocial factors predict levels and diurnal cycles of asthma symptomatology and peak flow. *Journal of Behavioral Medicine* 22: 179–193.
115. Lebowitz, M. D., Krzyzanowski, M., Quackenboss, J. J., and O'Rourke, M. K. (1997). Diurnal variation of PEF and its use in epidemiological studies. *European Respiratory Journal* 24: 49S–56S.
116. Meijer, G. G., Postma, D. S., van der Heide, S., de Reus, D. M., Koeter, G. H., Roorda, R. J., and van Aalderen, W. M. (1996). Seasonal variations in house dust mite influence the circadian peak expiratory flow amplitude. *American Journal of Respiratory Critical Care Medicine* 154: 881–884.
117. Burioka, N., Sako, T., Tomita, K., Miyata, M., Suyama, H., Igishi, T., and Shimizu, E. (2001). Theophylline chronotherapy of nocturnal asthma using bathyphase of circadian rhythm in peak expiratory flow rate. *Biomedicine and Pharmacotherapy* 55: 142–146.
118. Kondo, S. and Ito, M. (1997). Reevaluation of the relationship between within-day bronchial variability and bronchial responsiveness in asthmatic children. *Chronobiology International* 14: 61–69.
119. Dottorini, M. L., Tantucci, C., Peccini, F., Grassi, V., and Sorbini, C. A. (1996). Diurnal change of bronchial caliber and airway responsiveness in asthmatics during long-term treatment with long-acting β2 agonist salmeterol. *International Journal of Clinical Pharmacology and Therapeutics* 34: 438–443.
120. Kraft, M., Wenzel, S. E., Bettinger, C. M., and Martin, R. J. (1997). The effect of salmeterol on nocturnal symptoms, airway function, and inflammation in asthma. *Chest* 111: 1249–1254.
121. Troyanov, S., Ghezzo, H., Cartier, A., and Malo, J. L. (1994). Comparison of circadian variations using FEV1 and peak expiratory flow rates among normal and asthmatic subjects. *Thorax* 49: 775–780.
122. Karras, D. J., D'Alonzo, G. E., and Heilpern, K. L. (1995). Is circadian variation in asthma severity relevant in the emergency department? *Annals of Emergency Medicine* 26: 558–562.
123. Brenner, B. E., Chavda, K. K., Karakurum, M. B., Karras, D. J., and Camargo, C. A. (2001). Circadian differences among 4,096 emergency department patients with acute asthma. *Critical Care Medicine* 29: 1124–1129.
124. Reinberg, A., Gervais, P., Chaussade, M., Fraboulet, G., and Duburque, B. (1983). Circadian changes in effectiveness of corticosteroids in eight patients with allergic asthma. *Journal of Allergy and Clinical Immunology* 71: 425–433.
125. Reinberg, A., Pauchet, F., Ruff, F., Gervais, A., Smolensky, M. H., Levi, F., Gervais, P., Chaouat, D., Abella, M. L., and Zidani, R. (1987). Comparison of once-daily evening versus morning sustained release theophylline dosing for nocturnal asthma. *Chronobiology International* 4: 409–419.
126. Morrison, J., Pearson, S., and Dean, H. (1988). Parasympathetic nervous system in nocturnal asthma. *British Medical Journal* 296: 1427–1429.
127. Gaultier, L., Reinberg, A., and Motohashi, Y. (1988). Circadian rhythm in total pulmonary resistance of asthmatic children: Effects of a β agonist agent. *Chronobiology International* 5: 285–290.
128. Burioka, N., Miyata, M., Endo, M., Fukuoka, Y., Suyama, H., Nakasaki, H., Igawa, K., and Shimizu, E. (2005). Alteration of the circadian rhythm in peak expiratory flow of nocturnal asthma following nighttime transdermal β2 adrenoceptor agonist tulobuterol chronotherapy. *Chronobiology International* 22: 383–390.
129. Daan, S., Beersma, D. G. M., and Borbély, A. A. (1984). Timing of human sleep: Recovery process gated by a circadian pacemaker. *American Journal of Physiology* 246: R161–R178.
130. Achermann, P. (2004). The two-process model of sleep regulation revisited. *Aviation, Space, and Environmental Medicine* 75(3): A37–A43.
131. Parmeggiani, P. L. and Rabini, C. (1967). Modificazioni delle fasi del sonno nel gatto per esposizione di breve durata a differenti temperature ambientali. *Bollettino della Società Italiana di Biologia Sperimentale* 43: 1079–1083.
132. Schmidek, W. R., Hoshino, K., Schmidek, M., and Timo-Iaria, C. (1972). Influence of environmental temperature on the sleep-wakefulness cycle in the rat. *Physiology and Behavior* 8: 363–371.
133. Szymusiak, R. and Satinoff, E. (1981). Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate. *Physiology and Behavior* 26: 687–690.
134. Roussel, B., Turrillot, P., and Kitahama, K. (1984). Effect of ambient temperature on the sleep-waking cycle in two strains of mice. *Brain Research* 294: 67–73.
135. Muzet, A., Libert, J. P., and Candas, V. (1984). Ambient temperature and human sleep. *Experientia* 40: 425–429.
136. Kumar, D., Mallick, H. N., and Kumar, V. M. (2009). Ambient temperature that induces maximum sleep in rats. *Physiology and Behavior* 98: 186–191.
137. Raymann, R. J. E. M., Swaab, D. F., and Van Someren, E. J. W. (2004). Cutaneous warming promotes sleep onset. *American Journal of Physiology* 288: R1589–R1597.
138. Kräuchi, K., Knoblauch, V., Wirz-Justice, A., and Cajochen, C. (2006). Challenging the sleep homeostat does not influence the thermoregulatory system in men: Evidence from a nap vs. sleep-deprivation study. *American Journal of Physiology* 290: R1052–R1061.
139. Obál Jr., F., Rubicsek, G., Alföldi, P., Sáry, G., and Obál, F. (1985). Changes in the brain and core temperatures in relation to the various arousal states in rats in the light and dark periods of the day. *Pflügers Archiv* 404: 73–79.
140. Heller, H. C., Graf, R., and Rautenberg, W. (1983). Circadian and arousal state influences on thermoregulation in the pigeon. *American Journal of Physiology* 245: R321–R328.
141. Abrams, R. and Hammel, H. T. (1964). Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping. *American Journal of Physiology* 206: 641–646.
142. Glotzbach, S. F. and Heller, H. C. (1976). Central nervous regulation of body temperature during sleep. *Science* 194: 537–539.
143. Glotzbach, S. F. and Heller, H. C. (1984). Changes in the thermal characteristics of hypothalamic neurons during sleep and wakefulness. *Brain Research* 309: 17–26.
144. Schmidek, W. R., Zachariassen, K. E., and Hammel, H. T. (1983). Total calorimetric measurements in the rat: Influences of the sleep-wakefulness cycle and of environmental temperature. *Brain Research* 288: 261–271.
145. Walker, J. M., Walker, L. E., Harris, D. V., and Berger, R. J. (1983). Cessation of thermoregulation during REM sleep. *American Journal of Physiology* 244: R114–R118.
146. Hendricks, J. C. (1982). Absence of shivering in the cat during paradoxical sleep without atonia. *Experimental Neurology* 75: 700–710.

147. Amoros, C., Sagot, J. C., Libert, J. P., and Candas, V. (1986). Sweat gland response to local heating during sleep in man. *Journal de Physiology* 81: 209–215.
148. Shapiro, C. M., Moore, A. T., Mitchell, D., and Yodaiken, M. L. (1974). How well does man thermoregulate during sleep? *Experientia* 30: 1279–1281.
149. Sagot, J. C., Amoros, C., Candas, V., and Libert, J. P. (1987). Sweating responses and body temperatures during nocturnal sleep in humans. *American Journal of Physiology* 252: R462–R470.
150. Dewasmes, G., Bothorel, B., Candas, V., and Libert, J. P. (1997). A short-term poikilothermic period occurs just after paradoxical sleep onset in humans: Characterization changes in sweating effector activity. *Journal of Sleep Research* 6: 252–258.
151. Tayefeh, F., Plattnet, O., Sessler, D. I., Ikeda, T., and Marder, D. (1998). Circadian changes in the sweating-to-vasoconstriction interthreshold range. *Pflügers Archiv* 435: 402–406.
152. Borisenkov, M. F. (2011). The pattern of entrainment of the human sleep-wake rhythm by the natural photoperiod in the North. *Chronobiology International* 28: 921–929.
153. Miguel, M., de Oliveira, V. C., Pereira, D., and Pedrazzoli, M. (2014). Detecting chronotype differences associated to latitude: A comparison between Horne-Östberg and Munich Chronotype questionnaires. *Annals of Human Biology* 41: 105–108.
154. Sani, M., Refinetti, R., Jean-Louis, G., Pandi-Perumal, S. R., Durazo-Arvizu, R. A., Dugas, L. R., Kafensztok, R. et al. (2015). Daily activity patterns of 2316 men and women from five countries differing in socioeconomic development. *Chronobiology International* 32: 650–656.
155. Roenneberg, T., Kumar, C. J., and Merrow, M. (2007). The human circadian clock entrains to sun time. *Current Biology* 17: R44–R45.
156. Gates, A. I. (1916). Diurnal variations in memory and association. *University of California Publications in Psychology* 1: 323–344.
157. Refinetti, R. (1995). Persistence of synchronization of the daily rhythms of body temperature and sleep-wake in college students. *Biological Rhythm Research* 26: 532–540.
158. Van Reen, E., Sharkey, K. M., Roane, B. M., Barker, D., Seifer, R., Raffray, T., Bond, T. L., and Carskadon, M. A. (2013). Sex of college students moderates associations among bedtime, time in bed, and circadian phase angle. *Journal of Biological Rhythms* 28: 425–431.
159. Binkley, S., Tome, M. B., and Mosher, K. (1989). Weekly phase shifts of rhythms self-reported by almost feral human students in the USA and Spain. *Physiology and Behavior* 46: 423–427.
160. Steptoe, A., Peacey, V., and Wardle, J. (2006). Sleep duration and health in young adults. *Archives of Internal Medicine* 166: 1689–1692.
161. Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., and Merrow, M. (2007). Epidemiology of the human circadian clock. *Sleep Medicine Reviews* 11: 429–438.
162. Partinen, M. and Hublin, C. (2000). Epidemiology of sleep disorders. In: Kryger, M. H., Roth, T., and Dement, W. C. (Eds.), *Principles and Practice of Sleep Medicine*. Philadelphia, PA: Saunders, pp. 558–579.
163. Buysse, D. J., Carrier, J., Dew, M. A., Hall, M., Monk, T. H., Nowell, P., Reynolds, C. F., and Kupfer, D. J. (2001). Sleep disorders. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.), *Circadian Clocks* (*Handbook of Behavioral Neurobiology*, Vol. 12). New York: Kluwer/Plenum, pp. 645–683.
164. Hirshkowitz, M., Whiton, K., Albert, S. M., Alessi, C., Bruni, O., Don Carlos, L., Hazen, N. et al. (2015). National Sleep Foundation's sleep time duration recommendations: Methodology and results summary. *Sleep Health* 1: 40–43.
165. Cirelli, C. and Tononi, G. (2008). Is sleep essential? *PLoS Biology* 6: e216.
166. Czeisler, C. A. (2015). Duration, timing and quality of sleep are each vital for health, performance and safety. *Sleep Health* 1: 5–8.
167. Cappuccio, F. P., D'Elia, L., Strazzullo, P., and Miller, M. A. (2010). Sleep duration and all-cause mortality: A systematic review and meta-analysis of prospective studies. *Sleep* 33: 585–592.
168. Olders, H. (2003). What causes insomnia? *Scientific American* 289(4): 103.
169. Mercer, J. D., Bootzin, R. R., and Lack, L. C. (2002). Insomniacs' perception of wake instead of sleep. *Sleep* 25: 564–571.
170. Rosa, R. R. and Bonnet, M. H. (2000). Reported chronic insomnia is independent of poor sleep as measured by electroencephalography. *Psychosomatic Medicine* 62: 474–482.
171. Campbell, S. S., Murphy, P. J., Van Den Heuvel, C. J., Roberts, M. L., and Stauble, T. N. (1999). Etiology and treatment of intrinsic circadian rhythm sleep disorders. *Sleep Medicine Reviews* 3: 179–200.
172. Vignau, J., Dahlitz, M., Arendt, J., English, J., and Parkes, J. D. (1993). Biological rhythms and sleep disorders in man: The Delayed Sleep Phase Syndrome. In: Wetterberg, L. (Ed.), *Light and Biological Rhythms in Man*. Cambridge, U.K.: Pergamon, pp. 261–271.
173. Weitzman, E. D., Czeisler, C. A., Coleman, R. M., Spielman, A. J., Zimmerman, J. C., Dement, W., Richardson, G., and Pollack, C. P. (1981). Delayed sleep phase syndrome: A chronobiological disorder with sleep-onset insomnia. *Archives of General Psychiatry* 38: 737–746.
174. Alvarez, B., Dahlitz, M. J., Vignau, J., and Parkes, J. D. (1992). The delayed sleep phase syndrome: Clinical and investigative findings in 14 subjects. *Journal of Neurology, Neurosurgery, and Psychiatry* 55: 665–670.
175. Lack, L., Bailey, M., Lovato, N., and Wright, H. (2009). Chronotype differences in circadian rhythms of temperature, melatonin, and sleepiness as measured in a modified constant routine protocol. *Nature and Science of Sleep* 1: 1–8.
176. Jones, C. R., Campbell, S. S., Zone, S. E., Cooper, F., DeSano, A., Murphy, P. J., Jones, B., Czajkowski, L., and Ptácek, L. J. (1999). Familial advanced sleep-phase syndrome: A short-period circadian rhythm variant in humans. *Nature Medicine* 5: 1062–1065.
177. Toh, K. L., Jones, C. R., He, Y., Eide, E. J., Hinz, W. A., Virshup, D. M., Ptácek, L. J., and Fu, Y. H. (2001). An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* 291: 1040–1043.
178. Hu, Y., Padiath, Q. S., Shapiro, R. E., Jones, C. R., Wu, S. C., Saigoh, K., Ptácek, L. J., and Fu, Y. H. (2005). Functional consequences of a CKIδ mutation causing familial advanced sleep phase syndrome. *Nature* 434: 640–644.
179. Mistlberger, R. E., Yamazaki, S., Pendergast, J. S., Landry, G. J., Takumi, T., and Nakamura, W. (2008). Comment on "Differential rescue of light- and food-entrainable circadian rhythms". *Science* 322: 675.
180. He, Y., Jones, C. R., Fujiki, N., Xu, Y., Guo, B., Holder, J. L., Rossner, M. J., Nishino, S., and Fu, Y. H. (2009). The transcriptional repressor DEC2 regulates sleep length in mammals. *Science* 325: 866–870.

181. Morris, M., Lack, L., and Dawson, D. (1990). Sleep-onset insomniacs have delayed temperature rhythms. *Sleep* 13: 1–14.
182. Lack, L. and Wright, H. (1993). The effect of evening bright light in delaying the circadian rhythms and lengthening the sleep of early morning awakening insomniacs. *Sleep* 16: 436–443.
183. Lack, L. C., Mercer, J. D., and Wright, H. (1996). Circadian rhythms of early morning awakening insomniacs. *Journal of Sleep Research* 5: 211–219.
184. Chang, A. M., Reid, K. J., Gourineni, R., and Zee, P. C. (2009). Sleep timing and circadian phase in delayed sleep phase syndrome. *Journal of Biological Rhythms* 24: 313–321.
185. Terman, M., Lewy, A. J., Dijk, D. J., Boulos, Z., Eastman, C. I., and Campbell, S. S. (1995). Light treatment for sleep disorders: Consensus report. IV. Sleep phase and duration disturbances. *Journal of Biological Rhythms* 10: 135–147.
186. Sifton, D. W. (Ed.) (2003). *Physician's Desk Reference*, 57th edn. Montvale, NJ: Thomson PDR.
187. Mignot, E. (2013). The perfect hypnotic? *Science* 340: 36–38.
188. Czeisler, C. A., Richardson, G., Coleman, R., Zimmerman, J., Moore-Ede, M., Dement, W., and Weitzman, E. (1981). Chronotherapy: Resetting the circadian clocks of patients with delayed sleep phase insomnia. *Sleep* 4: 1–21.
189. Monk, T. H., Reynolds, C. F., Buysse, D. J., DeGrazia, J. M., and Kupfer, D. J. (2003). The relationship between lifestyle regularity and subjective sleep quality. *Chronobiology International* 20: 97–107.
190. Monk, T. H., Buysse, D. J., Billy, B. D., and DeGrazia, J. M. (2004). Using nine 2-h delays to achieve a 6-h advance disrupts sleep, alertness, and circadian rhythm. *Aviation, Space, and Environmental Medicine* 75: 1049–1057.
191. Lewy, A. J., Sack, R. L., and Singer, C. M. (1985). Treating phase typed chronobiologic sleep and mood disorders using appropriately timed bright artificial light. *Psychopharmacological Bulletin* 21: 368–372.
192. Rosenthal, N. E., Joseph-Vanderpool, J. R., Levendosky, A. A., Johnston, S. H., Allen, R., Kelly, K. A., Souêtre, E., Schultz, P. M., and Starz, K. (1990). Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. *Sleep* 13: 354–361.
193. Watanabe, T., Kajimura, N., Kato, M., Sekimoto, M., and Takahashi, K. (1999). Effects of phototherapy in patients with delayed sleep phase syndrome. *Psychiatry and Clinical Neurosciences* 53: 231–233.
194. Kirisoglu, C. and Guilleminault, C. (2004). Twenty minutes versus forty-five minutes morning bright light treatment on sleep onset insomnia in elderly subjects. *Journal of Psychosomatic Research* 56: 537–542.
195. Dahlitz, M., Alvarez, B., Vignau, J., English, J., Arendt, J., and Parkes, J. D. (1991). Delayed sleep phase syndrome response to melatonin. *Lancet* 337: 1121–1123.
196. Tzischinsky, O., Dagan, Y., and Lavie, P. (1993). The effects of melatonin on the timing of sleep in patients with delayed sleep phase syndrome. In: Touitou, Y., Arendt, J., and Pévet, P. (Eds.), *Melatonin and the Pineal Gland*. New York: Elsevier, pp. 351–354.
197. Zhdanova, I. V. and Wurtman, R. J. (1997). Efficacy of melatonin as a sleep-promoting agent. *Journal of Biological Rhythms* 12: 644–650.
198. Sack, R. L., Lewy, A. J., and Hughes, R. J. (1998). Use of melatonin for sleep and circadian rhythm disorder. *Annals of Medicine* 30: 115–121.
199. Lewy, A. J., Emens, J. S., Bernert, R. A., and Lefler, B. J. (2004). Eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation: Clinical implications. *Journal of Biological Rhythms* 19: 68–75.
200. U.S. Department of Health and Human Services (1999). *Mental Health: A Report of the Surgeon General*. Rockville, MD: National Institute of Mental Health.
201. Cowan, W. M., Kopnisky, K. L., and Hyman, S. E. (2002). The human genome project and its impact on psychiatry. *Annual Review of Neuroscience* 25: 1–50.
202. Kessler, R. C. and Zhao, S. (1999). The prevalence of mental illness. In: Horvitz, A. V., and Scheid, T. L. (Eds.), *A Handbook for the Study of Mental Health: Social Contexts, Theories, and Systems*. New York: Cambridge University Press, pp. 58–78.
203. Lykken, D. and Tellegen, A. (1996). Happiness is a stochastic phenomenon. *Psychological Science* 7: 186–189.
204. American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders*, 5th edn. Washington, DC: American Psychiatric Publishing.
205. American Psychological Association (2000). *Graduate Study in Psychology*, 33rd edn. Washington, DC: American Psychological Association.
206. Hollon, S. D., Thase, M. E., and Markowitz, J. C. (2002). Treatment and prevention of depression. *Psychological Science in the Public Interest* 3: 39–77.
207. Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: A review of supporting evidence. *American Journal of Psychiatry* 122: 509–522.
208. Masand, P. S. and Gupta, S. (1999). Selective serotonin-reuptake inhibitors: An update. *Harvard Review of Psychiatry* 7: 69–84.
209. Hiemke, C. and Hartter, S. (2000). Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacology and Therapeutics* 85: 11–28.
210. Kramer, M. S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J. J., Reines, S. A. et al. (1998). Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 281: 1640–1645.
211. Enserink, M. (1999). Can the placebo be the cure? *Science* 284: 238–240.
212. Lam, R. W. (2008). Addressing circadian rhythm disturbances in depressed patients. *Journal of Psychopharmacology* 22(7S): 13–18.
213. Courtet, P. and Olié, E. (2012). Circadian dimension and severity of depression. *European Neuropsychopharmacology* 22: S476–S481.
214. Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N. et al. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301: 805–809.
215. Duman, R. S. and Aghajanian, G. K. (2012). Synaptic dysfunction in depression: Potential therapeutic targets. *Science* 338: 68–72.
216. Bandura, A. (1969). *Principles of Behavior Modification*. New York: Holt, Rinehart and Winston.
217. Eysenck, H. J. (1952). The effects of psychotherapy: An evaluation. *Journal of Consulting Psychology* 16: 319–324.
218. Levitt, E. E. (1957). The results of psychotherapy with children: An evaluation. *Journal of Consulting Psychology* 21: 189–196.
219. Frank, J. D. (1979). The present status of outcome studies. *Journal of Consulting and Clinical Psychology* 47: 310–316.

220. Parloff, M. B. (1984). Psychotherapy research and its incredible credibility crisis. *Clinical Psychology Review* 4: 95–109.
221. Kopta, S. M., Lueger, R. J., Saunders, S. M., and Howard, K. I. (1999). Individual psychotherapy outcome and process research: Challenges leading to a greater turmoil or a positive transition? *Annual Review of Psychology* 50: 441–469.
222. Szasz, T. S. (1961). *The Myth of Mental Illness*. New York: Hoeber-Harper.
223. Foucault, M. (1972). *Histoire de la Folie à l'Âge Classique*. Paris, France: Gallimard.
224. Rosenhan, D. L. (1973). On being sane in insane places. *Science* 179: 250–258.
225. Pennebaker, J. W. and Graybeal, A. (2001). Patterns of natural language use: Disclosure, personality, and social integration. *Current Directions in Psychological Science* 10: 90–93.
226. Baker, T. B., McFall, R. M., and Shoham, V. (2009). Current status and future prospects of clinical psychology: Toward a scientifically principled approach to mental and behavioral health care. *Psychological Science in the Public Interest* 9: 67–103.
227. Lilienfeld, S. O. (2007). Psychological treatments that cause harm. *Perspectives on Psychological Science* 2: 53–70.
228. Özdemir, P. G., Boysan, M., Smolensky, M. H., Selvi, Y., Aydin, A., and Yilmaz, E. (2015). Comparison of venlafaxine alone versus venlafaxine plus bright light therapy combination for severe major depressive disorder. *Journal of Clinical Psychiatry* 76: e645–e654.
229. Nikitopoulou, G. and Crammer, J. L. (1976). Change in diurnal temperature rhythm in manic-depressive illness. *British Medical Journal* 272: 1311–1314.
230. Pflug, B., Johnsson, A., and Ekse, T. (1981). Manic-depressive states and daily temperature: Some circadian studies. *Acta Psychiatrica Scandinavica* 63: 277–289.
231. Wehr, T. A. and Wirz-Justice, A. (1982). Circadian rhythm mechanism in affective illness and in antidepressant drug action. *Pharmacopsychiatry* 15: 31–39.
232. Souetre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B., and Darcourt, G. (1988). Twenty-four-hour profiles of body temperature and plasma TSH in bipolar patients during depression and during remission and in normal control subjects. *American Journal of Psychiatry* 145: 1133–1137.
233. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K., and Takahashi, S. (1990). Circadian rhythms in depression. Part II. Circadian rhythms in inpatients with various mental disorders. *Journal of Affective Disorders* 18: 199–210.
234. Nagayama, H., Hasama, N., Tsuchiyama, K., Yamada, K., Ihara, J., and Yanagisawa, T. (1992). Circadian temperature and sleep-wake rhythms in depression. *Japanese Journal of Psychiatry and Neurology* 46: 244–245.
235. Yehuda, R., Teicher, M. H., Trestman, R. L., Levengood, R. A., and Siever, L. J. (1996). Cortisol regulation in posttraumatic stress disorder and major depression: A chronobiological analysis. *Biological Psychiatry* 40: 79–88.
236. Stampfer, H. G. (1998). The relationship between psychiatric illness and the circadian pattern of heart rate. *Australian and New Zealand Journal of Psychiatry* 32: 187–198.
237. Crasson, M., Kjiri, S., Colin, A., Kjiri, K., L'Hermite-Baleriaux, M., Ansseau, M., and Legros, J. J. (2004). Serum melatonin and urinary 6-sulfatoxymelatonin in major depression. *Psychoneuroendocrinology* 29: 1–12.
238. Armitage, R., Hoffman, R., Emslie, G., Rintelman, J., Moore, J., and Lewis, K. (2004). Rest-activity cycles in childhood and adolescent depression. *Journal of the Academy of Child and Adolescent Psychiatry* 43: 761–769.
239. Koenigsberg, H. W., Teicher, M. H., Mitropoulou, V., Navalta, C., New, A. S., Trestman, R., and Siever, L. J. (2004). 24-h monitoring of plasma norepinephrine, MHPG, cortisol, growth hormone and prolactin in depression. *Journal of Psychiatric Research* 38: 503–511.
240. Jones, S. H., Hare, D. J., and Evershed, K. (2005). Actigraphic assessment of circadian activity and sleep patterns in bipolar disorder. *Bipolar Disorders* 7: 176–186.
241. Moraes, C. A., Cambras, T., Diez-Noguera, A., Schimitt, R., Dantas, G., Levandovski, R., and Hidalgo, M. P. (2013). A new chronobiological approach to discriminate between acute and chronic depression using peripheral temperature, rest-activity, and light exposure parameters. *BMC Psychiatry* 13: art. 77.
242. Li, J. Z., Bunney, B. G., Meng, F., Hagenauer, M. H., Walsh, D. M., Vawter, M. P., Evans, S. J. et al. (2013). Circadian patterns of gene expression in the human brain and disruption in major depressive disorder. *Proceedings of the National Academy of Sciences of the United States of America* 110: 9950–9955.
243. Bullock, B. and Murray, G. (2014). Reduced amplitude of the 24-hr activity rhythm: A biomarker of vulnerability to bipolar disorder? *Clinical Psychological Science* 2: 86–96.
244. Kripke, D. F., Mullaney, D. J., Atkinson, M., and Wolf, S. (1978). Circadian rhythm disorders in manic-depressives. *Biological Psychiatry* 13: 335–351.
245. Goodwin, F. K., Wirz-Justice, A., and Wehr, T. A. (1982). Evidence that the pathophysiology of depression and the mechanism of action of antidepressant drugs both involve alterations in circadian rhythms. In: Costa, E. and Racagni, G. (Eds.), *Typical and Atypical Antidepressants: Clinical Practice*. New York: Raven, pp. 1–11.
246. Ehlers, C. L., Frank, E., and Kupfer, D. J. (1988). Social zeitgebers and biological rhythms. *Archives of General Psychiatry* 45: 948–952.
247. Szuba, M. P., Yager, A., Guze, B. H., Allen, E. M., and Baxter, L. R. (1992). Disruption of social circadian rhythms in major depression: A preliminary report. *Psychiatry Research* 42: 221–230.
248. Healy, D. and Waterhouse, J. M. (1995). The circadian system and the therapeutics of the affective disorders. *Pharmacology and Therapeutics* 65: 241–263.
249. Meyer, T. D. and Maier, S. (2006). Is there evidence for social rhythm instability in people at risk for affective disorders? *Psychiatry Research* 141: 103–114.
250. Jagannath, A., Peirson, S. N., and Foster, R. G. (2013). Sleep and circadian rhythm disruption in neuropsychiatric illness. *Current Opinion in Neurobiology* 23: 888–894.
251. Willner, P. (1984). The validity of animal models of depression. *Psychopharmacology* 83: 1–16.
252. Baltzer, V. and Weiskrantz, L. (1975). Antidepressant agents and reversal of diurnal activity cycles in the rat. *Biological Psychiatry* 10: 199–209.
253. Aschoff, J. (1989). Circadian activity rhythms in hamsters and rats treated with imipramine in the drinking water. *Chronobiologia* 16: 9–20.
254. Greco, A. M., Gambardella, P., Sticchi, R., D'Aponte, D., and de Franciscis, P. (1990). Chronic administration of imipramine antagonizes deranged circadian rhythm phases in individually housed rats. *Physiology and Behavior* 48: 67–72.
255. Refinetti, R. and Menaker, M. (1993). Effects of imipramine on circadian rhythms in the golden hamster. *Pharmacology, Biochemistry and Behavior* 45: 27–33.

256. Kripke, D. F. and Wyborney, V. G. (1980). Lithium slows rat circadian activity rhythms. *Life Sciences* 26: 1319–1321.
257. McEachron, D. L., Kripke, D. F., Sharp, F. R., Lewy, A. J., and McClellan, D. E. (1985). Lithium effects on selected circadian rhythms in rats. *Brain Research Bulletin* 15: 347–350.
258. Hafen, T. and Wollnik, F. (1994). Effect of lithium carbonate on activity level and circadian period in different strains of rats. *Pharmacology, Biochemistry and Behavior* 49: 975–983.
259. Nagayama, H. (1996). Chronic administration of imipramine and lithium changes the phase-angle relationship between the activity and core body temperature circadian rhythms in rats. *Chronobiology International* 13: 251–259.
260. Nascimento, N. F., Carlson, K. N., Amaral, D. N., Logan, R. W., and Seggio, J. A. (2015). Alcohol and lithium have opposing effects on the period and phase of the behavioral free-running activity rhythm. *Alcohol* 49: 367–376.
261. Wollnik, F. (1992). Effects of chronic administration and withdrawal of antidepressant agents on circadian activity rhythms in rats. *Pharmacology, Biochemistry and Behavior* 43: 549–561.
262. Possidente, B., Lumia, A. R., McEldowney, S., and Rapp, M. (1992). Fluoxetine shortens circadian period for wheel running activity in mice. *Brain Research Bulletin* 28: 629–631.
263. Duncan, W. C., Tamarkin, L., Sokolove, P. G., and Wehr, T. A. (1988). Chronic clorgyline treatment of Syrian hamsters: An analysis of effects on the circadian pacemaker. *Journal of Biological Rhythms* 3: 305–322.
264. Duncan, W. C. and Schull, J. (1994). The interaction of thyroid state, MAOI drug treatment, and light on the level and circadian pattern of wheel-running in rats. *Biological Psychiatry* 35: 324–334.
265. Hallonquist, J., Lindegger, M., and Mrosovsky, N. (1994). Rubidium chloride fuses split circadian activity rhythms in hamsters housed in bright constant light. *Chronobiology International* 11: 65–71.
266. Tapia-Osorio, A., Salgado-Delgado, R., Angeles-Castellanos, M., and Escobar, C. (2013). Disruption of circadian rhythms due to chronic constant light leads to depressive and anxiety-like behaviors in the rat. *Behavioural Brain Research* 252: 1–9.
267. Fonken, L. K. and Nelson, R. J. (2013). Dim light at night increases depressive-like responses in male C3H/HeNHsd mice. *Behavioural Brain Research* 243: 74–78.
268. Bedrosian, T. A., Vaughn, C. A., Galan, A., Daye, G., Weil, Z. M., and Nelson, R. J. (2013). Nocturnal light exposure impairs affective responses in a wavelength-dependent manner. *Journal of Neuroscience* 33: 13081–13087.
269. LeGates, T. A., Altimus, C. M., Wang, H., Lee, H. K., Yang, S., Zhao, H., Kirkwood, A., Weber, E. T., and Hattar, S. (2012). Aberrant light directly impairs mood and learning through melanopsin-expressing neurons. *Nature* 491: 594–598.
270. Kripke, D. F., Nievergelt, C. M., Joo, E. J., Shekhtman, T., and Kelsoe, J. R. (2009). Circadian polymorphisms associated with affective disorders. *Journal of Circadian Rhythms* 7: art. 2.
271. Kripke, D. F., Nievergelt, C. M., Tranah, G. J., Murray, S. S., Rex, K. M., Grizas, A. P., Hahn, E. K., Lee, H. J., Kelsoe, J. R., and Kline, L. E. (2013). FMR1, circadian genes and depression: Suggestive associations or false discovery? *Journal of Circadian Rhythms* 11: art. 3.
272. Lee, H. J., Woo, H. G., Greenwood, T. A., Kripke, D. F., and Kelsoe, J. R. (2013). A genome-wide association study of seasonal pattern mania identifies NF1A as a possible susceptibility gene for bipolar disorder. *Journal of Affective Disorders* 145: 200–207.
273. Mansour, H. A., Wood, J., Logue, T., Chowdari, K. V., Dayal, M., Kupfer, D. J., Monk, T. H., Devlin, B., and Nimgaonkar, V. L. (2006). Association study of eight circadian genes with bipolar I disorder, schizoaffective disorder and schizophrenia. *Genes, Brain and Behavior* 5: 150–157.
274. Mansour, H. A., Talkowski, M. E., Wood. J., Chowdari, K. V., McClain, L., Prasad, K., Montrose, D. et al. (2009). Association study of 21 circadian genes with bipolar I disorder, schizoaffective disorder, and schizophrenia. *Bipolar Disorders* 11: 701–710.
275. Lavebratt, C., Sjöholm, L. K., Soronen, P., Paunio, T., Vawter, M. P., Bunney, W. E., Adolfsson, R. et al. (2010). *CRY2* is associated with depression. *PLOS ONE* 5: e9407.
276. Sjöholm, L. K., Kovanen, L., Saarikoski, S. T., Schalling, M., Lavebratt, C., and Partonen, T. (2010). *CLOCK* is suggested to associate with comorbid alcohol use and depressive disorders. *Journal of Circadian Rhythms* 8: art. 1.
277. Pawlak, J., Dmitrzak-Weglarz, M., Maciukiewicz, M., Wilkosc, M., Leszczynska-Rodziewicz, A., Zaremba, D., Kapelski, P., and Hauser, J. (2015). Suicidal behavior in the context of disrupted rhythmicity in bipolar disorder: Data from an association study of suicide attempts with clock genes. *Psychiatry Research* 226: 517–520.
278. McCarthy, M. J., Nievergelt, C. M., Kelsoe, J. R., and Welsh, D. K. (2012). A survey of genomic studies supports association of circadian clock genes with bipolar disorder spectrum illnesses and lithium response. *PLOS ONE* 7: e32091.
279. Keers, R., Pedroso, I., Breen, G., Aitchison, K. J., Nolan, P. M., Cichon, S., Nöthen, M. M., Rietschel, M., Schalkwyk, L. C., and Fernandes, C. (2012). Reduced anxiety and depression-like behaviours in the circadian period mutant mouse Afterhours. *PLOS ONE* 7: e38263.
280. Jacobsen, F. M., Wehr, T. A., Sack, D. A., James, S. P., and Rosenthal, N. E. (1987). Seasonal affective disorder: A review of the syndrome and its public health implications. *American Journal of Public Health* 77: 57–60.
281. Chung, Y. S. and Daghestani, A. N. (1989). Seasonal affective disorder: Shedding light on a dark subject. *Postgraduate Medicine* 86: 309–314.
282. Harmatz, M. G., Well, A. D., Overtree, C. E., Kawamura, K. Y., Rosal, M., and Ockene, I. S. (2000). Seasonal variation of depression and other moods: A longitudinal approach. *Journal of Biological Rhythms* 15: 344–350.
283. Terman, M. (1988). On the question of mechanism in phototherapy for seasonal affective disorder: Considerations of clinical efficacy and epidemiology. *Journal of Biological Rhythms* 3: 155–172.
284. Geoffroy, P. A., Bellivier, F., Scott, J., and Etain, B. (2014). Seasonality and bipolar disorder: A systematic review, from admission rates to seasonality of symptoms. *Journal of Affective Disorders* 168: 210–223.
285. Albert, P. S., Rosen, L. N., Alexander, J. R., and Rosenthal, N. E. (1991). Effect of daily variation in weather and sleep on seasonal affective disorder. *Psychiatry Research* 36: 51–63.
286. Guillemette, J., Hébert, M., Paquet, J., and Dumont, M. (1998). Natural bright light exposure in the summer and winter in subjects with and without complaints of seasonal mood variations. *Biological Psychiatry* 44: 622–628.

287. Rosenthal, N. E., Levendosky, A. A., Skwerer, R. G., Joseph-Vanderpool, J. R., Kelly, K. A., Hardin, T., Kasper, S., DellaBella, P., and Wehr, T. A. (1990). Effects of light treatment on core body temperature in seasonal affective disorder. *Biological Psychiatry* 27: 39–50.
288. Magnusson, A. and Kristbjanarson, H. (1991). Treatment of seasonal affective disorder with high-intensity light: A phototherapy study with an Icelandic group of patients. *Journal of Affective Disorders* 21: 141–147.
289. Eastman, C. I., Gallo, L. C., Lahmeyer, H. W., and Fogg, L. F. (1993). The circadian rhythm of temperature during light treatment for winter depression. *Biological Psychiatry* 34: 210–220.
290. Stewart, J. W., Quitkin, F. M., Terman, M., and Terman, J. S. (1990). Is seasonal affective disorder a variant of atypical depression? Differential response to light therapy. *Psychiatry Research* 33: 121–128.
291. Wehr, T. A., Jacobsen, F. M., Sack, D. A., Arendt, J., Tamarkin, L., and Rosenthal, N. E. (1986). Phototherapy of seasonal affective disorder. *Archives of General Psychiatry* 43: 870–875.
292. Jacobsen, F. M., Wehr, T. A., Skwerer, R. A., Sack, D. A., and Rosenthal, N. E. (1987). Morning versus midday phototherapy of seasonal affective disorder. *American Journal of Psychiatry* 144: 1301–1305.
293. Lewy, A. J., Sack, R. L., Miller, K. S., and Hoban, T. M. (1987). Antidepressant and circadian phase-shifting effects of light. *Science* 235: 352–354.
294. Avery, D. H., Khan, A., Dager, S. R., Cox, G. B., and Dunner, D. L. (1990). Bright light treatment of winter depression: Morning versus evening light. *Acta Psychiatrica Scandinavica* 82: 335–338.
295. Ruhrmann, S., Kasper, S., Hawellek, B., Martinez, B., Höflich, G., Nickelsen, T., and Möller, H. J. (1998). Effects of fluoxetine *versus* bright light in the treatment of seasonal affective disorder. *Psychological Medicine* 28: 923–933.
296. Terman, M., Terman, J. S., and Ross, D. C. (1998). A controlled trial of timed bright light and negative air ionization for treatment of winter depression. *Archives of General Psychiatry* 55: 875–882.
297. Lewy, A. J., Bauer, V. K., Cutler, N. L., Sack, R. L., Ahmed, S., Thomas, K. H., Blood, M. L., and Jackson, J. M. L. (1998). Morning vs. evening light treatment of patients with winter depression. *Archives of General Psychiatry* 55: 890–896.
298. Terman, J. S., Terman, M., Lo, E. S., and Cooper, T. B. (2001). Circadian time of morning light administration and therapeutic response in winter depression. *Archives of General Psychiatry* 58: 69–75.
299. Avery, D. H., Kizer, D., Bolte, M. A., and Hellekson, C. (2001). Bright light therapy of subsyndromal seasonal affective disorder in the workplace: Morning vs. afternoon exposure. *Acta Psychiatrica Scandinavica* 103: 1–8.
300. Burgess, H. J., Fogg, L. F., Young, M. A., and Eastman, C. I. (2004). Bright light therapy for winter depression: Is phase advancing beneficial? *Chronobiology International* 21: 759–775.
301. Levendosky, A. A., Joseph-Vanderpool, J. R., Hardin, T., Sorek, E., and Rosenthal, N. E. (1991). Core body temperature in patients with seasonal affective disorder and normal controls in summer and winter. *Biological Psychiatry* 29: 524–534.
302. Koorengevel, K. M., Beersma, D. G. M., den Boer, J. A., and van den Hoofdakker, R. H. (2002). A forced desynchrony study of circadian pacemaker characteristics in seasonal affective disorder. *Journal of Biological Rhythms* 17: 463–475.
303. Koorengevel, K. M., Beersma, D. G. M., den Boer, J. A., and van den Hoofdakker, R. H. (2003). Mood regulation in seasonal affective disorder patients and healthy controls studied in forced desynchrony. *Psychiatry Research* 117: 57–74.
304. Glickman, G., Byrne, B., Pineda, C., Hauck, W. W., and Brainard, G. C. (2006). Light therapy for seasonal affective disorder with blue narrow-band light-emitting diodes (LEDs). *Biological Psychiatry* 59: 502–507.
305. Gooley, J. J., Rajaratnam, S. M. W., Brainard, G. C., Kronauer, R. E., Czeisler, C. A., and Lockley, S. W. (2010). Spectral responses of the human circadian system depend on the irradiance and duration of exposure to light. *Science Translational Medicine* 2: 31ra33.
306. Bhattacharjee, Y. (2007). Is internal timing key to mental health? *Science* 317: 1488–1490.
307. Golden, R. N., Gaynes, B. N., Ekstrom, R. D., Hamer, R. M., Jacobsen, F. M., Suppes, T., Wisner, K. L., and Nemeroff, C. B. (2005). The efficacy of light therapy in the treatment of mood disorders: A review and meta-analysis of the evidence. *American Journal of Psychiatry* 162: 656–662.
308. Terman, M. (1993). Overview: Light treatment and future directions of research. In: Wetterberg, L. (Ed.), *Light and Biological Rhythms in Man*. Cambridge, U.K.: Pergamon, pp. 421–436.
309. Schwartz, P. J., Brown, C., Wehr, T. A., and Rosenthal, N. E. (1996). Winter seasonal affective disorder: A follow-up study of the first 59 patients of the National Institute of Mental Health Seasonal Studies Program. *American Journal of Psychiatry* 153: 1028–1036.
310. Eastman, C. I., Young, M. A., Fogg, L. F., Liu, L., and Meaden, P. M. (1998). Bright light treatment of winter depression. *Archives of General Psychiatry* 55: 883–889.
311. Avery, D. H., Eder, D. N., Bolte, M. A., Hellekson, C. J., Dunner, D. L., Vitiello, M. V., and Prinz, P. N. (2001). Dawn simulation and bright light in the treatment of SAD: A controlled study. *Biological Psychiatry* 50: 205–216.
312. Martin, S. K. and Eastman, C. I. (2002). Sleep logs of young adults with self-selected sleep times predict the dim light melatonin onset. *Chronobiology International* 19: 695–707.
313. Laberge, L., Carrier, J., Lespérance, P., Lambert, C., Vitaro, F., Tremblay, R. E., and Montplaisir, J. (2000). Sleep and circadian phase characteristics of adolescent and young adult males in a naturalistic summertime condition. *Chronobiology International* 17: 489–501.
314. Shinkoda, H., Matsumoto, K., Park, Y. M., and Nagashima, H. (2000). Sleep-wake habits of schoolchildren according to grade. *Psychiatry and Clinical Neurosciences* 54: 287–289.
315. Ghata, J., Reinberg, A., Lagoguey, M., and Touitou, Y. (1977). Human circadian rhythms documented in May-June from three groups of young healthy males living respectively in Paris, Colombo and Sydney. *Chronobiologia* 4: 181–190.
316. Motohashi, Y. (1990). Desynchronization of oral temperature and grip strengths: Circadian rhythms in healthy subjects with irregular sleep-wake behavior. *Progress in Clinical and Biological Research* 341B: 57–63.
317. Horne, J. A. and Östberg, O. (1977). Individual differences in human circadian rhythms. *Biological Psychology* 5: 179–190.
318. Binkley, S., Tome, M. B., Crawford, D., and Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
319. Park, Y. M., Matsumoto, K., Seo, Y. J., and Shinkoda, H. (1997). Scores on morningness-eveningness and sleep habits of Korean students, Japanese students, and Japanese workers. *Perceptual and Motor Skills* 85: 143–154.

320. Medeiros, A. L. D., Mendes, D. B. F., Lima, P. F., and Araujo, J. F. (2001). The relationships between sleep-wake cycle and academic performance in medical students. *Biological Rhythm Research* 32: 263–270.
321. Roenneberg, T., Wirz-Justice, A., and Merrow, M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
322. Taillard, J., Philip, P., Chastang, J. F., and Bioulac, B. (2004). Validation of Horne and Ostberg morningness-eveningness questionnaire in a middle-aged population of French workers. *Journal of Biological Rhythms* 19: 76–86.
323. Giannotti, F., Cortesi, F., Sebastiani, T., and Ottaviano, S. (2002). Circadian preference, sleep and daytime behaviour in adolescence. *Journal of Sleep Research* 11: 191–199.
324. Bellamy, N., Sothern, R. B., and Campbell, J. (2004). Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. *Journal of Rheumatology* 31: 379–389.
325. Robilliard, D. L., Archer, S. N., Arendt, J., Lockley, S. W., Hack, L. M., English, J., Leger, D. et al. (2002). The 3111 *Clock* gene polymorphism is not associated with sleep and circadian rhythmicity in phenotypically characterized human subjects. *Journal of Sleep Research* 11: 305–312.

This page intentionally left blank

17 Pet Selection and Veterinary Medicine

17.1 PET SELECTION

In the three preceding chapters, we saw that knowledge of circadian physiology has potential applications in the optimal daily scheduling of physical and intellectual activities, in the planning of programs of space exploration, in the prevention and treatment of the malaises associated with transmeridian flights and shift work, and in many areas of human medicine, including the treatment of cardiovascular disease, cancer, asthma, sleep disorders, and depression. In this chapter, we will see that circadian physiology can also assist us in the selection of pets (Figure 17.1) and in the practice of veterinary medicine.

Pets are animals that humans keep for amusement or companionship. Many people enjoy spending time with pets, and psychological research has provided evidence that pet ownership buffers the owner from the deleterious cardiovascular effects of stressful situations.[1] Figure 17.2 shows the numbers of pets currently kept in the United States. The top graph indicates that dogs and cats are the pets preferred by most households. Dogs are more popular than cats, but, as indicated in the bottom graph, people who have cats have more of them, so that the total cat population (74 million cats) is slightly larger than the dog population (71 million dogs). Multiple ownership is even higher for fish, so that the fish population is almost as large as the dog population even though much fewer households have pet fishes. The choice of a pet involves multiple factors, such as cost, housing requirements, temperament, longevity, and "cuteness." One factor that many pet owners do not consider until after they have acquired the pet is diurnality or nocturnality (that is, whether the pet is active mostly during the day or mostly at night). This is where circadian physiology can provide assistance.

Figure 17.3 lists the eight most common pets grouped as diurnal or nocturnal species. Dogs (*Canis familiaris*) vary greatly in body size, according to breed. They are truly diurnal, can be housed either indoor or outdoor, and are affectionate and resourceful (as indicated by their nickname, "man's best friend"). Dogs can be adopted for a nominal fee at animal shelters or purchased from dog breeders at prices ranging from a few hundred to several thousand dollars (in the case of purebred dogs). The long process of domestication of dogs over the centuries apparently resulted in the selection of human-oriented canine characteristics. For instance, dogs—but not wolves or chimpanzees—can understand human communicative signals, such as the indication of location by finger pointing.[2] It has also been reported that when people choose purebred dogs as pets, they pick breeds with physical characteristics that resemble their own,[3] although the legitimacy of this finding has been questioned.[4] The most popular dog breeds in the United States are listed in Table 17.1. The top four breeds (Labrador, Rottweiler, German Shepherd, and Golden Retriever) are relatively large in size, but many breeds are quite small, such as Pomeranians, Chihuahuas, and some Poodle varieties.

Cats (*Felis catus*) are smaller than most dogs and can be trained to urinate and defecate in a *litter box*, which makes them convenient indoor pets for people who do not have the time to *walk the dog*. Cats can be very affectionate, but because they are nocturnal, they are often asleep when the pet owner is awake, which prevents frequent interactions. As a matter of fact, cats sleep about 16 hours per day, which gives humans the impression that they are always sleeping (Figure 17.4). People who desire a low-maintenance pet usually prefer cats to dogs. Like dogs, cats can be adopted for a nominal fee at animal shelters or purchased from cat breeders. Cats and dogs are very popular pets both in the United States and in Europe (Table 17.2).

The next two pets described in Figure 17.3 are birds—in the diurnal group—and rabbits—in the nocturnal group (Figure 17.5). Most pet birds are lovebirds (parakeets or budgerigars) and parrots. They are smaller than cats and dogs and are almost always kept in indoor cages. Although they are diurnal, their indifferent temperament makes them much less endearing than dogs to most people. Rabbits (*Oryctolagus cuniculus*) are technically crepuscular—not nocturnal—which usually allows them to be awake when their owners are awake. As furry mammals, they rank high in the "cuteness" scale. However, their indifferent temperament makes them good pets only for people who do not seek playful interaction with their pets.

Reptiles (lizards, snakes, turtles, and tortoises) and fishes are the other two common diurnal pets. Many people like them as pets, but others avoid them because the skin of reptiles and fishes is covered with scales rather than with fur or feathers. Among the nocturnal pets, ferrets (*Mustela putorius*) are very affectionate, whereas rodents (hamsters, gerbils, rats, mice, and guinea pigs) are more indifferent. Because most rodents are truly nocturnal, many rodent pet owners complain that their pets are always asleep during the day. Worse, because rodents are often provided with running wheels for entertainment, they run on the wheel all night long—thus disturbing the owner's sleep. An alternative for those who desire the convenience of a small rodent but require a diurnal species is the Nile grass rat (Figure 17.6). Nile grass rats (*Arvicanthis niloticus*) are small (120 g) diurnal rodents indigenous to north Africa.[5] Unfortunately, Nile grass rats are not just unusual as diurnal rodents; they are also unusual as pets—which means that they are *not* available at pet stores. If you want to obtain one, you may need to place a bid on eBay!

A pet that deserves special mention is the marmoset monkey (*Callithrix jacchus*). Marmosets are the smallest primates.



FIGURE 17.1 Furry friends. Dogs (*Canis familiaris*) and cats (*Felis catus*) are the favorite pets in most households. (Photo by R. Refinetti.)

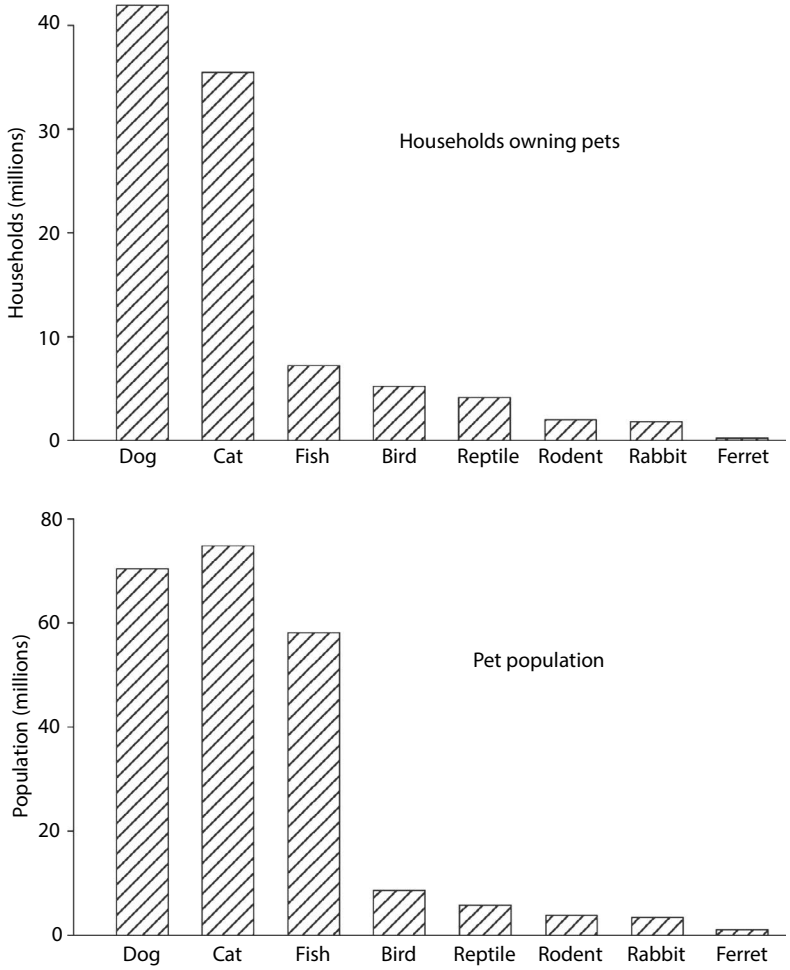


FIGURE 17.2 The most popular pets. The graphs show the number of U.S. households that own various species of pets (Panel A) and the total populations of these pets (Panel B). Although dogs are owned by the largest number of households, the populations of cats and fish are slightly larger because cat and fish owners tend to own multiple pets. (Data from *U.S. Pet Ownership and Demographics Sourcebook*, American Veterinary Medical Association, Schaumburg, IL, 2012).

Diurnal		Nocturnal	
Dog 	3–100 kg Indoor/outdoor 14–20 years Affectionate	Cat 	2–15 kg Indoor/outdoor 14–20 years Affectionate
Bird 	1/4–5 kg Cage 15–50 years Indifferent	Rabbit 	2–15 kg Indoor/outdoor 7–8 years Indifferent
Reptile 	1/4–20 kg Cage 8–15 years Indifferent	Ferret 	1–8 kg Indoor/cage 6–10 years Affectionate
Fish 	<1 kg Aquarium 30–40 years Indifferent	Rodent 	<1 kg Cage 2–8 years Indifferent

FIGURE 17.3 Characteristics of various pets. Four groups of diurnal (day-active) pets and four groups of nocturnal (night-active) pets are described. For each group, information about body size in kilograms (1 kg ≈ 2 lb), usual housing, longevity, and temperament is provided.

They are adorable as infants (Figure 17.7) and make excellent pets that are very affectionate and playful if adopted before weaning. They grow up to weigh less than 500 g (1 lb) as adults and need to be caged only for their own protection (that is, not to be stepped on by people or to run out of the house door and be eaten by an outdoor cat). They are fully diurnal and live 10 years on average. Originally from Brazil, they are bred in several locations in the United States.[6] One problem regarding marmosets is that many states in the United States (and some other countries) have laws against the adoption of primates as pets. Another problem is that uncastrated males can become aggressive after reaching full sexual maturity at about 2 years of age. Although they are too small to present a serious risk to humans, these aggressive males can produce skin lacerations and may need to be kept in a cage most of the time for the protection of humans in the house, especially children (Figure 17.8).

Finally, one pet that you are encouraged *not* to choose is a tiger (Figure 17.9). Tigers (*Panthera tigris*) are large, wild animals that, unlike dogs and cats, have *not* been domesticated and selectively bred for thousands of years. They can cause serious injury or death to humans. It is estimated that there are 10,000 tigers in the United States, many of them as pets.[7]

TABLE 17.1
Most Popular Dog Breeds in the United States

Ranking	Breed
1	Labrador Retriever
2	Rottweiler
3	German Shepherd
4	Golden Retriever
5	Beagle
6	Poodle
7	Cocker Spaniel
8	Dachshund
9	Pomeranian
10	Yorkshire Terrier
11	Dalmatian
12	Shih Tzu
13	Shetland Sheepdog
14	Chihuahua
15	Boxer

Sources: American Kennel Club, New York; American Veterinary Medical Association, Schaumburg, IL.

17.2 VETERINARY MEDICINE

Veterinary medicine is concerned with the prevention and treatment of disease in nonhuman animals. In addition to overseeing livestock, veterinary professionals in the United States care for over 70 million dogs at a cost of 8 billion dollars per year and perform numerous other tasks including food inspection and control of environmental hazards.[8] A small proportion of veterinarians is responsible for the health and well-being of horses, including race horses, which are a popular form of entertainment in many parts of the world (Figure 17.10).

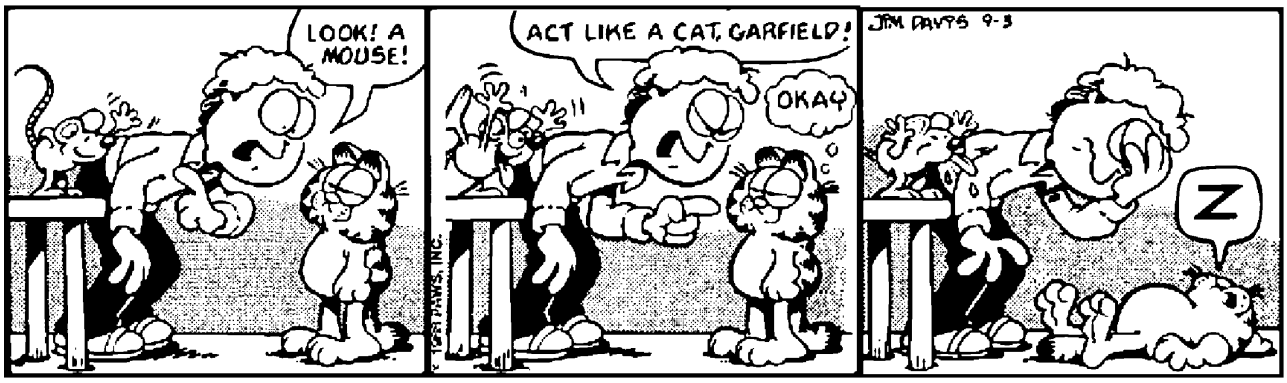


FIGURE 17.4 Cat and mouse. This comic strip of the syndicated cartoon *Garfield*, by Jim Davis, aptly illustrates the fact that cats sleep many more hours per day than other household pets. (From Garfield. Copyright 1988 Paws, Inc. Reprinted with permission of Universal Press Syndicate, Kansas City, MO.)

TABLE 17.2
Pet Population: Dogs and Cats

	Dogs	Cats
United States	71 million	74 million
Europe	75 million	90 million

Sources: Pet Food Institute, Washington, DC; European Pet Food Industry Federation, Brussels, Belgium, for the year 2013.



FIGURE 17.5 Cottontail and sparrow. Rabbits and birds are not uncommon pets. (Courtesy of National Image Library, United States Fish and Wildlife Service, Washington, DC.)



FIGURE 17.6 A Nile grass rat. This small diurnal rodent (*Arvicanthis niloticus*, 120 g) may be a good pet for some people. (Photo by R. Refinetti.)



FIGURE 17.7 An infant marmoset monkey. Marmoset monkeys (*Callithrix jacchus*) are the smallest primates, rarely weighing more than 500 g (1 lb) as adults. They are diurnal, playful, affectionate, and have physical features that are very attractive to humans. (Photo by R. Refinetti.)

As was the case for human medicine (Chapter 16), veterinary medicine can benefit from advances in circadian physiology. To start with, knowledge of the daily rhythmicity of body temperature can provide greater accuracy in the diagnosis of febrile states. Look at Figure 17.11, which shows the body temperature rhythm of a horse. This particular horse had a mean body temperature slightly higher than that of the average horse, but the daily oscillation around the mean is what is worth noting. The data shown have a mean level of 38.3°C and a range of oscillation of approximately 0.6°C. Should the daily oscillation be ignored, this horse would be misdiagnosed as febrile from 4 p.m. to 6 a.m. and as hypothermic at all other times of the day except for the two brief intervals during which the curve crosses the mean level. Of course, this would be true also for all other domestic animals. Figure 17.12 shows the normal body temperature ranges for the 10 animals most commonly encountered in veterinary practice. Birds (chicken and turkey) are consistently warmer than mammals, but there is considerable overlap in the normal ranges for the mammalian species. Generally, core temperatures below



FIGURE 17.8 Miniature King Kong. This fully grown marmoset monkey still weighs less than a pound and still has the looks of an endearing pet but must be kept in a cage because of occasional *temper tantrums* that might result in minor injury to his human caretakers. (Photo by R. Refinetti.)



FIGURE 17.9 Wild beast. Tigers (*Panthera tigris*) cannot be fully domesticated and are not safe pets. (Courtesy of National Image Library, United States Fish and Wildlife Service, Washington, DC.)

37.4°C characterize a hypothermic state, whereas temperatures above 39.9°C characterize a febrile state in any of the eight mammalian species. Notice that the lowest hypothermic threshold for these animals (37.4°C) is higher than the mean core temperature of humans (36.9°C).

Throughout this book, mention was made of many studies of circadian rhythms conducted on farm animals such as



FIGURE 17.10 Horse race. Racehorses constitute one of many groups of animals that require regular veterinary care. (Photo by Bill Tarpenning; Courtesy of United States Department of Agriculture's Photography Center, Washington, DC.)

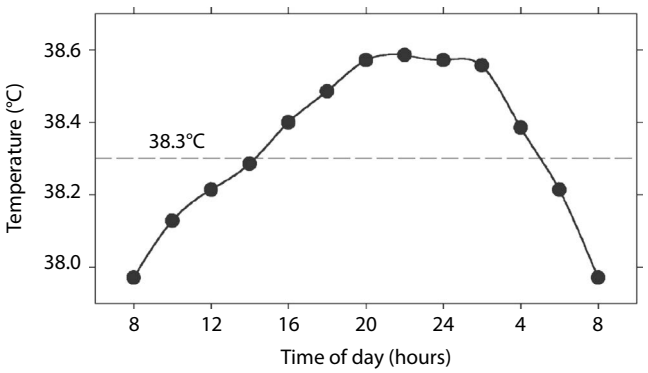


FIGURE 17.11 What is a fever? The graph shows the daily rhythm of rectal temperature of a normal healthy English Thoroughbred horse. Because the temperature oscillates from 37.9°C to 38.6°C daily, it makes no sense to use the mean value of 38.3°C (100.9°F) as a criterion for ascertaining the presence of a fever. (Data from Piccione, G. et al., *Biol. Rhythm Res.*, 33, 113, 2002.)

chicken,[9–23] sheep,[24–38] goat,[39–44] pig,[45–48] horse,[49–57] and cattle.[58–63] However, the number of studies is very small when compared to the number of studies conducted on laboratory animals. Furthermore, research on circadian rhythms in farm animals has too often lacked the rigor of scientific investigation found in laboratory research.[64,65] As a consequence, the exact parameters of circadian rhythms in farm animals have not been adequately determined. Yet, chronobiology of livestock will require controlled experiments similar to those conducted with laboratory animals. Refinements in research procedures are important not as a mere academic exercise but as a source of improvement in farming practices. Although the study of physiological processes under current farming practices may have immediate applicability, it does not provide the advancement in knowledge of fundamental processes that is necessary for the development of new, more efficient practices.

My research collaborator, Giuseppe Piccione (from the University of Messina, in Italy), is one of the few veterinarians engaged in serious research in circadian physiology

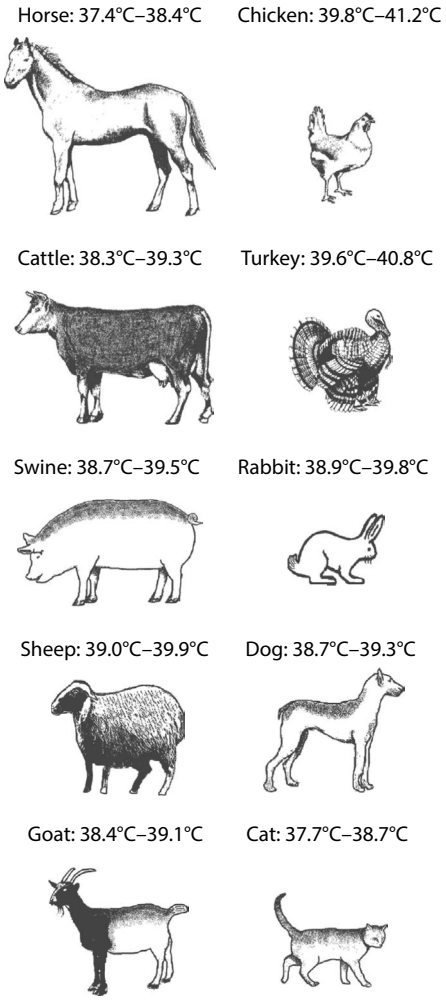


FIGURE 17.12 Normal range of body temperature. Shown are the normal ranges of oscillation of the body temperature rhythms of the 10 animals most commonly encountered in veterinary practice.

(Figure 17.13). One of many phenomena that we have investigated together is the disruption of the body temperature rhythm caused by parturition (i.e., by giving birth). Figure 17.14 shows the mean rectal temperatures of groups of mares (female horses) and ewes (female sheep) measured at dusk and dawn for several days after parturition. The mares were hyperthermic for a few days after giving birth, to such an extent that the dusk–dawn temperature difference was eliminated. The temperature of the ewes was less affected by parturition.[37]

Figure 17.15 shows simultaneous records of rectal temperature (with 2-hour resolution) and locomotor activity (with 1-minute resolution) of a nonpregnant horse maintained under a 24-hour light–dark cycle. A 1-day segment of the data is shown. As expected of a diurnal animal, the horse was much more active during the day than during the night. Rectal temperature was lowest at daybreak and rose slowly to achieve a peak in the early night. These records are representative of those obtained on other days in the same animal as well as in other horses. The slow rise of body temperature during the active hours resembles that seen in humans[66–85] and contrasts with the sudden rise upon awakening seen in rodents such as the rat.[86–104]



FIGURE 17.13 Giuseppe Piccione. This veterinary researcher from the University of Messina (Italy) is one of the few veterinarians in the world who specializes in the study of circadian rhythms. (Photo courtesy of Giuseppe Piccione.)

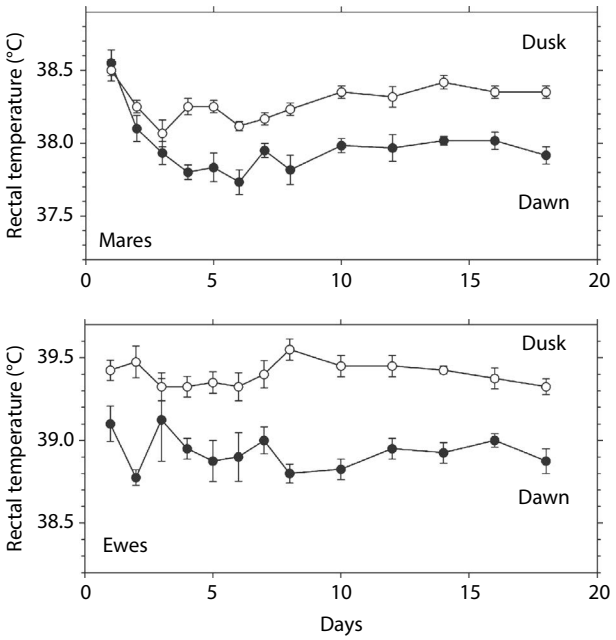


FIGURE 17.14 Maternity and circadian rhythms. The graphs show the mean rectal temperatures at dusk and dawn of female horses (mares) and female sheep (ewes) for 18 days after parturition. The data points correspond to the means (±SE) of 6 mares and 4 ewes. Animals gave birth on day 0. Notice that—except for a transient hyperthermia, particularly at dawn for the mares—the body temperature rhythm of the mothers (as estimated by the measurements at dusk and dawn) was not greatly affected by parturition. (Data from Piccione, G. et al., *J. Therm. Biol.*, 27, 333, 2002.)

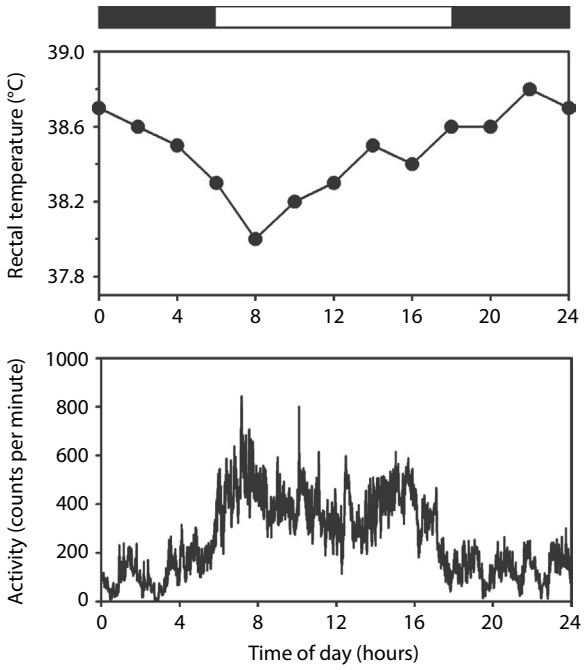


FIGURE 17.15 Rhythms of activity and body temperature of the horse. The graphs show 1-day segments of the records of body temperature (measured every 2 hours with a rectal probe) and locomotor activity (measured at 1-minute intervals with a data logger) of a representative female horse housed in a stall under a light–dark cycle (as indicated by the horizontal bar at the top). Unlike the pattern seen in laboratory rodents, body temperature continues to rise for several hours after the end of the phase of high locomotor activity. (Data courtesy of Giuseppe Piccione, University of Messina, Messina, Italy.)

I am aware of only six studies of the daily rhythm of body temperature of goats. Daily rhythmicity was found in all six studies, with acrophases ranging from 10 to 16 hours after sunrise across studies.[36,39–41,44,105] This means that body temperature peaked consistently toward the end of the day or beginning of the night. The 6-hour spread can be partially, though not fully, explained by the fact that different studies employed different photoperiods (i.e., different durations of the light phase of the light–dark cycle), which affects the timing of the acrophase. The average mean level of the temperature rhythm ranged from 38.5°C to 39.0°C in the six studies. This spread of 0.5°C is surprisingly narrow in view of the fact that ambient temperature was not thermostatically controlled in any of the studies. The fact that the mean level of body temperature varied only 0.5°C across studies conducted under different ambient temperatures, in some cases with large daily oscillations, attests to the accuracy of the thermoregulatory system of the goat. Finally, the average daily range of excursion of the temperature rhythm calculated in each study varied from 0.4°C to 1.9°C. This large interstudy variability can be attributed mostly to the variability in ambient temperature. The 1.9°C range of excursion was observed in a study in which ambient temperature oscillated by as much as 20°C daily.[41] Another study found a range of excursion of 0.4°C when the animals were tested outdoors in the (mild) winter and of 1.1°C when the animals were tested in the (hot) summer.[39] While the use of natural photoperiods and natural variations in ambient temperature may provide a naturalistic research environment, it also prevents the rigorous study of rhythmic parameters that can be achieved in controlled laboratory experiments.[98]

As mentioned in Chapter 4, the horse is one of various mammals that exhibit reproductive seasonality. In the northern hemisphere, mares breed only from April to September.[106] As in humans, the age of a horse is counted by the calendar year of birth, regardless of the exact birth date. Thus, horse breeders have an interest in having foals that are born early in the year. More than 60 years ago, it was shown that the breeding season can be advanced by manipulation of the photoperiod to which the mares are exposed.[107] Because day length is shorter in the winter, early spring can be simulated by artificial light presented early in the day (prior to sunrise) and late in the day (after sunset).[108] The procedure amounts to a phase advance of the zeitgeber that entrains the circannual rhythm of reproduction of the mares.

Manipulations of the light–dark cycle can also be used to synchronize the daily rhythm of oviposition (egg laying) of domestic fowl (chicken and quail). Although the oviposition rhythm cannot be entrained in all hens (see Chapter 9), most hens will lay an egg a day under a variety of 24-hour photoperiods.[14,109,110] To save energy costs in a chicken farm, light–dark cycles with as little as 2 hours of light per day can be used.[14] An interesting idea to increase productivity is to implement a light–dark cycle shorter than 24 hours. If the oviposition rhythm could be entrained, for example, by a 21-hour cycle, the farmer could obtain an egg from each hen every 21 hours instead of every 24 hours. The rhythm does seem to be entrained by a 21-hour cycle, but egg production per cycle is reduced, thus resulting in no net gain.[14]

In cows, the estrous cycle lasts 21–25 days and involves oscillations in both activity and body temperature.[111,112] A rise in body temperature of 0.2°C–0.6°C is observed on the day of estrous,[111–116] although different studies describe this rise as lasting only a few hours[112,114,116] or through the whole luteal phase.[111,113] The use of body temperature records to predict ovulation (as measured by ultrasonography or blood progesterone levels) has a detection rate of 80%–87% and a false-positive rate of 10%–17%.[112,114,116]

Livestock production is an industry of colossal magnitude. Figure 17.16 shows the annual production of the four major sources of meat in the United States. In the top graph, you can see that about the same amounts of cattle meat (beef) and chicken meat are produced each year (20 billion kg, or 44 billion lb), with swine meat (pork) and turkey meat coming in third and fourth places. However, because chickens are much lighter than the other animals, many more chickens must be slaughtered to make up the same amount of meat (Panel B). Each year, almost 9 billion chickens are slaughtered in the United States alone. Table 17.3 shows how much meat an average U.S. resident eats each year. Given the consumption of about 26 kg of chicken meat per year, and considering that one chicken yields about 2 kg of meat, one can easily calculate that the average American eats 13 chickens each year. What we call *meat* is muscle tissue, such as the muscle in

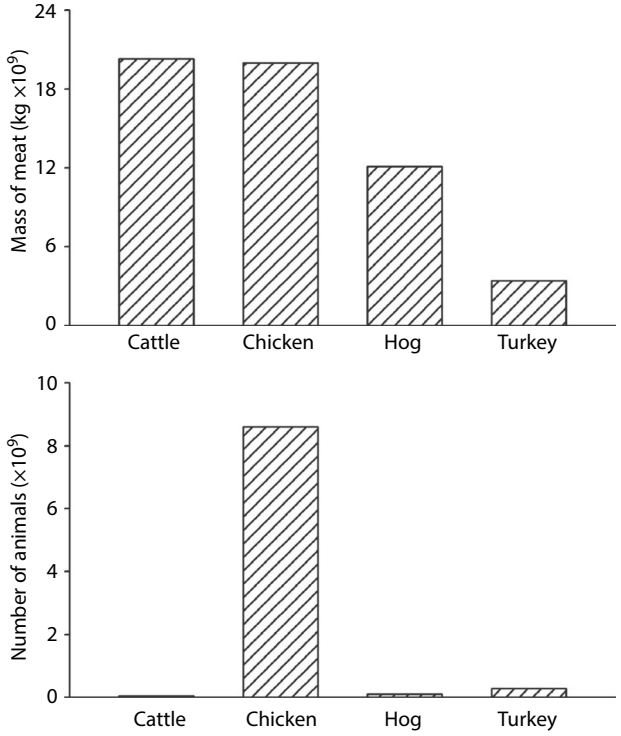


FIGURE 17.16 Livestock production. The graphs show the annual production of the four major sources of meat in the United States in terms of mass of meat (Panel A) and of number of animals (Panel B). The production of chicken meat (20 billion kg) is comparable to that of cattle meat; however, because a chicken's mass is only 0.4% of that of a cow, the number of chickens killed each year (8.6 billion) is much larger than that of cattle (35.7 million). (Data from *Livestock Slaughter—2002 Summary*, National Agricultural Statistics Service, Washington, DC, 2003; *Poultry Production and Value—2002 Summary*, National Agricultural Statistics Service, Washington, DC, 2003.)

TABLE 17.3
Consumption of Meat in the United States

Meat	kg/person/year	lb/person/year
Chicken	26.5	58.4
Beef	24.7	54.5
Pork	19.2	42.4
Fish	6.8	14.9

Source: *The World Almanac and Book of Facts*, Simon & Schuster, New York, 2014.

chicken breast. Muscle that performs much work (such as that in cattle legs) is usually hard and must be cooked slowly for many hours in order to be easily eaten. Muscle that performs little work (such as that in the back of cows) is naturally tender.

Although the health of farm animals while they are being raised is a major responsibility of veterinarians, transportation to slaughter houses is no small matter. Road transport of livestock can cause stress, injury, fatigue, and morbidity as a result of limited access to food and water during the transportation process, of exposure to extreme climatic conditions, noise, and toxins, as well as of poor handling and of mixing with unfamiliar animals.[117] Less than 1% of animals die while being transported, but because of the large number of animals that are transported, the absolute number of deaths is significant. For instance, in the United States, about 220,000 swines arrive dead at the destination each year, and comparable death rates are observed in other countries.[118]

Cattle (Figure 17.17) are used for the production of not only meat (beef and veal) but also milk (dairy products). Additionally, they are used as draft animals (pulling carts and plows) in many locations. Other products from cattle include leather and dung (the latter for manure). Refined red-meat eaters can distinguish a number of beef cuts, the primal cuts being the chuck, brisket, shank, rib, plate, flank, round, short loin, sirloin, top sirloin, bottom sirloin, and tenderloin. The tenderloin, which is the most tender cut, is either removed as a separate subprimal (filet mignon) or cut bone-in as part of the T-bone and porterhouse loin steaks. The term "cattle" refers to two species, *Bos taurus* and *Bos indicus*, that were domesticated about 10,000 years ago. The most common species in Europe and the Americas is *B. taurus*.[119]

Chickens (Figure 17.18) contribute both meat and eggs to the human food supply. In commercial poultry production, there are two types of chickens—an egg type and a meat type, with a number of breeds within each type. The egg type (or *layer*) and the meat type (or *broiler*) both produce eggs and meat, but layers are more efficient at producing eggs, whereas broilers are more efficient at growing, thus being more efficient at producing meat. The two types of chickens belong to the same species, *Gallus domesticus*.

Pigs (Figure 17.19) and turkeys (Figure 17.20) contribute almost exclusively meat to the human food supply. Pig meat is sold under various names, principally under the names pork, ham, and bacon. All domestic pigs belong to the same species, *Sus scrofa*. Roasted whole turkeys are extremely popular in the United States for two national holidays: Thanksgiving and Christmas. Domestic turkeys belong to the species *Meleagris gallopavo*.

Sheep (Figure 17.21) provide meat (lamb and mutton), fleece (wool), and, less commonly, milk. Because people raised in cities are frequently unaware of the sources of various products, it should be added that sheep are the source of one of the major fibers used as fabric for clothing (wool). The other major source of textile fiber is cotton, which is a plant, not an animal product. There are many breeds of domestic sheep, but they all belong to one species, *Ovis aries*.

With the increase in human population and the rising demand for animal products, larger and more specialized livestock production units have been developed. These units require added efficiency to balance the narrow margin of profit, and veterinarians must give consideration to various practical issues, such as the cost of losing a certain percentage of the calf crop, or the cost of rebreeding a cow with low reproductive capacity.[8]

Some veterinarians work in zoological parks (Figure 17.22), although few of them are employed exclusively by the zoos.



FIGURE 17.17 Grazing cattle. Cattle is the number one source of edible meat in the United States. (Courtesy of Agricultural Research Service, United States Department of Agriculture, Washington, DC.)

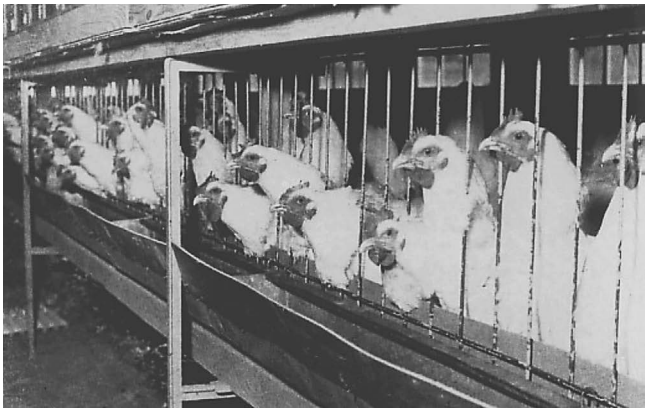


FIGURE 17.18 Chicken farm. Although production of chicken meat is slightly lower than production of beef (cattle meat), more chickens are slaughtered each year than any other farm animal in the United States. (Image courtesy of Giuseppe Piccione.)



FIGURE 17.19 Sow with piglet. Pork (swine meat) is the third most produced meat in the United States. (Photo by Scott Bauer; Courtesy of Agricultural Research Service, United States Department of Agriculture, Washington, DC.)

Most zoo veterinarians are private practitioners who have a contract with the zoo to provide care to the captive animals.[8] Here, knowledge of circadian physiology is helpful not only in the regular treatment of disease but also in the implementation of exhibits for nocturnal animals. Several zoos have exhibit halls with reversed light–dark cycles to provide visitors with the opportunity to observe nocturnal animals (mostly mammals) during the active phase of their daily cycle. For example, the New York Zoological Park opened its World of Darkness exhibit in 1969,[120] the Berlin Zoological Park (in Germany) opened its Nocturnal House exhibit in 1975,[121] and the Bristol Zoological Park (in England) opened its Twilight World exhibit in 1996.[122] From personal experience, I can add that the U.S. National Zoological Park (in Washington, DC) keeps its nocturnal small animals under reversed light–dark cycles—even if it can be difficult to find the animals in the dark exhibit rooms! Proper organization of these exhibits requires knowledge of the extent of nocturnality of the species as well as knowledge of the physical characteristics of the photic environment (photoperiod length, illuminance levels, spectral composition, etc.).

Whereas physicians frequently have to address the problem of transmission of diseases from animals to humans (which is called "zoonosis"), veterinarians often have to address the problem of *inverse zoonosis*, or the transmission of diseases from humans to animals. About 43% of reported cases of disease transmission from humans to animals are related to wildlife, 37% to livestock, and 20% to companion animals.[123]

Over 45,000 veterinarians in the United States are involved in private clinical practice, and 60%–70% of them specialize in small animals, such as cats and dogs[8] (Figure 17.23).



FIGURE 17.20 Turkey farm. Turkey meat is the fourth most produced meat in the United States. (Photo by Scott Bauer; Courtesy of Agricultural Research Service, United States Department of Agriculture, Washington, DC.)



FIGURE 17.22 At the zoo. Shown is the Brick Elephant House, built in 1903 at the U.S. National Zoological Park. (Courtesy of Smithsonian Institution Archives: Record Unit 95, Box 47, Folder 13, Neg. # 15533, Washington, DC.)



FIGURE 17.21 Sheep farm. Sheep are farmed for both meat and wool. (Courtesy of Agricultural Research Service, United States Department of Agriculture, Washington, DC.)



FIGURE 17.23 My puppy. Many veterinarians specialize in the health care of household pets such as this Toy Poodle. (Photo by R. Refinetti.)

Here again, knowledge of circadian physiology can be very helpful in the prevention and treatment of disease. As one example, surgical procedures for spaying, removal of tumors, or extirpation of anal glands are quite common in small-animal practice. Thus, adequate anesthesia is required. The combination of ketamine and midazolam (or diazepam), which is commonly used in veterinary anesthesia, produces longer sedation during the inactive phase of the circadian cycle than during the active phase.[124] Thus, the anesthetic dose must be adjusted to ensure optimal action at different times of the day.

SUMMARY

1. The selection of a pet involves many factors, such as cost, appearance, housing requirements, temperament, longevity, and diurnality or nocturnality. Most people prefer diurnal pets and can enjoy dogs, birds, reptiles, and fishes. If nocturnality of the pet is an undesired characteristic, pets such as cats, rabbits, ferrets, and rodents may not be good choices.
2. Veterinarians care for the health of animals in private clinical practice as well as in industrial settings. Knowledge of circadian physiology can help veterinarians in general to improve health care and can assist industrial veterinarians and farmers in the improvement of livestock management.

EXERCISES

17.1 Circadian discrepancy between pets and pet owners. The program **Health**, which you have used in the exercises in the preceding chapters, has a section on the compatibility between pets and pet owners (second choice from the bottom in the main menu). This issue has been addressed in the text (Section 17.1), but the program provides a simple, automatic evaluation of compatibility. Check it out.

17.2 No more exercises! If you have made it all the way to this last chapter, you have already worked very hard and now deserve a break. If you have not yet listened to the *Bioclock Rhapsody* (the program whose icon is the second from the right in the Circadian banner), you may do it now. Although this piece sounds like a studio recording, it is entirely synthesized. All instruments and vocals were digitally synthesized on a computer. The only human being involved in the project was the composer (R. Refinetti). More than just entertainment, this is a work of voice and instrument synthesis. The lyrics are pertinent to circadian physiology. If you are curious about the computer software used in the production of the piece, a full list follows. ***Vocals:*** Digital audio synthesizers using the University of Edinburgh's *Festival Speech Synthesis System* and Oregon Health & Science University's *Flinger Singing Voice Synthesis System*. ***Instruments:*** MIDI synthesizers using Voyetra's *Music Write Plus*, Ars-Nova's *Songworks II,* and Voyetra's *Digital Orchestrator Pro*. ***Mixing:*** MIDI-to-WAV conversions using FMJ Software's *Awave Studio.*

SUGGESTIONS FOR FURTHER READING

Fogle, B. (2000). *The New Encyclopedia of the Dog.* New York: D.K. Publishing.

With a little over 400 pages, this general-audience book written by a veterinarian provides plenty of information about dog breeds to satisfy the needs of prospective pet owners without overwhelming the reader with details that belong in a specialized veterinary book.

Kahn, C. M. (Ed.) (2010). *Merck Veterinary Manual*, 10th edn. West Trenton, NJ: Merck & Co.

With over 2800 pages, this introductory book for veterinary students is a comprehensive treatise on veterinary medicine, covering practically everything from animal behavior to zoonoses.

Bassert, J. M. and Thomas, J. (Eds.) (2013). *Clinical Textbook for Veterinary Technicians,* 8th edn. Philadelphia, PA: W.B. Saunders.

At 1500 pages, this textbook is, by design, not as comprehensive as the *Merck Manual*. However, it provides the essential material needed by veterinary technicians.

WEBSITES TO EXPLORE

American Animal Hospital Association: https://www.aaha.org/.
American Kennel Club: http://www.akc.org/.
American Veterinary Medical Association: http://www.avma.org/.
Department of Animal Science at Oklahoma State University: http://www.ansi.okstate.edu/.
Federation of Veterinarians of Europe: http://www.fve.org/.
International Livestock Research Institute: https://www.ilri.org/.
Pets' Mart: http://www.petsmart.com/.

REFERENCES

1. Allen, K. (2003). Are pets a healthy pleasure? The influence of pets on blood pressure. *Current Directions in Psychological Science* 12: 236–239.
2. Hare, B., Brown, M., Williamson, C., and Tomasello, M. (2002). The domestication of social cognition in dogs. *Science* 298: 1634–1636.
3. Roy, M. M. and Christenfeld, N. J. S. (2004). Do dogs resemble their owners? *Psychological Science* 15: 361–363.
4. Levine, D. W. (2005). Do dogs resemble their owners? A reanalysis of Roy and Christenfeld. *Psychological Science* 16: 83–84.
5. Refinetti, R. (2004). The Nile grass rat as a laboratory animal. *Lab Animal* 33(9): 54–57.
6. Abbott, D. H., Barnett, D. K., Colman, R. J., Yamamoto, M. E., and Schultz-Darken, N. J. (2003). Aspects of common marmoset basic biology and life history important for biomedical research. *Comparative Medicine* 53: 339–350.
7. Lemonick, M. D. (2003). Never trust a tiger. *Time* 162(16): 63–64.
8. Swope, R. E. (2001). *Opportunities in Veterinary Medicine Careers*. Chicago, IL: VGM Career Books.
9. Winget, C. M., Averkin, E. G., and Fryer, T. B. (1965). Quantitative measurement by telemetry of ovulation and oviposition in the fowl. *American Journal of Physiology* 209: 853–858.
10. Winget, C. M. and Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
11. Aschoff, J. and von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
12. Aschoff, J. and Von Saint Paul, U. (1976). Brain temperature in the unanesthetized chicken: Its circadian rhythm of responsiveness to light. *Brain Research* 101: 1–9.
13. Bobr, L. W. and Sheldon, B. L. (1977). Analysis of ovulation-oviposition patterns in the domestic fowl by telemetry measurement of deep body temperature. *Australian Journal of Biological Sciences* 30: 243–257.
14. Bhatti, B. M. and Morris, T. R. (1978). Entrainment of oviposition in the fowl using light-dark cycles. *British Poultry Science* 19: 333–340.
15. Kadono, H. and Besch, E. L. (1980). Influence of laying cycle on body temperature rhythm in the domestic hen. In: Tanabe, Y. (Ed.), *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin, Germany: Springer, pp. 91–99.
16. Kadono, H., Besch, E. L., and Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
17. Michels, H., Herremans, M., and Decuypere, E. (1985). Light-dark variations of oxygen consumption and subcutaneous temperature in young *Gallus domesticus*: Influence of ambient temperature and depilation. *Journal of Thermal Biology* 10: 13–20.

18. Schenda, J. and Vollrath, L. (2000). Single-cell recordings from chick pineal glands in vitro reveal ultradian and circadian oscillations. *Cellular and Molecular Life Sciences* 57: 1785–1792.
19. Kasahara, T., Okano, T., Haga, T., and Fukada, Y. (2002). Opsin-G_{11}-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *Journal of Neuroscience* 22: 7321–7325.
20. Willbold, E., Huhn, J., Korf, H. W., Voisin, P., and Layer, P. G. (2002). Light-dark and circadian melatonin rhythms are established de novo in re-aggregates of the embryonic chicken retina. *Developmental Neuroscience* 24: 504–511.
21. Chong, N. W., Chaurasia, S. S., Haque, R., Klein, D. C., and Iuvone, P. M. (2003). Temporal-spatial characterization of chicken clock genes: Circadian expression in retina, pineal gland, and peripheral tissues. *Journal of Neurochemistry* 85: 851–860.
22. Bailey, M. J., Beremand, P. D., Hammer, R., Bell-Pedersen, D., Thomas, T. L., and Cassone, V. M. (2003). Transcriptional profiling of the chick pineal gland, a photoreceptive circadian oscillator and pacemaker. *Molecular Endocrinology* 17: 2084–2095.
23. Zawilska, J. B., Berezinska, M., Lorenc, A., Skene, D. J., and Nowak, J. Z. (2004). Retinal illumination phase shifts the circadian rhythm of serotonin *N*-acetyltransferase activity in the chicken pineal gland. *Neuroscience Letters* 360: 153–156.
24. Bligh, J., Ingram, D. L., Keynes, R. D., and Robinson, S. G. (1965). The deep body temperature of an unrestrained Welsh mountain sheep recorded by a radiotelemetric technique during a 12-month period. *Journal of Physiology* 176: 136–144.
25. Recabarren, S. E., Vergara, M., Llanos, A. J., and Serón-Ferré, M. (1987). Circadian variation of rectal temperature in newborn sheep. *Journal of Developmental Physiology* 9: 399–408.
26. Mohr, E. and Krzywanek, H. (1990). Variations of core-temperature rhythms in unrestrained sheep. *Physiology and Behavior* 48: 467–473.
27. Mohr, E. G. and Krzywanek, H. (1995). Endogenous oscillator and regulatory mechanisms of body temperature in sheep. *Physiology and Behavior* 57: 339–347.
28. Houghton, D. C., Young, I. R., and McMillen, I. C. (1995). Evidence for hypothalamic control of the diurnal rhythms in prolactin and melatonin in the fetal sheep during late gestation. *Endocrinology* 136: 218–223.
29. Parraguez, V. H., Valenzuela, G. J., Vergara, M., Ducsay, C. A., Yellon, S. M., and Serón-Ferré, M. (1996). Effect of constant light on fetal and maternal prolactin rhythms in sheep. *Endocrinology* 137: 2355–2361.
30. Guerin, M. V. and Matthews, C. D. (1998). Alterations of estrous activity in the ewe by circadian-based manipulation of the endogenous pacemaker. *Journal of Biological Rhythms* 13: 60–69.
31. Parraguez, V. H., Sales, F., Valenzuela, G. J., Vergara, M., Catalán, L., and Serón-Ferré, M. (1998). Diurnal changes in light intensity inside the pregnant uterus in sheep. *Animal Reproduction Science* 52: 123–130.
32. Lincoln, G. A., Rhind, S. M., Pompolo, S., and Clarke, I. J. (2001). Hypothalamic control of photoperiod-induced cycles in food intake, body weight, and metabolic hormones in rams. *American Journal of Physiology* 281: R76–R90.
33. Lowe, T. E., Cook, C. J., Ingram. J. R., and Harris, P. J. (2001). Impact of climate on thermal rhythm in pastoral sheep. *Physiology and Behavior* 74: 659–664.
34. Souza, M. I. L., Bicudo, S. D., Uribe-Velásquez, L. F., and Ramos, A. A. (2002). Circadian and circannual rhythms of T3 and T4 secretions in Polwarth-Ideal rams. *Small Ruminant Research* 46: 1–5.
35. Lincoln, G., Messager, S., Andersson, H., and Hazlerigg, D. (2002). Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: Evidence for an internal coincidence timer. *Proceedings of the National Academy of Sciences of the United States of America* 99: 13890–13895.
36. Piccione, G., Caola, G., and Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
37. Piccione, G., Caola, G., and Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.
38. Lincoln, G. A., Andersson, H., and Hazlerigg, D. (2003). Clock genes and the long-term regulation of prolactin secretion: Evidence for a photoperiodic/circannual timer in the pars tuberalis. *Journal of Neuroendocrinology* 15: 390–397.
39. Jessen, C. and Kuhnen, G. (1996). Seasonal variations of body temperature in goats living in an outdoor environment. *Journal of Thermal Biology* 21: 197–204.
40. Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D., and Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflügers Archiv* 436: 659–666.
41. Ayo, J. O., Oladele, S. B., Ngam, S., Fayomi, A., and Afolayan, S. B. (1998). Diurnal fluctuations in rectal temperature of the Red Sokoto goat during the harmattan season. *Research in Veterinary Science* 66: 7–9.
42. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2001). Seasonal variation in endogenous serum melatonin profiles in goats: A difference between spring and fall? *Journal of Biological Rhythms* 16: 254–263.
43. Alila-Johansson, A., Eriksson, L., Soveri, T., and Laakso, M. L. (2003). Serum cortisol levels in goats exhibit seasonal but not daily rhythmicity. *Chronobiology International* 20: 65–79.
44. Piccione, G., Caola, G., and Refinetti, R. (2003). Circadian rhythms of body temperature and liver function in fed and food-deprived goats. *Comparative Biochemistry and Physiology A* 134: 563–572.
45. Ingram, D. L. and Mount, L. E. (1973). The effects of food intake and fasting on 24-hourly variations in body temperature in the young pig. *Pflügers Archiv* 339: 299–304.
46. Verstegen, M. W. A., van der Hel, W., Duijghuisen, R., and Geers, R. (1986). Diurnal variation in thermal demand of growing pigs. *Journal of Thermal Biology* 11: 131–135.
47. Kemp, B., de Greef-Lammers, F. J., Verstegen, M. W. A., and van der Hel, W. (1990). Some aspects of daily pattern in thermal demand of breeding boars. *Journal of Thermal Biology* 15: 103–108.
48. Henken, A. M., Brandsma, H. A., van der Hel, W., and Verstegen, M. W. A. (1993). Circadian rhythm in heat production of limit-fed growing pigs of several breeds kept at and below thermal neutrality. *Journal of Animal Science* 71: 1434–1440.
49. Gill, J. (1991). A new method for continuous recording of motor activity in horses. *Comparative Biochemistry and Physiology A* 99: 333–341.

50. Hamra, J. G., Kamerling, S. G., Wolfsheimer, K. J., and Bagwell, C. A. (1993). Diurnal variation in plasma ir-β-endorphin levels and experimental pain thresholds in the horse. *Life Sciences* 53: 121–129.
51. Irvine, C. H. G. and Alexander, S. L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology* 11: 227–238.
52. Guerin, M. V., Deed, J. R., Kennaway, D. J., and Matthews, C. D. (1995). Plasma melatonin in the horse: Measurements in natural photoperiod and in acutely extended darkness throughout the year. *Journal of Pineal Research* 19: 7–15.
53. Piccione, G., Caola, G., and Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
54. Piccione, G., Caola, G., and Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
55. Piccione, G., Assenza, A., Grasso, F., and Caola, G. (2004). Daily rhythm of circulating fat soluble vitamin concentration (A, D, E, and K) in the horse. *Journal of Circadian Rhythms* 2: art. 3.
56. Piccione, G., Bertolucci, C., Foà, A., and Caola, G. (2004). Influence of fasting and exercise on the daily rhythm of serum leptin in the horse. *Chronobiology International* 21: 405–417.
57. Refinetti, R., and Piccione, G. (2005). Intra- and inter-individual variability in the circadian rhythm of body temperature of rats, squirrels, dogs, and horses. *Journal of Thermal Biology* 30: 139–146.
58. Bligh, J. and Harthoorn, A. M. (1965). Continuous radiotelemetric records of the deep body temperature of some unrestrained African mammals under near-natural conditions. *Journal of Physiology* 176: 145–162.
59. Araki, C. T., Nakamura, R. M., and Kam, L. W. G. (1987). Diurnal temperature sensitivity of dairy cattle in a naturally cycling environment. *Journal of Thermal Biology* 12: 23–26.
60. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A., and Littledike, E. T. (1990). Measuring physiological responses of animals to environmental stressors using a microcomputer-based portable datalogger. *Journal of Animal Science* 68: 2658–2665.
61. Macaulay, A. S., Hahn, G. L., Clark, D. H., and Sisson, D. V. (1995). Comparison of calf housing types and tympanic temperature rhythms in Holstein calves. *Journal of Dairy Science* 78: 856–862.
62. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L., and Bitman, J. (1999). Circadian and ultradian rhythms of body temperature and peripheral concentrations of insulin and nitrogen in lactating dairy cows. *Domestic Animal Endocrinology* 16: 41–55.
63. Piccione, G., Caola, G., and Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
64. Hahn, G. L. (1989). Body temperature rhythms in farm animals: A review and reassessment relative to environmental influences. In: Driscoll, D. and Box, E. O. (Eds.), *Proceedings of the 11th ISB Congress*. The Hague, the Netherlands: SPB Academic Publishing, pp. 325–337.
65. Piccione, G. and Refinetti, R. (2003). Thermal chronobiology of domestic animals. *Frontiers in Bioscience* 8: 258–264.
66. Kleitman, N. and Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
67. Mellette, H. C., Hutt, B. K., Askovitz, S. I., and Horvath, S. M. (1951). Diurnal variations in body temperature. *Journal of Applied Physiology* 3: 665–675.
68. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.
69. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.
70. Aschoff, J., Gerecke, U., and Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
71. Aschoff, J., Gerecke, U., and Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
72. Marotte, H. and Timbal, J. (1981). Circadian rhythm of temperature in man: Comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
73. Weitzman, E. D., Moline, M. L., Czeisler, C. A., and Zimmerman, J. C. (1982). Chronobiology of aging: Temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
74. Gander, P. H., Connell, L. J., and Graeber, R. C. (1986). Masking of the circadian rhythm of heart rate and core temperature by the rest-activity cycle in man. *Journal of Biological Rhythms* 1: 119–135.
75. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
76. Scales, W. E., Vander, A. J., Brown, M. B., and Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
77. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K., and Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
78. Cisse, F., Martineaud, R., and Martineaud, J. P. (1991). Circadian cycles of central temperature in hot climate in man. *Archives Internationales de Physiologie, de Biochimie et de Biophysique* 99: 155–159.
79. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T., and Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
80. Lee, Y. H. and Tokura, H. (1993). Circadian rhythm of human rectal and skin temperatures under the influences of three different kinds of clothing. *Journal of Interdisciplinary Cycle Research* 24: 33–42.
81. Pollak, C. P. and Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
82. Kräuchi, K. and Wirz-Justice, A. (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *American Journal of Physiology* 267: R819–R829.
83. Van Dongen, H. P. A., Kerkhof, G. A., and Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
84. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C., and Van Hoecke, J. (2001). Nycthemeral variations in core temperature and heart rate: Continuous cycling exercise versus continuous rest. *International Journal of Sports Medicine* 22: 553–557.

85. Gradisar, M. and Lack, L. (2004). Relationships between the circadian rhythms of finger temperature, core temperature, sleep latency, and subjective sleepiness. *Journal of Biological Rhythms* 19: 157–163.
86. Roussel, B., Chouvet, G., and Debilly, G. (1976). Rythmes circadiens des températures internes et ambiance thermique chez le rat. *Pflügers Archiv* 365: 183–189.
87. Thornhill, J. A., Hirst, M., and Gowdey, C. W. (1978). Measurement of diurnal core temperatures of rats in operant cages by AM telemetry. *Canadian Journal of Physiology and Pharmacology* 56: 1047–1050.
88. Honma, K. and Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
89. Meinrath, M. and D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
90. Kittrell, E. M. W. and Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.
91. Berkey, D. L., Meeuwsen, K. W., and Barney, C. C. (1990). Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. *American Journal of Physiology* 258: R743–R749.
92. Morley, R. M., Conn, C. A., Kluger, M. J., and Vander, A. J. (1990). Temperature regulation in biotelemetered spontaneously hypertensive rats. *American Journal of Physiology* 258: R1064–R1069.
93. Refinetti, R., Ma, H., and Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
94. Shido, O., Sakurada, S., and Nagasaka, T. (1991). Effect of heat acclimation on diurnal changes in body temperature and locomotor activity in rats. *Journal of Physiology* 433: 59–71.
95. Shido, O., Sakurada, S., Kohda, W., and Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
96. Gordon, C. J. (1994). 24-Hour control of body temperature in rats. I. Integration of behavioral and autonomic effectors. *American Journal of Physiology* 267: R71–R77.
97. Li, H. and Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
98. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
99. Yang, Y. and Gordon, C. J. (1996). Ambient temperature limits and stability of temperature regulation in telemetered male and female rats. *Journal of Thermal Biology* 21: 353–363.
100. Mistlberger, R. E., Lukman, H., and Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: Assessment and intervention. *Appetite* 30: 255–267.
101. Ikeda, M. and Inoué, S. (1998). Simultaneous recording of circadian rhythms of brain and intraperitoneal temperatures and locomotor and drinking activities in the rat. *Biological Rhythm Research* 29: 142–150.
102. Gordon, C. J. and Rezvani, A. H. (2001). Genetic selection of rats with high and low body temperatures. *Journal of Thermal Biology* 26: 223–229.
103. Ray, B., Mallick, H. N., and Kumar, V. M. (2004). Changes in thermal preference, sleep-wakefulness, body temperature and locomotor activity of rats during continuous recording for 24 hours. *Behavioural Brain Research* 154: 519–526.
104. Leon, L. R., Walker, L. D., DuBose, D. A., and Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: Impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
105. Mphahlele, N. R., Fuller, A., Roth, J., and Kamerman, P. R. (2004). Body temperature, behavior, and plasma cortisol changes induced by chronic infusion of *Staphylococcus aureus* in goats. *American Journal of Physiology* 287: R863–R869.
106. Nagy, P., Guillaume, D., and Daels, P. (2000). Seasonality in mares. *Animal Reproduction Science* 60: 245–262.
107. Burkhardt, J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *Journal of Agricultural Science* 37: 64–68.
108. Palmer, E., Driancourt, M. A., and Ortavant, R. (1982). Photoperiodic stimulation of the mare during winter anoestrus. *Journal of Reproduction and Fertility* 32(S): 275–282.
109. Konishi, T. (1980). Circadian rhythm of ovipositional time in Japanese quail. In: Tanabe, Y. (Ed.), *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin, Germany: Springer, pp. 79–90.
110. Zivkovic, B. D., Underwood, H., and Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: Role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
111. Lewis, G. S. and Newman, S. K. (1984). Changes throughout estrous cycles of variables that might indicate estrus in dairy cows. *Journal of Dairy Science* 67: 146–152.
112. Redden, K. D., Kennedy, A. D., Ingalls, J. R., and Gilson, T. L. (1993). Detection of estrus by radiotelemetric monitoring of vaginal and ear skin temperature and pedometer measurements of activity. *Journal of Dairy Science* 76: 713–721.
113. Wrenn, T. R., Bitman, J., and Sykes, J. F. (1958). Body temperature variations in dairy cattle during the estrous cycle and pregnancy. *Journal of Dairy Sciences* 41: 1071–1076.
114. Fordham, D. P., Rowlinson, P., and McCarthy, T. T. (1988). Oestrus detection in dairy cows by milk temperature measurement. *Research in Veterinary Science* 44: 366–374.
115. Rajamahendran, R., Robinson, J., Desbottes, S., and Walton, J. S. (1989). Temporal relationships among estrus, body temperature, milk yield, progesterone and luteinizing hormone levels, and ovulation in dairy cows. *Theriogenology* 31: 1173–1182.
116. Kyle, B. L., Kennedy, A. D., and Small, J. A. (1998). Measurement of vaginal temperature by radiotelemetry for the prediction of estrus in beef cows. *Theriogenology* 49: 1437–1449.
117. Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S., Shand, P., González, L. A., and Crowe, T. G. (2012). Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. *Meat Science* 92: 227–243.
118. Ritter, M. J., Ellis, M., Berry, N. L., Curtis, S. E., Anil, L., Berg, E., Benjamin, M. et al. (2009). Transport losses in market weight pigs: I. A review of definitions, incidence, and economic impact. *Professional Animal Scientist* 25: 404–414.

119. Price, E. O. (2002). *Animal Domestication and Behavior*. New York: CABI Publishing.
120. House, H. B. and Doherty, J. G. (1975). The World of Darkness at the New York Zoological Park. *International Zoo Yearbook* 15: 31–34.
121. Klös, U. (1977). The new nocturnal house at the West Berlin Zoo. *International Zoo Yearbook* 17: 229–231.
122. Partridge, J. (1997). The twilight world of Bristol Zoo. *International Zoo News* 44(2): 80–84.
123. Messenger, A. M., Barnes, A. N., and Gray, G. C. (2014). Reverse zoonotic disease transmission (zooanthroponosis): A systematic review of seldom-documented human biological threats to animals. *PLOS ONE* 9: e89055.
124. Rebuelto, M., Ambros, L., Waxman, S., and Montoya, L. (2004). Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. *Chronobiology International* 21: 591–600.

This page intentionally left blank

Appendix A: Assessment of Learning

If you would like to test how much you learned by reading this book, the short examination below (with 20 multiple-choice questions) may help you. There is approximately one question per chapter. The correct answers can be found at the end.

Question 1: How long ago was reliable evidence for the existence of endogenous (internal) 24-hour rhythmicity first recorded in an animal?
A. 1 year
B. 10 years
C. 25 years
D. 50 years
E. 100 years

Question 2: The word *circadian* was not created until the 1950s. Who introduced the word?
A. Colin Pittendrigh
B. Franz Halberg
C. Jürgen Aschoff
D. Albert Einstein
E. Jean-Jacques de Mairan

Question 3: Which of the following descriptions does *not* apply to circadian rhythmicity?
A. A process that can be found in bacteria, plants, fungi, and animals
B. A process that cycles in intervals of approximately 24 hours
C. A process that is rhythmic only in the presence of environmental cycles
D. A process that can be synchronized by environmental cycles
E. A process that can be disrupted by prolonged exposure to constant darkness

Question 4: What is an *actogram*?
A. A unit of mass equivalent to 10^{-15} g
B. A machine that records circadian rhythms
C. A cyclic nightmare
D. A plot of activity over time
E. A plant used in early studies of circadian rhythms

Question 5: A woman's menstrual cycle is an example of
A. Circadian rhythm
B. Infradian rhythm
C. Ultradian rhythm
D. All of the above
E. None of the above

Question 6: What is a *race tube*?
A. An inflatable float used in water-park rides
B. A device for monitoring running speed of mice
C. A cylinder with white, yellow, and brown bands
D. A device for the monitoring of microbial growth
E. None of the above

Question 7: The period (cycle length) of circadian rhythms is determined
A. Mostly by nature (genetic inheritance)
B. Mostly by nurture (history of environmental exposure)
C. Equally by nature and nurture
D. By neither nature nor nurture
E. All of the above

Question 8: According to the *nonparametric theory of entrainment*, entrainment (synchronization) of circadian rhythms by environmental stimuli is achieved by
A. The slowing down or speeding up of the clock
B. Unknown parameters
C. Discrete daily phase shifts
D. Little green men hiding behind the bushes
E. Genetic mutation

Question 9: What is *food-anticipatory activity*?
A. The preprandial secretion of insulin
B. The postprandial secretion of saliva
C. The expression of displeasure about a meal
D. A psychological phenomenon related to expectation of outcomes
E. A behavioral pattern typical of entrainment by food restriction schedules

Question 10: How long ago is circadian rhythmicity believed to have evolved?
A. Several trillion years
B. Several billion years
C. Several million years
D. Several thousand years
E. Several hundred years

Question 11: Why are nocturnal organisms active at night?
A. Because their eyes are specialized for night vision
B. Because their circadian system is unresponsive to light
C. Because their active phase is genetically timed to subjective night
D. A and C
E. B and C

Question 12: Sleep is controlled
A. By a homeostatic (restorative) system
B. By the circadian system
C. By both a homeostatic system and the circadian system
D. By neither a homeostatic system nor the circadian system
E. By elves hidden in the brain

Question 13: The circadian system of mammals is sensitive to ambient illumination that reaches
A. The rods and cones in the retina of the eye
B. Specialized photosensitive ganglion cells in the retina
C. The skin
D. A and C
E. A and B

Question 14: The master circadian pacemaker in the mammalian brain is located in the
A. Suprachiasmatic nucleus
B. Preoptic area
C. Thalamus
D. Hippocampus
E. Visual cortex

Question 15: The suprachiasmatic nucleus can be anatomically and functionally subdivided into two regions. These two regions differ because
A. One is mostly ventrally located, whereas the other is mostly dorsally located
B. One is the target of many projections from extrahypothalamic sites, whereas the other is not
C. One contains many intrinsically rhythmic neurons, whereas the other contains few
D. All of the above
E. None of the above

Question 16: The molecular mechanism responsible for circadian rhythmicity can best be characterized as
A. Being part of the cell cycle
B. Consisting of a single transcriptional loop
C. Consisting of a single translational loop
D. Being a feedbackless process
E. Consisting of one or more transcriptional/translational loops

Question 17: Which of the following pairs of taxonomic groups and clock genes is mismatched?
A. Bacteria—*kai*
B. Plants—*toc1*
C. Fungi—*bmal1*
D. Invertebrates—*per*
E. Vertebrates—*clk*

Question 18: Which of the following conditions is *not* common to jet lag and the malaise associated with shift work?
A. Inadequate phase relationship between the internal clock and the external cycle of sunlight and darkness
B. Inadequate phase relationship between the internal clock and the schedule of activity and rest
C. Difficulty in sleeping at the scheduled sleep time
D. Drowsiness during the scheduled work time
E. None of the above

Question 19: A persistent phase delay of the internal clock is most likely the cause of which of these diseases?
A. Lung cancer
B. Syphilis
C. Narcolepsy
D. Sleep-onset insomnia
E. Sleep-termination insomnia

Question 20: In a human adult, an oral temperature of 36.9°C (98.4°F)
A. Clearly indicates a state of fever
B. Clearly indicates the absence of fever
C. Provides no helpful information for the diagnosis of fever
D. Is suggestive of a light fever at night but not in the afternoon
E. None of the above

Correct answers:
1. E
2. B
3. C
4. D
5. B
6. D
7. A
8. C
9. E
10. B
11. D
12. C
13. E
14. A
15. D
16. E
17. C
18. A
19. D
20. D

Appendix B: Dictionary of Circadian Physiology

B.1 INTRODUCTION

This dictionary consists of an English dictionary of circadian physiology, an abbreviated table of equivalency for eight other languages, and tables of units of measurement (including conversion factors for units that are not recognized by the International System of Units).

Dictionary entries were compiled and adapted from previously published glossaries that can be found in the following sources:

- Aschoff, J. (Ed.) (1981). *Biological Rhythms*. New York: Plenum.
- Binkley, S. (1997). *Biological Clocks: Your Owner's Manual*. Amsterdam, the Netherlands: Harwood Academic.
- Dunlap, J. C., Loros, J. J., and DeCoursey, P. J. (Eds.) (2004). *Chronobiology: Biological Timekeeping*. Sunderland, MA: Sinauer.
- Halberg, F. and Watanabe, H. (Eds.) (1992). *Chronobiology and Chronomedicine*. Tokyo, Japan: Medical Review.
- Mercer, J. (Ed.) (2001). Glossary of terms for thermal physiology. *Japanese Journal of Physiology* 51: 245–280.

Whenever possible, different meanings for the same word are indicated in the same entry. When this is not possible, separate entries followed by numerical superscripts are used. In either case, each entry is followed by its pronunciation in parentheses and by its etymology in square brackets. The alphabetical order of entries follows the order of the combined Greek and Roman alphabets, as indicated in the following table:

	Greek			Roman		
1	1	A	α	1	A	a
2	2	B	β	2	B	b
3	3	Γ	γ			
4				3	C	c
5	4	Δ	δ	4	D	d
6	5	E	ε	5	E	e
7				6	F	f
8	6	Z	ζ			
9	7	H	η			
10	8	Θ	θ			
11				7	G	g
12				8	H	h
13	9	I	ι	9	I	i
14				10	J	j
15	10	K	κ	11	K	k
16	11	Λ	λ	12	L	l
17	12	M	μ	13	M	m
18	13	N	ν	14	N	n
19	14	Ξ	ξ			
20	15	O	ο	15	O	o
21	16	Π	π	16	P	p
22				17	Q	q
23	17	P	ρ	18	R	r
24	18	Σ	σ	19	S	s
25	19	T	τ	20	T	t
26	20	Υ	υ	21	U	u
27	21	Φ	ϕ			
28	22	X	χ			
29	23	Ψ	ψ			
30	24	Ω	ω			
31				22	V	v
32				23	W	w
33				24	X	x
34				25	Y	y
35				26	Z	z

The spelling of words was set according to the norms of American English. For proper British, Canadian, and other English spellings, adjustments may be necessary (such as *centre* for *center*, *colour* for *color*, *to analyse* for *to analyze*, and *defence* for *defense*). Orthography, phonology, and etymology were derived from the following sources:

- *The American Heritage Dictionary of the English Language*, 4th edn. Boston, MA: Houghton Mifflin, 2000.
- *New Webster's Dictionary of the English Language*. New York: Delair, 1986.
- *Academic Press Dictionary of Science and Technology*. San Diego, CA: Academic Press, 1992.
- *PDR Medical Dictionary*. Montavale, NJ: Medical Economics, 1995.

The language equivalency table that follows the dictionary was compiled with the assistance of Frank Scheer (Dutch), Patrick Vuillez (French), the late Franz Halberg (German), Giuseppe Piccione (Italian), Shizufumi Ebihara (Japanese), John Fontenele Araújo (Portuguese), George Katinas (Russian), and Diego Golombek (Spanish). Japanese terms were entered as approximate Kanji transcriptions. Russian terms were entered as transliterations of the Cyrillic alphabet.

Official units of measurement and conversion factors were compiled from the following sources:

- *CRC Handbook of Chemistry and Physics*, 66th edn. Boca Raton, FL: CRC Press, 1985.
- *Publication Manual of the American Psychological Association*, 5th edn. Washington, DC: American Psychological Association, 2001.

The abbreviations and phonetic symbols used in the dictionary are as follows:

Abbreviations

adj.	adjective
adv.	adverb
Cf.	confer (see also)
n.	noun
pl.	plural
v.	verb

Phonetic symbols

Symbol	Examples
a	p**a**t, **a**pple
ä	f**a**ther, d**a**rk
â	d**ay**, cl**ay**
å	ab**ou**t, dr**ou**ght
æ	**ai**r, b**ear**, p**e**t, r**e**d
b	**b**ride, bi**b**
¢	**ch**arm, **ch**ur**ch**
d	**d**ea**d**, call**ed**
e	b**ee**, t**ea, ea**r, p**ie**r
f	**f**ive, **ph**ase, rou**gh**
g	**g**irl, **g**rade
h	**h**at, **h**ome
î	**a**bout, it**e**m, ed**i**ble, gall**o**p
ï	p**i**t, g**i**ve
í	p**ie**, b**y**
j	**j**ourney, **j**u**dg**e
k	**k**eep, **c**at, fli**ck**
l	**l**ove, a**l**ong
m	**m**other, da**mn**
n	**n**ice, sudde**n**
ñ	thi**ng**, lovi**ng**
ó	p**o**t, cl**o**t
œ	t**oe**, gl**ow**
ò	c**au**ght, cl**aw**, f**or**
ö	n**oi**se, ann**oy**
p	**p**lace, hel**p**
r	**r**odent, **r**e**ar**
s	**s**ex, **s**au**ce**
š	**sh**op, di**sh**
t	**t**ime, walk**ed**
þ	**th**ing, **th**eory
ð	**th**e, **th**ere
u	b**oo**k, l**oo**k
ú	f**oo**l, pr**oo**f
û	c**u**t, **u**rge, **i**rm
v	**v**alid, ner**ve**
w	**w**ash, a**w**ake
y	**y**es, **y**ard
z	**z**ebra, **x**ylem
ž	vi**s**ion, plea**s**ure
ˈ	*Indicates primary stress*
ˌ	*Indicates secondary stress*
·	*Delimits syllables*

B.2 ENGLISH DICTIONARY

α (alˈ·fî) [First letter of the Greek alphabet] *n.* **1.** The segment of a circadian cycle during which the organism is active. **2.** The level of significance of a statistical test. // *Cf.* ρ.

Acclimation (a'·klî·mâˈ·šîn) [French *acclimater*, acclimate + Latin *ion*, suffix of process] *n.* A process consisting of physiological or behavioral changes occurring within the lifetime of an organism that reduce the strain caused by experimentally induced changes in particular climatic factors such as ambient temperature and photoperiod. // *v.* = **acclimate** // *adj.* = **acclimated**. // *Cf.* Acclimatization and Adaptation.

Acclimatization (î·klï'·mî·tî·zâˈ·šîn) [French *acclimater*, acclimate + Latin *ion*, suffix of process] *n.* A process consisting of physiological or behavioral changes occurring within the lifetime of an organism that reduce the strain caused by the naturally occurring changes in climatic factors associated with the seasons. // *v.* = **acclimatize** // *adj.* = **acclimatized**. // *Cf.* Acclimation and Adaptation.

Acrophase (aˈ·krî·fâz') [Greek *akros*, extreme + *phasis*, appearance] *n.* The time at which the peak of a rhythm occurs. *Note:* Originally, "acrophase" referred to the phase angle of the peak of a cosine wave fitted to the raw data of a rhythm (time series); when the term is applied to the actual rhythm, the acrophase will likely vary from cycle to cycle. *Unit of measurement:* hours (h) or degrees of circumference (°) in relation to an absolute or arbitrary reference. *Caveat:* The official units of time and of plane angle in the International System of Units are, respectively, the second and the radian. // *Cf.* Cosinor and Peak.

Action spectrum (ak'·šîn·spækˈ·trîm) [Latin *agere*, do + *ion*, suffix of process + *specere*, look at, appearance] *n.* A function relating the effect of electromagnetic radiation to the wavelength of the radiation. // *pl.* = **action spectra**.

Actogram (akˈ·tœ·gram') [Latin *agere*, do + Greek *gramma*, letter] *n.* The graphical display of a time series along two time axes. The duration of a cycle (or predicted duration of a cycle) determines the length of each plot line. Successive cycles are plotted on successive lines. *Note:* On each line, values may be plotted in digital format (all-or-none data points) or analog format (using compressed Y axes). // *Cf.* Cartesian plot.

Adaptation (a'·dap·tâˈ·šîn) [Latin, *adaptare*, fit to + *ion*, suffix of process] *n.* **1.** A decrease in the responsiveness of a sensory mechanism resulting from previous or continuing stimulation. **2.** A process consisting of physiological or behavioral changes, occurring within the lifetime of an organism or as a result of genetic selection in a species, that reduce the strain caused by climatic or nonclimatic changes in the environment. // *v.* = **adapt** // *adj.* = **adaptive**, **adapted**, **adaptable**.

Adenosine (ad/·n·î·sïn') [Greek *aden*, gland + Greek *arabikos* → English (gum) arabic → ribose → osine] *n.* A compound consisting of adenine combined with ribose, one of four nucleoside units in RNA and presumed natural sleep-inducing substance.

Ad libitum (ad·lïb/·tîm) [Latin *ad libitum*, at pleasure] *adj.* Freely available. // *adv.* = **ad libitum**. // *Abbreviation:* **ad lib**.

Aftereffects (af /·tîr·ï·fækts') [Old English *æfter*, after + Latin *effectus*, accomplished] *n. pl.* Characteristics of a rhythm that result from previous stimulation, such as the longer free-running period observed after entrainment by a zeitgeber with period longer than that of the organism.

Alpha, *see* **α**.

Amplitude (am/·plï·túd') [Latin *amplus*, large] *n.* The difference between the peak (or trough) and the mean value of a wave. *Note:* For symmetrical waves, the amplitude is half the value of the range of oscillation. // *Cf.* Range of oscillation.

Anticipatory activity (an·tï·sî·pî·tò'·re·ak·tï/·vï·te) [Latin *anticipare*, take before + *agere* (*act*), do + *itas*, quality suffix] *n.* Activity exhibited prior to the initiation of a stimulus that is believed to be responsible for the activity. *Note:* In circadian physiology, the term *anticipatory* can be misleading. The organism's activity anticipates (precedes) the presentation of the synchronizing stimulus. However, there is no evidence that the anticipation is a volitional psychological state distinct from the phase angle of entrainment determined by the properties of the pacemaker and the zeitgeber.

Arrhythmia (î·rïð/·me·î) [Greek *a*, negation prefix + *rhuthmos*, rhythm] *n.* Absence of rhythmicity in a process that is normally rhythmic. // *adj.* = **arrhythmic**.

Astronomical time (as·trî·nó'·mï·kîl·tím/) [Greek *astron*, star + *nomos*, law + Old English *tima*, time] *n.* A standard of time based on astronomical observations. // *Cf.* Atomic time, Solar time, and Sidereal time.

Atomic time (î·tóm'·îk·tím/) [Greek *atomos*, indivisible + Old English *tima*, time] *n.* A standard of time based on the number of oscillations of the cesium-133 atom as its electrons move from high to low energy levels. *Unit of measurement:* second (s). // *Cf.* Astronomical time and Second.

Bathyphase (ba/·þï·fâz') [Greek *bathus*, deep + *phasis*, appearance] *n.* The time at which the trough of a rhythm occurs. *Note:* This term is very rarely used. *Unit of measurement:* hours (h) or degrees of circumference (°) in relation to an absolute or arbitrary reference. *Caveat:* The official units of time and of plane angle in the International System of Units are, respectively, the second and the radian. // *Cf.* Acrophase.

Biorhythm (bí'·î·rïð/·îm) [Greek *bios*, life + *rhuthmos*, rhythm] *n.* A biological rhythm. *Note:* This term should be avoided because of its association with the unscientific theory of Fliess and Swoboda. // *adj.* = **biorhythmic**.

Bistability (bí'·stî·bï/·lï·te) [Latin *bis*, twice + *stabilis*, stable] *n.* The property of showing entrainment to either the dawn component or the dusk component of a skeleton photoperiod. // *adj.* = **bistable**. // *Cf.* Skeleton photoperiod.

Bmal1, see **Cycle²**.

Cartesian plot (kär·te/·žîn·plót') [Renati *Cartesio* (René Descartes) + Old English *plot*, a spot] *n.* The graphical representation of a time series on Cartesian coordinates (with time in the abscissa and the pertinent variable in the ordinate). // *Cf.* Plexogram.

Cathemeral (kî·þæm/·îr·îl) [Greek *kata*, through + *hemera*, day] *adj.* Occurring or functioning sporadically through the day, without clear 24-hour rhythmicity. // *Cf.* Daily and Circadian.

Chi-square periodogram (kí'·skwær'·pïr·e·ód/·œ·gram') [Twenty-second letter of the Greek alphabet + Latin *quadrum*, a square + Greek *periodos*, circuit + *gramma*, letter] *n.* An implementation of the Enright periodogram that utilizes the χ^2 distribution to determine the statistical significance of spectral power. // *Cf.* Enright periodogram.

Chronobiology (krœ'·nî·bí·ó/·lî·je) [Greek *khronos*, time + *bios*, life + *logos*, discourse] *n.* The scientific study of biological rhythms. // *adj.* = **chronobiological**.

Chronome (krœ/·nœm') [Greek *khronos*, time + *nomos*, rule] *n.* The full complex of rhythms and temporal trends in an organism. The chronome consists of a multifrequency spectrum of rhythms, trends, and residual structures, including intermodulations within and among physiological variables as well as changes with maturation and aging. // *adj.* = **chronomic**.

Chronotherapy (krœ'·nî·þæ/·rî·pe) [Greek *khronos*, time + *therapeuein*, treat medically] *n.* The treatment of disease based on principles of chronobiology. *adj.* = **chronotherapeutic**. // *Cf.* Chronobiology.

Circadian (sîr·kâ/·de·în) [Latin *circa*, around + *dies*, day] *adj.* Occurring or functioning in cycles of approximately 24 hours. *Note 1:* For most researchers of circadian rhythms, the definition of *circadian* must include the requirement of endogenous generation (as determined by the ability to freerun in constant conditions). Accordingly, the use of the term *circadian* in connection with nycthemeral rhythms whose endogenous nature has not been ascertained is acceptable only if there is a justifiable assumption of endogenesis. *Note 2:* Some researchers make the additional demand that a circadian rhythm be entrainable by a zeitgeber with a period in the circadian range (approximately 19–28 hours). // *Cf.* Nycthemeral and Daily.

Circadian hour (sîr·kâ'·de·în·år/) [Latin *circa*, around + *dies*, day + Greek *hora*, time] *n.* The unit of time corresponding to 1/24 of the duration of a circadian cycle.

Circadian time (sîr·kâ'·de·în·tím/) [Latin *circa*, around + *dies*, day + Old English *tima*, time] *n.* A standard of time

based on the free-running period of a rhythm (oscillation). *Note:* By convention, the onset of activity of diurnal organisms defines circadian time zero (CT 0). The onset of activity of nocturnal organisms defines circadian time twelve (CT 12). *Unit of measurement:* circadian hours (h) or degrees of circumference (°). *Caveat:* The official units of time and of plane angle in the International System of Units are, respectively, the second and the radian. // *Cf.* Circadian hour and Zeitgeber time.

Circalunar (sïr'·kâ·lú/·nîr) [Latin *circa*, around + *luna*, moon] *adj.* Occurring or functioning in cycles of approximately one lunar cycle (29.5 days).

Circannual (sîr·ka/·nyú·îl) [Latin *circa*, around + *annus*, year] *adj.* Occurring or functioning in cycles of approximately one year. *Note 1:* For most researchers of biological rhythms, the definition of *circannual* must include the requirement of endogenous generation (as determined by the ability to freerun in constant conditions). Accordingly, the use of the term *circannual* in connection with annual rhythms whose endogenous nature has not been ascertained is acceptable only if there is a justifiable assumption of endogenesis. *Note 2:* Some researchers make the additional demand that a circannual rhythm be entrainable by a zeitgeber with a period in the circannual range (approximately 8–16 months).

Circatidal (sïr'·kâ·tí/·dl) [Latin *circa*, around + Old English *tyd*, division of time] *adj.* Occurring or functioning in cycles of approximately one ocean tide (usually 12 hours and 25 minutes).

Civil time (sï'·vîl·tím/) [Latin *civis*, citizen + Old English *tima*, time] *n.* The standard system of time measurement based on solar time, with a mean solar day beginning at midnight. *Unit of measurement:* hours, calendar. // *Cf.* Solar time and Solar day.

Civil twilight (sï'·vîl·twí/·lít) [Latin *civis*, citizen + Middle English *twylyghte*, twilight] *n.* The time of day between sunset (or sunrise) and the moment when the Sun is 6° beneath the horizon. *Other denominations:* nautical twilight (12°) and astronomical twilight (18°).

Clk, *see* **Clock²**.

Clock¹ (klók) [Medieval Latin *clocca*, bell] *n.* A functional entity that indicates or records the time of day, usually by dividing the Earth's period of rotation into equal time intervals.

Clock² (klók) [**C**ircadian **L**ocomotor **O**utput **C**ycles **K**aput] *n.* A gene that is an essential element in the molecular mechanism of circadian rhythmicity in animals. *Abbreviation:* **Clk**.

Cold-induced thermogenesis (kœld'·ïn·dúst'·þûr'·mœ·jæ/·nï·sïs) [Old English *ceald*, cold + Latin *inducere*, induce + Greek *therme*, heat + *genesis*, production] *n.* An increase in the rate of metabolic heat production, usually of endothermic animals, in response to cold exposure. *Note:* There are two forms of cold-induced thermogenesis: shivering (which results from increased contractile activity of skeletal muscles) and nonshivering thermogenesis (which results from other thermogenic mechanisms such as the activation of brown adipose tissue in mammals). *Unit of measurement:* watt (W).

Constant routine (kón'·stînt·rú·ten/) [Latin *constare*, stand firm + French *route*, route] *n.* A research protocol intended to unmask the endogenous rhythmicity of physiological processes in human subjects. It usually involves constant bed rest under constant illumination with frequent, equally spaced meals.

Cosinor (kœ/·sí'·nîr) [**Cosin**e vect**or**] *n.* A procedure for the analysis of biological rhythms based on the fitting of a cosine wave to the raw data. *Note:* A basic cosinor procedure fits a single cosine function with a presumed period (anticipated on the basis of prior experience) to the time series and provides estimates of mesor, amplitude, and acrophase. More complex cosinor procedures utilize a fundamental function and one or more harmonics.

Crepuscular (krï·pûs/·kyî·lîr) [Latin *crepusculum*, twilight] *adj.* Active mostly at dusk, at dawn, or at dusk and dawn. *Note:* Crepuscular is the adjective for the noun *crepuscule*. Matutinal crepuscule refers to dawn, and vespertinal crepuscule corresponds to dusk. An organism active only at dusk is said to be *vespertinally crepuscular* (although this expression is rarely used). // *Cf.* Dawn and Dusk.

Crest, *see* **Peak**.

Cry, *see* **Cryptochrome**.

Cryptochrome (krïp/·tî·krœm') [Greek *kruptos*, hidden + *khroma*, color] *n.* A gene (or family of genes) that is an essential element in the molecular mechanism of circadian rhythmicity in animals. In plants and invertebrates, the product of the *Cryptochrome* gene serves as a photic transducer in the entrainment pathway; in vertebrates, it is a core component of the clock mechanism. *Abbreviation:* **Cry**.

CT, *see* **Circadian time**.

Cyc, *see* **Cycle²**.

Cycle¹ (sí/·kîl) [Greek *kuklos*, circle] *n.* **1.** A single occurrence of a periodically repeated phenomenon. **2.** A periodically repeated sequence of events. *Note:* Meaning 2 is synonymous with *rhythm*, although by tradition the reproductive rhythm is called the "estrous cycle" (or *menstrual cycle*), not the *estrous rhythm*. // *adj.* = **cyclic**. // *Cf.* Estrous and Rhythm.

Cycle² (sí/·kîl) [Greek *kuklos*, circle] *n.* A gene that is an essential element in the molecular mechanism of circadian rhythmicity in animals. The name *Cycle* is usually restricted to invertebrates. In vertebrates, the names *Bmal1* (**B**rain and **m**uscle **A**RNT-**l**ike protein **1**) and *Mop3* (**M**ediator **o**f **p**aramutation **3**) are used. *Abbreviation:* **Cyc**.

Daily (dâ/·le) [Old English *dæglic*, daily] *adj.* Having the duration of a day (24 hours). *Note:* As defined here,

daily is synonymous with *nycthemeral*. However, *nycthemeral* is a more precise term because *daily* may also mean "happening every day" or "happening once a day" in other contexts. // *n.* = **day**. // *Cf.* Circadian and Nycthemeral.

Dawn (dòn) [Old English *dagung*, dawn] *n.* The time each morning at which daylight begins. // *Cf.* Dusk and Crepuscular.

Day (dâ) [Old English *dæg*, day] *n.* **1.** The 24-hour interval between two successive sunrises on Earth (*solar day*). **2.** The interval of time between dawn and dusk. *Note:* The dual meaning of this term creates ambiguity in scientific language. However, tradition prevents the elimination of either meaning. Therefore, caution should be used to identify the intended meaning. When needed, terms such as "daylight hours" and "photophase" can be used to refer to meaning 2. // *adj.* = **daily** (meaning 1) or **diurnal** (meaning 2). // *Cf.* Lunar day, Sidereal day, and Solar day.

Daylight-saving time (dâ'·lít·sâ'·vïñ·tím′) [Old English *dæg*, day + *leoht*, light + Late Latin *salvare*, save + Old English *tima*, time] *n.* Time during which clocks are set an hour or more ahead of standard time to provide more daylight at the end of the working day (from March to November in the Northern hemisphere). *Note:* In the United States, three-letter abbreviations are used to indicate daylight-saving time according to time zones, for example, EDT (Eastern Daylight-saving Time) vs. EST (Eastern Standard Time). // *Cf.* Time zone.

DD (de'·de′) [**D**ark–**d**ark cycle] *n.* Constant (continuous) darkness. // *Cf.* LD and LL.

Dead zone (dæd′·zœn') [Old English *dead*, dead + Greek *zone*, girdle] *n.* The section of a phase–response curve in which the stimulus evokes a null response. // *Cf.* Phase-response curve.

Delta phi, *see* **Δϕ**.

Desynchronization (de·sïn′·krî·nî·zâ'·šîn) [Latin *de*, removal prefix + Greek *sunkhronos*, synchronous + Latin *ion*, suffix of process] *n.* Loss of synchrony between a rhythm and its zeitgeber (*external desynchronization*) or between two rhythms within an organism (*internal desynchronization*). // *v.* = **desynchronize** // *adj.* = **desynchronized**.

Diapause (dí′·î·pòz') [Greek *dia*, through + *pausis*, a pause] *n.* A period of inactivity in arthropods during which growth stops.

Diel, see **Nycthemeral**.

Diurnal (dí·ûr′·nîl) [Latin *diurnus*, diurnal] *adj.* Occurring or active during the daytime. *Note:* In traditional English, *diurnal* may also mean *daily*. This second meaning should be avoided in circadian physiology to prevent ambiguity. // *n.* = **daytime**. // *Cf.* Daily and Nocturnal.

Double plot (dû′·bîl·plót') [Latin *duplus*, double + Old English *plot*, a spot] *n.* An actogram with two cycles per line. The second cycle on a line is the same as the first cycle on the following line. // *v.* = **double plot**. // *adj.* = **double plotted**. // *Cf.* Actogram.

Dusk (dûsk) [Old English *dox*, dusk] *n.* The time each evening at which daylight ends. // *Cf.* Dawn and Crepuscular.

Δϕ (dæl'·tî·fï′) [Fourth and twenty-first letters of the Greek alphabet] *n.* Phase shift of a biological rhythm (oscillation). *Cf.* Phase shift.

Ectothermy (æk'·tî·þûr′·me) [Greek *ektos*, outside + *therme*, heat] *n.* The pattern of temperature regulation of organisms in which body temperature depends mainly on the behaviorally controlled exchange of heat with the environment. // *adj.* = **ectothermic**. // *n.* = **ectotherm**. // *Cf.* Endothermy.

Electromagneticspectrum (ï·læk'·trœ·mag·næ'·tîk·spæk′·trîm) [Greek *elektron*, amber + *Magnes lithos*, stone of Magnesia + Latin *spectrum*, appearance] *n.* The entire range of electromagnetic radiation that includes, in order of increasing wavelength, cosmic rays, gamma rays, x-rays, ultraviolet radiation, visible light, infrared radiation, microwaves, and radio waves.

Endogenous (æn·dó′·jî·nîs) [Greek *endon*, within + *genesis*, origin] *adj.* Originating within an organism. // *Cf.* Exogenous.

Endothermy (æn'·dœ·þûr′·me) [Greek *endo*, inside + *therme*, heat] *n.* The pattern of temperature regulation of organisms in which body temperature depends on a high and controlled rate of metabolic heat production. *Note:* In physiology, endothermy refers to the *production* of heat within an organism; in chemistry, it refers to the *uptake* of heat from an outside source during a chemical reaction. // *adj.* = **endothermic**. // *n.* = **endotherm**. // *Cf.* Ectothermy.

Enright periodogram (ïn'·rít·pïr·e·ód′·œ·gram') [James Thomas *Enright* + Greek *periodos*, circuit + *gramma*, letter] *n.* A mathematical procedure for the determination of periodicity in time series with equally spaced data points. The Enright periodogram is based on the variances of different segments of the time series sequentially aligned by period. // *Cf.* Periodogram.

Entrain (æn·trân′) [Latin *in*, in + Old French *trainer*, to drag] *v.* To synchronize a self-sustaining oscillation (or oscillator). *Usage:* The zeitgeber *entrains* the organism's clock (it "drags" the clock). In the passive voice, the organism is *entrained by* the zeitgeber. However, many authors have used the verb in a reverse transitivity: the organism *entrains to* the zeitgeber (meaning that the organism "enters the train" [or "succumbs to the dragger"]). // *n.* = **entrainment** // *adj.* = **entrained**. // *Cf.* Entrainment.

Entrainment (æn·trân′·mînt) [Latin *in*, in + Old French *trainer*, to drag + Latin *mentum*, noun suffix] *n.* The synchronization of a self-sustaining oscillation (such as a circadian rhythm) by a forcing oscillation (the zeitgeber). Under conditions of steady entrainment, the period of the self-sustaining oscillation

conforms to that of the zeitgeber, and there is a stable phase relationship between the two of them. // *v.* = **entrain**. // *Cf.* Entrain.

Estivation (æs'·tî·vâ/·šîn) [Latin *aestivus*, of summer + *ion*, suffix of process] *n.* A state of summer lethargy, with a reduction in body temperature and metabolism, in organisms that are temperature regulators when active. // *v.* = **estivate**. // *adj.* = **estivating**. // *Cf.* Hibernation.

Estrous (æs/·trîs) [Greek *oistros*, frenzy] *adj.* Relating to the reproductive cycle of nonprimates. *Usage:* **Estrus** (*n.*) is one of the stages of the **estrous** cycle. // *Cf.* Menstrual.

Exogenous (æk·só/·jî·nîs) [Greek *exo*, outside of + *genesis*, origin] *adj.* Originating outside an organism. // *Cf.* Endogenous.

ϕ (fi/) [Twenty-first letter of the Greek alphabet] *n.* Phase of a biological rhythm (oscillation). // *Cf.* Phase.

Food-entrainable oscillator (fúd'·æn·trân'·î·bîl·ó/·sî·lâ'·tîr) [Old English *foda*, food + Old French *trainer*, to drag + Latin *oscillum*, swing] *n.* A putative circadian pacemaker that can be entrained by a schedule of food restriction but not by a light–dark cycle.

Fourier analysis (fu'·re·â'·î·na/·lï·sïs) [Jean Baptiste *Fourier* + Greek *analusis*, a dissolving] *n.* A mathematical procedure for the determination of periodicity in time series with equally spaced data points. Fourier analysis is based on the decomposition of the time series into periodic components described by sine and cosine functions. // *Cf.* Periodogram.

Freerun (fre/·rûn) [Old English *freo*, free + *rinnan*, run] *n.* The state of a self-sustaining oscillation (rhythm) in the absence of effective zeitgebers or other environmental agents that may affect the period of the oscillation. // *v.* = **freerun** (fre rûn/). // *adj.* = **free-running**.

Free-running period (fre'·rûn'·îñ·pïr/·e·îd) [Old English *freo*, free + *rinnan*, run + Greek *periodos*, circuit] *n.* The period of an oscillation (rhythm) in the freerun state. // *Cf.* Freerun and Period.

Frequency[1] (fre/·kwîn·se) [Latin *frequentia*, multitude] *n.* The number of times a specified phenomenon occurs within a specified time interval. *Note:* Frequency is the reciprocal of **period** (f = 1/P). *Unit of measurement:* hertz (Hz). // *Cf.* Period.

Frequency[2] (fre/·kwîn·se) [Latin *frequentia*, multitude] *n.* A gene that is an essential element in the molecular mechanism of circadian rhythmicity in fungi. *Abbreviation:* **Frq**.

FRP, *see* **Free-running period**.

Frq, *see* **Frequency[2]**.

Heterothermy (hæ'·tî·rœ·þûr/·me) [Greek *heteros*, other + *therme*, heat] *n.* The pattern of temperature regulation (of an endothermic species) in which the variation in body core temperature (either daily or seasonally) exceeds the species' limits of homeothermy. // *adj.* = **heterothermic**. // *n.* = **heterotherm**. // *Cf.* Endothermy and Homeothermy.

Hibernation (hí'·bîr·nâ/·šîn) [Latin *hibernare*, pass the winter + *ion*, suffix of process] *n.* A state of winter lethargy, with a reduction in body temperature and metabolism, in organisms that are homeothermic temperature regulators when active. // *v.* = **hibernate** // *adj.* = **hibernating**. // *Cf.* Estivation.

Hibernaculum (hí'·bîr·na/·kî·lîm) [Latin *hibernare*, pass the winter + *culum*, suffix of place] *n.* A place where one hibernates. // *pl.* = **hibernacula**. // *Cf.* Hibernation.

Homeostasis (hœ'·me·œs·tâ/·sïs) [Greek *homoios*, constant + *stasis*, standing] *n.* The regulatory process that ensures the relative constancy of the internal environment of an organism despite variations in the external environment. // *adj.* = **homeostatic**.

Homeothermy (hœ'·me·œ·þûr/·me) [Greek *homoios*, constant + *therme*, heat] *n.* The pattern of temperature regulation (of an endothermic species) in which the variation in body core temperature (either daily or seasonally) is maintained within a narrow range despite much larger variations in ambient temperature. *Note:* There is not an exact definition of the limits of homeothermy. For any given species, the permissible range of variation must be at least as wide as the range of oscillation of the circadian rhythm of body temperature, which is as narrow as 0.5°C in dogs and as wide as 6°C in tree shrews. // *adj.* = **homeothermic**. // *n.* = **homeotherm**. // *Cf.* Poikilothermy.

Hypothermia (hí'·pî·þûr/·me·î) [Greek *hypo*, beneath + *therme*, heat] *n.* The condition of an organism when body core temperature is below the normal range of oscillation. *Note:* Hypothermia may be *regulated* (e.g., during torpor and hibernation) or *forced* (when heat loss exceeds the capacity for heat production). // *adj.* = **hypothermic**.

Illuminance (ï·lú/·mî·nîns') [Latin *lumen*, light] *n.* Density of the flow of energy, traveling in the form of electromagnetic waves as perceived by the human eye per unit time, incident on a surface. *Unit of measurement:* lux (lx) // *Cf.* Luminance.

Infradian (ïn·frâ/·de·în) [Latin *infra*, beneath + *dies*, day] *adj.* Occurring or functioning with a frequency lower than circadian (i.e., with a period longer than circadian). // *Cf.* Circadian and Ultradian.

Insomnia (ïn·sóm/·ne·î) [Latin *in*, negation prefix + *somnus*, sleep] *n.* Chronic inability to sleep. *Onset insomnia:* inability to initiate sleep at the usual sleep time; *termination insomnia:* inability to maintain sleep until the end of the usual sleep interval. // *adj.* = **insomniac**.

Interval timer, *see* **Timer**.

Irradiance (ï·râ/·de·îns') [Latin *radiare*, radiate] *n.* Density of the flow of energy, traveling in the form of electromagnetic waves per unit time, incident on a surface. *Unit of measurement:* $W \cdot m^{-2}$ // *Cf.* Radiance.

Jet lag (jæt/·lag') [Latin *jactare*, throw out + Old English *lag*, last person] *n.* A malaise associated with the

disruption of bodily rhythms caused by high-speed air travel across time zones. // *adj.* = **jet-lagged**.

KaiABC (kí'·â·be·se/) [Japanese *kai*, cycle + first, second, and third letters of the Roman alphabet] *n.* A cluster of genes that is an essential element in the molecular mechanism of circadian rhythmicity in cyanobacteria.

LD (æl'·de/) [**L**ight–**d**ark cycle] *n.* A schedule of illumination consisting of a regular alternation of light and darkness each day. // *Cf.* LL and DD.

Light (lít) [Old English *leoht*, light] *n.* The visible segment of the electromagnetic spectrum (the interval of wavelengths from 400 to 700 nm). *Unit of measurement: see* **Illuminance**, **Luminance**, **Irradiance**, and **Radiance**. // *Cf.* Electromagnetic spectrum.

Light–dark cycle (lít'·därk·sí/·kîl) [Old English *leoht*, light + *deorc*, dark + Greek *kuklos*, circle] *n.* A schedule of illumination consisting of a regular alternation of light and darkness each day. // *Cf.* T cycle.

Light-entrainable oscillator (lít'·æn·trân'·î·bîl·ó/·sî·lâ'·tîr) [Old English *leoht*, light + Old French *trainer*, to drag + Latin *oscillum*, swing] *n.* A circadian pacemaker that can be entrained by a light–dark cycle.

Limit cycle (lï/·mït·sí/·kîl) [Latin *limes*, limit + Greek *kuklos*, circle] *n.* The trajectory in phase space around which the values of state variables (oscillating components) change.

LL (æl'·æl/) [**L**ight–**l**ight cycle] *n.* Constant (continuous) illumination. // *Cf.* LD and DD.

Lomb–Scargle periodogram (lóm'·skär'·gîl·pïr·e·ód/·œ·gram') [N. R. *Lomb* + Jeffrey D. *Scargle* + Greek *periodos*, circuit + *gramma*, letter] *n.* A mathematical procedure derived from Fourier analysis and used for spectral analysis of time series with unequally spaced data points. // *Cf.* Fourier analysis and Periodogram.

Luminance (lú/·mî·nîns) [Latin *lumen*, light] *n.* Luminous intensity per unit area leaving, passing through, or arriving at a surface in a given direction. *Unit of measurement:* $cd \cdot m^{-2}$ // *Cf.* Luminous intensity and Illuminance.

Luminous intensity (lú'·mî·nîs·ïn·tæn/·sï·te) [Latin *lumen*, light + *intensus*, intended] *n.* Flow of energy traveling in the form of electromagnetic waves, as perceived by the human eye, per unit time per unit solid angle. *Unit of measurement:* candela (cd).

Luminosity (lú'·mî·nós/·î·te) [Latin *lumen*, light] *n.* A nontechnical term referring to the intensity of illumination. *Note:* In optics, luminosity refers to the quality of giving light; in astrophysics, it refers to the absolute brightness of a celestial object. // *v.* = **illuminate** // *adj.* = **illuminated** // *Cf.* Luminance and Illuminance.

Lunar day (lú/·nîr·dâ') [Latin *luna*, moon + Old English *dæg*, day] *n.* **1.** The 24.8-hour interval during which the Earth completes one rotation on its axis with respect to the Moon. **2.** The 29.5-day interval between one sunrise and the next for an observer at a location on the Moon (which is equivalent to the time it takes the Moon to make one complete orbit around the Earth and come back to the same phase). // *Cf.* Sidereal day and Solar day.

Masking (mas/·kîñ) [Medieval Latin *masca*, specter] *n.* Disruption in the expression of overt rhythms caused by an external agent without a direct effect on the period or phase of a pacemaker. // *v.* = **mask** // *adj.* = **masked** // *Cf.* Entrainment and Relative coordination.

Mean level (men·læ/·vîl) [Latin *medius*, middle + *libra*, a balance] *n.* A measure of central tendency of the distribution of instantaneous values of an oscillating variable (the average value around which the variable oscillates). *Note:* The mean level is often computed as the arithmetic mean of all measured values in one cycle. // *Cf.* Mesor.

Menstrual (mæn/·strú·îl) [Latin *menstrua*, menses] *adj.* Relating to the reproductive cycle of primates. // *Cf.* Estrous.

Mesor (mæ/·zîr) [**M**idline-**E**stimating **S**tatistic **o**f **R**hythm] *n.* An estimate of central tendency of the distribution of values of an oscillating variable (the average value around which the variable oscillates). The mesor is a circadian rhythm-adjusted mean based on the parameters of a cosine function fitted to the raw data. *Note:* When a process is known to be rhythmic—and the data points are not equidistant or the sample size is small—the mesor often provides a more appropriate unbiased estimator of central tendency than does the arithmetic mean of the raw data. // *Cf.* Mean level.

Metabolic rate (mæ'·tî·ból/·ïk·rât') [Greek *metabole*, change + Latin *ratus*, reckoned] *n.* Amount of metabolic activity per unit time. *Note:* In physiology, the amount of metabolic activity is usually determined as the amount of heat released by the metabolic process (or as the amount of oxygen consumed under aerobic conditions). *Unit of measurement:* watt (W).

Monochromatic (mó'·nî·krœ·ma/·tïk) [Greek *monos*, single + *khroma*, color] *adj.* Pertaining to light of a single or narrow-band wavelength.

Mop3, *see* **Bmal1**.

Morning–evening typology (mòr'·nïñ·ev'·nïñ·tí·pól/·î·je) [Old English *morgen*, morning + *æfen*, evening + Greek *tupos*, impression + *logos*, discourse] *n.* An arbitrary classification of organisms into morning types, evening types, and intermediary types according to their usual phase of entrainment under a light–dark cycle.

Nadir (nâ/·dïr') [Arabic *nazir* (*as samt*), opposite (the zenith)] *n.* The lowest value of an oscillatory function. *Note:* This term is only occasionally used in circadian physiology as a synonym of trough (of a rhythm). // *Cf.* Trough.

Nocturnal (nók·tûr/·nîl) [Latin *nocturnus*, at night] *adj.* Occurring or active during the nighttime. // *n.* = **night**, **nighttime**. // *Cf.* Diurnal.

Nonphotic (nón'·fœ/·tïk) [Latin *non*, negation prefix + Greek *phos*, light] *adj.* Of or relating to stimuli other than light. // *Cf.* Photic.

Nycthemeral (nïk·þæm/·îr·îl) [Greek *nychthemeron*, a night and a day] *adj.* Having the duration of a day (24 hours). *Note:* The term *diel* has occasionally been used as a synonym of nycthemeral. // *Alternative spelling:* **nyctohemeral**. // *Cf.* Daily and Circadian.

Offset (òf/·sæt) [Neologism as antonym of *onset*] *n.* The end of α. // *Cf.* α^1 and Onset.

Onset (òn/·sæt) [Old English *on*, on + *settan*, set] *n.* The beginning of α. // *Cf.* α^1 and Offset.

Oscillator (ó/·sî·lâ'·tîr) [Latin *oscillum*, swing] *n.* An entity capable of generating a periodic variation in the value of a physical or logical quantity, especially a regular variation above and below some mean value. // *v.* = **oscillate** // *adj.* = **oscillatory** // *n.* = **oscillation**. // *Cf.* Pacemaker.

Pacemaker (pâs/·mâ'·kîr) [Latin *passus*, pace + Old English *macian*, make] *n.* A functional entity capable of generating endogenous rhythmicity and of imposing this rhythmicity on one or more other entities. *Note:* A *pacemaker* is an *oscillator*, but not all oscillators are pacemakers. // *v.* = **oscillate**, **set the pace** // *adj.* = **pacemaking**, **oscillatory**. // *Cf.* Oscillator.

Parametric[1] (pa'·rî·mæt/·rïk) [Greek *para*, beside + *metron*, measure] *adj.* Dependent on the parameters (particularly intensity and duration) of the zeitgeber. *Note:* In strict terms, all biological responses are parametric. However, the expression *nonparametric entrainment* is often used to denote entrainment that is primarily due to the timing (rather than the intensity or duration) of the synchronizing stimulus. *Antonym:* **nonparametric**.

Parametric[2] (pa'·rî·mæt/·rïk) [Greek *para*, beside + *metron*, measure] *adj.* Dependent on the parameters (usually the mean and variance) of a distribution. *Note:* In inferential statistics, the most common parametric tests are the *t* test and the analysis of variance (ANOVA). *Antonym:* **nonparametric**.

PAS domain (päs'·dœ·mân/) [**P**er + **A**RNT + **S**im + Latin *dominus*, lord] *n.* A sequence of approximately 270 amino acids found in most of the central proteins involved in the molecular mechanism of the circadian clock in eukaryotes. (*Per*, period; *ARNT*, aryl hydrocarbon receptor nuclear transporter; *Sim*, single-minded protein)

PDF[1] (pe·de·æf/) [**P**ortable **D**ocument **F**ormat] *n.* A computer file format used to present documents in a manner independent of application software, hardware, and operating systems. *Note:* Until 2008, PDF was a proprietary format controlled by Adobe Systems.

PDF[2] (pe·de·æf/) [**P**igment **D**ispersing **F**actor] *n.* A gene important for circadian output coordination in insects. *Note:* PDF is also the abbreviation for the **p**eptide **def**ormylase gene.

Peak (pek) [Middle English *pike*, peak] *n.* The point of culmination of an oscillatory function. *Synonym:* **crest**.

Per, *see* **Period[2]**.

Period[1] (pïr/·e·îd) [Greek *periodos*, circuit] *n.* The time elapsed for one complete oscillation or cycle (the distance in time between two consecutive peaks [or troughs, etc.] of a recurring wave). *Note 1*: Period is the reciprocal of **frequency** (P = 1/f). *Note 2*: Period has a second, less precise meaning in English (*period* = an interval of time). To avoid ambiguity, this second meaning should be avoided in circadian physiology. *Interval* is usually an adequate substitute. *Unit of measurement:* hours (h), seconds (s), days (d), etc. *Caveat:* The official unit of time in the International System of Units is the second. // *n.* = **periodicity**. // *Cf.* Frequency.

Period[2] (pïr/·e·îd) [Greek *periodos*, circuit] *n.* A gene (or family of genes) that is an essential element in the molecular mechanism of circadian rhythmicity in animals. *Abbreviation:* **Per**.

Periodogram (pïr·e·ód/·œ·gram') [Greek *periodos*, circuit + *gramma*, letter] *n.* A function relating periodic components of a time series to their spectral power. *Note:* There are many types of periodograms (e.g., Fourier, Enright, and Lomb–Scargle). If the type of periodogram is not specified, the periodogram is usually assumed to be a Fourier periodogram (i.e., a periodogram obtained by Fourier analysis). The term *periodogram* was first used in 1898 to describe a squared function of the sine and cosine terms of a Fourier transform.

Phase (fâz) [Greek *phasis*, appearance] *n.* **1.** The relative angular displacement between a periodic quantity and a reference angle (*synonym:* **phase angle**). **2.** A distinct stage of a process (such as the luteal phase of the estrous cycle).

Phase angle (fâz'·añ/·gîl) [Greek *phasis*, appearance + Latin *angulus*, angle] *n.* The relative angular displacement between a periodic quantity and a reference angle.

Phase shift (fâz'·šïft/) [Greek *phasis*, appearance + Old English *sciftan*, arrange] *n.* A discrete displacement of an oscillation along the time axis. *Cf.* Transients.

Phase–response curve (fâz'·rïs·póns'·kûrv/) [Greek *phasis*, appearance + Latin *responsum*, response + *curvus*, curve] *n.* A graphical description of how the magnitude of phase shifts induced by single stimuli depends on the phase at which the stimuli are presented.

Phenology (fe·nól/·î je) [Greek *phainein*, show + *logia*, speak] *n.* The study of seasonally periodical cycles in living organisms (particularly the dates of first occurrence of biological events in their annual cycle).

Phi, *see* **ϕ**.

Photic (fœ/·tïk) [Greek *phos*, light] *adj.* Of or relating to light. // *Cf.* Nonphotic.

Photoperiod (fœ'·tœ·pïr/·e·îd) [Greek *phos*, light + *periodos*, circuit] *n.* The duration of the illuminated segment of a light–dark cycle. *Usage*: A "long photoperiod"

refers to a light–dark cycle that has a light segment longer than the dark segment. // *Cf.* Photophase and Scotophase.

Photoperiodism (fœ'·tœ·pïr/·e·îd·îsm') [Greek *phos*, light + *periodos*, circuit + *ismos*, noun suffix] *n.* The property of being affected by changes in photoperiod. // *adj.* = **photoperiodic**. // *Cf.* Photoperiod.

Photophase (fœ/·tœ·fâz') [Greek *phos*, light + *phasis*, appearance] *n.* The illuminated segment of a light–dark cycle. // *Cf.* Photoperiod and Scotophase.

Photoreceptor (fœ'·tœ·rï·sæp/·tîr) [Greek *phos*, light + Latin *recipere*, receive] *n.* A structure or molecule that detects light. *Note:* In the mammalian retina, there are three classes of photoreceptors: rods, cones, and photosensitive ganglion cells.

Photorefractory (fœ'·tœ·rï·frak/·tî·re) [Greek *phos*, light + Latin *refringere*, break up] *adj.* Temporarily (or permanently) nonresponsive to light. // *n.* = **photorefractoriness**.

Phototaxis (fœ'·tœ·tak/·sïs) [Greek *phos*, light + *taxis*, arrangement] *n.* Movement of an organism toward or away from a source of light. // *adj.* = **phototactic**. // *Cf.* Phototropism.

Phototherapy (fœ'·tœ·þæ/·rî·pe) [Greek *phos*, light + *therapeuein*, treat medically] *n.* Treatment of disease by the use of light. // *adj.* = **phototherapeutic**.

Phototropism (fœ·tó/·trî·pïz' îm) [Greek *phos*, light + *trope*, a turning] *n.* Plant movement or orientation in response to the location of a source of light. // *adj.* = **phototropic**. // *Cf.* Phototaxis.

Pineal gland (pí'·ne·îl·gland/) [Latin *pinea*, pine cone + *glans*, acorn] *n.* A small endocrine gland lying between the superior colliculi (in the brain) and secreting the hormone melatonin.

Plexogram (plæk/·sî·gram') [Latin *plectere*, plait + Greek *gramma*, letter] *n.* The graphical display of the temporal progression of a biological variable. // *Cf.* Cartesian plot.

Poikilothermy (pö·kï/·lî·þûrme') [Greek *poikilos*, various + *therme*, heat] *n.* The pattern of temperature regulation (of an ectothermic species) in which body core temperature exhibits great variability as the result of variations in ambient temperature. *Note:* Many poikilothermic animals can minimize the influence of environmental temperature on body temperature by the use of behavioral responses. // *adj.* = **poikilothermic**. // *n.* = **poikilotherm**. // *Cf.* Homeothermy.

Power spectrum (på'·îr·spæk/·trîm) [Vulgar Latin *potere*, be able + Latin *spectrum*, appearance] *n.* The distribution of variances associated with the periodic components of a time series.

Psi, *see* **ψ**.

Q_{10} (kyú'·tæn/) [**Q**uotient for **10**°C difference] *n.* The ratio of the rate of a physiological process at a particular temperature to the rate at a temperature 10°C lower. *Note 1:* For most physiological processes, $Q_{10} \approx 2$. *Note 2:* Q_{10} values are of very little use if the logarithm of the rate is not an approximately linear function of temperature.

ρ (rœ) [Seventeenth letter of the Greek alphabet] *n.* The segment of a circadian cycle during which the organism is inactive. // *Cf.* α^1.

Radiance (râ/·de·îns') [Latin *radiare*, radiate] *n.* Radiant intensity per unit area leaving, passing through, or arriving at a surface in a given direction. *Unit of measurement:* $W \cdot sr^{-1} \cdot m^{-2}$. // *Cf.* Radiant intensity and Irradiance.

Radiant intensity (râ'·de·înt'·ïn·tæn/·sï·te) [Latin *radiare*, radiate + *intensus*, intended] *n.* Flow of energy traveling in the form of electromagnetic waves per unit time per unit solid angle. *Unit of measurement:* $W \cdot sr^{-1}$.

Range of entrainment (rânj'·îv·æn·trân/·mînt) [Old French *rangier*, put in a row + Old English *of*, of + Latin *in*, in + Old French *trainer*, to drag + Latin *mentum*, noun suffix) *n.* The range of periods of a zeitgeber capable of entraining a self-sustaining oscillation (rhythm). *Note:* Consistently with the transitivity of the verb *entrain*, the *range of entrainment* is a property of the zeitgeber, not of the organism. However, the same zeitgeber can have different ranges of entrainment for different organisms, which means that the range of entrainment is also a property of the organism (and, therefore, one can speak of the "range of entrainment of an organism"). // *Cf.* Entrain.

Range of oscillation (rânj'·îv·ó'·sî·lâ/·šîn) [Old French *rangier*, put in a row + Old English *of*, of + Latin *oscillum*, swing + *ion*, suffix of process] *n.* The extent of variation of an oscillatory process (the difference between the maximum and minimum observed values). // *Cf.* Amplitude.

Relative coordination (ræ'·lî·tïv·kœ·òr'·dnâ/·šîn) [Latin *relativus*, relative + *co*, prefix of association + *ordinatus*, put in order + *ion*, suffix of process] *n.* Partial entrainment of a rhythm (oscillatory process) by a zeitgeber, where the zeitgeber affects the period and phase of the pacemaker but not with enough strength to establish steady entrainment. // *Cf.* Entrainment and Masking.

Retino-hypothalamic tract (ræt'·nœ·hí'·pœ·þî·lam'· ïk·trakt/) [Medieval Latin *retina*, retina + Greek *hypo*, under + *thalamos*, a bedroom + Latin *tractus*, drawn] *n.* The monosynaptic pathway that connects the retina (in the eye) to the hypothalamus (in the diencephalon).

Rho, *see* **ρ**.

Rhythm (rï/·ðîm) [Greek *rhuthmos*, rhythm] *n.* A periodically repeated sequence of events. // *adj.* = **rhythmic**. // *Cf.* Cycle.

Robustness (rœ·bûst/·nîs) [Latin *robus*, strength] *n.* **1.** Tolerance of a statistical test to violations of its assumptions. **2.** The strength (stationarity) of a biological rhythm.

Second (sæ/·kînd) [Medieval Latin *pars secunda*, small part] *n.* **1.** The base unit of time of the International System of Units, defined as the duration of 9,192,631,770 periods of the radiation corresponding to the transition between two hyperfine levels of the ground state of the cesium-133 atom. **2.** A unit of angular measure equal to 1/60 of a minute (which is 1/60 of a degree). // *Cf.* Time.

Scotophase (skœ'·tœ·fâz/) [Greek *scotos*, darkness + *phasis*, appearance] *n.* The dark segment of a light–dark cycle. // *Cf.* Photophase.

Self-sustaining oscillation (sælf'·sîs·tâ'·nîñ·ó'·sî·lâ/·šîn) [Old English *self*, self + Latin *sustinere*, hold up + *oscillum*, swing] *n.* An oscillation that can be sustained without external support. *Antonym:* **damped oscillation**. *Usage:* Pacemakers are capable of producing self-sustaining oscillations. Timers can produce only damped oscillations. // *Cf.* Pacemaker and Timer.

Set point (sæt/·pönt) [Old English *settan*, set + Latin *punctus*, pricked] *n.* A reference signal used by a homeostatic system to adjust the regulated variable. The difference between the set point and the current value of the regulated variable determines the magnitude of the error signal and, consequently, directs the corrective action to be taken. // *Cf.* Homeostasis.

Shift work (šïft/·wûrk) [Old English *sciftan*, arrange + *weorc*, work] *n.* A work schedule involving nontraditional working hours, usually during the evening and night.

Sidereal day (sí·dï'·re·îl·dâ') [Latin *sidus*, star + *alis*, characterization suffix + Old English *dæg*, day] *n.* The 23.9-hour interval during which the Earth completes one rotation on its axis with respect to the stars. // *Cf.* Lunar day and Solar day.

Sidereal time (sí·dï'·re·îl·tím/) [Latin *sidus*, star + *alis*, characterization suffix + Old English *tima*, time] *n.* A standard of time based on the apparent movement of the celestial sphere (i.e., the perceived sphere that contains the stars). *Unit of measurement:* hours, calendar. // *Cf.* Solar time.

Singularity (sïñ'·gyî·la/·rï·te) [Latin *singularis*, singular + *itas*, state suffix] *n.* The point of equilibrium in a limit cycle. // *Cf.* Limit cycle.

Skeleton photoperiod (skæ'·lï·tîn·fœ'·tœ·pïr/·e·îd) [Greek *skeletos*, dried up + *phos*, light + *periodos*, circuit] *n.* A light–dark cycle whose photophase consists only of brief photic stimulation at dawn, at dusk, or at dawn and dusk. // *Cf.* T cycle and Light–dark cycle.

Slave oscillator (slâv'·ó/·sî·lâ'·tîr) [Latin *sclavus*, Slav + *oscillum*, swing] *n.* An oscillator that is driven or entrained by another oscillator. // *Cf.* Oscillator.

Solar day (sœ/·lîr·dâ') [Latin *sol*, sun + Old English *dæg*, day] *n.* The 24.0-hour interval between one sunrise and the next for an observer at a location on Earth. *Note*: Because the solar day is not constant, *solar day* usually means **mean solar day** (the mean duration of a solar day). // *Cf.* Lunar day and Sidereal day.

Solar time (sœ/·lîr·tím') [Latin *sol*, sun + Old English *tima*, time] *n.* A standard of time based on the apparent movement of the Sun in the sky (or the movement of a "fictitious" Sun that moves uniformly along the celestial equator). *Unit of measurement:* hours, calendar. // *Cf.* Sidereal time.

Spectral analysis (spæk'·trîl·î·na/·lï·sïs) [Latin *spectrum*, appearance + Greek *analusis*, a dissolving] *n.* A mathematical procedure that evaluates the contribution of putative periodic components of a time series to the actual temporal profile of the series. // *Cf.* Fourier analysis.

Spectral power (spæk'·trîl·på/·îr) [Latin *spectrum*, appearance + Vulgar Latin *potere*, be able] *n.* The relative magnitude of the variance associated with a period (or frequency) in the power spectrum of a time series. // *Cf.* Power spectrum.

Splitting (splï/·tïñ) [Middle Dutch *splitten*, split] *n.* The separation of a rhythm into two independent components. *Note:* Some researchers believe that the two components of a split circadian rhythm are controlled by distinct oscillators, often called the "morning" and "evening" oscillators. // *v.* = **split** // *adj.* = **splitted**.

Spring tide (sprïñ/·tíd) [Old English *springan*, spring, stretch + *tyd*, division of time] *n.* A tide of increased range that occurs about twice monthly at the new and full phases of the Moon.

State variable (stât'·var/·e·î·bîl) [Latin *status*, state + *variare*, vary] *n.* In a limit cycle, a variable that must be defined in order for the oscillatory state of the system to be known. *Cf.* Limit cycle.

Subjective day (sîb·jæk/·tïv·dâ') [Latin *subjectivus*, subjective + Old English *dæg*, day] *n.* The segment of a circadian cycle during the freerun state that corresponds to the illuminated segment during entrainment by a light–dark cycle. Metaphorically, the organism or pacemaker "thinks" that subjective day is the daylight segment of a day. // *Cf.* Day and Subjective night.

Subjective night (sîb·jæk/·tïv·nít') [Latin *subjectivus*, subjective + Old English *niht*, night] *n.* The segment of a circadian cycle during the freerun state that corresponds to the dark segment during entrainment by a light–dark cycle. Metaphorically, the organism or pacemaker "thinks" that subjective night is the night segment of a day. // *Cf.* Subjective day.

Suprachiasmatic nucleus (sú'·prî·kí·îz·ma'·tïk·nú/·kle·îs) [Latin *supra*, above + Greek *chiasma*, crossing + Latin *nucleus*, kernel] *n.* A small group of nerve cells lying in the ventral hypothalamus and possessing the properties of a circadian pacemaker. // *pl.* = **suprachiasmatic nuclei**.

Synchronization (sïn'·krî·nï zâ/·šîn) [Greek *sunkhronos*, synchronous + Latin *ion*, suffix of process] *n.* **1.** The action of causing two or more processes to proceed at the same rate. **2.** The action of causing two or more processes to start at the same time (or to coincide in

their phase of oscillation). *Note:* Meaning 1 is usually synonymous with *entrainment*, although synchronization may be due to masking instead. // *v.* = **synchronize**. // *adj.* = **synchronized**. // *n.* = **synchronizer**. // Cf. Entrainment and Masking.

Synchronizer, *see* **Zeitgeber**.

Synchrony (sïn′·krî·ne) [Greek *sunkhronos*, synchronous] *n.* Simultaneous occurrence. *Usage:* Generally, *synchrony* is a state, whereas *synchronization* is a process. // *adj.* = **synchronous**, **synchronized** // *Cf.* Synchronization.

τ (tå) [Nineteenth letter of the Greek alphabet] *n.* Period of a biological rhythm (oscillation). // *Cf.* T.

T (te) [Twentieth letter of the Roman alphabet] *n.* Period of a zeitgeber. // *Cf.* τ.

T cycle (te′·sí′·kîl) [Twentieth letter of the Roman alphabet + Greek *kuklos*, circle] *n.* A schedule of illumination consisting of a regular alternation of light and darkness with a period different from 24 hours. *Note:* T cycles often have a very short photophase, but a short photophase is not a required property. // *Cf.* Light–dark cycle and Skeleton photoperiod.

Tau, *see* **τ**.

Temperature compensation (tæm′·pîr·î·¢ur′·kóm′·pîn·sâ′·šîn) [Latin *temperare*, temper + *compensare*, weigh together + *ion*, suffix of process] *n.* The property of preserving the rate of a biological process as the surrounding temperature changes. // *adj.* = **temperature-compensated**.

Thermogenesis (þûr′·mœ·jæ′·nï·sïs) [Greek *therme*, heat + *genesis*, production] *n.* Metabolic heat production. *Note:* There are four forms of thermogenesis in animals: obligatory thermogenesis (responsible for *basal metabolic rate*), diet-induced thermogenesis, cold-induced thermogenesis, and voluntary thermogenesis (resulting from exercise or external work). *Unit of measurement:* watt (W).

Thermotaxis (þûr′·mœ·tak′·sïs) [Greek *therme*, heat + *taxis*, arrangement] *n.* Movement of an organism toward or away from a source of heat. // *adj.* = **thermotactic**.

Tim, *see* **Timeless**.

Time (tím) [Old English *tima*, time] *n.* A nonspatial continuum in which events occur in apparently irreversible succession. *Note:* The current standard of time is *atomic time*. // *adj.* = **temporal**. // *Cf.* Atomic time.

Timeless (tím′·lïs) [Old English *tima*, time + *leas*, without] *n.* A gene that is an essential element in the molecular mechanism of circadian rhythmicity in some animals. *Abbreviation:* **Tim**.

Timer (tí′·mîr) [Old English *tima*, time + *er*, performer suffix] *n.* A device that measures the passage of time. // *Cf.* Clock[1].

Time series (tím′·sïr′·ez) [Old English *tima*, time + Latin *serere*, join] *n.* A set of observations of values in chronological order that is used to determine the effect of time on the values observed. // *pl.* = **time series**.

Time zone (tím′·zœn′) [Old English *tima*, time + Greek *zone*, girdle] *n.* Any of the 24 longitudinal divisions of Earth's surface in which a standard time is kept. The standard time of each zone is the mean astronomical time of one of 24 meridians, 15° apart, extending east and west of the Greenwich meridian (in England). *Note:* Within the United States territory, there are eight official time zones: Atlantic, Eastern, Central, Mountain, Pacific, Alaska, Hawaii-Aleutian, and Samoa.

Torpor (tòr′·pîr) [Latin *torpere*, be stiff] *n.* A state of inactivity and reduced responsiveness to stimuli associated with a reduction in metabolism and body temperature. // *adj.* = **torpid**.

Transients (tran′·ze·înts) [Latin *transire*, go over] *n. pl.* Temporary oscillatory states between two steady states. *Note:* Transients are often observed after phase shifts of circadian rhythms.

Trough (tròf) [Old English *trog*, trough] *n.* The lowest value of an oscillatory function.

Twilight, *see* **Civil twilight** and **Crepuscular**.

Type 0 resetting (típ′·zï′·rœ·re·sæt′·îñ) [Greek *tupos*, impression + Arabic *sifr*, cipher + Latin *re*, undoing prefix + Old English *settan*, set + *ung*, action suffix] *n.* A strong resetting of a pacemaker in which, on average, the new phase is the same regardless of when in the cycle the resetting stimulus was applied. *Cf.* Type 1 resetting.

Type 1 resetting (típ′·wûn′·re·sæt′·îñ) [Greek *tupos*, impression + Old English *an*, one + Latin *re*, undoing prefix + Old English *settan*, set + *ung*, action suffix] *n.* A weak resetting of a pacemaker in which, on average, the new phase is a function primarily of the phase at which the resetting stimulus was applied. *Cf.* Type 0 resetting.

Ultradian (ûl·trâ′·de·în) [Latin *ultra*, beyond + *dies*, day] *adj.* Occurring or functioning with a frequency higher than circadian (i.e., with a period shorter than circadian). // *Cf.* Circadian and Infradian.

ϕ (fï) [Twenty-first letter of the Greek alphabet] *n.* Phase angle of a biological rhythm (oscillation) in relation to an internal or external reference point. // *Cf.* ψ.

χ^2 periodogram, *see* **Chi-square periodogram**.

ψ (sí) [Twenty-third letter of the Greek alphabet] *n.* Phase angle of a biological rhythm (oscillation) in reference to the zeitgeber. // *Cf.* Phase angle.

Wave (wâv) [Old English *wafian*, wave) *n.* **1.** A uniformly advancing disturbance in a medium. **2.** The graphical representation of the prototypical cycle of a rhythm. // *Cf.* Cycle and Rhythm.

Wave form (wâv′·fòrm′) [Old English *wafian*, wave + Latin *forma*, form] *n.* The shape of a wave (such as square and sinusoid). // *Cf.* Wave.

Wave length (wâv′·læñkþ′) [Old English *wafian*, wave + *lengthu*, length] *n.* The distance between two successive peaks (or troughs, etc.) of a regular wave. *Alternative spelling:* **wavelength**. *Unit of measurement:* meter (m). // *Cf.* Period.

Zeitgeber (zít/·gâ'·bîr) [German *Zeit*, time + *geben*, to give] *n*. A synchronizing agent (a stimulus capable of resetting a pacemaker or synchronizing a self-sustaining oscillation). *Note 1:* As a German noun, Zeitgeber should always be capitalized. However, as an English noun of German origin, it need not be capitalized. *Note 2:* The zeitgeber "gives" the *local time*, not the *ability to keep time* (which the organism already possesses). // *v.* = **entrain**, **synchronize** // *adj.* = **entrained**, **synchronized**.

Zeitgeber hour (zít'·gâ·bîr·år/) [German *Zeit*, time + *geben*, to give + Greek *hora*, time] *n*. The unit of time corresponding to 1/24 of the period of a zeitgeber.

Zeitgeber time (zít'·gâ·bîr·tím/) [German *Zeit*, time + *geben*, to give + Old English *tima*, time] *n*. A standard of time based on the period of a zeitgeber. *Note:* Under standard light–dark cycles, the time of lights on usually defines zeitgeber time zero (ZT 0) for diurnal organisms and the time of lights off defines zeitgeber time twelve (ZT 12) for nocturnal animals. *Unit of measurement:* zeitgeber hours (h) or degrees of circumference (°). *Caveat:* The official units of time and of plane angle in the International System of Units are, respectively, the second and the radian. // *Cf.* Circadian time.

Zenith (ze/·nïþ) [Arabic *samt* (*arra's*), path (over the head)] *n*. The point of culmination of an oscillatory function. *Note:* This term is only occasionally used in circadian physiology as a synonym of peak (of a rhythm). // *Cf.* Peak.

ZT, *see* **Zeitgeber time**.

B.3 LANGUAGE EQUIVALENCY

Acrophase (*n.*)	Dutch	Acrofase
	French	Acrophase
	German	Acrophase
	Italian	Acrofase
	Japanese	Choten iso
	Portuguese	Acrofase
	Russian	Akrofaza
	Spanish	Acrofase
Amplitude (*n.*)	Dutch	Amplitude
	French	Amplitude
	German	Amplitude
	Italian	Ampiezza
	Japanese	Shinpuku
	Portuguese	Amplitude
	Russian	Amplituda
	Spanish	Amplitud
Circadian (*adj.*)	Dutch	Circadiaan
	French	Circadien
	German	Circadian
	Italian	Circadiano
	Japanese	Gaijitsu
	Portuguese	Circadiano
	Russian	Cirkadiannyi
	Spanish	Circadiano
Circannual (*adj.*)	Dutch	Circannueel
	French	Circannuel
	German	Circannual
	Italian	Circannuale
	Japanese	Gainen
	Portuguese	Circanual
	Russian	Cirkannualnyi
	Spanish	Circanual
Cosinor (*n.*)	Dutch	Cosinor
	French	Cosinor
	German	Cosinor
	Italian	Cosinor
	Japanese	Cosinor
	Portuguese	Cosinor
	Russian	Kosinor
	Spanish	Cosinor
Entrainment (*n.*)	Dutch	Entrainering
	French	Entraînement
	German	Mitnahme
	Italian	Entrainment
	Japanese	Docho
	Portuguese	Arrastamento
	Russian	Zatiagivanie
	Spanish	Sincronización
Freerun (*n.*)	Dutch	Freerun (Vrij loop)
	French	Libre cours
	German	Freilauf
	Italian	Corsa libera
	Japanese	Jiyu keizoku
	Portuguese	Livre curso
	Russian	Svobodno-tekushyi (*adj.*)
	Spanish	Libre curso
Infradian (*adj.*)	Dutch	Infradiaan
	French	Infradien
	German	Infradian
	Italian	Infradiano
	Japanese	Infradian
	Portuguese	Infradiano
	Russian	Infradiannyi
	Spanish	Infradiano
Light–dark cycle (*n.*)	Dutch	Lich-donker cyclus
	French	Cycle lumière-obscurité
	German	Licht-Dunkel-Wechsel
	Italian	Ciclo luce-buio
	Japanese	Meian shuki
	Portuguese	Ciclo claro-escuro
	Russian	Cikl svet-temnota
	Spanish	Ciclo luz-oscuridad
Mean level (*n.*)	Dutch	Gemiddelde
	French	Niveau moyen
	German	Gleichwert
	Italian	Livello medio
	Japanese	Heikin reberu
	Portuguese	Nível médio
	Russian	Srednii uroven
	Spanish	Nivel medio

(*Continued*)

Mesor (*n.*)	Dutch	Mesor
	French	Mésor
	German	Mesor
	Italian	Mesor
	Japanese	Mesor
	Portuguese	Mesor
	Russian	Mezor
	Spanish	Mesor
Pacemaker (*n.*)	Dutch	Pacemaker
	French	Pacemaker
	German	Schrittmacher
	Italian	Pacemaker (segnapassi)
	Japanese	Pacemaker
	Portuguese	Marcapasso
	Russian	Peismeker
	Spanish	Marcapasos
Period (*n.*)	Dutch	Periode
	French	Période
	German	Periode
	Italian	Periodo
	Japanese	Shuki
	Portuguese	Período
	Russian	Period
	Spanish	Período
Phase (*n.*)	Dutch	Fase
	French	Phase
	German	Phase
	Italian	Fase
	Japanese	Iso
	Portuguese	Fase
	Russian	Faza
	Spanish	Fase
Phase shift (*n.*)	Dutch	Fase verschuiving
	French	Déphasage
	German	Phasenverschiebung
	Italian	Phase shift (slittamento di fase)
	Japanese	Iso heni
	Portuguese	Mudança de fase
	Russian	Sdvig fazy
	Spanish	Cambio de fase
Phase–response curve (*n.*)	Dutch	Fase respons curve
	French	Courbe de réponse de phase
	German	Phase-Response-Kurve
	Italian	Phase–response curve (curva fase risposta)
	Japanese	Iso hanno kyokusen
	Portuguese	Curva de resposta dependente de fase
	Russian	Krivaia fazovogo otveta
	Spanish	Curva de respuesta de fase
Photoperiod (*n.*)	Dutch	Fotoperiode
	French	Photopériode
	German	Photoperiode
	Italian	Fotoperiodo
	Japanese	Koshuki
	Portuguese	Fotoperíodo
	Russian	Svetovaia faza cikla
	Spanish	Fotoperíodo
Range of oscillation (*n.*)	Dutch	Bereik van oscillatie
	French	Étendue de l'oscillation
	German	Schwingungsbreite
	Italian	Range di oscillazione
	Japanese	Shinpuku haba
	Portuguese	Limite de oscilação
	Russian	Razmakh kolebanyi
	Spanish	Rango de oscilación
Ultradian (*adj.*)	Dutch	Ultradiaan
	French	Ultradien
	German	Ultradian
	Italian	Ultradiano
	Japanese	Ultradian
	Portuguese	Ultradiano
	Russian	Ultradiannyi
	Spanish	Ultradiano
Waveform (*n.*)	Dutch	Golfvorm
	French	Forme d'onde
	German	Wellenform
	Italian	Forma d'onda
	Japanese	Wave form
	Portuguese	Forma de onda
	Russian	Forma volny
	Spanish	Forma de onda
Zeitgeber (synchronizer) (*n.*)	Dutch	Tijdgever (synchronisator)
	French	Zeitgeber (synchroniseur)
	German	Zeitgeber (synchronisator)
	Italian	Zeitgeber (sincronizzatore)
	Japanese	Docho inshi
	Portuguese	Zeitgeber (sincronizador)
	Russian	Tseitgeber (synkhronizator)
	Spanish	Zeitgeber (sincronizador)

B.4 UNITS OF MEASUREMENT

B.4.1 Base Units

The International System of Units recognizes seven base units associated with the seven fundamental physical quantities. The seven quantities, as well as the names and symbols of the units used to measure them, are as follows:

Quantity	Name	Symbol
Amount of substance (î·mânt'·îv·sûb/·stîns)	mole (mœl)	mol
Electric current (ï·læk'·trĭk·kûr/·înt)	ampere (am/·pïr)	A
Length (læñkþ)	meter (me/·tîr)	m
Luminous intensity (lú'·mî·nîs·ïn·tæn/·sï·te)	candela (kan·dæl/·î)	cd
Mass (mas)	kilogram (kĭ//·lî·gram')	kg
Thermodynamic temperature (þûr'·mœ·dí·nam'·ïk·tæm/·pî·rî·¢ur')	kelvin (kæl/·vïn)	K
Time (tím)	second (sæ/·kînd)	s

Note: In common language, as well as in some traditional scientific usage (such as in the expressions *body weight* or *atomic weight*), the term *weight* is often incorrectly used in place of *mass*. Mass and weight are numerically the same only when the acceleration due to gravity is 1 $m \cdot s^{-2}$ (which is not the case on Earth).

B.4.2 Order of Magnitude

When the numerical value of a quantity is awkwardly small or large (e.g., 10^{-12} kg or 10^{18} m), the unit of measurement may be modified with standard prefixes indicating the order of magnitude in steps of 10^3. The following prefixes are recognized:

Factor	Prefix	Symbol
10^{18}	exa (æk′·sî)	E
10^{15}	peta (pæ′·tî)	P
10^{12}	tera (tæ′·rî)	T
10^{9}	giga (gï′·gî)	G
10^{6}	mega (mæ′·gî)	M
10^{3}	kilo (kï′·lî)	k
10^{0}	[base unit]	
10^{-3}	milli (mï′·lî)	m
10^{-6}	micro (mí′·krî)	μ
10^{-9}	nano (na′·nî)	n
10^{-12}	pico (pï′·kî)	p
10^{-15}	femto (fæm′·tî)	f
10^{-18}	atto (a′·tî)	a

It should be noted that, although the official base unit of mass is the *kilogram*, the *gram* is considered the base unit for the purpose of prefixing. Thus, 10^{-3} kg should be indicated as 1 g, not as 1 mkg. Also, prefixes for 10^{-2} (centi, c), 10^{-1} (deci, d), 10^{1} (deka, da), and 10^{2} (hecto, h) are accepted though not encouraged and are used for units such as the centimeter (cm).

It is also important to indicate that there is no international standard for the symbol used to separate the noninteger part of a real number. For example, the number resulting from the division of 7 by 2 is indicated as "3.5" in the United States but as "3,5" in most of the rest of the world. This can lead to serious misunderstandings, such as for the number 8,000—is it 8×10^3 or is it 8 with three decimal places of precision? One cannot answer the question without knowing the nationality of the person who asked it. Assuming the American notation, the following are examples of usage of factor prefixes: 1,000 m = 1 km, 9.5×10^7 g = 95 Mg, and 0.0032 A = 3.2 mA. It should be pointed out that the use of prefixes is facultative. For example, the amount of substance equal to 0.000000007 mol may be expressed either as 7 nmol or as 7×10^{-9} mol.

B.4.3 Derived Units

Derived units can be expressed as combinations of base units or of previously derived units. Some of the derived units have been assigned specific names and symbols, whereas others remain unnamed, as shown in the following table:

Quantity	Name	Symbol	Equivalent in Base Units	Equivalent in Derived Units
Acceleration			$m \cdot s^{-2}$	
Activity (radionuclide)	becquerel (bæ'·kî·ræl′)	Bq	s^{-1}	
Capacitance	farad (fa′·rîd)	F	$m^{-2} \cdot kg^{-1} \cdot s^4 \cdot A^2$	$C \cdot V^{-1}$
Electric charge	coulomb (kú′·lóm')	C	$A \cdot s$	
Electric potential	volt (vœlt)	V	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-1}$	$W \cdot A^{-1}$
Electric resistance	ohm (œm)	Ω	$m^2 \cdot kg \cdot s^{-3} \cdot A^{-2}$	$V \cdot A^{-1}$
Energy, work, heat	joule (júl, jål)	J	$m^2 \cdot kg \cdot s^{-2}$	$N \cdot m$
Force	newton (nyú′·tîn)	N	$m \cdot kg \cdot s^{-2}$	$J \cdot m^{-1}$
Frequency	hertz (hûrts)	Hz	s^{-1}	
Illuminance	lux (lûks)	lx		$lm \cdot m^{-2}$
Irradiance			$kg \cdot s^{-3}$	$W \cdot m^{-2}$
Luminance			$cd \cdot m^{-2}$	$lm \cdot m^{-2} \cdot sr^{-1}$
Luminous flux	lumen (lú′·mîn)	lm		$cd \cdot sr$
Magnetic flux	weber (vâ′·bîr)	Wb	$V \cdot s$	
Plane angle	radian (râ′·de·în)	rad	$m \cdot m^{-1}$	
Power, radiant flux	watt (wót)	W	$m^2 \cdot kg \cdot s^{-3}$	$J \cdot s^{-1}$
Pressure	pascal (pas·kal′)	Pa	$m^{-1} \cdot kg \cdot s^{-2}$	$N \cdot m^2$
Radiance				$W \cdot sr^{-1} \cdot m^{-2}$
Solid angle	steradian (stæ·râ′·de·în)	sr	$m^2 \cdot m^{-2}$	
Temperature (Celsius)	degree Celsius (dï·gre'·sæl′·se·îs)	°C	K – 273.15	
Thermal conductivity				$W \cdot m^{-1} \cdot K^{-1}$
Velocity			$m \cdot s^{-1}$	
Volume	liter (le′·tîr)	L	m^3 (1 L = 1 dm^3)	

B.4.4 Conversions

Conversion factors for the most common units that are not recognized by the International System of Units are given in the table that follows. Some conversion factors are exact but others are not. If precision greater than that shown is needed, one of the original sources should be consulted. Also, a few of the conversion factors are provided for illustrative purposes only, as they are not currently used. This is the case of units for amount of substance and for time. For example, although the amount of oxygen consumed by an organism should be expressed in moles, it is universally expressed in liters (a unit of volume, not of amount of substance). Likewise, although time should be expressed in seconds, no one refers to an hour as 3.6 ks.

Quantity	Unit	Conversion
Amount of substance	gram of salt	1 g NaCl = 17.112 mmol
	liter of oxygen	1 L O_2 (STPD) = 44.646 mmol[a]
Energy, work, heat	British thermal unit	1 btu = 1.055 kJ
	calorie	1 cal = 4.1868 J[b]
	erg	1 erg = 0.1 μJ
	liter of oxygen (metabolism)	1 L O_2 (STPD) ≈ 20.083 kJ[a]
Force	dyne	1 dyn = 10 μN
	pound force	1 lb = 4.44822 N
Illuminance	footcandle	1 ft-c = 10.76391 lx
Length	ångström	1 Å = 0.1 nm
	inch	1 in = 2.54 cm
	foot	1 ft = 0.3048 m
	mile	1 mi = 1.609347 km
Luminance	footlambert	1 ft-L = 3.426359 $cd \cdot m^{-2}$
Mass	ounce	1 oz = 28.34952 g
	pound (United States)	1 lb = 0.453592 kg
Plane angle	degree of arc	1° = 17.45329 mrad
Power	calories per second	1 cal/s = 4.1868 W
	horsepower	1 hp = 0.74570 kW
	kilocalories per hour	1 kcal/h = 1.163 W
Pressure	atmosphere	1 atm = 101.325 kPa
	millimeters of mercury	1 mm Hg = 133.3224 Pa
	pounds per square inch	1 psi = 6.89476 kPa
Velocity	miles per hour	1 mph = 1.609347 kph
Volume	fluid ounce (United States)	1 fl oz = 29.57353 mL
	pint	1 pt = 0.473177 L
	gallon	1 gal = 3.785412 L
Temperature	degree Fahrenheit	°F = 1.8 × °C + 32
Time	minute	1 min = 60 s
	hour	1 h = 3.6 ks
	year	1 yr ≈ 31.5 Ms

[a] STPD stands for standard temperature and pressure (0°C, 760 mm Hg) for dry air.

[b] In food packaging for human consumption (or commercial animal diets), caloric values are reported in calories but are actually given in kilocalories. Thus, for example, a cereal bar reporting a caloric value of 140 cal contains approximately 586 kJ.

This page intentionally left blank

Appendix C: Organisms Used

Organisms cited by name and discussed in some detail in the main text are included in the Index at the end of the book. All organisms from which data were used—whether they appear in the main text, tables, figures, collective citations, or software—are listed here in alphabetical order of Latin names, followed by common English names and major phylogenetic groups. Except for the orders Monotremata and Marsupialia (within the class Mammalia), phylogenetic groups are indicated at the class level or above, in English. The program **Organism**, which is part of the software package that accompanies this book, provides a convenient way to convert English names to Latin names, and vice versa.

Latin Name	English Name	Group
Abutilon theophrasti	Velvetleaf	Plant
Acanthis flammea[a]	Common redpoll	Bird
Acanthis hornemanni	Hoary redpoll	Bird
Acanthopagrus australis	Yellowfin bream	Fish
Acheta domestica	House cricket	Insect
Acinonyx jubatus	Cheetah	Mammal
Acomys cahirinus	Common spiny mouse	Mammal
Acomys russatus	Golden spiny mouse	Mammal
Acrocephalus scirpaceus	Eurasian reed warbler	Bird
Acropora millepora	Coral	Cnidarian
Aethomys namaquensis	Bush rat	Mammal
Aethopyga christinae	Fork-tailed sunbird	Bird
Agelaius phoeniceus	Red-winged blackbird	Bird
Agelena consociata	Gabon spider	Arachnid
Agriolimax reticulatus	Grey field slug	Mollusk
Alaemon alaudipes	Hoopoe lark	Bird
Alces alces	Moose	Mammal
Alectoris chukar	Chukar partridge	Bird
Alligator mississippiensis	American alligator	Reptile
Alopex lagopus	Arctic fox	Mammal
Amadina erythrocephala	Red-headed finch	Bird
Amaranthus retroflexus	Redroot pigweed	Plant
Amblyrhynchus cristatus	Galapagos marine iguana	Reptile
Ambrosia artemisiifolia	Common ragweed	Plant
Ameiurus melas	Black bullhead	Fish
Ammospermophilus leucurus	Antelope ground squirrel	Mammal
Amphibolurus nuchalis	Central netted dragon	Reptile
Amphibolurus vitticeps	Bearded dragon	Reptile
Anas barbariae	Duck	Bird
Anas platyrhynchos	Mallard	Bird
Androctonus australis	Scorpion	Arachnid
Anguilla anguilla	European eel	Fish
Anolis carolinensis	Green anole	Reptile
Anopheles gambiae	Malaria mosquito	Insect
Anous stolidus	Brown noddy	Bird
Anser anser	Common goose	Bird
Antechinomys laniger	Kultarr	Marsupial
Antechinus stuartii	Brown antechinus	Marsupial
Antennaria alpina	Alpine everlasting	Plant
Antheraea pernyi	Chinese oak silk moth	Insect
Antidorcas marsupialis	Springbok	Mammal
Aotus azarai	Owl monkey	Mammal
Aotus lemurinus	Owl monkey	Mammal

(*Continued*)

Latin Name	English Name	Group
Aotus trivirgatus	Night monkey	Mammal
Apis cerana	Wax bee	Insect
Apis dorsata	Giant bee	Insect
Apis mellifera	Honeybee	Insect
Apis mellifica	Black bee	Insect
Aplodontia rufa	Mountain beaver	Mammal
Aplysia californica	Marine snail	Mollusk
Apodemus flavicollis	Yellow-necked field mouse	Mammal
Apodemus mystacinus	Broad-toothed field mouse	Mammal
Apodemus sylvaticus	Wood mouse	Mammal
Aporrhais occidentalis	Marine snail	Mollusk
Aptenodytes forsteri	Emperor penguin	Bird
Aptenodytes patagonicus	King penguin	Bird
Apteronemobius asahinai	Mangrove cricket	Insect
Apus apus	Common swift	Bird
Arabidopsis thaliana	Mustard plant	Plant
Arbacia punctulata	Purple-spined sea urchin	Echinoderm
Arctocephalus pusillus	Southern fur seal	Mammal
Arenaria peploides	Sandwort	Plant
Armadillidium nasatum	Pill bug	Crustacean
Armadillidium vulgare	Pill bug	Crustacean
Arvicanthis ansorgei	Sudanian grass rat	Mammal
Arvicanthis niloticus	Nile grass rat	Mammal
Arvicola sapidus	Water vole	Mammal
Astacus astacus	Crayfish	Crustacean
Asterias forbesi	Common sea star	Echinoderm
Asterocampa leilia	Desert hackberry butterfly	Insect
Astyanax mexicanus	Mexican tetra	Fish
Atta sexdens	Sauba ant	Insect
Auriparus flaviceps	Verdin	Bird
Autographa gamma[b]	Gamma moth	Insect
Baeolophus griseus	Juniper titmouse	Bird
Baiomys taylori	Pygmy mouse	Mammal
Bellicositermes bellicosus	Termite	Insect
Bellicositermes natalensis	Termite	Insect
Bettongia gaimardi	Tasmanian bettong	Marsupial
Biston betularia	Peppered moth	Insect
Blaberus craniifer	Death's head cockroach	Insect
Blarina brevicauda	Short-tailed shrew	Mammal
Bombyx mori	Domestic silkworm	Insect
Bombus terrestris	Bumblebee	Insect
Boreogadus saida	Polar cod	Fish
Bos taurus	Domestic cattle	Mammal
Brachiosaurus brancai	Dinosaur (extinct)	Reptile
Brassica kaber	Wild mustard	Plant
Bufo cognatus	Great Plains toad	Amphibian
Bufo ictericus	Yellow cururu toad	Amphibian
Bufo marinus	Cane toad	Amphibian
Bulla gouldiana	California bubble	Mollusk
Burramys parvus	Mountain pygmy possum	Marsupial
Buthus occitanus	Scorpion	Arachnid
Caenorhabditis elegans	Roundworm	Nematode
Cairina moschata	Duck	Bird
Calcarius lapponicus	Lapland longspur	Bird
Calcinus laevimanus	Hermit crab	Crustacean
Calidris canutus	Red knot	Bird

(*Continued*)

Latin Name	English Name	Group
Calidris mauri	Western sandpiper	Bird
Calidris melanotos	Pectoral sandpiper	Bird
Calidris pusilla	Semipalmated sandpiper	Bird
Calliergon sarmentosum	Twiggy spear-moss	Plant
Callipepla gambelii	Gambel's quail	Bird
Callithrix jacchus	Common marmoset	Mammal
Callorhinus ursinus	Northern fur seal	Mammal
Callospermophilus lateralis[c]	Golden-mantled ground squirrel	Mammal
Cambarus bartonii	Crayfish	Crustacean
Cambarus latimanus	Crayfish	Crustacean
Camelus dromedarius	Camel	Mammal
Campanula rotundifolia	Harebell	Plant
Camponotus compressus	Carpenter ant	Insect
Camponotus mus	Carpenter ant	Insect
Camponotus rufipes	Carpenter ant	Insect
Campylorhynchus brunneicapillus	Cactus wren	Bird
Canis familiaris	Domestic dog	Mammal
Canis lupus	Wolf	Mammal
Canis mesomelas	Black-backed jackal	Mammal
Capra hircus	Domestic goat	Mammal
Capreolus capreolus	Western roe deer	Mammal
Carassius auratus	Goldfish	Fish
Carcinus maenas	European green crab	Crustacean
Carduelis chloris	Green finch	Bird
Carduelis flammea[a]	Common redpoll	Bird
Carduelis spinus	Siskin	Bird
Carpodacus mexicanus	House finch	Bird
Cassiope tetragona	White Arctic mountain heather	Plant
Castor canadensis	North American beaver	Mammal
Catharus ustulatus	Swainson's thrush	Bird
Catostomus commersoni	White sucker	Fish
Catostomus tahoensis	Tahoe sucker	Fish
Cavia porcellus	Guinea pig	Mammal
Cebus albifrons	Ring-tail monkey	Mammal
Cephalophus monticola	Blue duiker	Mammal
Cercartetus concinnus	Dormouse possum	Marsupial
Cercartetus lepidus	Dormouse possum	Marsupial
Cercartetus nanus	Eastern pigmy possum	Marsupial
Cervus elaphus	Red deer	Mammal
Cetraria islandica	Iceland moss	Plant
Chaetodipus intermedius[d]	Rock pocket mouse	Mammal
Charadrius dubius	Little ringed plover	Bird
Cheirogaleus crossleyi	Crossley's dwarf lemur	Mammal
Cheirogaleus sibreei	Sibree's dwarf lemur	Mammal
Chelonia mydas	Green sea turtle	Reptile
Chelydra serpentina	Snapping turtle	Reptile
Chenopodium album	Common lambsquarter	Plant
Chenopodium rubrum	Red goosefoot	Plant
Chlorocebus aethiops	Vervet monkey	Mammal
Christinus marmoratus	Marbled gecko	Reptile
Chrysemys scripta	Turtle	Reptile
Chrysocyon brachyurus	Maned wolf	Mammal
Chrysophrys auratus	Australian snapper	Fish
Cicindela hybrida	Tiger beetle	Insect
Citheronia sepulcralis	Pine devil moth	Insect
Cladonia alpestris	Reindeer lichen	Plant

(*Continued*)

Latin Name	English Name	Group
Cladonia rangiferina	Reindeer lichen	Plant
Cladonia sylvatica	Reindeer lichen	Plant
Clethrionomys glareolus	Bank vole	Mammal
Clethrionomys rutilus	Red-backed vole	Mammal
Clunio marinus	Marine midge	Insect
Clunio tsushimensis	Marine midge	Insect
Cnemidophorus gularis	Texas spotted whiptail lizard	Reptile
Cnemidophorus laredoensis	Laredo striped whiptail lizard	Reptile
Colaphellus bowring	Cabbage beetle	Insect
Colias eurytheme	Orange sulfur butterfly	Insect
Colius striatus	Speckled mousebird	Bird
Colossendeis robusta	Sea spider	Chelicerate
Columba livia	Pigeon	Bird
Conolophus pallidus	Galapagos land iguana	Reptile
Cordylus vittifer	Transvaal girdled lizard	Reptile
Corvus brachyrhynchos	Common crow	Bird
Coturnix coturnix	Quail	Bird
Crangon crangon	Brown shrimp	Crustacean
Cricetulus griseus	Chinese hamster	Mammal
Cricetulus migratorius	Ratlike hamster	Mammal
Cricetus cricetus	European hamster	Mammal
Crocuta crocuta	Spotted hyena	Mammal
Crotalus cerastes	Colorado desert sidewinder	Reptile
Crotalus mitchellii	Southwestern speckled rattlesnake	Reptile
Crotaphytus collaris	Collard lizard	Reptile
Cryptomys damarensis	Damara mole rat	Mammal
Cryptomys darlingi	Mashona mole rat	Mammal
Cryptomys hottentotus	Highveld mole rat	Mammal
Ctenomys knighti	Catamarca tuco-tuco	Mammal
Cuculus canorus	Common cuckoo	Bird
Cyclorana platycephala	Water-holding frog	Amphibian
Cydia pomonella	Codling moth	Insect
Cynomys leucurus	White-tailed prairie dog	Mammal
Cynomys ludovicianus	Black-tailed prairie dog	Mammal
Cynops pyrrhogaster	Japanese newt	Amphibian
Cyprinodon nevadensis	Tecopa pupfish	Fish
Cyprinus carpio	Carp	Fish
Cystophora cristata	Hooded seal	Mammal
Danio rerio	Zebrafish	Fish
Daphnia longispina	Water flea	Crustacean
Dasycercus cristicauda	Mulgara	Marsupial
Dasypus novemcinctus	Nine-banded armadillo	Mammal
Dasyuroides byrnei	Kowari	Marsupial
Dasyurus viverrinus	Eastern quoll	Marsupial
Delichon urbica	Northern house martin	Bird
Delphinapterus leucas	Beluga whale	Mammal
Dentex dentex	Common dentex	Fish
Dermochelys coriacea	Leatherback turtle	Reptile
Desmodillus auricularis	Short-tailed gerbil	Mammal
Dicentrarchus labrax	Sea bass	Fish
Diceros bicornis	Black rhinoceros	Mammal
Dicrostonyx groenlandicus	Collared lemming	Mammal
Didelphis marsupialis	American opossum	Marsupial
Didelphis virginiana	Virginia opossum	Marsupial
Dimorphostylis asiatica	Zooplankton	Crustacean
Dipodomys deserti	Desert kangaroo rat	Mammal

(*Continued*)

Latin Name	English Name	Group
Dipodomys ingens	Giant kangaroo rat	Mammal
Dipodomys merriami	Merriam's kangaroo rat	Mammal
Dipodomys spectabilis	Banner-tailed kangaroo rat	Mammal
Dipsosaurus dorsalis	Desert iguana	Reptile
Dissostichus mawsoni	Giant Antarctic cod	Fish
Drepanocladus aduncus	Kneiff's hook-moss	Plant
Drosophila melanogaster	Fruit fly	Insect
Drosophila pseudoobscura	Fruit fly	Insect
Dryomys laniger	Woolly dormouse	Mammal
Dugesia dorotocephala	Planarian	Flatworm
Dugesia tigrina	Planarian	Flatworm
Dusicyon thous	Crab-eating fox	Mammal
Dusicyon vetulus	Hoary fox	Mammal
Echinochloa crusgalli	Barnyardgrass	Plant
Elaphurus davidianus	Père David's deer	Mammal
Eleginus navaga	Navaga	Fish
Elephantulus edwardi	Elephant shrew	Mammal
Elephas maximus	Asian elephant	Mammal
Emerita talpoida	Sand crab	Crustacean
Empetrum nigrum	Black crowberry	Plant
Epilobium angustifolium	Fireweed	Plant
Epirrita autumnata	Norwegian autumnal moth	Insect
Eptesicus fuscus	Big brown bat	Mammal
Equisetum arvense	Field horsetail	Plant
Equisetum sylvaticum	Wood horsetail	Plant
Equus asinus	Donkey	Mammal
Equus caballus	Domestic horse	Mammal
Equus hemionus	Asiatic wild ass	Mammal
Equus przewalskii	Wild horse	Mammal
Erinaceus europaeus	Eurasian hedgehog	Mammal
Escherichia coli	*E. coli*	Bacterium
Eschrichtius robustus	Gray whale	Mammal
Etheostoma flabellare	Fantail darter	Fish
Etheostoma nigrum	Johnny darter	Fish
Eublepharis macularius	Leopard gecko	Reptile
Eulemur fulvus	White-fronted lemur	Mammal
Eupelmus orientalis	Eupelmidae wasp	Insect
Eupelmus vuilleti	Eupelmidae wasp	Insect
Euphausia pacifica	Zooplankton	Crustacean
Eurydice pulchra	Speckled sea louse	Crustacean
Eutamias merriami	Merriam's chipmunk	Mammal
Eutamias minimus	Least chipmunk	Mammal
Eutamias sibiricus	Asian chipmunk	Mammal
Falco sparverius	American kestrel	Bird
Falco subbuteo	Eurasian hobby	Bird
Felis catus	Domestic cat	Mammal
Felis serval	Serval	Mammal
Formica rufa	Red wood ant	Insect
Formica ulkei	Red ant	Insect
Fringilla coelebs	Chaffinch	Bird
Fringilla montifringilla	Brambling	Bird
Funambulus palmarum	Striped palm squirrel	Mammal
Funambulus pennanti	Striped palm squirrel	Mammal
Fundulus grandis	Gulf killifish	Fish
Fundulus heteroclitus	Mummichog	Fish
Fundulus majalis	Striped killifish	Fish

(*Continued*)

Latin Name	English Name	Group
Galaxias maculatus	Inanga	Fish
Gallotia galloti	Tenerife lizard	Reptile
Gallus domesticus	Chicken	Bird
Gammarus oceanicus	Sandhopper	Crustacean
Gasterosteus aculeatus	European stickleback	Fish
Gazella thomsonii	Thomson's gazelle	Mammal
Gekko gecko	Tokay gecko	Reptile
Geochelone elephantus	Galapagos tortoise	Reptile
Geolycosa godeffroyi	Wolf spider	Arachnid
Georychus capensis	Cape mole rat	Mammal
Gerbillurus paeba	Southern pygmy gerbil	Mammal
Gerbillurus setzeri	Southern pygmy gerbil	Mammal
Gerbillurus tytonis	Southern pygmy gerbil	Mammal
Gerbillurus vallinus	Southern pygmy gerbil	Mammal
Gerbillus campestris	Northern pygmy gerbil	Mammal
Gerbillus dasyurus	Northern pygmy gerbil	Mammal
Gerbillus gerbillus	Northern pygmy gerbil	Mammal
Gerris paludum	Water strider	Insect
Gillichthys mirabilis	Mud sucker	Fish
Giraffa camelopardalis	Giraffe	Mammal
Glaucidium perlatum	Pearl-spotted owlet	Bird
Glaucomys volans	Flying squirrel	Mammal
Glis glis	Edible dormouse	Mammal
Glyphiulus cavernicolus	Glyphiulus	Millipede
Glyptonotus antarcticus	Giant Antarctic isopod	Crustacean
Gnathonemus leopoldianus[e]	Elephant fish	Fish
Gnathopogon caerulescens	Honmoroko	Fish
Gonyaulax polyedra[f]	Plankton	Protist
Gopherus agassizii	Desert tortoise	Reptile
Gorilla gorilla	Gorilla	Mammal
Grus canadensis	Sandhill crane	Bird
Gryllus bimaculatus	Cricket	Insect
Halobacterium salinarum	*H. salinarum*	Archeon
Hantzschia virgata	Alga	Protist
Helianthus annuus	Sunflower	Plant
Heloderma suspectum	Gila monster	Reptile
Hesperiphona vespertina	Evening grosbeak	Bird
Heterocephalus glaber	Naked mole rat	Mammal
Heteropneustes fossilis	Catfish	Fish
Hipposideros speoris	Schneider's roundleaf bat	Mammal
Hirundo rustica	Barn swallow	Bird
Homo sapiens	Human	Mammal
Hoplosternum thoracatum[g]	Hoplo catfish	Fish
Hyalophora cecropia	Cecropia moth	Insect
Hydra littoralis	Freshwater polyp	Coelenterate
Hydropsyche contubernalis	Caddisfly	Insect
Hylocichla mustelina	Wood thrush	Bird
Hylocichla ustulata	Swainson's thrush	Bird
Ictalurus natalis	Yellow bullhead	Fish
Ictalurus nebulosus	Brown bullhead	Fish
Ictidomys tridecemlineatus[h]	Thirteen-lined ground squirrel	Mammal
Iguana iguana	Green iguana	Reptile
Imbrasia belina	Mopane worm	Insect
Ischnura elegans	Damselfly	Insect
Isoodon auratus	Golden bandicoot	Marsupial
Isoodon macrourus	Short-nosed bandicoot	Marsupial

(*Continued*)

Latin Name	English Name	Group
Isoodon obesulus	Southern brown bandicoot	Marsupial
Ixodes scapularis	Deer tick	Arachnid
Kalanchoe daigremontiana	Mother of thousands	Plant
Lacerta viridis	European green lizard	Reptile
Lacerta vivipara	Viviparous lizard	Reptile
Lagenorhynchus acutus	Atlantic white-sided dolphin	Mammal
Lagopus lagopus	Willow grouse	Bird
Lagopus mutus	Rock ptarmigan	Bird
Lama pacos	Alpaca	Mammal
Lampyris noctiluca	European glow-worm	Insect
Lanius excubitor	Great grey shrike	Bird
Lapara coniferarum	Southern pine sphinx	Insect
Larus argentatus	Herring gull	Bird
Larus canus	Mew gull	Bird
Lasiorhinus latifrons	Hairy-nosed wombat	Marsupial
Leiobunum vittatum	Harvestman	Arachnid
Leipoa ocellata	Mallee fowl	Bird
Lemmus lemmus	Norwegian lemming	Mammal
Lemna perpusilla	Duckweed	Plant
Lemniscomys barbarus	Striped mouse	Mammal
Lepismachilis y-signata	Bristletail	Insect
Lepomis cyanellus	Green sunfish	Fish
Lepomis gibbosus	Pumpkinseed	Fish
Lepomis macrochirus	Bluegill	Fish
Lepus americanus	Snowshoe hare	Mammal
Lepus arcticus	Arctic hare	Mammal
Lepus californicus	Black-tailed jack rabbit	Mammal
Leucophaea maderae	Madeira cockroach	Insect
Leuresthes tenuis	California grunion	Fish
Lexodonta africana	African elephant	Mammal
Limulus polyphemus	Horseshoe crab	Crustacean
Lingulodinium polyedrum[f]	Plankton	Protist
Litoria aurea	Green and golden bell frog	Amphibian
Litoria caerulea	Green tree frog	Amphibian
Lobaria linita	Cabbage lung	Plant
Lobipes lobatus	Northern phalarope	Bird
Locusta migratoria	Migratory locust	Insect
Lolium perenne	Perennial ryegrass	Plant
Lucilia sericata	Blow fly	Insect
Lumbricus terrestris	Nightcrawler earthworm	Annelid
Lycaon pictus	African wild dog	Mammal
Lycopersicon esculentum	Common tomato plant	Plant
Lycopodium annotinum	Stiff clubmoss	Plant
Lycosa tarantula	Wolf spider	Arachnid
Lymnaea stagnalis	Pond snail	Mollusk
Lynx canadensis	Canadian lynx	Mammal
Lynx lynx	Eurasian lynx	Mammal
Macaca arctoides[i]	Stump-tailed monkey	Mammal
Macaca cyclopis	Taiwanese macaque	Mammal
Macaca fascicularis	Cynomolgus monkey	Mammal
Macaca fuscata	Japanese macaque	Mammal
Macaca mulatta	Rhesus monkey	Mammal
Macaca nemestrina	Pig-tailed macaque	Mammal
Macaca radiata	Bonnet macaque	Mammal
Macaca speciosa[i]	Stump-tailed monkey	Mammal
Macrobrachium acanthurus	Freshwater prawn	Crustacean

(*Continued*)

Latin Name	English Name	Group
Macropus giganteus	Eastern grey kangaroo	Marsupial
Macropus rufus	Red kangaroo	Marsupial
Macroscelides proboscideus	Round-eared elephant shrew	Mammal
Malacosoma americanum	Eastern tent caterpillar	Insect
Manduca sexta	Tobacco hornworm	Insect
Marcusenius leopoldianus[e]	Elephant fish	Fish
Marmota flaviventris	Yellow-bellied marmot	Mammal
Marmota marmota	Alpine marmot	Mammal
Marmota monax	Woodchuck	Mammal
Megalechis thoracata[g]	Hoplo catfish	Fish
Megascolex mauritii	Earthworm	Annelid
Meleagris gallopavo	Turkey	Bird
Melopsittacus undulatus	Parakeet (lovebird)	Bird
Mephitis mephitis	Striped skunk	Mammal
Meriones crassus	Fat jird	Mammal
Meriones unguiculatus	Mongolian gerbil	Mammal
Mesocricetus auratus	Golden (Syrian) hamster	Mammal
Mesocricetus brandti	Turkish hamster	Mammal
Microcebus murinus	Gray mouse lemur	Mammal
Microcebus myoxinus	Pygmy mouse lemur	Mammal
Micropterus salmoides	Largemouth black bass	Fish
Microtus agrestis	Field vole	Mammal
Microtus arvalis	Common vole	Mammal
Microtus cabrerae	Cabrera vole	Mammal
Microtus californicus	California vole	Mammal
Microtus guentheri	Levant vole	Mammal
Microtus montanus	Montane vole	Mammal
Microtus oeconomus	Root vole	Mammal
Microtus pennsylvanicus	Meadow vole	Mammal
Mimosa pudica	Sensitive plant	Plant
Miniopterus schreibersii	Long-fingered bat	Mammal
Mirounga leonina	Southern elephant seal	Mammal
Mogera kobeae	Large Japanese mole	Mammal
Molossus molossus	Molossid bat	Mammal
Monodelphis domestica	Gray short-tailed opossum	Marsupial
Mus booduga	Field mouse	Mammal
Mus musculus	Domestic mouse	Mammal
Mus platythrix	Spiny mouse	Mammal
Mus spretus	Wild mouse	Mammal
Mus terricolor	Earth-colored mouse	Mammal
Muscicapa striata	Spotted flycatcher	Bird
Mustela erminea	Short-tail weasel	Mammal
Mustela putorius	Ferret	Mammal
Mustela vison	Mink	Mammal
Myocastor coypus	Coypu	Mammal
Myopus schisticolor	Wood lemming	Mammal
Myotis lucifugus	Little brown bat	Mammal
Myrmecobius fasciatus	Numbat	Marsupial
Myuchelys bellii	Western saw-shelled turtle	Reptile
Nannospalax ehrenbergi[j]	Mediterranean blind mole rat	Mammal
Nassa obsoleta	Mud snail	Mollusk
Nasua nasua	Coati	Mammal
Navodon scaber[k]	Leatherjacket	Fish
Nectarinia famosa	Malachite sunbird	Bird
Nelumbo nucifera	Sacred lotus	Plant
Neobatrachus centralis	Trilling frog	Amphibian

(*Continued*)

Latin Name	English Name	Group
Nephelopsis obscura	Leech	Annelid
Nephrops norvegicus	Norway lobster	Crustacean
Neurospora crassa	Bread mold	Fungus
Ningaui yvonneae	Ningaui	Marsupial
Notemigonus crysoleucas	Golden shiner	Fish
Nothobranchius furzeri	Nothobranchius	Fish
Notonecta glauca	Backswimmer	Insect
Notothenia coriiceps	Black rockcod	Fish
Nyctereutes procyonoides	Raccoon dog	Mammal
Nyctophilus bifax	Eastern long-eared bat	Mammal
Nyctophilus geoffroyi	Lesser long-eared bat	Mammal
Octodon bridgesi	Bridges' degu	Mammal
Octodon degus	Degu	Mammal
Odocoileus hemionus	Mule deer	Mammal
Odocoileus virginianus	White-tailed deer	Mammal
Oenanthe oenanthe	Northern wheatear	Bird
Oncorhynchus mykiss	Rainbow trout	Fish
Ondatra zibethicus	Muskrat	Mammal
Oniscus asellus	Wood louse	Crustacean
Operophtera brumata	Norwegian winter moth	Insect
Orcinus orcha	Killer whale	Mammal
Orconectes immunis	Crayfish	Crustacean
Oreochromis niloticus	Nile tilapia	Fish
Ornithorhynchus anatinus	Platypus	Monotreme
Oryctolagus cuniculus	Domestic rabbit	Mammal
Oryx beisa	Oryx	Mammal
Oryzomys palustris	Marsh rice rat	Mammal
Otolemur garnettii	Greater bush baby	Mammal
Otus senegalensis	African scops owl	Bird
Ovibos moschatus	Muskox	Mammal
Ovis aries	Domestic sheep	Mammal
Ovis musimon	Mouflon	Mammal
Oxyria digyna	Mountain sorrel	Plant
Pachnoda marginata	Goldsmith beetle	Insect
Pachyptila desolata	Antarctic prion	Bird
Pachyuromys duprasi	Fat-tailed gerbil	Mammal
Padda oryzivora	Java sparrow	Bird
Palaemonetes vulgaris	Common shore shrimp	Crustacean
Pan troglodytes	Chimpanzee	Mammal
Panthera leo	Lion	Mammal
Panthera tigris	Tiger	Mammal
Panulirus interruptus	Red spiny lobster	Crustacean
Papio anubis	Olive baboon	Mammal
Papio hamadryas	Hamadryas baboon	Mammal
Papio papio	Western baboon	Mammal
Papio ursinus	Chacma baboon	Mammal
Paralvinella sulfincola	Polychaete worm	Annelid
Paramecium aurelia	Paramecium	Protist
Paramecium caudatum	Paramecium	Protist
Paraserianthes lophantha	Crested wattle	Plant
Parika scaber[k]	Leatherjacket	Fish
Parmelia centrifuga	Lichen	Plant
Parus atricapillus	Black-capped chickadee	Bird
Passer domesticus	House sparrow	Bird
Pavo muticus	Peacock (pheasant)	Bird
Pelecanoides georgicus	South Georgia diving petrel	Bird

(*Continued*)

Latin Name	English Name	Group
Pelecanoides urinatrix	Common diving petrel	Bird
Peltigera canina	Dog lichen	Plant
Periplaneta americana	American cockroach	Insect
Perisoreus canadensis	Gray jay	Bird
Perla burmeisteriana	Stonefly	Insect
Perognathus intermedius[d]	Rock pocket mouse	Mammal
Perognathus longimembris	Little pocket mouse	Mammal
Peromyscus californicus	California mouse	Mammal
Peromyscus eremicus	Cactus mouse	Mammal
Peromyscus leucopus	White-footed mouse	Mammal
Peromyscus maniculatus	Deer mouse	Mammal
Peromyscus polionotus	Beach mouse	Mammal
Petaurus breviceps	Australian sugar glider	Marsupial
Phaeodactylum tricornutum	Alga	Protist
Phalangium opilio	Harvestman	Arachnid
Phalaropus fulicarius	Red phalarope	Bird
Phascolarctos cinereus	Koala	Mammal
Phaseolus multiflorus	Runner bean plant	Plant
Phaseolus vulgaris	Common bean plant	Plant
Phasianus colchicus	Ring-necked pheasant	Bird
Philodendron selloum	Cut-leaf philodendron	Plant
Philoscia muscorum	Wood louse	Crustacean
Phoca groelandica	Harp seal	Mammal
Phoca hispida	Ringed seal	Mammal
Phodopus sungorus	Siberian (Djungarian) hamster	Mammal
Phoenicurus phoenicurus	Common redstart	Bird
Phormia regina	Black blowfly	Insect
Phrynosoma cornutum	Texas horned lizard	Reptile
Phyciodes tharos	Pearly crescentspot	Insect
Phylloscopus trochilus	Willow warbler	Bird
Physella heterostropha	Freshwater snail	Mollusk
Pica pica	Magpie	Bird
Pithecia pithecia	White-faced saki	Mammal
Planigale gilesi	Flat-skulled marsupial mouse	Marsupial
Platichthys flesus	Flounder	Fish
Platynereis dumerilii	Marine ragworm	Annelid
Plecoglossus altivelis	Ayu	Fish
Plethodon cinereus	Red-backed salamander	Amphibian
Pleuronectes platessa	Plaice	Fish
Plodia interpunctella	Indian meal moth	Insect
Plusia gamma[b]	Gamma moth	Insect
Pluvialis apricaria	Eurasian golden plover	Bird
Podarcis muralis	Common wall lizard	Reptile
Podarcis sicula	Ruin lizard	Reptile
Podargus strigoides	Tawny frogmouth	Bird
Poecile gambeli	Mountain chickadee	Bird
Polygonum pensylvanicum	Pennsylvania smartweed	Plant
Polygonum viviparum	Alpine smartweed	Plant
Pongo pygmaeus	Orangutan	Mammal
Porcellio laevis	Wood louse	Crustacean
Porcellio scaber	Wood louse	Crustacean
Proarna bergi	Cicada	Insect
Proarna insignis	Cicada	Insect
Procambarus bouvieri	Crayfish	Crustacean
Procambarus clarkii	Crayfish	Crustacean
Procambarus spiculifer	Crayfish	Crustacean

(*Continued*)

Latin Name	English Name	Group
Procyon lotor	Raccoon	Mammal
Progne subis	Purple martin	Bird
Protophormia terraenovae	Blow fly	Insect
Psammomys obesus	Fat sand rat	Mammal
Pseudantechinus macdonnellensis	Fat-tailed antechinus	Marsupial
Pseudechis porphyriacus	Australian blacksnake	Reptile
Pseudopidorus fasciata	Zygenid moth	Insect
Puffinus pacificus	Wedge-tailed shearwater	Bird
Pygoscelis adeliae	Adelie penguin	Bird
Python molurus	Indian python	Reptile
Quiscalus quiscula	Common grackle	Bird
Ramalina alludens	Lichen	Plant
Ramphastos toco	Toco toucan	Bird
Rana catesbeiana	Bullfrog	Amphibian
Rana esculenta	Frog	Amphibian
Rangifer tarandus	Reindeer	Mammal
Ranunculus pygmaeus	Dwarf buttercup	Plant
Raphicerus campestris	Steenbok	Mammal
Rattus norvegicus	Laboratory rat	Mammal
Reithrodontomys megalotis	Harvest mouse	Mammal
Rhabdomys pumilio	Four-striped grass mouse	Mammal
Rhamdia quelen	South American silver catfish	Fish
Rhaucus vulneratus	Harvestman	Arachnid
Rhinopithecus brelichi	Guizhou snub-nosed monkey	Mammal
Riparia riparia	Bank swallow (Sand martin)	Bird
Rivulus marmoratus	Mangrove killifish	Fish
Rutilus rutilus	Roach	Fish
Saccharomyces cerevisiae	Baker's yeast	Fungus
Saccostomus campestris	Pouched mouse	Mammal
Saduria entomon	Saduria	Crustacean
Saimiri sciureus	Squirrel monkey	Mammal
Salix herbacea	Dwarf willow	Plant
Salmo gairdneri	Rainbow trout	Fish
Salvelinus alpinus	Arctic char	Fish
Salvelinus fontinalis	Speckled trout	Fish
Sapajus apella	Capuchin monkey	Mammal
Sarcophaga argyrostoma	Flesh fly	Insect
Sarcophilus harrisii	Tasmanian devil	Marsupial
Sauromatum guttatum	Voodoo lily	Plant
Saxicola rubetra	Whinchat	Bird
Saxicola torquata	Stonechat	Bird
Saxifraga cernua	Nodding saxifrage	Plant
Scaphiopus couchii	Couch's spadefoot toad	Amphibian
Scaphiopus hammondii[l]	Western spadefoot toad	Amphibian
Scardafella inca	Inca dove	Bird
Sceloporus jarrovi	Spiny lizard	Reptile
Sceloporus magister	Spiny lizard	Reptile
Sceloporus occidentalis	Spiny lizard	Reptile
Sceloporus undulatus	Spiny lizard	Reptile
Sceloporus variabilis	Spiny lizard	Reptile
Schistocerca gregaria	Desert locust	Insect
Sciurus carolinensis	Gray squirrel	Mammal
Sekeetamys calurus	Bushy-tailed gerbil	Mammal
Selasphorus rufus	Rufous hummingbird	Bird
Semotilus atromaculatus	Creek chub	Fish
Sephanoides sephanoides	Chilean hummingbird	Bird

(*Continued*)

Latin Name	English Name	Group
Serinus canaria	Canary	Bird
Serinus serinus	Serin	Bird
Sesarma haematocheir	Red-clawed crab	Crustacean
Sesarma reticulatum	Heavy marsh crab	Crustacean
Setaria faberi	Giant foxtail	Plant
Setaria glauca	Yellow foxtail	Plant
Setaria viridis	Green foxtail	Plant
Siganus canaliculatus	Seagrass rabbitfish	Fish
Siganus doliatus	Pencil-streaked rabbitfish	Fish
Sigmodon hispidus	Cotton rat	Mammal
Siphlonurus armatus	Mayfly	Insect
Sminthopsis crassicaudata	Fat-tailed dunnart	Marsupial
Sminthopsis macroura	Stripe-faced dunnart	Marsupial
Solanum tuberosum	Potato plant	Plant
Solea senegalensis	Senegal sole	Fish
Somateria mollissima	Common eider	Bird
Sorex minutus	Pygmy shrew	Mammal
Sorex vagrans	Vagrant shrew	Mammal
Spalacopus cyanus	Coruro	Mammal
Spalax ehrenbergi[j]	Mediterranean blind mole rat	Mammal
Sparrmannia flava	Beetle	Insect
Sparus aurata	Gilthead seabream	Fish
Spea hammondii[l]	Western spadefoot toad	Amphibian
Spermestes cucullatus	Bronze mannikin	Bird
Spermophilus beecheyi[m]	California ground squirrel	Mammal
Spermophilus citellus	European ground squirrel	Mammal
Spermophilus lateralis[c]	Golden-mantled ground squirrel	Mammal
Spermophilus parryii	Arctic ground squirrel	Mammal
Spermophilus richardsonii[n]	Richardson's ground squirrel	Mammal
Spermophilus saturatus	Golden-mantled ground squirrel	Mammal
Spermophilus tereticaudus[o]	Round-tailed ground squirrel	Mammal
Spermophilus tridecemlineatus[h]	Thirteen-lined ground squirrel	Mammal
Spermophilus undulatus	Arctic ground squirrel	Mammal
Spermophilus xanthoprymnus	Anatolian ground squirrel	Mammal
Spheniscus magellanicus	Magellanic penguin	Bird
Sticta laciniata	Lichen	Plant
Stoliczia abbotti	Crab	Crustacean
Streptococcus lactis	*S. lactis*	Bacterium
Streptopelia risoria	Barbary dove	Bird
Streptopelia turtur	European turtle-dove	Bird
Struthio camelus	Ostrich	Bird
Sturnus vulgaris	European starling	Bird
Sula granti	Nazca boobie	Bird
Suncus murinus	House musk shrew	Mammal
Sus scrofa	Domestic pig	Mammal
Sylvia borin	Garden warbler	Bird
Sylvilagus floridanus	Eastern cottontail	Mammal
Symplocarpus foetidus	Eastern skunk cabbage	Plant
Syncerus caffer	Buffalo	Mammal
Synechococcus elongatus	Cyanobacteria	Bacterium
Syngnathus fuscus	Pipefish	Fish
Tachyglossus aculeatus	Echidna	Monotreme
Taeniopygia guttata	Zebra finch	Bird
Tamarindus indica	Tamarind tree	Plant
Tamias sibiricus	Siberian chipmunk	Mammal
Tamias striatus	Eastern American chipmunk	Mammal

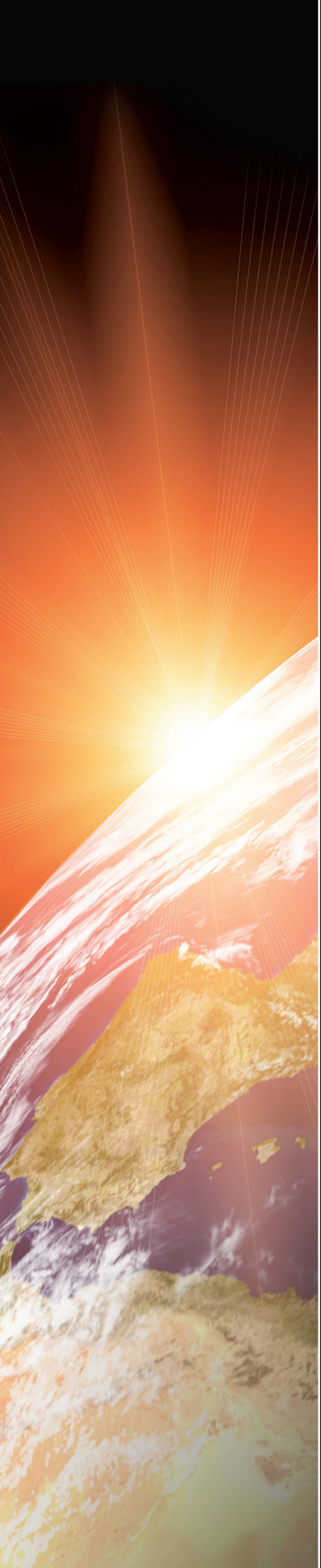
(*Continued*)

Latin Name	English Name	Group
Tamiasciurus hudsonicus	Red squirrel	Mammal
Tarentola boettgeri	Canary Island gecko	Reptile
Tatera leucogaster	Bushveld gerbil	Mammal
Taterillus petteri	Small naked-soled gerbil	Mammal
Taurotragus oryx	Common eland	Mammal
Tautogolabrus adspersus	Cunner	Fish
Tenrec ecaudatus	Tenrec	Mammal
Teratoscincus przewalskii	Frog-eyed gecko	Reptile
Terrapene carolina	Eastern American box turtle	Reptile
Testudo elephantopus	Galapagos tortoise	Reptile
Thalarctos maritimus[p]	Polar bear	Mammal
Thalassarche chrysostoma	Grey-headed albatross	Bird
Thallomys nigricauda	Black-tailed tree rat	Mammal
Thallomys paedulcus	Acacia rat	Mammal
Thamnomys surdaster	Congo tree rat	Mammal
Thamnophis sirtalis	Garter snake	Reptile
Thomomys bottae	Western pocket gopher	Mammal
Thunnus alalunga	Albacore	Fish
Thunnus albacares	Yellowfin tuna	Fish
Thunnus obesus	Bigeye tuna	Fish
Thunnus orientalis	Pacific bluefin tuna	Fish
Thunnus thynnus	Atlantic bluefin tuna	Fish
Thymelicus lineola	European skipper	Insect
Tiliqua rugosa	Australian sleepy lizard	Reptile
Tiliqua scincoides	Blue-tongued lizard	Reptile
Tinca tinca	Tench	Fish
Toxostoma dorsale	Crissal thrasher	Bird
Tremarctos ornatus	Andean bear	Mammal
Trematomus bernacchii	Emerald rock cod	Fish
Trematomus borchgrevinki	Antarctic cod	Fish
Triaenodon obesus	Whitetip reef shark	Fish
Triatoma infestans	Kissing bug	Insect
Trichosurus vulpecula	Brush-tailed possum	Marsupial
Trigonoscelis gigas	Black-bodied beetle	Insect
Triturus carnifex	Italian crested newt	Amphibian
Tupaia belangeri	Tree shrew	Mammal
Tupaia glis	Tree shrew	Mammal
Tupinambis teguixin	Tupinambis	Reptile
Turdus migratorius	Robin	Bird
Turdus viscivorus	Mistle thrush	Bird
Tursiops aduncus	Pacific bottle-nosed dolphin	Mammal
Tursiops truncatus	Atlantic bottle-nosed dolphin	Mammal
Tyrannosaurus rex	Dinosaur (extinct)	Reptile
Tyto alba	Barn owl	Bird
Uca crenulata	Mexican fiddler crab	Crustacean
Uca pugilatur	Atlantic sand fiddler crab	Crustacean
Uca pugnax	Atlantic marsh fiddler crab	Crustacean
Uca uruguayensis	Southwestern Atlantic fiddler crab	Crustacean
Uma notata	Colorado fringe-toed lizard	Reptile
Umbilicaria proboscidea	Navel lichen	Plant
Urocitellus richardsonii[n]	Richardson's ground squirrel	Mammal
Urocolius macrourus	Blue-naped mousebird	Bird
Urodacus armatus	Scorpion	Arachnid
Ursus americanus	Black bear	Mammal
Ursus arctos	Brown bear	Mammal
Ursus maritimus[p]	Polar bear	Mammal

(*Continued*)

Latin Name	English Name	Group
Ursus spelaeus	Cave bear (extinct)	Mammal
Uta stansburiana	Side-blotched lizard	Reptile
Varanus salvator	Common water monitor	Reptile
Varanus scalaris	Spotted tree monitor	Reptile
Vespa crabro	Hornet	Insect
Vespadelus pumilus	Eastern forest bat	Mammal
Vombatus ursinus	Common wombat	Marsupial
Vorhisia vulpes	Vorhisia (extinct)	Fish
Vulpes vulpes	Red fox	Mammal
Wyeomyia smithii	Pitcher-plant mosquito	Insect
Xantusia riversiana	Island night lizard	Reptile
Xenopus laevis	African clawed frog	Amphibian
Xerospermophilus tereticaudus[o]	Round-tailed ground squirrel	Mammal
Yersinia pestis	Plague	Bacterium
Zoarces viviparus	Eel-pout	Fish
Zonotrichia leucophrys	White-crowned sparrow	Bird

[a] *Acanthis flammea* is better known as *Carduelis flammea.*
[b] *Plusia gamma* is also known as *Autographa gamma.*
[c] *Spermophilus lateralis* has been renamed as *Callospermophilus lateralis.*
[d] *Perognathus intermedius* is better known as *Chaetodipus intermedius.*
[e] *Gnathonemus leopoldianus* is also known as *Marcusenius leopoldianus.*
[f] *Gonyaulax polyedra* has been renamed as *Lingulodinium polyedrum.*
[g] *Hoplosternum thoracatum* has been renamed as *Megalechis thoracata.*
[h] *Spermophilus tridecemlineatus* has been renamed as *Ictidomys tridecemlineatus.*
[i] *Macaca speciosa* has been renamed as *Macaca arctoides.*
[j] *Nannospalax ehrenbergi* is also known as *Spalax ehrenbergi.*
[k] *Navodon scaber* is also known as *Parika scaber.*
[l] *Scaphiopus hammondii* has been renamed as *Spea hammondii.*
[m] The genus *Spermophilus* was formerly called *Citellus.*
[n] *Spermophilus richardsonii* has been renamed as *Urocitellus richardsonii.*
[o] *Spermophilus tereticaudus* has been renamed as *Xerospermophilus tereticaudus.*
[p] *Thalarctos maritimus* is better known as *Ursus maritimus.*



Life Sciences

CIRCADIAN PHYSIOLOGY

Third Edition

Circadian rhythms influence most of our life activities, notably getting up and going to sleep every day. This new edition of **Circadian Physiology** delves into the mechanisms surrounding how these rhythms work, the physiology and biology behind them, and the latest research on this cutting-edge field. The book also discusses a wide variety of practical topics including the prevention of jet lag, management of shift work, sleep disorders, depression, and the optimal times for physical and intellectual activities.

To facilitate its use as a textbook, the book contains summaries, suggestions for further reading, links to pertinent websites, and exercises at the end of each chapter. It also includes software for data analysis, tutorials, and simulation programs discussed throughout the text. In addition, more than a thousand references have been added, resulting in a total of more than 6000 references.

The text has been thoroughly updated as a result of major advances in recent years. This edition adds discussion on the anatomical and physiological characterization of photoresponsive ganglionic cells in the mammalian retina, molecular circadian clocks in cells of multiple organs other than the hypothalamus and the pineal gland, and advances in the understanding of post-translational mechanisms in the operation of the molecular clock.

Praise for the Previous Edition

"... the text remains one of the few comprehensive and scientific introductions to circadian physiology ..."

—Roy Shephard, MD, PhD, Applied Physiology,
University of Toronto

K15022

CRC Press
Taylor & Francis Group
an informa business
www.crcpress.com

6000 Broken Sound Parkway, NW
Suite 300, Boca Raton, FL 33487
711 Third Avenue
New York, NY 10017
2 Park Square, Milton Park
Abingdon, Oxon OX14 4RN, UK

ISBN: 978-1-4665-1497-3
90000
9 781466 514973