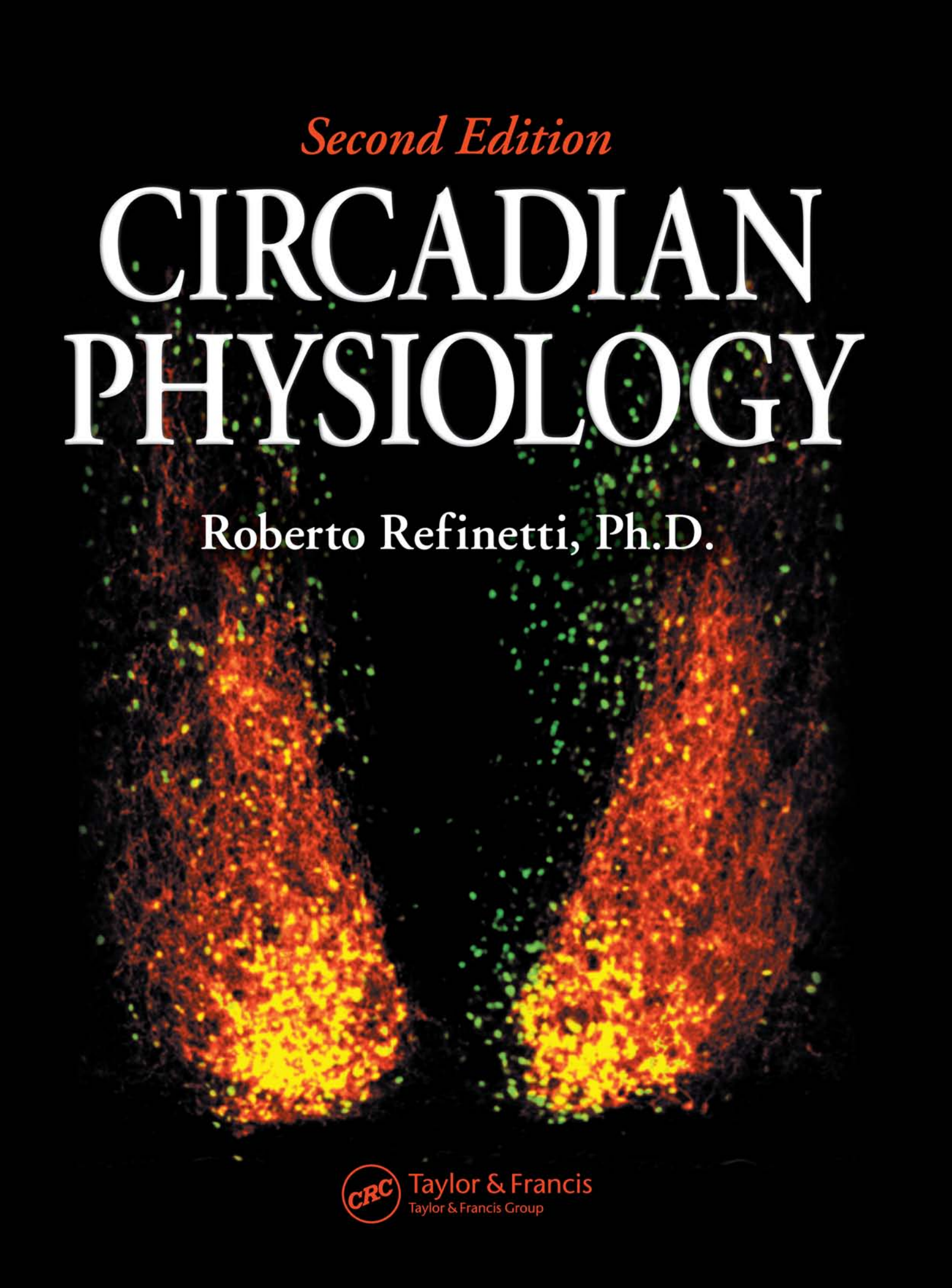
Second Edition

CIRCADIAN PHYSIOLOGY

Roberto Refinetti, Ph.D.



CRC Taylor & Francis
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Boca Raton London New York

A CRC title, part of the Taylor & Francis imprint, a member of the Taylor & Francis Group, the academic division of T&F Informa plc.

Published in 2006 by
CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-10: 0-8493-2233-2 (Hardcover)
International Standard Book Number-13: 978-0-8493-2233-4 (Hardcover)
Library of Congress Card Number 2005050123

Library of Congress Cataloging-in-Publication Data

Refinetti, Roberto.
Circadian physiology / by Roberto Refinetti.--2nd ed.
p. ; cm.
Includes bibliographical references and indexes.
ISBN 0-8493-2233-2 (alk. paper)
1. Circadian rhythms.
[DNLM: 1. Circadian Rhythm--physiology. QT 167 R332c 2005] I. Title.

QP84.6.R534 2005
571.7'7--dc22 2005050123

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http://www.crcpress.com

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Preface

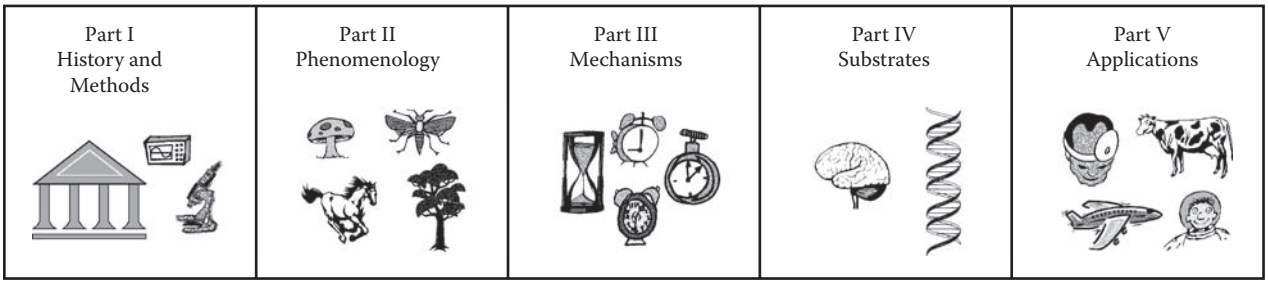
It has been 6 years since the publication of the first edition of *Circadian Physiology.* Based on sales figures and comments from readers, it seems clear that the book achieved its goal of serving as a concise but rigorous review of basic and applied research on circadian rhythms. Its accessible language and minimal requirement of background knowledge have allowed it to serve both as a brief handbook for experienced life scientists expanding their research efforts into the study of circadian rhythms and as a short textbook for undergraduate and graduate students.

Several excellent books on circadian rhythms have been published in the past 6 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 a 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.

This second edition of *Circadian Physiology* not only updates the material covered in the original one — incorporating many new experimental findings, such as the discovery of new retinal photoreceptors, the identification of several non-hypothalamic circadian pacemakers, and the elucidation of genomic and proteomic mechanisms of biological timing — but also expands its scope. With 184 pages and 13 figures, the first edition had to omit much of the detailed information required for the acquisition of in-depth knowledge of the field. The present edition, with over 700 pages, 700 figures, and 5,000 bibliographic references, can aspire to be a true handbook of circadian physiology without giving up the important features of accessible language and minimal requirement of background knowledge. This edition can be more effective than the first one as a textbook for undergraduate students, more comprehensive as a handbook for life scientists, more educational as a trade book for general readers, and more pragmatic as a reference text for medical, psychological, and veterinary practitioners. Of course, no book can provide truly exhaustive coverage of a scientific discipline. 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.

To facilitate its use as a textbook, this book contains summaries, suggestions for further readings, directions to pertinent web sites, and exercises at the end of each chapter. A CD-ROM included in the book provides a suite of 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 — as well as 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 web site (www.circadian.org) and to use the e-mail link to send me queries about specific issues.

The organization of this edition is similar to that of the first edition, which was praised by several reviewers. The book is divided into 5 parts, each with several chapters (see Figure). The first part covers historical and methodological topics in the study of circadian rhythms. The second part 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. The third part addresses the physiological mechanisms, both endogenous and environmental, that control circadian rhythms. The fourth part provides a look into the physical substrates of circadian rhythms at the level of organs, cells, and molecules. Finally, the fifth part 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.

Some readers have pointed out to me that the conciseness of the first edition was one of its most valuable features. For these readers, the expanded second edition may not be as attractive as the first one. However, I believe that the readability, not the brevity, of the first edition was its major asset, and I strived to make the second edition just as readable as the first one — if not more so. As a matter of fact, the highly interdisciplinary nature of the study of circadian rhythms makes this study not only exciting but also challenging. The breath of life-sciences background required in this enterprise practically eliminates the learning advantage that researchers experienced in other areas might have over bright but unexperienced undergraduate students. Consequently, it is quite appropriate to write *Circadian Physiology* as a book 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 (and, when it does, additional background material is provided). Still, individuals at different stages of their careers, and individuals in different occupations, will most likely have a greater interest in some parts of the book than in others. Thus, although I strongly recommend that the book be read from beginning to end, I provide the following table with what I believe to be the most interesting chapters for different audiences:

Chapter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
General readers	•			•	•				•					•	•	•	•
Life scientists		•	•	•	•	•	•	•	•	•	•	•	•				
Medical practitioners				•	•					•	•	•	•		•	•	•
Future chronobiologists	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•

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. Preparation of class schedules can be facilitated by consultation of the table below. The length of each chapter is indicated as the approximate number of text words (in thousands).

Part	**I**			**II**		**III**					**IV**			**V**			
Chapter	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Length	9	12	13	12	12	10	16	5	16	14	6	11	9	6	7	10	3

Inspection of the table readily suggests a possible schedule of classes: one chapter per week for the first 13 weeks and two chapters per week for the last two weeks. Extra time for additional activities would be available on weeks 8 and 11 (when the chapters are relatively short). Of course, the professor should take into consideration not only the length but also the complexity of the material in each chapter. As much as I tried to make all chapters equally readable, readers with different backgrounds may find some chapters to be "denser" than others.

I hope that all readers — novices as well as experts — will enjoy and benefit from reading this book 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 bibliographic references — but have also clearly conveyed the importance and the fascination of past and current studies on the all-encompassing process of circadian rhythmicity.

About the Author

Roberto Refinetti is a physiological psychology professor and circadian physiology researcher at the University of South Carolina. He received his doctoral degree from the University of California at Santa Barbara in 1987 and subsequently conducted postdoctoral research at the Center for Biological Timing at the University of Virginia. His research program in circadian physiology, which concentrates on the integration of circadian and homeostatic mechanisms, is funded by the National Science Foundation and the National Institutes of Health. Refinetti is Editor-in-Chief of the *Journal of Circadian Rhythms* and co-editor of the journal *Sexuality & Culture*. His web site is www.circadian.org. He can be reached by e-mail at refinetti@circadian.org.

Acknowledgments

Many people assisted me in the monumental task of preparing this book. First and foremost, I would like to thank the three women in my life — my wife, my daughter, and my mother — for their continuing support of my academic endeavors. Past mentors and collaborators — including Dora Ventura (University of São Paulo), Harry Carlisle and Steven Horvath (University of California, Santa Barbara), Evelyn Satinoff (University of Illinois), Michael Menaker (University of Virginia), and Giuseppe Piccione and Giovanni Caola (University of Messina) — were instrumental in the development of my research career. Intellectual exchanges with numerous students who worked in my laboratory over the years — especially Aaron Osborne, Candice Brown, and Adam Shoemaker — 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 section, 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 the late Professor Jürgen Aschoff and with Professor Franz Halberg, both pioneers in the field of circadian rhythms, helped me gain a broader historical perspective of the field. Professor Halberg has been a constant source of professional and personal support for me for the past three years, and I will never be able to thank him enough.

For financial support of my research program, I thank the National Institute of Mental Health and the National Science Foundation. For comments on the use of the first edition of *Circadian Physiology* as a textbook, I thank Ralph Mistlberger (Simon Fraser University) and William Timberlake (Indiana University). I thank also 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. Special thanks are due to Daniela Lupi (Imperial College London) for the microphotograph of the suprachiasmatic nucleus that appears on the cover of the book.

Finally, this book would not have been published if it were not for the superb work of the staff at CRC Press. I am especially appreciative of the support and encouragement provided by Barbara Ellen Norwitz and the technical assistance provided by Helena Redshaw and Mimi Williams.

Software Installation

A CD-ROM 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. The **Setup** program will automatically install the software package in personal computers running under Windows 95, Windows 98, Windows Me, Windows XP, or more recent versions. For installation in network computers (Windows NT, Windows 2000, and more recent versions), users should consult their network administrator, who should read the **Readme** file in the distribution disk. Memory and and disk space requirements are moderate (40 Mb of RAM and 80 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 three of the programs. Individual users with personal computers limited in memory and disk space may consult the **Readme** file to learn how to perform an installation that will require as little as 20 Mb of RAM and 2 Mb of free disk space (see also the *Troubleshooting* section below).

Procedure. Insert the *Circadian Physiology* CD-ROM in your CD-ROM drive. If the drive is set to automatically read the CD-ROM, **Setup** will start automatically. Otherwise, navigate to the CD-ROM 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 below. The icon looks like this: 

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, 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 Table 1 below. 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 9) are given in the main text of Chapter 3.

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 1). If you plan to analyze your own data sets, you must be aware that the data analysis programs (i.e., programs 1 through 9) 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): a time tag and the value to be plotted or analyzed. The time tag must be in 24-h clock mode (e.g., 22.5 for 10:30 P.M.). If the file contains more than one day, the clock must be reset to 0 every day at midnight. 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 Table 2.

TABLE 1
Programs

No.	Name	Description	Chapter
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	Freerun	Demonstration of free-running rhythms	12
11	Wave	Tutorial on periodic processes	3
12	Entrain	Tutorial on entrainment of circadian rhythms	7
13	PRC	Compilation of phase-response curves	7, 8
14	Model	Computer model of circadian pacemaker	6, 7, 8
15	Jet-lag	How to minimize jet lag	15
16	Health	How to control your own clock	14–17
17	SayIt	How to pronounce circadian physiology terms	1, 2
18	Bioclock	Listen to music (*Bioclock Rhapsody*)	17
19		Close the banner program	

TABLE 2
Data Files

File	Time Tag?	Length	Description
A01.txt	No	7 days, 6-min resolution	Body temperature (°C) of a Richardson's ground squirrel
A02.txt	No	8 days, 6-min resolution	Body temperature (°C) of a degu (noisy record)
A03.txt	No	36 days, 6-min resolution	Running-wheel activity (revolutions per 6 min) of a golden hamster
A04.txt	No	29 days, 6-min resolution	Running-wheel activity (revolutions per 6 min) of a golden hamster
A05.txt	No	42 days, 6-min resolution	Body temperature (°C) of a laboratory rat
A06.txt	No	19 days, 6-min resolution	Locomotor activity (beam breaks per 6 min) of a pill bug
A07.txt	No	6 days, 6-min resolution	Heat production (W) of a fat-tailed gerbil
A08.txt	No	20 days, 6-min resolution	Computer-generated cosine wave, no noise
A09.txt	No	20 days, 6-min resolution	Computer-generated cosine wave, 60% noise
A10.txt	No	20 days, 6-min 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-min resolution	Body temperature (°C) of a fat-tailed gerbil
A17.txt	No	7 days, 6-min resolution	Body temperature (°C) of a tree shrew
A18.txt	Yes	1 day	Locomotor activity (counts per 6 min) of a 13-lined ground squirrel
A19.txt	Yes	1 day	Body temperature (°C) of a man
A20.txt	No	34 days, 6-min resolution	Running-wheel activity of a domestic mouse with a light-induced phase shift on day 23
A21.txt	No	29 days, 6-min resolution	Running-wheel activity of a domestic mouse with a light-induced phase shift on day 14
A22.txt	No	43 days, 6-min resolution	Running-wheel activity of a Nile grass rat transferred from DD to LD on day 26
A23.txt	No	30 days, 6-min resolution	Running-wheel activity of a golden hamster under LD 7:5 (LD included in the file)
A24.txt	No	33 days, 6-min resolution	Running-wheel activity of a domestic mouse transferred from LD to DD on day 17
A25.txt	No	10 days, 6-min resolution	Computer-generated cosine wave with periodicities of 24 and 12 hours
A26.txt	No	10 days, 6-min resolution	Computer-generated cosine wave with periodicities of 24, 12, 10, and 6 hours
A27.txt	No	10 days, 6-min 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

Problem	Solution
Nothing happened when I placed the installation CD-ROM in the CD-ROM drive	The **autorun** function of your CD-ROM drive is probably disabled. You must either enable it or access the CD-ROM directly by navigating to it using the tools in the Taskbar.
The software installation failed	Most likely, you are trying to install the software on a network computer. Call your network administrator and ask him/her to read the **Readme** file in the CD-ROM. If the installation failed in a stand-alone computer, you may consult the **Readme** file yourself. If you have at least 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 three *wav* files (which will save tens of megabytes but will also prevent using the programs **SayIt** and **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 (the Circadian folder, unless you designated a different folder during installation of the program) and double-clicking on the **Circadian** icon. You may also create a Shortcut yourself. First locate the **Circadian** program. Then right-click on the **Circadian** 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. If you wish, you may move the banner to the bottom of the screen using the toggle switch (the up and down arrows at the right end of 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 my data set	Make sure that the data file is in the correct format (see specifications above). 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.
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.).
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 web site (www.microsoft.com).
In the program SayIt, some words have spurious characters	Encoding of characters does not have a universal standard. SayIt uses 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 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 800 x 600 pixels or greater. Color settings should not be a problem (a 16-bit color scheme is sufficient).
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.
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.
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.

Part I

History and Methods

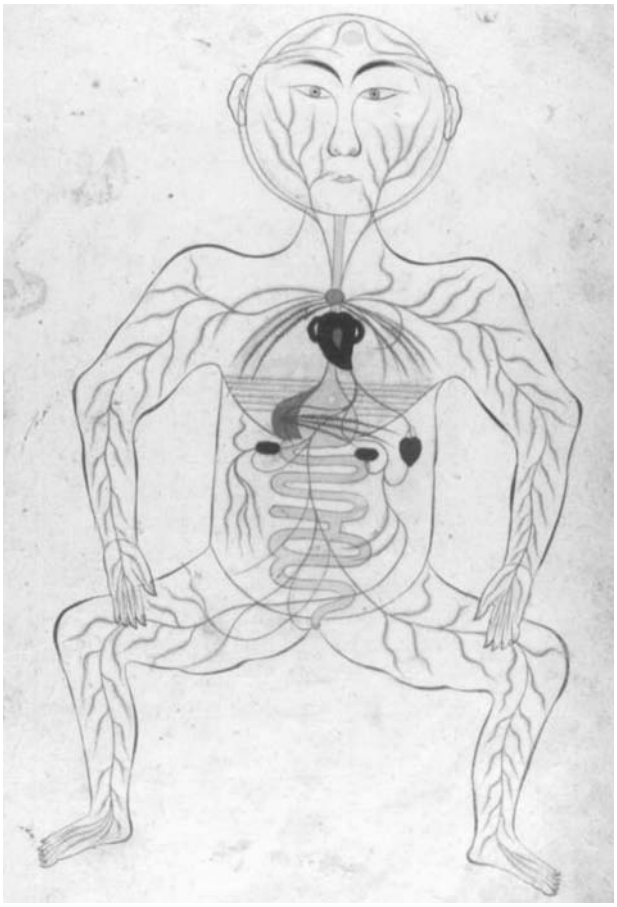


Illustration of human anatomy drawn by Persian physician Mansur ibn Mohammed in 1396. (Image courtesy of the Clendening Library at the University of Kansas Medical Center.)

1 Early Research on Circadian Rhythms

CHAPTER OUTLINE

1.1 Remote Past
1.2 20th Century
1.3 Current Trends
1.4 Ethics of Animal Research

1.1 REMOTE PAST

This chapter discusses the history of circadian physiology from ancient times to the present. *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] Physiological processes are dependent on anatomical and biochemical factors and constitute the physical basis of behavior. Physiology, therefore, incorporates anatomy, endocrinology, molecular biology, pharmacology, neuroscience, and psychology. *Circadian physiology* deals with the temporal organization of vital processes in the course of a day. Circadian physiology is integrative biology at its best: it combines functions in both the spatial and temporal dimensions. The conceptual and practical importance of circadian physiology is first discussed in Section 1.3; the discussion continues throughout this book.

Written records of observations in circadian physiology are limited to the few millennia since the invention of written language. However, early humans likely were aware of daily variations in physiological processes. At the very least, they must have recognized daily rhythmicity in the environment and its impact on their own daily cycle of wake and sleep. The creation of clocks and calendars is evidence of this awareness. 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 human-made clock. The Egyptians erected obelisks used as sundials more than 5500 years ago.[2] About 3000 years ago, the Chaldeans, in Mesopotamia, created a sophisticated nondecimal time measurement system,[3] from which our own system is derived. The Chaldean day, however, was divided into 12 long hours instead of the shorter 24 hours we adopt today. A decree issued in France in 1793 established a decimal division of the day, but the decree was revoked 2 years later.[4] Except for this brief diversion, the partition of a day into 24 hours and an hour into 60 minutes has been a global standard for centuries.

FIGURE 1.1 A 19th-century sundial in Budapest, Hungary. Sundials are one of oldest instruments devised by humankind to measure the passage of time. (Source: Photograph by Pavel Marek. Courtesy of Miroslav Broz, Czech Republic.)

Use of the system today differs around the world. In the United States, only scientists and the military use a true 24-hour system; businesses and ordinary citizens follow a double 12-hour system with 12 hours before noon (*ante meridiem* [A.M.]) and 12 hours after noon (*post meridiem* [P.M.]), 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). American military notation usually omits the colon between hours and minutes (that is, 5:35 P.M. = 1735 hours).

Jürgen Aschoff, a prominent 20th-century circadian physiologist whose contributions are discussed later in this chapter, identified the Greek poet *Archilochus* (675–635 B.C.) as the author of the oldest written record of observations in circadian physiology.[5] The verses of Archilochus remain only as fragments today.[6] Aschoff alluded to the fragment shown in Figure 1.3. The critical passage is

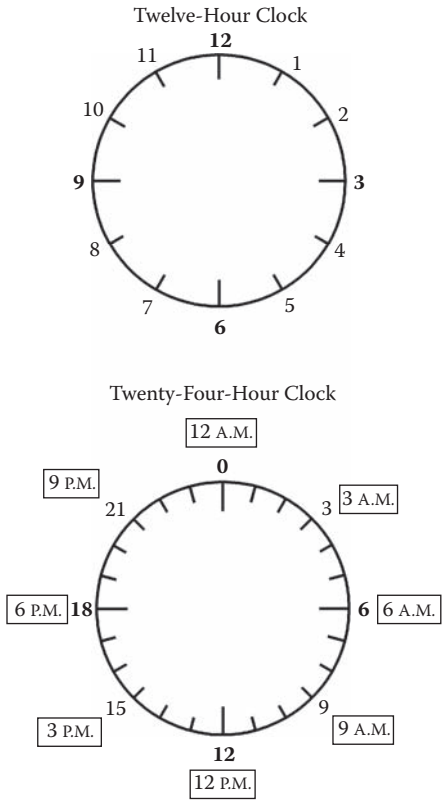


FIGURE 1.2 Diagrams of a 12-hour clock and a 24-hour clock. Although analog clocks almost always have 12-hour dials, a full day has twice as many hours. In most of the world, time of day is expressed according to a 24-hour clock. In the United States, a day is divided into 12 hours before noon (*ante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

the last sentence, which can be translated as: "Recognize what sort of rhythm governs man." The historical problem is what Archilochus meant by the term *rhythm* (ρυσμος). The fragment advises the reader to stand and fight in life, not to openly rejoice after 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. The same verse has been translated with the word *motion* instead of *rhythm*: "A measured motion governs man."[7] Based on these translations, it is inaccurate to identify Archilochus as the author of the oldest written record of observations in circadian physiology.

An unambiguous written record of observations in circadian physiology dates to the 4th century B.C., 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.[8] Androsthenes traveled through North Africa and India, where he observed the daily movement of the leaves of the tamarind tree (*Tamarindus indica*). Androsthenes noticed that tamarind leaves exhibit an impressive daily cycle of movement, in which the leaves move up during the day and down at night. Although 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.

The great physicians *Hippocrates* and *Galen* also made noteworthy observations of daily rhythmicity.[5,9] Hippocrates (460–370 B.C.), the Greek healer heralded as the father of medicine, noted periodic physiological processes, such as the recurrence of fever in 24-hour intervals. Galen (130–200 A.D.), 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 realized that daily physiological rhythms may be caused not only by environmental factors (such as the alternation of day and night) but also by an endogenous clock (that is, by a process that takes place inside the organism and persists in the absence of daily environmental cycles).

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.[10–12] Mairan (1678–1771), a

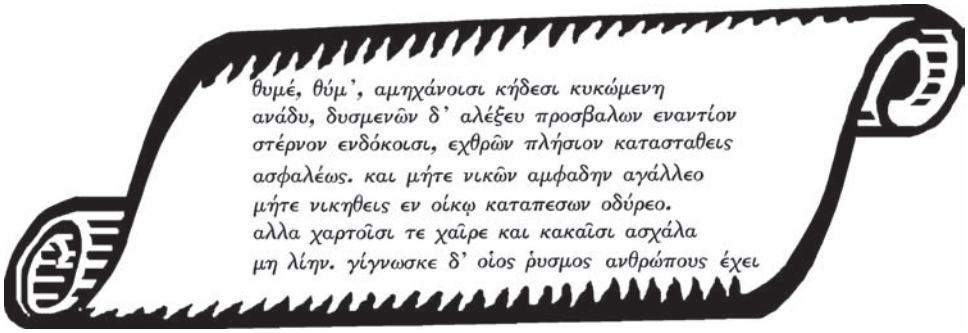


FIGURE 1.3 Is it "Greek" to you? It *is* Greek. This is a fragment of a poem written around 650 B.C. by the Greek poet Archilochus. It talks about the rhythms of life.

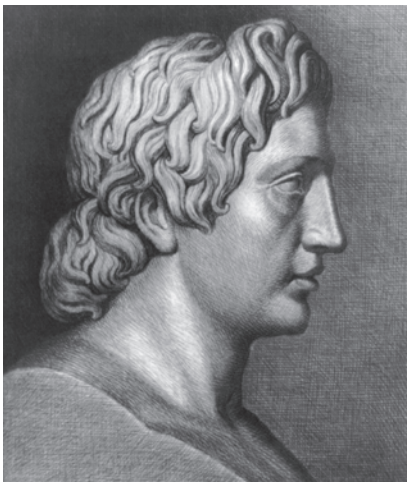


FIGURE 1.4 Alexander the Great (356–323 B.C.). The great Greek (Macedonian) general conquered most of the civilized world in the 4th century B.C. One of the many ship captains in his fleet was Androsthenes of Thasus, who wrote the first description of daily movements in plants. (Source: Library of Congress, Washington, DC.)

French astronomer, observed the *sensitive* plant (*Mimosa pudica*), which folds up its leaves 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 in the morning and folded in the evening.[13] This indicated that the daily rhythm of leaf-folding does not require a daily rhythm of sunlight. This observation alone, however, does *not* demonstrate the existence of endogenous rhythmicity. Other environmental factors besides light might have caused the leaves to open up. Mairan's report to the French Royal Academy of Sciences concluded that "the sensitive plant perceives the sun without seeing it,"[13] thus conceding that the persistent rhythmicity had an exogenous cause.

Christoph Wilhelm Hufeland (Figure 1.7) was more of a circadian physiologist than Mairan, although he also lacked an explicit notion of endogenous rhythmicity. A German physician, Hufeland (1762–1836) created the discipline of *macrobiotics*, the study of the prolongation of life.[14] In his acclaimed 1797 book, *The Art of Prolonging Life*, he expressed many concepts of physiological rhythmicity and noted that the 24-hour period of the Earth's revolution is reflected in organic life and appears in all human diseases.[15] His contemporary, *Julien Joseph Virey* (Figure 1.8), wrote the first book (his doctoral dissertation in medical school) dedicated to daily rhythmicity in physiological processes.[16] Virey (1775–1846), a French pharmacist, 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*.[9] He believed that circadian rhythms were endogenously generated, but his research was restricted to the careful description of daily rhythms in diseases and mortality.[17]

The honor of first describing research that demonstrated the endogenous nature of circadian rhythms belongs to *Augustin Pyramus de Candolle* (Figure 1.9). A renowned Swiss botanist, Candolle (1778–1841) studied the rhythm

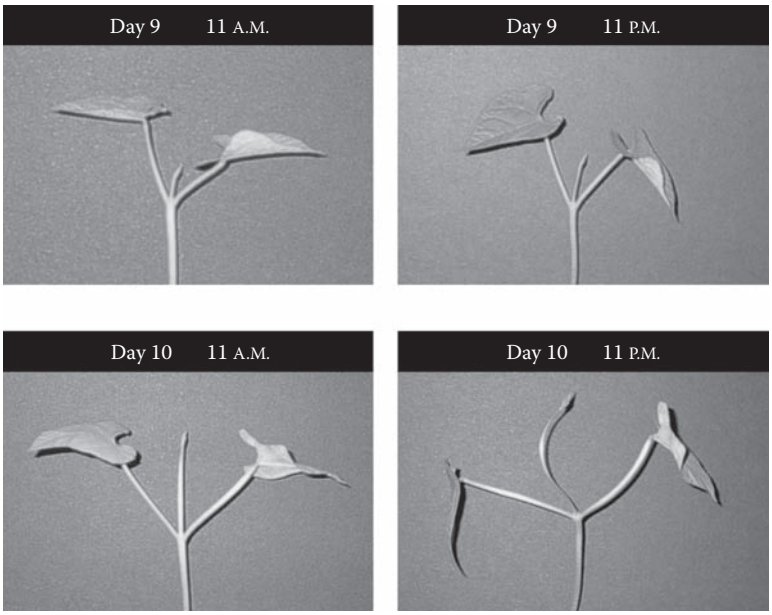


FIGURE 1.5 The "sleep" cycle of the bean plant. The leaves of the common bean plant (*Phaseolus vulgaris*) rise during the day and drop at night. (Source: Photographs and montage by R. Refinetti.)

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

FIGURE 1.7 Christoph Wilhelm Hufeland (1762–1836). As part of a larger book, this German physician wrote the first systematic account of daily rhythmicity in human physiology. (Source: Clendening Library, University of Kansas Medical Center, Kansas City, Kansas.)

of the 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. Candolle, however, noticed that the period of the rhythm (i.e., the duration of the cycle) was shorter than 24 hours.[18] This finding was important because, if some uncontrolled geophysical factor were responsible for the rhythm, the period of the rhythm should have been 24 hours. 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. In this way, Candolle effectively demonstrated the existence of an endogenous circadian clock. The location of this endogenous clock and its

FIGURE 1.8 Julien Joseph Virey (1775–1846). The doctoral dissertation of this French pharmacist was the first book devoted to biological rhythms. (Source: Library of the National Academy of Medicine, Paris, France.)

FIGURE 1.9 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). (Source: Library of the Russian Academy of Sciences, Moscow, Russia.)

method of operation remained unknown for another 100 years.

Many other researchers investigated biological rhythms during the 19th century, although not all conducted scientific observations. One example of speculative theory is that of *biorhythms,* developed late in the 19th 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 consist of three natural cycles within the human body that affect

us physically, emotionally, and intellectually.[19–22] 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 1.10). The different length of the three cycles causes 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). A person's disposition on any given day, then, will be a composite of the states of the three rhythms. By calculating and studying biorhythms, an individual is supposedly capable of knowing what to expect each day and, therefore, is capable of avoiding bad experiences.

A central problem with the theory of biorythms is that it ignores the notion of biological variability. 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 a person's bedtime expresses regularity with variability. You probably go to bed at about the same time each night (say, eleven o'clock or midnight), but that time varies — rarely do you keep to your bedtime with the accuracy of minutes (and certainly not of seconds). Variability is an essential feature of biological processes,[23] to such an extent that absence of variability is often a sign of disease.[24] 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.

Unlike Fliess and Swoboda, physician John Davy collected real data. He recorded his own body temperature (under the tongue) in the morning and evening every day for 9 consecutive months in 1844.[25] Figure 1.11 shows a 1-month segment of his data. His temperature goes up and down reliably each day but it also varies considerably from one day to the next. Taking only two measurements per day did not provide Davy with enough data to look closely at the daily oscillation of his temperature. Twenty-two years later, physician William Ogle recorded his own temperature several times a day for several months.[26] As can be seen in Figure 1.12, 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[27,28] and other animals[29–32] through the end of the 19th century.

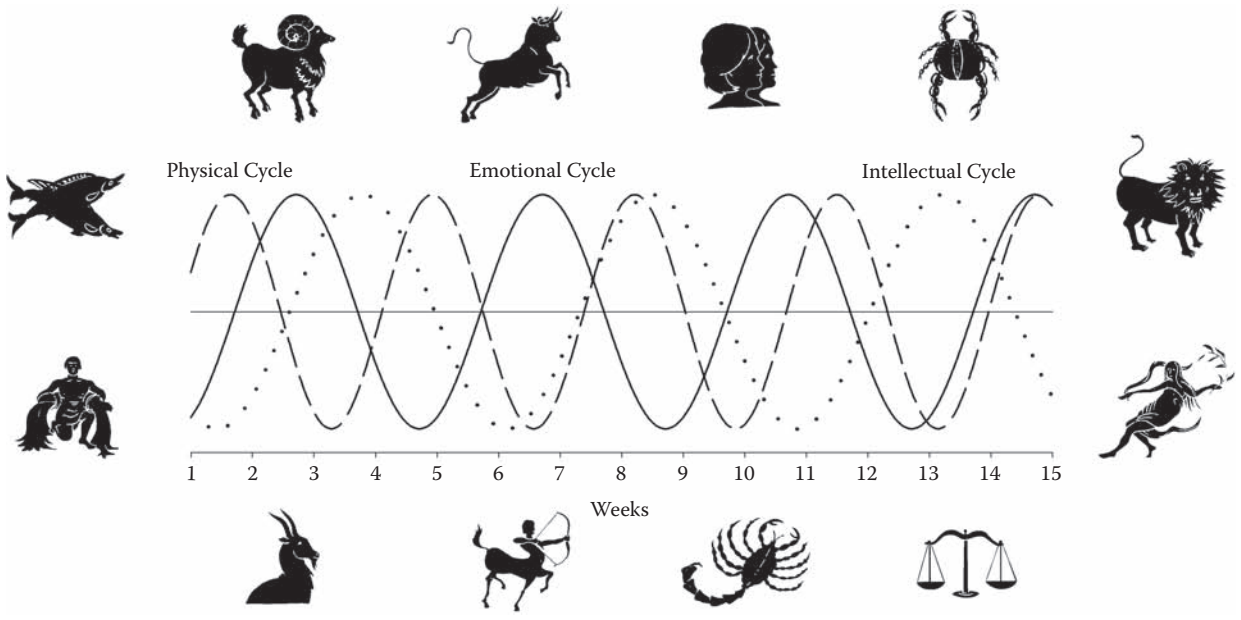


FIGURE 1.10 Biorhythms and horoscope? The concept of biorhythms was developed by W. Fliess and H. Swoboda in the late 1800s. Although they claimed no connection with the signs of the zodiac, their notion of biorhythms was just as unscientific as horoscopes are. (Sources: Crawley, J. (1996). *The Biorhythm Book*. Boston: Journey. Signs of the zodiac after Fisher, D. & Bragonier, R. (1981). *What's What*. Maplewood, NJ: Hammond.)

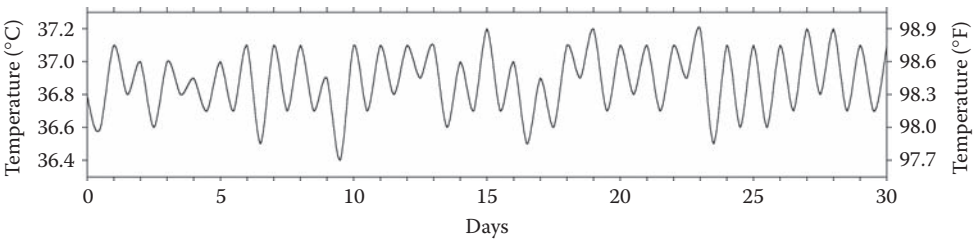


FIGURE 1.11 A real biological rhythm. In 1844, British physician John Davy made accurate measurements of the day-to-day variation of his own body temperature. (Source: Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.)

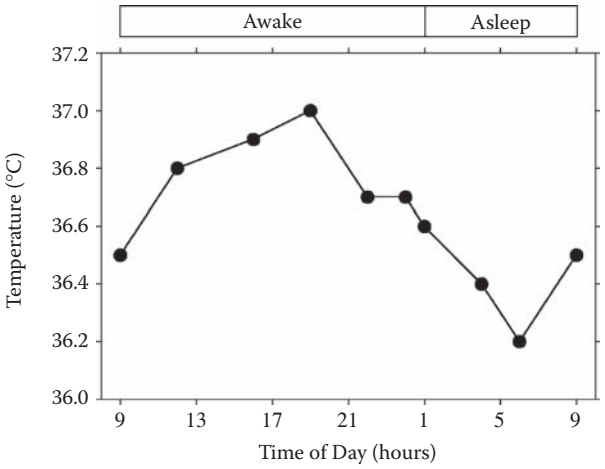


FIGURE 1.12 An early record of the daily rhythm of body temperature. In 1865, physician William Ogle conducted measurements of his own oral temperature with temporal resolution high enough to allow the characterization of a daily rhythm. (Source: Ogle, W. (1866). On the diurnal variations in the temperature of the human body in health. *St. George's Hospital Reports* 1: 221–245.)

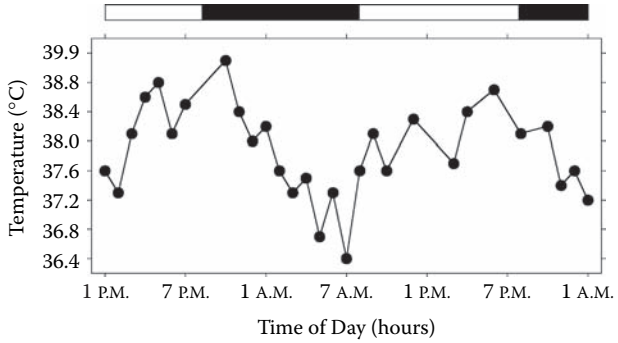


FIGURE 1.13 Old records of body temperature of a monkey. From 1902 to 1905, Simpson and Galbraith conducted numerous measurements of the body temperature of rhesus monkeys, as exemplified in these records from Monkey #31. The light and dark bars at the top of the figure indicate the approximate duration of the light and dark phases of the prevailing light–dark cycle. (Source: Simpson, S. & 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.)

1.2 20th CENTURY

The 20th century witnessed a surge in sophisticated research on circadian rhythms. 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.[33] An example of their experimental results in rhesus monkeys is shown in Figure 1.13. At about the same time, Francis Benedict, in Connecticut, and Arthur Gates, in California, conducted detailed measurements of the body temperature rhythm[34] and of daily variations in memory[35] in human subjects.

In 1926 Maynard Johnson, in Illinois, provided the first demonstration of the endogenous nature of circadian rhythms in an animal species.[36] Johnson studied the rhythm of locomotor activity (that is, 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 1.14, 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 are 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 endogenous, not exogenous. This issue is 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*).[37] Representative records are shown in Figure 1.15. Despite considerable random variation, clear daily rhythmicity can be seen. Rogers and Greenbank did not investigate whether the growth rhythm was endogenously generated, but their

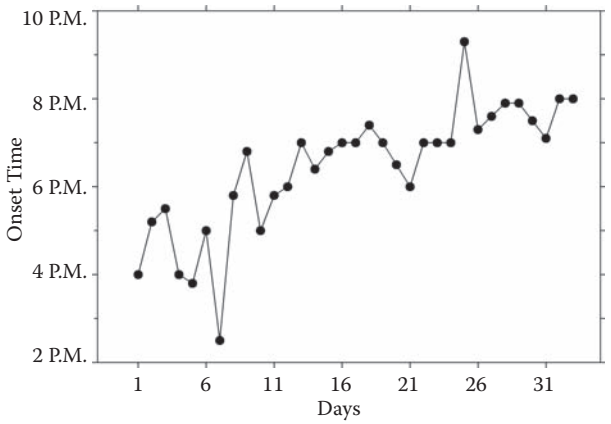


FIGURE 1.14 "Free-running" mouse. In 1925, Maynard Johnson documented a circadian rhythm of locomotor activity in deer mice (*Peromyscus leucopus*) maintained in constant darkness. The rhythm exhibited a period longer than 24 hours and, therefore, could not be attributed to geophysical factors. "Onset time" refers to the time each day when the mouse initiated activity. (Source: Johnson, M. S. (1926). Activity and distribution of certain wild mice in relation to biotic communities. *Journal of Mammalogy* 7: 245–277.)

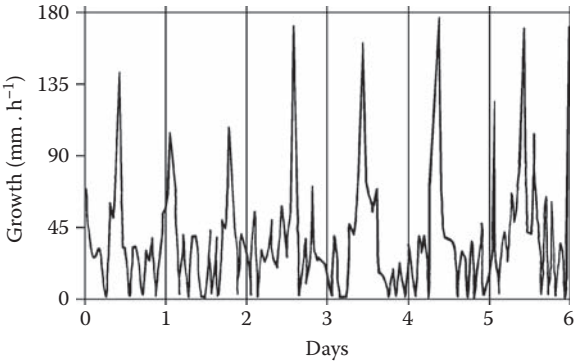


FIGURE 1.15 Daily rhythmicity in prokaryotes. In 1929, Rogers and Greenbank demonstrated the existence of daily rhythmicity in the growth of bacteria, which are prokaryotes (i.e., organisms whose cells do not have a separate nucleus). Except in the second of the 6 days shown, clear daily peaks of growth can be seen. (Source: Rogers, L. A. & Greenbank, G. R. (1930). The intermittent growth of bacterial cultures. *Journal of Bacteriology* 19: 181–190.)

study was important because it showed daily rhythmicity in a *prokaryotic* organism (that is, a unicellular organism without a membrane separating the nucleus from the cytoplasm). These findings implied that daily rhythmicity is not restricted to more complex *eukaryotic* organisms and, therefore, is probably a characteristic of all life on Earth. We return to this topic in Chapter 9.

Before the end of the 1930s, enough knowledge on daily rhythms was available to justify a literature review of the topic[38] and to stimulate discussion of potential medical uses of this knowledge.[39] An influential researcher at this time was German botanist *Erwin Bünning* (Figure 1.16). 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 essential for the synchronization of circadian rhythms to the environmental light–dark cycle.[40] Bünning's contribution to circadian physiology also included writing the first comprehensive book in the field, *The Physiological Clock*. The book was originally published in German in 1958[41] and later in three English editions, the last of which appeared in 1973.[42]

FIGURE 1.16 Erwin Bünning (1906–1990). This German botanist, whose central research interest was the mechanism of photoperiodism, made significant contributions to the study of circadian rhythms in the 20th century. (Source: Botanical Archive, University of Hamburg, Germany.)

Two other mid-20th-century researchers deserve special mention: Curt Richter (1894–1988), a psychology professor at Johns Hopkins University who conducted extensive research on circadian rhythms in laboratory animals and human patients,[43] and Nathaniel Kleitman (1895–1999), the renowned investigator at the University of Chicago, who studied the physiology of sleep and circadian rhythms in humans.[44] In the 1950s, many investigators began to concentrate their full-time efforts on research in circadian physiology. Three individuals, Jürgen Aschoff, Franz Halberg, and Colin Pittendrigh, became so influential that they can be called the *forefathers* of modern circadian physiology.

Jürgen Aschoff (Figure 1.17) 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

FIGURE 1.17 Jürgen Aschoff (1913–1998). This German physiologist was a leader in the development of the study of circadian rhythms in the 20th century. (Source: Reprinted with permission from Sage Publications. (1994). *Journal of Biological Rhythms* 9(3):187.)

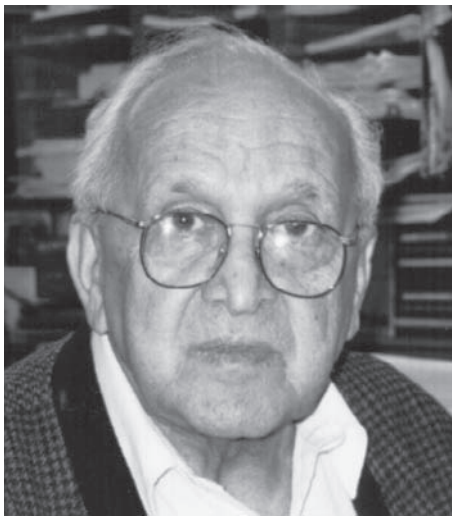


FIGURE 1.18 Franz Halberg (1919–). This American physician (originally from Romania) created the terms *circadian* and *chronobiology* and has been the foremost advocate of the establishment of chronobiology as a separate discipline. (Source: Photograph courtesy of Franz Halberg.)

switched to the study of circadian rhythms.[45] 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*[46,47] was a driving force in circadian physiology for decades. His thorough and exhaustive reviews of the literature in circadian physiology[48–50] served as invaluable guides to numerous researchers. I met Aschoff 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,[51] but his legacy lives on. This book cites over 30 of his articles.

Franz Halberg (Figure 1.18) 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*[52] and *chronobiology.*[53] 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.[54] Although the medical applications of circadian physiology were his main concern,[55–57] he conducted a great deal of basic research as well.[58–61] Halberg was still alive and productive when I wrote this book, and I had the chance to consult with him about historical and technical matters.

Colin S. Pittendrigh (Figure 1.19) was born in Whatley Bay, England, in 1919 and moved to the United States as a graduate student. He spent the first 20 years of his

FIGURE 1.19 Colin Pittendrigh (1919–1996). This American biologist was a leader in the development of the study of circadian rhythms in the 20th century. (Source: Reprinted with permission from *Annual Review of Physiology.* (1993). 55:16**,** www.annualreviews.org.)

faculty career at Princeton University, in New Jersey, and then relocated to Stanford University, in California. A "clock watcher" at heart,[62] he strived to understand how the operation of a physical oscillator could explain circadian rhythmicity in animals. Most of our current understanding of the operation of the circadian clock is derived from his work with flies[63,64] and rodents.[65–68] I met Pittendrigh 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,[69] but his contribution to circadian physiology is everlasting, as shown in Chapter 7.

The personal and professional interactions between the three forefathers were not always cordial and productive. Aschoff recognized Halberg's leading role in the development of circadian physiology[5] and Pittendrigh's insights into mechanisms of circadian organization.[49] Pittendrigh acknowledged some of Halberg's contributions[62] and credited Aschoff with important discoveries.[67] Halberg recognized the contributions of both Aschoff and Pittendrigh.[70] However, a great divide characterized the field during the second half of the 20th century.

Researchers split into two groups referred to as the *clocks* faction and the *chronome* faction. As shown in Table 1.1, Pittendrigh headed the clocks faction, which concerned itself mainly with the mechanisms of biological timing. The chronome faction (named for 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 groups. 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 into print,[71,72] the animosity was clearly revealed by sociologists who looked at the "disciplinary stake" of chronobiology.[73] A textbook recently published by eminent members of the clocks faction presented Pittendrigh and Aschoff as the "founders of chronobiology" with no mention of Halberg. This is especially noteworthy because the book is entitled *Chronobiology*,[11] and it was Halberg who created the term[53] and forcefully promoted the creation of the new discipline against Pittendrigh's objections.[73]

Antagonisms are not peculiar to circadian physiology — or to science more generally. In the musical arts, for example, the renowned classical composer and conductor Rymsky-Korsakov satirically said this about Ludwig von Beethoven: "His music abounds in countless leonine leaps of orchestral imagination, but his technique, viewed in detail, remains much inferior to his titanic conception."[74]

Over the years, small but sincere attempts have been made to reconcile the two factions. In 1960 a conference held at Cold Spring Harbor Laboratory (in Long Island, New York) brought together Bünning, Aschoff, Halberg, Pittendrigh, and others under one roof,[75] although the factions were not yet strongly divided. Thirty-five years later, a conference organized at Dartmouth Medical School by members of the clocks faction (Figure 1.20) welcomed members of the chronome faction. Most participants were members of the clocks faction, however, and therefore, the conference resembled dozens of other conferences held over the years. 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, D.C. (Figure 1.21) — included basic researchers as well as medical practitioners, and provided the opportunity for individuals with quite different professional interests to exchange ideas. 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.[76]

Members of the chronome faction often feel that the clocks faction wastes time on esoteric questions instead of addressing important real-life issues. Members of the clocks faction feel that the chronome faction conducts sloppy research that fails to address the intricacies of the biological clock. Because each faction judges the other by its own values, it is difficult to reach a consensus. Members of both factions agree, however, that peer-recognition of one's work and the ability to obtain research funds are objective measures of professional achievement, so that these two criteria can be used to evaluate the merits of each faction.

The extent of a researcher's peer-recognition can be estimated by the number of times that the researcher's work is cited in publications by other authors. The *Science Citation Index* (produced by Thomson Scientific [formerly the Institute for Scientific Information], in Philadelphia) shows that as of August 2004, Aschoff had 8900 citations, Halberg had 9200 citations, and Pittendrigh had 6800 citations. Although Halberg had more total citations than Pittendrigh, he had fewer citations per published article (11 as compared with 42). This means that overall he is cited more often than Pittendrigh, but his articles are not individually considered as "important" as Pittendrigh's

TABLE 1.1
Characteristics of the Two Main Factions in Chronobiology in the 20th 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 to 1994)

FIGURE 1.20 Where is Waldo? As in the popular book series *Where's Waldo?*, you may have a hard 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. (Source: Photograph courtesy of Jay Dunlap and Jennifer Loros, the meeting organizers.)

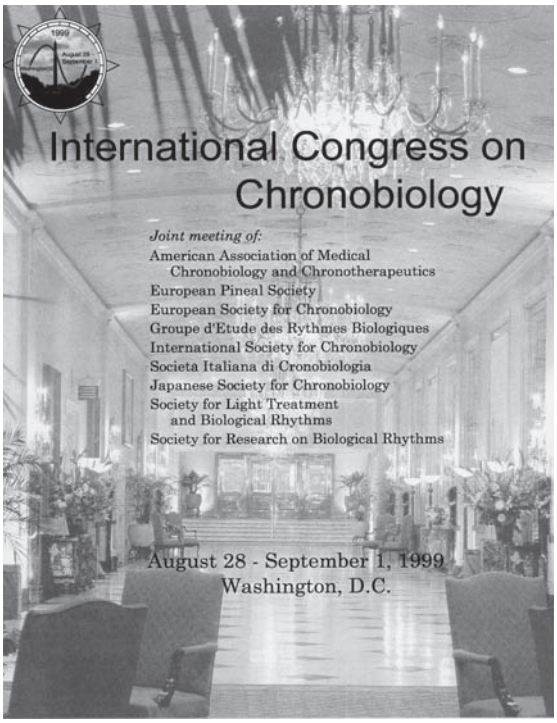


FIGURE 1.21 First major attempt to unify the field. An International Congress on Chronobiology, held in Washington, D.C., in 1999, was the first major attempt to unify the field of studies of biological rhythms. (Source: Image cover of Congress program.)

publications. The fact that Pittendrigh's individual articles are cited more often may reflect his focus on specific topics. In his articles, Halberg often digressed into far-reaching subjects, including the concept of "astrochronobiology."[57,77] I once told him that this reminded me of the concept of "orgasmic energy," a crazy idea of psychoanalyst Wilhelm Reich, according to whom the energy of sexual orgasms permeates the universe.[78] Halberg's reply was not "Oops, maybe I should be more reticent," but something like "Oh yes, poor Reich, he was ridiculed for being an open-minded scientist!"

Basic information about research grants awarded in the United States by the National Institutes of Health (the major source of research funding in the country) is freely available through the Computer Retrieval of Information on Scientific Projects (CRISP). Between 1972 (the first year available) and 1996 (the year of Pittendrigh's death), CRISP lists 81 grants awarded to Franz Halberg and 17 grants awarded to Colin Pittendrigh. Between 1972 and 2004, CRISP lists 20 grants for William Hrushesky, a major researcher in the chronome faction,[79–82] and 32 grants for Joseph Takahashi, a major researcher in the clocks faction.[83–86]

In terms of peer-recognition and research funding, the objective measures of professional achievement agreed to by both factions, the chronome and clock groups are

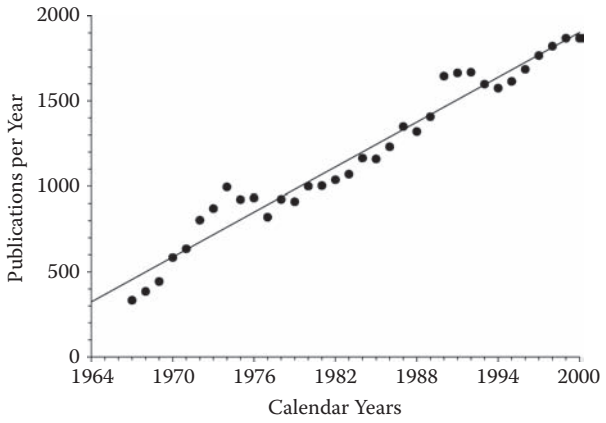


FIGURE 1.22 Growth in the number of journal articles on circadian rhythms published during the last 35 years of the 20th century. The number of articles on circadian rhythms catalogued by PubMed (U.S. National Library of Medicine) grew from fewer than 200 articles per year in 1965 to almost 2000 articles per year in the year 2000. (Source: PubMed database searched by R. Refinetti in August 2003 targeting the term *circadian* in any searchable field year-by-year.)

equally meritorious, and they are both unjustified in degrading each other.

1.3 CURRENT TRENDS

Work in circadian physiology during the 21st century builds upon the progress made during the second half of the 20th century. As shown in Figure 1.22, the number of published journal articles in circadian physiology grew from fewer than 200 articles per year in 1965 to almost 2000 articles per year in the year 2000. The growth was linear in terms of yearly publication rates, resulting in an exponential growth in terms of cumulative rates — exponential growth being typical of progressive research fields.[87] 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 50,000. Because the yield of database searches is greatly reduced by limitations in coverage, indexing, and retrieval,[88–91] we can estimate the actual number of existing journal articles in circadian physiology to be around 100,000. Although this book cites only 5000 of these articles, I did my best to ensure that the most significant articles were included. Citation analysis has shown that the most significant scientific literature appears in a small core of journals.[92] I took very seriously the task of constructing a coherent picture out of thousands of disperse pieces of information.[93–95]

Before we move on to an overview of the major topics of current research in circadian physiology, we must pause briefly to ensure that we do not lose sight of the "big picture" — that is, how circadian physiology fits in with other areas of scientific research. For example, in colleges and universities, human knowledge is often organized into four major categories: the humanities, the natural sciences, the social sciences, and the various graduate professional specializations (Figure 1.23). Biology falls within the natural sciences. Subdivisions of biology are often based on the life forms under study (botany, zoology, and microbiology) or on the processes involved (anatomy, endocrinology, neuroscience, and so on). Biology can also be subdivided 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

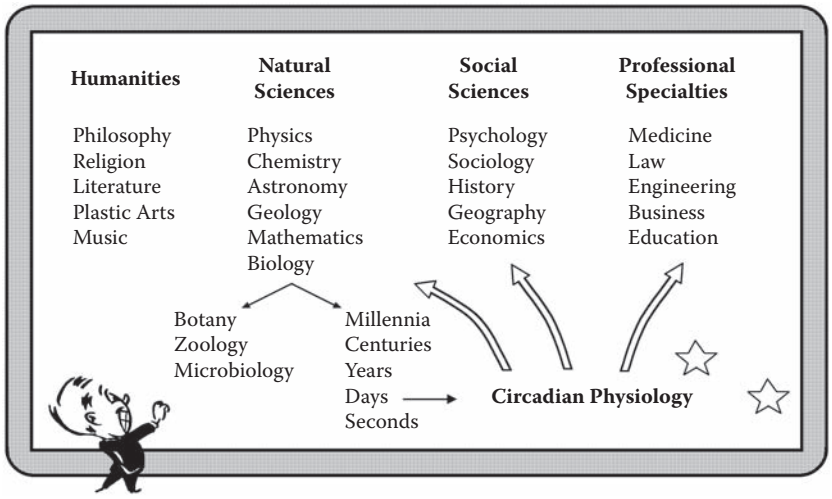


FIGURE 1.23 The "big picture." Circadian physiology has a definite place in the universe of human knowledge, and its implications are widespread.

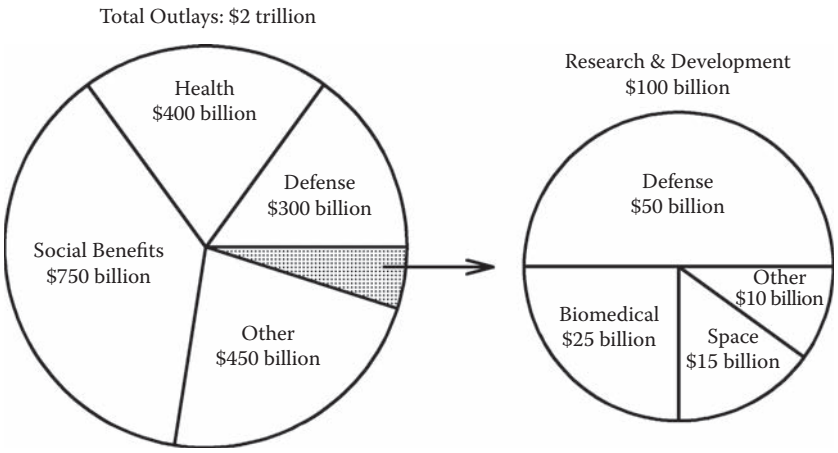


FIGURE 1.24 The money chest. Shown is the breakdown of outlays in the U.S. federal budget (year 2002). (Sources: *TIME Almanac 2004* (2003). Des Moines, IA: TIME Books; Malakoff, D. (2002). War effort shapes U.S. budget, with some program casualties. *Science* 295: 952–954.)

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 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 provides essential information about the temporal structure of the processes investigated by psychologists, sociologists, economists, and many others. Part V of this book demonstrates that 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 group called Circadian Rhythm (*Internal Clock*, 1998; *Over Under Everything*, 2001), a 1993 album called *Circadian Rhythyms* [sic] by New Age musician Colin Chin, and a 1997 album called *Circadian Symphony* by David Cohen. In the plastic arts, the "Inside Time" series of paintings by artist Julie Newdoll (www.brushwithscience.com) further shows the influence of circadian physiology on the humanities.

The "big picture" also includes the allocation of money for scientific research. In 2002, the federal budget of the United States surpassed $2 trillion in expenditures.[96] As indicated in Figure 1.24, 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). Only 5% of the outlays were related to research and development (R&D), but in such a large budget, 5% was still an enormous amount of money ($100 billion). As shown on the right side of Figure 1.24, defense (military) research accounted for half of the R&D budget. Half of the civilian research budget was allocated to biomedical research ($25 billion), a large part of it routed through the National Institutes of Health.[97] What proportion of the $25 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 14 million citations at the end of 2003. Of these citations, 46,000 could be retrieved by the term *circadian*. Thus, it can be conservatively estimated that 0.3% of all biomedical research deals with circadian rhythms. This allows us to estimate a federal investment of $75 million in circadian research in the United States each year. Considering that the U.S. economy corresponds to 20% of the world's economy,[96] but also that most other countries allocate smaller proportions of their budget to R&D,[98] a rough estimate of investment in circadian research worldwide is $230 million per year. This figure does not include funding from private sources or the salaries of investigators paid by their university employers.

Although recent research in circadian physiology involves all aspects of circadian organization — as is described in detail throughout this book — five topics are currently receiving special attention. These "hot topics" are indicated in Figure 1.25. Topic 1 refers to the molecular mechanisms (genes, proteins, and their interactions) underlying the operation of the master circadian clock located at the base of the mammalian brain (the *suprachiasmatic nucleus*).[99–102] Topic 2 relates to how a small gland located deep inside the brain (the *pineal gland*) helps the circadian clock adapt to the environmental cycle of light and darkness.[103–106] Topic 3 has to do with the nature and location of specialized cells in the eyes (the *photoreceptors*) that provide information about light to the circadian clock.[107–110] Topic 4 refers to how stimuli other

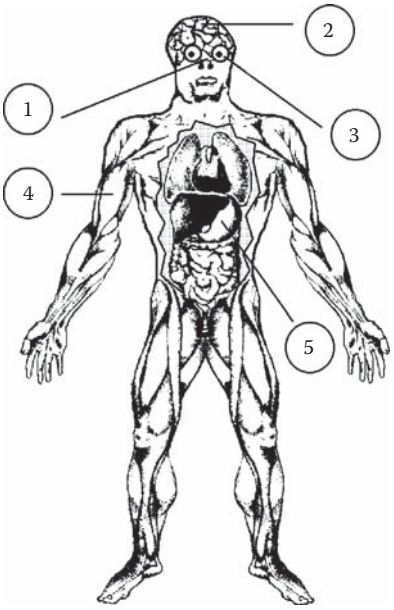


FIGURE 1.25 A new Frankenstein creation? No, this is not a new Frankenstein creation. It is just an exaggerated diagram indicating topics of current research in circadian physiology. See text for details.

than light (such as physical exercise) affect the circadian clock.[111–114] Topic 5 relates to the search for other circadian clocks in the body besides the master clock in the brain.[115–118] Reports of research in these and other topics are published in a variety of professional journals, including journals specialized in biological rhythms. Currently, three print journals specialize in biological rhythms: *Biological Rhythm Research*, *Chronobiology International*, and the *Journal of Biological Rhythms* (Figure 1.26). In 2003, an electronic journal specializing in circadian rhythms, the *Journal of Circadian Rhythms* (Figure 1.27), began publication.

Researchers with a primary interest and expertise in the mechanisms of biological timing conduct a significant amount of research in circadian physiology. Other life science investigators, who have a secondary interest in circadian physiology, also perform a considerable amount of relevant research. In a recent 12-month interval, Thomson Scientific cataloged 1227 journal articles in circadian physiology authored by 3330 researchers. As shown in Figure 1.28, 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 better understand these numbers, I conducted a more detailed study to identify the major players in the game of circadian physiology, as reported in Table 1.2. Of course, number of publications per se is not a meaningful figure, but the professional prestige of a researcher has been shown to correlate highly with the number of his/her publications.[119] Table 1.2 should not be read as a ranking of prestige among circadian physiologists; however, it provides an objective measure of the productivity of prestigious researchers in the field.

In the following paragraphs, I briefly discuss five of the researchers listed in Table 1.2. As we discuss people's research activities, the reader may wonder if I could include some personal gossip to spice up the text. Unfortunately, most scientists are "nerds" who prefer a laboratory experiment to a wild party, and those few who are "party animals" keep their private lives secret. To satisfy bored readers, I may have to resort to my own personal history to provide some form of gossip. This would be the story of a university professor who, as a single man in his early 30s, fell in love with an exceptionally bright, great-looking graduate student, had a steaming romance with her, and ended up losing his job because of it.[120] Romances between university professors and graduate students, as well as other romances that appear to involve a conflict of interests, are often depicted benignly in Hollywood motion pictures.[121] However, in the last two decades, at least in the United States, these relationships have been condemned and proscribed,[122–125] despite reasoned arguments in defense of the freedom of association between consenting adults.[126–129]

French endocrinologist *Paul Pévet* (Figure 1.29) currently is the most prolific circadian physiologist. Pévet works at the Louis Pasteur University, in Strasbourg, and conducts research on the neuroendocrinology of circadian rhythms in mammals; in particular, he investigates the role of the pineal gland.[130–132] *Urs Albrecht* (Figure 1.30), a Swiss biochemist, 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). He works at the University of Fribourg (in Switzerland), where he conducts research on the neural and molecular aspects of circadian rhythms in vertebrates.[99,133,134] *Serge Daan* (Figure 1.31), a professor at the University of Groningen (in the Netherlands) since 1975, obtained his doctorate at the University of Amsterdam and worked with both Aschoff in Germany and Pittendrigh in the United States. His research involves multiple aspects of the neurobiology and ecology of circadian rhythms in various life forms.[135–137] *Ken-ichi Honma* (Figure 1.32) is 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.[138–140] *Michael Menaker* (Figure 1.33) is a professor of biology at the University of Virginia. After completing his doctorate with Pittendrigh at Princeton University, he worked as a postdoctoral fellow at Harvard University and a professor at the University of Texas (Austin) and the

TABLE 1.2
The Most Prolific Circadian Physiologists Today[a]

Rank	Author	Institution	Specialty	Articles
1	Paul Pévet	Louis Pasteur University, France	Endocrinology of circadian rhythms in mammals	43
2	Hitoshi Okamura	Kobe University, Japan	Molecular biology of circadian clocks in mammals	38
3	Steve A. Kay	Scripps Research Institute, U.S.A.	Molecular biology of circadian clocks in plants	33
3	Shigenobu Shibata	Waseda University, Japan	Molecular biology of circadian clocks in mammals	33
5	Franz Halberg	University of Minnesota, U.S.A.	Circadian rhythms and health care in humans	29
6	Ramón C. Hermida	University of Vigo, Spain	Circadian control of blood pressure in humans	27
6	Francis Lévi	University of Paris, France	Chronotherapy of cancer (basic and applied research)	27
8	Urs Albrecht	University of Fribourg, Switzerland	Neurobiology of circadian rhythms in vertebrates	26
8	Charles A. Czeisler	Harvard University, U.S.A.	Control of sleep and circadian rhythms in humans	26
10	Yvan Touitou	Pitié-Salpêtrière Hospital, France	Endocrinology of circadian rhythms in humans	24
11	Ruud M. Buijs	Inst. for Brain Research, Netherlands	Neurobiology of circadian rhythms in vertebrates	23
11	Katsuya Nagai	Osaka University, Japan	Molecular biology of circadian clocks in mammals	23
13	Steven M. Reppert	University of Massachusetts, U.S.A.	Molecular biology of circadian clocks in animals	22
14	Serge Daan	Groningen University, Netherlands	Neurobiology and ecology of circadian rhythms	21
15	Jay C. Dunlap	Dartmouth College, U.S.A.	Molecular biology of circadian clocks in fungi	19
16	Ken-ichi Honma	Hokkaido University, Japan	Neurobiology of circadian rhythms	18
16	Daniel F. Kripke	Univ. of California at San Diego, U.S.A.	Control of sleep and circadian rhythms in humans	18
16	Michael Menaker	University of Virginia, U.S.A.	Neurobiology of circadian rhythms	18
19	Russell G. Foster	Imperial College London, U.K.	Neurobiology of circadian photoreceptors	17
19	Andries Kalsbeek	Inst. for Brain Research, Netherlands	Neurobiology of circadian rhythms in vertebrates	17

[a] This table was compiled in two main steps. The Research Alert service of Thomson Scientific (formerly, Institute for Scientific Information, Philadelphia, PA) was used to compile a list of authors who published journal articles containing the word *circadian* in the title or as an indexed key word from November 2002 to October 2003. This 12-month interval was the most recent time period available at the time this table was compiled. Number of publications during the most recent 12-month interval was chosen as a criterion of recency in research activity. Over 3000 authors published articles containing the word *circadian* in the title or as an indexed key word during this interval. The 50 authors with the highest numbers of publications were moved to the second step.

In the second step, the PubMed database (U.S. National Library of Medicine, Washington, DC) was used to retrieve all publications of these 50 authors that contained the word *circadian* in any searchable field (title, abstract, or key words) from January 2000 to October 2003. This 46-month interval corresponded to all years of the 21st century (plus the last year of the 20th century) at the time this table was compiled. Authors who published more than 50% of their articles with another author were considered to be members of that author's research team and were not individually listed. This table lists the top 20 authors in the PubMed search.

FIGURE 1.26 The three print journals specializing in biological rhythms. Currently, three print journals specialize in biological rhythms: *Biological Rhythm Research* (published by Swets & Zeitlinger), *Chronobiology International* (published by Taylor & Francis), and the *Journal of Biological Rhythms* (published by Sage Science Press).

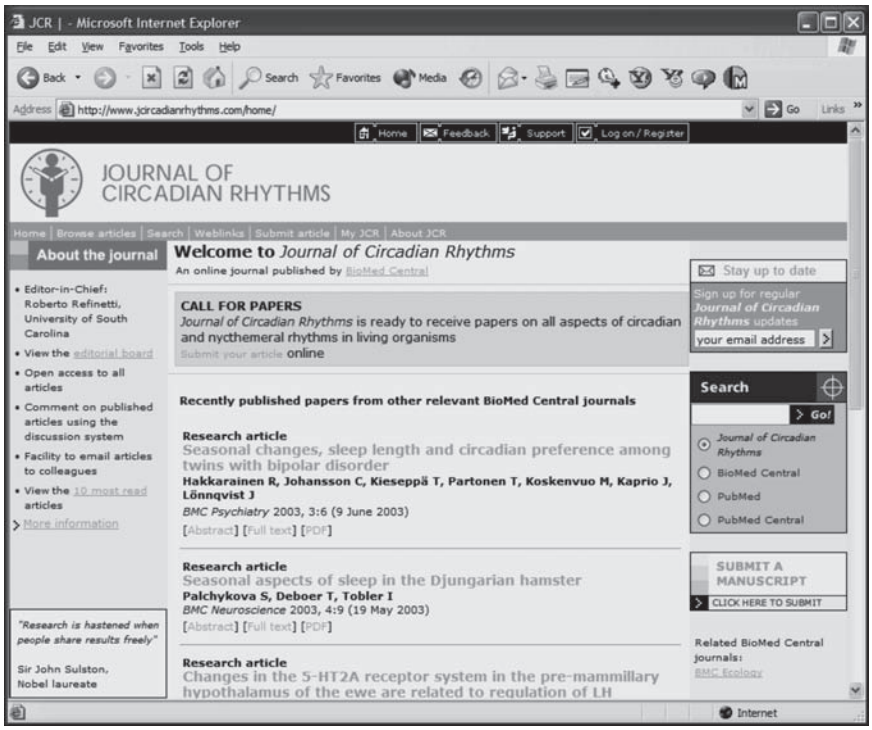


FIGURE 1.27 The *Journal of Circadian Rhythms.* The first journal specializing in circadian rhythms was launched in 2003. The *Journal of Circadian Rhythms*, published by BioMed Central, is an open-access electronic journal (with no print version) in which articles are freely available to readers worldwide upon publication. The URL is www.JCircadianRhythms.com.

FIGURE 1.28 How many people publish research articles on circadian rhythms? In a recent 12-month interval, 3330 authors published journal articles containing the word *circadian* in the title or as an indexed key word. The figure shows the number of articles published by each author. (Source: Research Alert service of Thomson Scientific (formerly, Institute for Scientific Information, Philadelphia, PA). Search conducted by R. Refinetti for the 12-month interval between November 2002 and October 2003.)

FIGURE 1.29 Paul Pévet (1945–). This French neurobiologist, who specializes in the endocrinology of circadian rhythms in mammals, was the most prolific circadian physiologist in the first years of the 21st century. (Source: Photograph courtesy of Paul Pévet.)

University of Oregon before the University of Virginia recruited him to chair its biology department. He has served as doctoral and postdoctoral adviser to numerous researchers, including the author of this book. His research involves a wide range of phenomena related to biological timing in various life forms.[141–143]

Many researchers in addition to those shown in Table 1.2 specialize in circadian physiology. Table 1.3 lists 425 researchers who authored four or more articles in the field during a recent 2-year interval. The list includes three of five scientists awarded membership in the National

FIGURE 1.30 Urs E. Albrecht (1962–). This Swiss biochemist specializes in the molecular neurobiology of circadian rhythms in vertebrates. (Source: Photograph courtesy of Urs Albrecht.)

FIGURE 1.31 Serge Daan (1940–). This Dutch physiologist specializes in the neurobiology and ecology of circadian rhythms in animals. (Source: Photograph courtesy of Serge Daan.)

Academy of Sciences of the United States in 2003 for research related to circadian rhythms: Jeffrey Hall (Brandeis University), Michael Rosbash (also from Brandeis University), and Joseph Takahashi (Northwestern University). The other two inductees were Anthony Cashmore (University of Pennsylvania) and Woodland Hastings (Harvard University). All of the researchers listed in Table 1.3 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.

To provide more detailed information about major research centers in circadian physiology, I conducted a PubMed search for all articles retrieved by the term *circadian* published during a recent 5-year interval in the journals *Science* and *Nature*. I restricted the search to these

FIGURE 1.32 Ken-ichi Honma (1946–). This Japanese medical physiologist specializes in the neurobiology of circadian rhythms in vertebrates. (Source: Photograph courtesy of Ken-ichi Honma.)

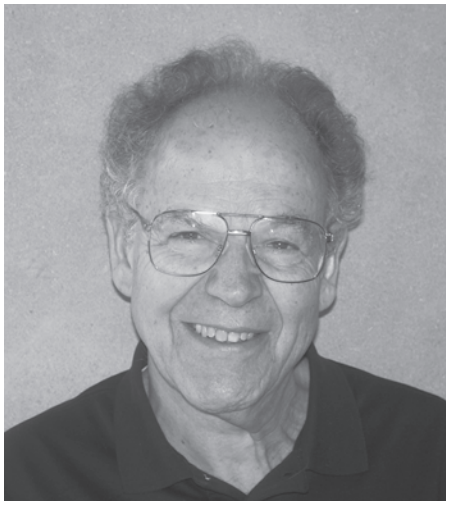


FIGURE 1.33 Michael Menaker (1934–). This American biologist specializes in the neurobiology of circadian rhythms in vertebrates. (Source: Photograph courtesy of Rebecca Arrington.)

two journals because they are the two most prestigious journals in biological research. Although many institutions from around the world were represented in the articles retrieved, only seven institutions appeared in four or more articles in the 5-year window. These seven institutions are located in the United States, as indicated in Figure 1.34. The figure shows the names and locations of the institutions and indicates their areas of greater expertise in terms of experimental subjects (plants, invertebrates, vertebrates, or humans). Institutions with excellent performance that ranked below my arbitrary selection criterion included the Johns Hopkins University School of Medicine and Northwestern University in the United States,

Imperial College London in the United Kingdom, and Kobe University School of Medicine in Japan. My own institution, a satellite campus of the University of South Carolina (Figure 1.35), does not have a major research center in circadian physiology and cannot host postdoctoral or sabbatical visitors. However, my laboratory is quite active, and I welcome inquiries about potential collaborations with researchers from other institutions.

1.4 ETHICS OF ANIMAL RESEARCH

Much research in circadian physiology is conducted with nonhuman animal subjects. Although a small fraction of this research is directed at improvements in veterinary care, the major goal is to improve *human* health. *Vivisection*, or experimentation with living organisms, is performed in animals for the benefit of humankind. Simply put, some research procedures are too harmful to conduct on human subjects; so, we use animals instead (see Figure 1.36). Of course, we also use animals (and plants and fungi, for that matter) because they provide the opportunity to study 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.

If we use animals as experimental subjects in biomedical research because we think it is inhumane to use humans, we may wonder whether the use of animals is also inhumane. The manifesto of the modern antivivisection movement was Peter Singer's 1975 book, *Animal Liberation*.[144] Although Singer 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.[145–148] Editors of biomedical journals felt the strength of the movement and wrote editorials about it.[149–152] Embarrassed by their depiction as animal torturers by the activists, biomedical researchers overreacted by imposing on themselves strict rules for the use of animals in research.[153–156] On one hand, this course of affairs was positive because it showed that researchers were willing to compromise and also because it improved the quality of biomedical research by forcing scientists with sloppy animal maintenance habits to shape up. On the other hand, it reinforced the misconception that antivivisectionism is a philosophy that merely opposes the mistreatment of research animals. Once the question of mistreatment was 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.[157–162] 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.[163] His main argument was that there is no logical reason to attribute moral rights to humans and not to animals.[164] 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.[157–162] 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.[165]

To address this issue, we must start 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 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 (55%) consists of rats and mice — two rodent species considered pests in most of the world.

In the United States, an estimated 20 million animals are used in biomedical research each year.[166] 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. Have scientists gone mad? No, they have not. Surprising as it may be to some readers, 20 million animals are not too many animals in the larger scheme of life. As shown in Figure 1.38, this number is lower than the number of cats and dogs euthanized in animal shelters each year and is dwarfed by the number of chickens killed each year to feed us.

Although some people would like to think that biomedical research is a major form of animal exploitation, a little reflection about the real world may show otherwise. Let us start with pets. We certainly love our pets and do not wish them any harm. 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. Many people 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 a work 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

TABLE 1.3
Contemporary Circadian Physiologists[a]

Abraham, U.
Achermann, P.
Aguilar-Roblero, R.
Ahmad, A.M.
Aida, K.
Akerstedt, T.
Akiyama, M.
Albers, H.E.
Albrecht, U.
Albus, H.
Allada, R.
Allen, C.N.
Alonso, I.
Amir, S.
Ancoli-Israel, S.
Andersson, H.
Antle, M.C.
Araki, T.
Arendt, J.
Avivi, A.
Ayala, D.E.
Azama, T.
Beaule, C.
Beersma, D.G.
Bellingham, J.
Bell-Pedersen, D.
Berezinska, M.
Berger, M.
Bertolucci, C.
Biello, S.M.
Bliwise, D.L.
Block, G.D.
Boari, B.
Boivin, D.B.
Borbely, A.A.
Bradley, T.D.
Brainard, G.C.
Brandstatter, R.
Bruguerolle, B.
Buganov, A.A.
Buijs, R.M.
Bult-Ito, A.
Burgess, H.J.
Butcher, G.Q.
Buysse, D.J.
Cahill, G.M.
Cajochen, C.
Calvo, C.
Cambras, T.
Cano, P.
Caola, G.
Cardinali, D.P.
Cassone, V.M.
Cermakian, N.
Challet, E.
Cheng, P.
Claustrat, B.
Coen, C.W.
Colwell, C.S.
Coon, S.L.
Cornelissen, G.
Cortelli, P.
Costa, R.
Covelo, M.
Covic, A.
Cugini, P.
Cutler, D.J.
Czeisler, C.A.
Daan, S.
Dardente, H.
Davenne, D.
Davidson, A.J.
Davis, S.J.
Dawson, D.
De la Iglesia, H.O.
De Rosa, R.
Deboer, T.
Delaunay, F.
Diez-Noguera, A.
Dijk, D.J.
Dinges, D.F.
Djurhuus, J.C.
Dominguez, M.J.
Dryer, S.E.
Dubocovich, M.L.
Dunlap, J.C.
Earnest, D.J.
Eastman, C.I.
Ebihara, S.
Elliott, J.A.
Ellison, M.C.
Esposito, K.
Esquifino, A.I.
Evans, J.A.
Fahrenkrug, J.
Fernandez, J.R.
Ferreyra, G.A.
Filipski, E.
Fliers, E.
Foa, A.
Focan, C.
Forslund, A.
Forslund, J.
Foster, R.G.
Foulkes, N.S.
Franken, P.
Fraser, W.D.
Frolich, M.
Fujimoto, K.
Fukada, Y.
Fukuhara, C.
Fukunaga, K.
Garidou, M.L.
Gauer, F.
Gauthier, A.
Giebultowicz, J.M.
Gillette, M.U.
Giugliano, D.
Glass, J.D.
Glossop, N.R.
Goldbeter, A.
Golden, S.S.
Goldsmith, D.J.
Golombek, D.A.
Gorman, M.R.
Granda, T.G.
Green, C.B.
Gualdiero, P.
Halberg, F.
Hall, J.C.
Hamada, T.
Hannibal, J.
Hardin, P.E.
Harmar, A.J.
Hastings, M.H.
Hayashi, M.
He, Q.
Helfrich-Forster, C.
Hendricks, J.C.
Hermida, R.C.
Herzog, E.D.
Higuchi, S.
Hirshkowitz, M.
Hofman, M.A.
Hogenesch, J.B.
Holmback, U.
Honma, K.
Honma, S.
Hori, T.
Horikawa, K.
Hozawa, A.
Iglesias, M.
Iigo, M.
Ikeda, M.
Illnerova, H.
Imai, Y.
Ingram, C.D.
Inoue, Y.
Inouye, S.T.
Ishida, N.
Isojima, Y.
Ito, S.
Itri, J.
Iuvone, P.M.
Iwanaga, H.
Iwasaki, H.
James, F.O.
Johnson, C.H.
Kalsbeek, A.
Kamada, H.
Kangawa, K.
Kario, K.
Katinas, G.
Kato, T.
Kato, Y.
Kawamoto, T.
Kay, S.A.
Kennaway, D.J.
Kikuya, M.
King, V.M.
Kirschbaum, C.
Klein, D.C.
Klosen, P.
Kobayashi, E.
Kobayashi, H.
Kojima, M.
Kondo, T.
Korf, H.W.
Koyanagi, S.
Kozak, M.
Kozma-Bognar, L.
Kraft, M.
Krauchi, K.
Kriegsfeld, L.J.
Kripke, D.F.
Krueger, J.M.
Kubo, K.
Kyriacou, C.P.
Larue, J.
Laudet, V.
Laudon, M.
Lee, C.
Leloup, J.C.
Lemmer, B.
Lennernas, M.
LeSauter, J.
Levi, F.
Levine, J.D.
Li, X.
Lin, C.
Liu, J.
Liu, J.H.

(continued)

TABLE 1.3 (CONTINUED)
Contemporary Circadian Physiologists

Liu, R.Y.
Liu, Y.
LiWang, A.C.
Lonnqvist, J.
Lopez, J.E.
Loros, J.J.
Lowden, A.
Lucas, R.J.
Malan, A.
Mancia, G.
Manfredini, R.
Mangel, S.C.
Mantzoros, C.S.
Marfella, R.
Martin, R.J.
Masana, M.I.
Masson-Pevet, M.
Matsubara, M.
Matsushika, A.
Maywood, E.S.
McClung, C.R.
McMahon, D.G.
Meijer, J.H.
Menaker, M.
Menet, J.S.
Merrow, M.
Michael, T.P.
Michel, S.
Michimata, M.
Mignot, E.
Millar, A.J.
Mistlberger, R.E.
Mitchell, J.W.
Mittag, M.
Miyazaki, K.
Mizuno, T.
Mizusawa, K.
Mojon, A.
Monden, M.
Monk, T.H.
Morin, L.P.
Morita, Y.
Mormont, M.C.
Morse, D.
Mortola, J.P.
Mrosovsky, N.
Nagai, K.
Nagano, M.
Nagy, F.
Nakahama, K.
Nakamichi, N.
Nevo, E.
Nishino, S.
Noshiro, M.
Novak, C.M.
Nowak, J.Z.
Obrietan, K.
Ogilvie, M.D.
Ohdo, S.
Ohkawa, S.
Oishi, K.
Okabayashi, N.
Okamura, H.
Okano, T.
Okumura, N.
Oster, H.
Otsuka, K.
Pack, A.I.
Panda, S.
Parati, G.
Partonen, T.
Perfetto, F.
Pévet, P.
Piccione, G.
Pickard, G.E.
Pickering, T.G.
Piggins, H.D.
Pijl, H.
Poirel, V.J.
Porkka-Heiskanen, T.
Potten, C.S.
Prolo, P.
Provencio, I.
Pyza, E.
Ralph, M.R.
Redon, J.
Refinetti, R.
Reilly, T.
Reiter, R.J.
Reppert, S.M.
Ribelayga, C.
Riemann, D.
Rivkees, S.A.
Roelfsema, F.
Roenneberg, T.
Rogers, N.L.
Romijn, J.A.
Rosato, E.
Rosbash, M.
Rouyer, F.
Ruby, N.F.
Rye, D.B.
Saboureau, M.
Sakamoto, K.
Salamatina, L.V.
Salome, P.A.
Sancar, A.
Sassone-Corsi, P.
Sato, S.
Sauman, I.
Scheer, F.A.
Schibler, U.
Schwartz, W.J.
Schwartzkopff, O.
Sehgal, A.
Sei, H.
Selmaoui, B.
Sesboue, B.
Shapiro, C.M.
Sharma, V.K.
Shen, S.
Sher, L.
Shibata, S.
Shigeyoshi, Y.
Shimada, K.
Shimizu, T.
Silver, R.
Simonneaux, V.
Singh, R.K.
Skene, D.J.
Sladek, M.
Smale, L.
Sothern, R.B.
Staiger, D.
Stanewsky, R.
Stehle, J.H.
Stein, P.K.
Stenberg, D.
Stephan, F.K.
Straume, M.
Sumova, A.
Sutherland, E.R.
Suzuki, H.
Suzuki, M.
Suzuki, T.
Swaab, D.F.
Tabata, M.
Tabata, S.
Takahashi, J.S.
Takahashi, K.
Takahashi, T.
Tan, Y.
Tanaka, K.
Tarquini, R.
Tei, H.
Thompson, C.L.
Tobler, I.
Todo, T.
Tokura, H.
Tomioka, K.
Tosini, G.
Touitou, Y.
Trinder, J.
Tufik, S.
Turek, F.W.
Uchiyama, M.
Ueda, H.R.
Vakonakis, I.
Van der Horst, G.T.
Van Gelder, R.N.
Van Reeth, O.
Van Someren, E.J.
Vivien-Roels, B.
Voderholzer, U.
Vollrath, L.
Von Gall, C
Vora, J.P.
Wang, L.
Wang, Y.
Wang, Z.
Wang, Z.R.
Warman, G.R.
Watanabe, M.
Watanabe, T.
Watanabe, Y.
Waterhouse, J.
Weaver, D.R.
Weiner, W.W.
Weinreb, R.N.
Weller, J.L.
White, W.B.
Whitmore, D.
Wiechmann, A.F.
Wirz-Justice, A.
Wisor, J.P.
Witte, K.
Wright, K.P., Jr.
Yagita, K.
Yamadera, H.
Yamaguchi, S.
Yamamoto, Y.
Yamashino, T.
Yamazaki, S.
Yan, L.
Yang, Y.
Yano, M.
Yasuo, S.
Yoshimura, T.
Young, M.W.
Youngstedt, S.D.
Zawilska, J.B.
Zheng, X
Zhou, JN

[a] This list of 425 researchers includes all authors with four or more publications listed in the PubMed database (U.S. National Library of Medicine, Washington, DC) containing the word *circadian* in any searchable field (title, abstract, or key words) in the 2-year interval between June 2002 and June 2004.

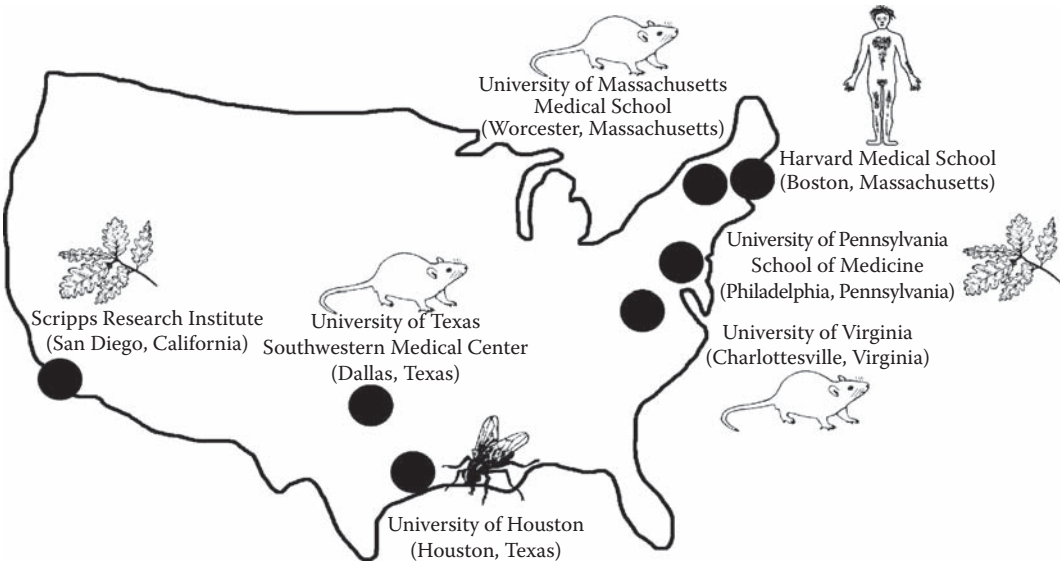


FIGURE 1.34 The most prestigious research institutions in circadian physiology. The seven most prestigious institutions, as determined by the number of publications in the elite journals *Science* and *Nature*, are all located in the United States. The most commonly used experimental subjects (plants, invertebrates, vertebrates, or humans) in each institution are indicated. (Source: PubMed database (U.S. National Library of Medicine) searched by R. Refinetti in October 2003. The search targeted all articles published in the journals *Science* and *Nature* with the word *circadian* in any searchable field during the 5-year interval between October 1998 and September 2003. Although many articles have authors from multiple institutions, PubMed lists only the institution of the senior author of each article.)

FIGURE 1.35 Carolina in my mind. The satellite campus of the University of South Carolina in the small town of Walterboro is home to the author's Circadian Rhythm Laboratory. (Source: Photograph by R. Refinetti.)

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) and, for all projects that receive federal funding, research methods must conform to detailed guidelines set by the Public Health Service. Every research project must be preapproved by an ethics committee.[167–169] The Institutional Animal Care and Use Committees (IACUC)[170] 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.

Biomedical researchers are 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,[171] 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.[172] 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.[173] Whether we realize it or not, we accept the sacrifice of a small group for the common good, even when it is a small group of humans.

You may feel that Heffner's analogy is faulty because people share equally the benefits and the costs of driving a car, whereas only nonhumans pay the price of research

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. (Source: © 1984 by Brant Parker and Johnny Hart. Reproduced by permission of John L. Hart FLP and Creators Syndicate Inc.)

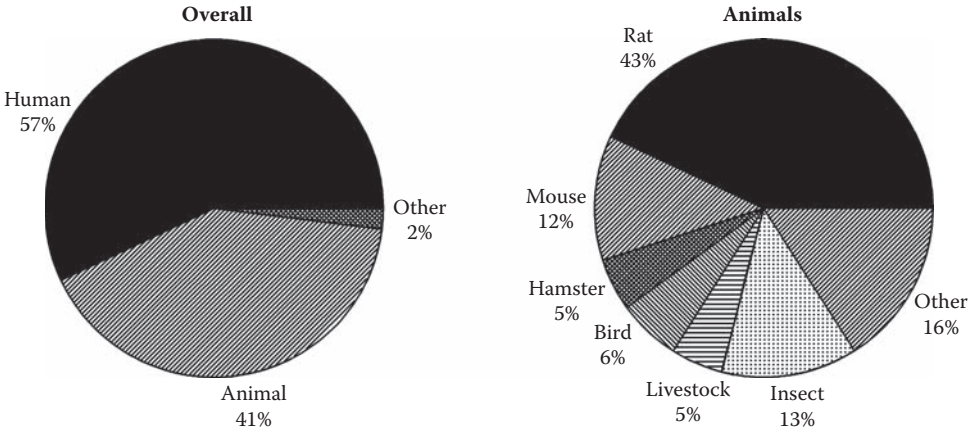


FIGURE 1.37 Proportions of experimental subjects in studies of circadian rhythms. More than half of all studies dealing with circadian rhythms are conducted on human subjects. More than half of the studies conducted in animals involve rats and mice. (Source: PubMed database searched by R. Refinetti in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

to benefit human health. This is not true, however. 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.[173] (Chapter 16 identifies heart disease, cancer, and other illnesses as the leading cause of death of older adults.) Still, you might argue that every person who does not die young benefits from motor vehicles throughout his or her life, whereas a rat never becomes a human being and never benefits from advances in human medicine. However, of the 42,401 deaths due to motor-vehicle accidents in the United States in 1999, 28,552 involved male victims.[173] 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.

You may argue that only people who choose to get into an automobile share the risk of dying in an accident, while 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 decline to zero deaths at young ages. In 1999 alone, 834 children under 5 years of age died in motor-vehicle accidents in the United States.[173] These children did not choose to get into an automobile. Embarrassing as these figures may be, they clearly show that the decision to sacrifice a number of animals to improve the life conditions of a larger number of humans is a moral decision at least equivalent to other moral decisions we make daily. We may not be comfortable with some of the ethical decisions 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.[174]

Some readers may take my arguments backwards, decide to become vegetarians, and refuse medical treatment for serious diseases (because the treatment was developed through biomedical research in animals). I remind them that the kingdom Animalia is only a small fraction of the diversity of life on Earth. They 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,

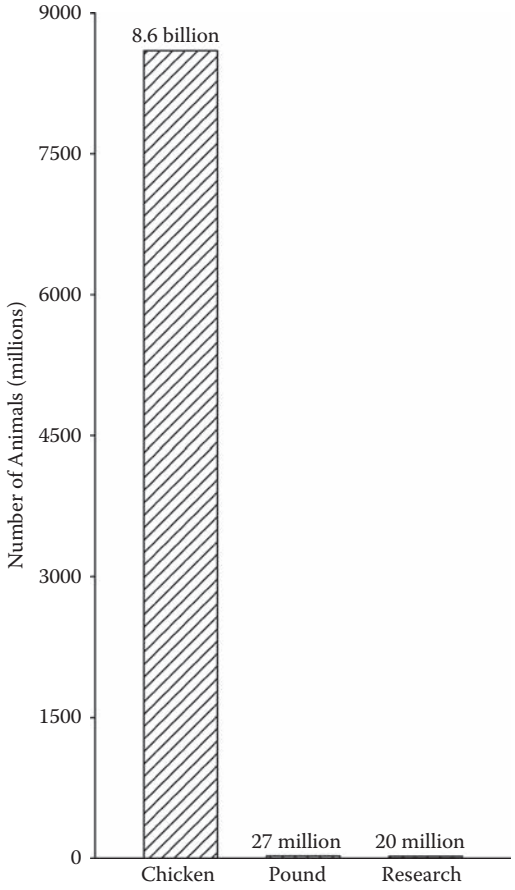


FIGURE 1.38 Comparative figures of animal use by humans. The figure shows the approximate number of animals killed each year in the United States in three sectors: chicken (used as food), pound (cats and dogs euthanized in animal shelters), and research (mostly rats and mice used in biomedical research). (Sources: *Poultry Production and Value — 2002 Summary.* (2003). Washington, DC: National Agricultural Statistics Service; Nicoll, C.S. (1991). A physiologist's views on the animal rights/liberation movement. *The Physiologist* 34(6): 303–315.)

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 20th century, thanks especially to the research and tactical efforts of Jürgen Aschoff (1913–1998), Franz Halberg (1919–), 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.

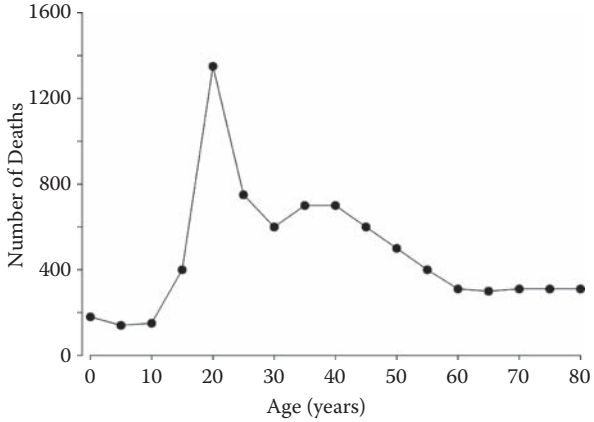


FIGURE 1.39 Deaths due to motor-vehicle accidents in the United States. The figure shows the actual number of human deaths resulting from motor-vehicle accidents in the year 1999 for various ages (ages between multiples of 5 are not shown). (Source: U.S. National Safety Council. (2002). *Injury Facts:*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

EXERCISES

Exercise 1.1 Pronunciation of researchers' names

If you have not installed yet the software package that accompanies this book, this is a good time to do it. Follow the instructions in the Software Installation section. Once the package is installed, double-click on the Circadian icon to open the program banner. Then select the SayIt program (the second icon from the right, just to the left of the music icon). This program provides the pronunciation of the names of the various circadian physiologists introduced in Chapter 1. Click on the down-arrow in the top drop-down menu (People), then choose the name that you want to hear. Repeat the procedure for each name you want to hear. The pronunciations are guided by the rules of American English and by peculiarities of international usage. *Note:* The second and third drop-down menus contain terms introduced in the next chapter.

Exercise 1.2 Daily leaf movement of bean plant

In Section 1.1, you learned 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 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 filled with soil. (Thin plastic cups will not work because they turn over when the plants grow.) 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 they are close to a window so that they get light during the day but not at night. Grow the plants until the pair of leaves above the cotyledons is almost fully expanded. (This will take 1 to 3 weeks, depending on ambient temperature and day length.) At this point, you will also need a protractor, a plastic or cardboard semicircular instrument used for measuring angles. You can buy one at any school-supply store. To start the observations, choose the best plant, then 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, to avoid disturbing the light–dark cycle.) When you have recorded measurements for at least 3 days, 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.

Exercise 1.3 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. 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). Try to record your temperature every hour for 2 or more consecutive days. Occasionally, you may also use an alarm clock to wake you up in the middle of the night for nocturnal measurements. (Don't do this too often; otherwise, you may disturb the body's clock.) When you have recorded measurements for at least 2 days, draw a graphic showing temperature (Y axis) as a function of time (X axis). You should be able to observe a clear daily rhythm.

SUGGESTIONS FOR FURTHER READING

No single book is dedicated specifically to the history of circadian physiology. However, information about major developments in the 20th century can be obtained from books written by researchers who were active in the field during that time. These books include:

Bünning, E. (1973). ***The Physiological Clock*** **(3rd Edition). New York: Springer.** First published in German in 1958 (*Die Physiologische Uhr*), this scholarly book was probably the earliest to summarize a large body of research on biological rhythms from a variety of investigators. This 3rd 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, F. A., Jr., 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 oriented more toward nonspecialists.

Brady, J. (1979). ***Biological Clocks.*** **Baltimore, MD: University Park Press.** The first book explicitly written as 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, easy-to-read 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.

WEB SITES TO EXPLORE

European Society for Chronobiology:
http://www.far.ub.es/~crono/

Japanese Society for Chronobiology:
http://wwwsoc.nii.ac.jp/jsc/index-e.html

NIH Office of Laboratory Animal Welfare:
http://grants.nih.gov/grants/olaw/olaw.htm

Society for Light Treatment and Biological Rhythms:
http://www.sltbr.org

Society for Research on Biological Rhythms (USA):
http://www.srbr.org

LITERATURE CITED

1. Jobe, P. C., Adams-Curtis, L. E., Burks, T. F., Fuller, R. W., Peck, C. C., Ruffolo, R. R., Snead, O. C., & 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. & Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency*, 2nd Edition. Mineola, NY: Dover.
3. Burns, E. M. (1973). *Western Civilizations: Their History and Their Culture*, 8th Edition. New York: Norton.
4. Audoin, C. & Guinot, B. (2001). *The Measurement of Time* (Trans. by S. Lyle). Cambridge, UK: Cambridge University Press.
5. Aschoff, J. (1974). Speech after dinner. *Chronobiologia* 1(S): 483–493.
6. Mulroy, D. (1992). *Early Greek Lyric Poetry*. Ann Arbor, MI: University of Michigan Press.
7. Davenport, G. (1964). *The Fragments of Archilocos*. Berkeley, CA: University of California Press.
8. Bretzl, H. (1903). *Botanische Forschungen des Alexanderzuges*. Leipzig: B. G. Teubner.
9. Virey, J. J. (1819). Périodicité: recherches sur les causes des mouvemens périodiques dans l'économie animale. In: *Dictionaire des Sciences Médicales, Tome 40*. Paris: C. L. F. Panckoucke, pp. 419–429.
10. Bünning, E. (1964). *The Physiological Clock: Endogenous Diurnal Rhythms and Biological Chronometry*. New York: Academic Press.
11. Dunlap, J. C., Loros, J. J. & DeCoursey, P. J. (2004). *Chronobiology: Biological Timekeeping*. Sunderland, MA: Sinauer.
12. Foster, R. G. & Kreitzman, L. (2004). *Rhythms of Life: The Biological Clocks that Control the Daily Lives of Every Living Thing*. London: Profile.
13. Mairan, J. J. D. de (1729). Observation botanique. In: *Histoire de l'Académie Royale des Sciences, Année 1729*. Paris: Imprimerie Royale (1731), pp. 35.
14. 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.
15. Hufeland, C. W. (1797). *Die Kunst das Menschliche Leben zu Verlängern*. Jena, Germany: Akademische Buchhandlung.
16. 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: Didot Jeune.
17. Reinberg, A. E., Lewy, H., & Smolensky, M. (2001). The birth of chronobiology: Julien Joseph Virey 1814. *Chronobiology International* 18: 173–186.
18. Candolle, A. P. de (1832). *Physiologie Végétale*. Paris: Béchet.
19. Wernli, H. J. (1960). *Biorhythm: A Scientific Exploration into the Life Cycles of the Individual*. New York: Crown.
20. Smith, R. E. (1976). *The Complete Book of Biorhythm Life Cycles*. New York: Aardvark.
21. Crawley, J. (1996). *The Biorhythm Book*. Boston: Journey.
22. Gittelson, B. (1996). *Biorhythm: A Personal Science*. New York: Warner.
23. Elowitz, M. B., Levine, A. J., Siggia, E. D., & Swain, P. S. (2002). Stochastic gene expression in a single cell. *Science* 297: 1183–1186.
24. Goldberger, A. L., Rigney, D. R., & West, B. J. (1990). Chaos and fractals in human physiology. *Scientific American* 262(2): 42–49.
25. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
26. Ogle, W. (1866). On the diurnal variations in the temperature of the human body in health. *St. George's Hospital Reports* 1: 221–245.
27. 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.
28. 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.
29. Chossat, C. (1843). Recherches expérimentales sur l'inanition. *Annales des Sciences Naturelles, Série 2* 20: 293–326.
30. 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.
31. Kiesel, A. (1894). Untersuchungen zur Physiologie des facettirten Auges. *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften (Abtheilung 3)* 103: 97–139.
32. Hobday, F. (1896). Notes on physiological temperatures. *Journal of Comparative Pathology and Therapeutics* 9: 286–314.
33. Simpson, S. & 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.

34. 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.
35. Gates, A. I. (1916). Diurnal variations in memory and association. *University of California Publications in Psychology* 1: 323–344.
36. Johnson, M. S. (1926). Activity and distribution of certain wild mice in relation to biotic communities. *Journal of Mammalogy* 7: 245–277.
37. Rogers, L. A. & Greenbank, G. R. (1930). The intermittent growth of bacterial cultures. *Journal of Bacteriology* 19: 181–190.
38. Welsh, J. H. (1938). Diurnal rhythms. *Quarterly Review of Biology* 13: 123–139.
39. Jores, A. (1936). Rhythmikphysiologie und -pathologie des Menschen. *Naturwissenschaften* 24: 408–412.
40. Bünning, E. (1936). Die endonome Tagesrhythmik als Grundlage der photoperiodischen Reaktion. *Berichte der Deutchen Botanischen Gesellschaft* 54: 590–607.
41. Bünning, E. (1958). *Die Physiologische Uhr*. Berlin: Springer.
42. Bünning, E. (1973). *The Physiological Clock: Circadian Rhythms and Biological Chronometry*, 3rd Edition. New York: Springer.
43. Richter, C. P. (1965). *Biological Clocks in Medicine and Psychiatry.* Springfield, IL: Charles C Thomas.
44. Kleitman, N. (1963). *Sleep and Wakefulness*. Chicago: University of Chicago Press.
45. Aschoff, J. (1990). From temperature regulation to rhythm research. *Chronobiology International* 7: 179–186.
46. Aschoff, J., Gerecke, U., & Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
47. Aschoff, J. & Wever, R. (1976). Human circadian rhythms: a multioscillatory system. *Federation Proceedings* 35: 2326–2332.
48. Aschoff, J. (1966). Circadian activity pattern with two peaks. *Ecology* 47: 657–662.
49. Aschoff, J. (1979). Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
50. 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.
51. Daan, S. & Gwinner, E. (1998). Obituary: Jürgen Aschoff (1913–98). *Nature* 396: 418.
52. 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.
53. Halberg, F. (1969). Chronobiology. *Annual Review of Physiology* 31: 675–725.
54. Ahlgren, A. & Halberg, F. (1990). *Cycles of Nature: An Introduction to Biological Rhythms*. Washington, DC: National Science Teachers Association.
55. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K., & Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *Journal-Lancet* 71: 312–319.
56. Kumagai, Y., Shiga, T., Sunaga, K., Cornélissen, G., Ebihara, A. & Halberg, F. (1992). Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 19: 43–58.
57. Halberg, F., Cornélissen, G., Watanabe, Y., Otsuka, K., Fiser, B., Siegelova, J., Mazankova, V., Maggioni, C., Sothern, R. B., Katinas, G. S., Syutkina, E. V., Burioka, N., & Schwartzkopff, O. (2001). Near 10-year and longer periods modulate circadians: intersecting antiaging and chronoastrobiological research. *Journal of Gerontology* 56A: M304–M324.
58. Halberg, F., Zander, H. A., Houglum, M. W., & Mühlemann, H. R. (1954). Daily variations in tissue mitoses, blood eosinophils and rectal temperatures of rats. *American Journal of Physiology* 177: 361–366.
59. Halberg, F. & 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.
60. Nelson, W., Scheving, L., & Halberg, F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *Journal of Nutrition* 105: 171–184.
61. Powell, E. W., Halberg, F., Pasley, J. N., Lubanovic, W., Ernsberger, P., & Scheving, L. E. (1980). Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *Journal of Thermal Biology* 5: 189–196.
62. Pittendrigh, C. S. (1993). Temporal organization: reflections of a Darwinian clock-watcher. *Annual Review of Physiology* 55: 17–54.
63. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences USA* 40: 1018–1029.
64. Pittendrigh, C. S. (1966). The circadian oscillation in *Drosophila psudoobscura* pupae: a model for the photoperiod clock. *Zeitschrift für Pflanzenphysiologie* 54: 275–307.
65. Pittendrigh, C. S. & 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.
66. Daan, S. & 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.
67. Daan, S. & 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.
68. Pittendrigh, C. S. & Daan, S. (1976). A functional analysis of circadian pacemakers in nocturnal rodents: IV. Entrainment: pacemaker as clock. *Journal of Comparative Physiology* 106: 291–331.

69. Menaker, M. (1996). Colin S. Pittendrigh (1918–96). *Nature* 381: 24.
70. Halberg, F., Cornélissen, G., Katinas, G., Syutkina, E.V., Sothern, R.B., Zaslavskaya, R., Halberg, F., Watanabe, Y., Schwartzkopff, O., Otsuka, K., Tarquini, R., Frederico, P., & Siggelova, J. (2003). Transdisciplinary unifying implications of circadian findings in the 1950s. *Journal of Circadian Rhythms* 1: art. 2.
71. Cornélissen, G., Halberg, F., Sánchez-ed-la-Peña, S., Jinyi, W., & Carandente, F. (1988). The need for both macroscopy and microscopy in dealing with spectral structure. *Chronobiologia* 15: 323–327.
72. Turek, F. W. (1988). Do circadian biologists and chronopharmacologists talk the same "language"? *Annual Review of Chronopharmacology* 4: 205–208.
73. Cambrosio, A. & Keating, P. (1983). The disciplinary stake: the case of chronobiology. *Social Studies of Science* 13: 323–353.
74. Rimsky-Korsakov, N. (1964). *Principles of Orchestration* (Trans. by E. Agate). New York: Dover.
75. Bünning, E. (Org.) (1960). *Cold Spring Harbor Symposia on Quantitative Biology, Volume 25: Biological Clocks*. Cold Spring Harbor, NY: The Biological Laboratory.
76. Honma, K. & Shibata, S. (2003). Announcement: First World Congress of Chronobiology. *Chronobiology International* 20: 739.
77. Nintcheu-Fata, S., Katinas, G., Halberg, F., Cornélissen, G., Tolstykh, V., Michael, H. N., Otsuka, K., Schwartzkopff, O., & Bakken, E. (2003). Chronomics of tree rings for chronoastrobiology and beyond. *Biomedicine and Pharmacotherapy* 57: 24s–30s.
78. Reich, W. (1961). *The Function of the Orgasm*. New York: Noonday.
79. Hrushesky, W. J. (1985). Circadian timing of cancer chemotherapy. *Science* 228: 73–75.
80. Sothern, R. B., Rhame, F., Suarez, C., Fletcher, C., Sackett-Lundeen, L., Haus, E., & Hrushesky, W. J. M. (1990). Oral temperature rhythmometry and substantial within-day variation in zidovudine levels following steady-state dosing in human immunodeficiency virus (HIV) infection. *Progress in Clinical and Biological Research* 351A: 67–76.
81. Tzannis, S. T., Hrushesky, W. J., Wood, P. A., & Przybycien, T. M. (1996). Irreversible inactivation of interleukin 2 in a pump-based delivery environment. *Proceedings of the National Academy of Sciences USA* 93: 5460–5465.
82. Bjarnason, G. A., Jordan, R. C. K., Wood, P. A., Li, Q., Lincoln, D. W., Sothern, R. B., Hrushesky, W. J. M., & Ben-David, Y. (2001). Circadian expression of clock genes in human oral mucosa and skin. *American Journal of Pathology* 158: 1793–1801.
83. Takahashi, J. S. & Menaker, M. (1980). Interaction of estradiol and progesterone: effects on circadian locomotor rhythm of female golden hamsters. *American Journal of Physiology* 239: R497–R504.
84. 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., & Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264: 719–725.
85. Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamazaki, S., Zemenides, P. D., Ralph, M. R., Menaker, M., & Takahashi, J. S. (2000). Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* 288: 483–491.
86. Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M., Hong, H. K., Oh, W. J., Yoo, O. J., Menaker, M., & Takahashi, J. S. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences USA* 101: 5339–5346.
87. Price, D. J. S. (1986). *Little Science, Big Science and Beyond*. New York: Columbia University Press.
88. Snow, B. & Ifshin, S. L. (1984). Online database coverage of forensic medicine. *Online* 8(2): 37–43.
89. McCain, K. W., White, H. D., & Griffith, B. C. (1987). Comparing retrieval performance in online data bases. *Information Processing and Management* 23: 539–553.
90. Barber, J., Moffat, S., & Wood, F. (1988). Case studies of the indexing and retrieval of pharmacology papers. *Information Processing and Management* 24: 141–150.
91. Refinetti, R. (1990). Retrieval performance in online search in thermal physiology. *Computers and Biomedical Research* 23: 32–36.
92. Garfield, E. (1996). The significant scientific literature appears in a small core of journals. *Scientist* 10(17): 13–14.
93. Refinetti, R. (1990). In defense of the least publishable unit. *FASEB Journal* 4: 128–129.
94. Refinetti, R. (1989). Information processing as a central issue in philosophy of science. *Information Processing and Management* 25: 583–584.
95. Refinetti, R. (1999). Keeping up with the research literature through reprint requests. *Scientist* 13(12): 13.
96. Brunner, B. (Ed.) (2003). *TIME Almanac 2004*. Des Moines, IA: TIME Books.
97. Malakoff, D. (2002). War effort shapes U.S. budget, with some program casualties. *Science* 295: 952–954.
98. May, R. M. (1997). The scientific wealth of nations. *Science* 275: 793–796.
99. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U., & 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 USA* 99: 11718–11723.
100. Lincoln, G., Messager, S., Andersson, H., & 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 USA* 99: 13890–13895.

101. Barnes, J. W., Tischkau, S. A., Barnes, J. A., Mitchell, J. W., Burgoon, P. W., Hickok, J. R., & Gillette, M. U. (2003). Requirement of mammalian *timeless* for circadian rhythmicity. *Science* 302: 439–442.
102. Chen, Y. & Tan, E. C. (2004). Identification of human *Clock* gene variants by denaturing high-performance liquid chromatography. *Journal of Human Genetics* 49: 209–214.
103. Brandstätter, R., Kumar, V., Abraham, U., & 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 USA* 97: 12324–12328.
104. Hunt, A. E., Al-Ghoul, W. M., Gillette, M. U., & 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.
105. Schuhler, S., Pitrosky, B., Kirsch, R., & Pévet, P. (2002). Entrainment of locomotor activity rhythm in pinealectomized adult Syrian hamsters by daily melatonin infusion. *Behavioural Brain Research* 133: 343–350.
106. Karolczak, M., Burbach, G. J., Sties, G., Korf, H. W., & Stehle, J. H. (2004). Clock gene mRNA and protein rhythms in the pineal gland of mice. *European Journal of Neuroscience* 19: 3382–3388.
107. Berson, D. M., Dunn, F. A., & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073.
108. Ruby, N. F., Brennan, T. J., Xie, X., Cao, V., Franken, P., Heller, H. C., & O'Hara, B. F. (2002). Role of melanopsin in circadian responses to light. *Science* 298: 2211–2213.
109. Hattar, S., Lucas, R. J., Mrosovsky, N., Thompson, S., Douglas, R. H., Hankins, M. W., Lem, J., Biel, M., Hofmann, F., Foster, R. G., & Yau, K. W. (2003). Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424: 76–81.
110. Panda, S., Provencio, I., Tu, D. C., Pires, S. S., Rollag, M. D., Castrucci, A. M., Pletcher, M. T., Sato, T. K., Wiltshire, T., Andahazy, M., Kay, S. A., Van Gelder, R. N., & Hogenesch, J. B. (2003). Melanopsin is required for non-image-forming photic responses in blind mice. *Science* 301: 525–527.
111. Miyazaki, T., Hashimoto, S., Masubuchi, S., Honma, S., & Honma, K. (2001). Phase-advance shifts of human circadian pacemaker are accelerated by daytime physical exercise. *American Journal of Physiology* 281: R197–R205.
112. Buxton, O. M., Lee, C. W., L'Hermite-Balériaux, M., Turek, F. W., & 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.
113. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J., & 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.
114. Barger, L. K., Wright, K. P., Hughes, R. J., & Czeisler, C. A. (2004). Daily exercise facilitates phase delays of circadian melatonin rhythm in very dim light. *American Journal of Physiology* 286: R1077–R1084.
115. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M., & Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685.
116. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., & 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.
117. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H., & 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.
118. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H., & Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.
119. Simonton, D. K. (1988). *Scientific Genius: A Psychology of Science*. New York: Cambridge University Press.
120. Refinetti, R. (2001). Sexual correctness in Academia: the case of a professor. *Sexuality & Culture* 5(2): 91–94.
121. Refinetti, R. (2000). Cinematic depiction of conflicts of interest in romantic relationships. *Sexuality and Culture* 4(1): 61–74.
122. Dziech, B. W. & Weiner, L. (1984). *The Lecherous Professor.* Boston: Beacon.
123. Lane, A. J. (1998). "Consensual" relations in the academy: gender, power, and sexuality. *Academe* 84(5): 24–31.
124. Irvine, L. (1997). A "consensual" relationship. In: Sandler, B. R. & Shoop, R. J. (Eds.). *Sexual Harassment on Campus*. Boston: Allyn and Bacon, pp. 234–247.
125. Stamler, V. L. & Stone, G. L. (1998). *Faculty-Student Sexual Involvement: Issues and Interventions*. Thousand Oaks, CA: Sage.
126. Keller, E. A. (1988). Consensual amorous relationships between faculty and students: the constitutional right to privacy. *Journal of College and University Law* 15: 21–42.
127. Dank, B. M. & Fulda, J. S. (1997). Forbidden love: student-professor romances. *Sexuality and Culture* 1: 107–130.
128. Bellas, M. L. & Gossett, J. L. (2001). Love or the "lecherous professor": consensual sexual relationships between professors and students. *Sociological Quarterly* 42: 529–558.
129. Jafar, A. (2003). Consent or coercion? Sexual relationships between college faculty and students. *Gender Issues* 21(1): 43–58.

130. Gauer, F., Schuster, C., Poirel, V. J., Pévet, P., & 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.
131. Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., van der Vliet, J., van Heijningen, C., Simonneaux, V., Pévet, P., & Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. *European Journal of Neuroscience* 17: 221–228.
132. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J., & 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.
133. Albrecht, U., Zheng, B., Larkin, D., Sun, Z. S., & Lee, C. C. (2001). *mPer1* and *mPer2* are essential for normal resetting of the circadian clock. *Journal of Biological Rhythms* 16: 100–104.
134. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U., & 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.
135. Hut, R. A., van Oort, B. E. H., & 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.
136. Oklejewicz, M., Daan, S., & Strijkstra, A. M. (2001). Temporal organisation of hibernation in wild-type and *tau* mutant Syrian hamsters. *Journal of Comparative Physiology B* 171: 431–439.
137. Daan, S. & Oklejewicz, M. (2003). The precision of circadian clocks: assessment and analysis in Syrian hamsters. *Chronobiology International* 20: 209–221.
138. Honma, S. & Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
139. Shirakawa, T., Honma, S., Katsuno, Y., Oguchi, H., & Honma, K. (2000). Synchronization of circadian firing rhythms in cultured rat suprachiasmatic neurons. *European Journal of Neuroscience* 12: 2833–2838.
140. Honma, S., Nakamura, W., Shirakawa, T., & 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.
141. Loudon, A. S. I., Ihara, N., & 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.
142. Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y., & Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science* 291: 490–493.
143. Davidson, A. J., Poole, A. S., Yamazaki, S., & Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain and Behavior* 2: 32–39.
144. Singer, P. (1975). *Animal Liberation: A New Ethics for Our Treatment of Animals*. New York: Avon.
145. Erickson, D. (1990). Blood feud: researchers begin fighting back against animal-rights activists. *Scientific American* 262(6): 17–18.
146. Zola-Morgan, S. (1994). Animals in Research Committee monitors changing focus of activists. *Neuroscience Newsletter* 25(5): 15–16.
147. 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.
148. Samuels, W. M. (1990). Activist pleads Nolo Contendere to charge of attempted murder. *Physiologist* 33: 51.
149. Korner, P. I. (1984). Medicine and the animal liberation movement. *Medical Journal of Australia* 141: 773–775.
150. Koshland, Jr., D. E. (1989). Animal rights and animal wrongs. *Science* 243: 1253.
151. White, R. J. (1988). Animal rights versus human rights. *Surgical Neurology* 30: 410–411.
152. Conn, P. M. & Parker, J. (1998). Animal rights: reaching the public. *Science* 282: 1417.
153. Bulger, R. E. (1987). Use of animals in experimental research: a scientist's perspective. *Anatomical Record* 219: 215–220.
154. Dresser, R. (1988). Standards for animal research: looking at the middle. *Journal of Medicine and Philosophy* 13: 123–143.
155. Johnson, D. (1990). Animal rights and human lives: time for scientists to right the balance. *Psychological Science* 1: 213–214.
156. McCance, I. (1989). The number of animals. *News in Physiological Sciences* 4: 172–176.
157. Kaplan, J. (1988). The use of animals in research. *Science* 242: 839–840.
158. Nicoll, C. S. & Russell, S. M. (1991). Mozart, Alexander the Great, and the animal rights/liberation philosophy. *FASEB Journal* 5: 2888–2892.
159. Hatch, O. G. (1987). Biomedical research. *American Psychologist* 42: 591–592.
160. Maas, G. A. (1994). Public relations and animal research. *Lab Animal* 23(4): 28–31.
161. Nicoll, C. S. & Russell, S. M. (1989). Animal research vs. animal rights. *FASEB Journal* 3: 1668–1671.
162. Botting, J. H. & Morrison, A. R. (1997). Animal research is vital to medicine. *Scientific American* 276(2): 83–85.
163. Plous, S. (1991). An attitude survey of animal rights activists. *Psychological Science* 2: 194–196.
164. Singer, P. (1990). The significance of animal suffering. *Behavioral and Brain Sciences* 13: 9–12.
165. Refinetti, R. (1990). The real issue in the antivivisection controversy. *Science, Technology, and Human Values* 25: 122–123.
166. Shalev, M. (1997). Animals used in research, 1973–1995. *Lab Animal* 26(1): 14–16.

167. United States Congress (1966). Animal Welfare Act. *United States Code*, Title 7, Sections 2131–2156.
168. Animal and Plant Health Inspection Service (USDA). *Code of Federal Regulations*, Title 9, Chapter 1, Subchapter A. Riverdale, MD: United States Department of Agriculture.
169. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. Washington, DC: National Academy Press.
170. Podolsky, M. L. & Lukas, V. S. (1999). *The Care and Feeding of an IACUC*. Boca Raton, FL: CRC Press.
171. Herzog Jr., H. A. (1988). The moral status of mice. *American Psychologist* 43: 473–474.
172. Heffner, H. (2001). Animal research in the college classroom. *APS Observer* 14(3): 5, 31.
173. National Safety Council (2002). *Injury Facts 2002 Edition*. Itasca, IL: National Safety Council.
174. Sartre, J. P. (1948). *Existentialism and Humanism* (Trans. by P. Mairet). London: Methuen.

2 Research Methods in Circadian Physiology

CHAPTER OUTLINE

2.1 The Scientific Method
2.2 Research on Populations and Organisms
2.3 Research on Organs, Cells, and Molecules
2.4 Research on the Environment

2.1 THE SCIENTIFIC METHOD

Research in circadian physiology is 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 21st century. These individuals most likely will 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 intents,[4–7] the next section examines it more closely.

2.1.1 Philosophy and Science

Since at least the late 1800s, most scientists and lay citizens have supported 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 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; and
3. A hierarchy unites the various sciences, and this hierarchy runs up from mathematics and physics to chemistry, to biochemistry, to cell biology, and to physiology.

FIGURE 2.1 Auguste Comte (1798–1857). This French philosopher created the doctrine of positivism and the discipline of sociology. (Source: Maison d'Auguste Comte, Paris, France.)

Comte had a relatively idiosyncratic view in which *sociology* (the science he created) should be at the top of the hierarchy.[8] This view was similar to that of Plato, thousands of years earlier, who 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 an influential philosophy around the world. The influence was so strong that the positivist motto ("Order and Progress") was included on a national flag (Figure 2.2).

The first of the three assumptions (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 get to know it (Panel A). Relativism 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? We could look from both

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." (Source: Pauwels, G. J. (1987). *Atlas Geográfico Melhoramentos, 50th Edition*. São Paulo: Melhoramentos.)

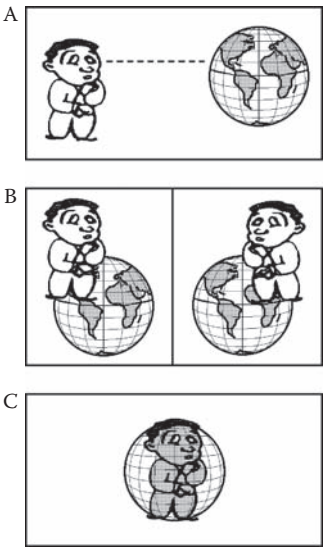


FIGURE 2.3 Three different worldviews. The drawings symbolize the three main epistemological perspectives: realism (A), relativism (B), and dialectics (C).

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

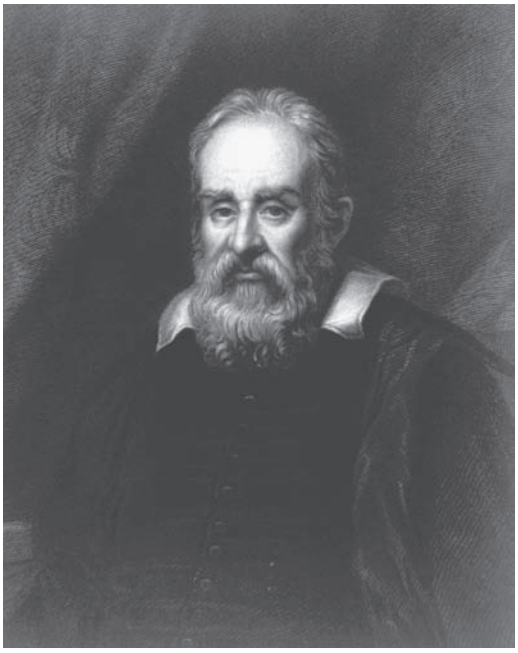


FIGURE 2.5 Galileo Galilei (1564–1642). This famous Italian astronomer and physicist endorsed epistemological realism. (Source: Library of Congress, Washington, DC.)

sides and then combine the information into a single real world. But, if two worldviews are possible, how can we be sure that there aren't more than two views? And what if there are infinite views? If infinite views exist, we cannot possibly find out what the "real" world is. We are forced to admit that the doctrine of realism is untenable!

If this reasoning is starting to sound too abstract to you, consider a simple but concrete example. 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. If you choose the white color as the background, you 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 human sensory experiences — but how can one know it, if it is beyond 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

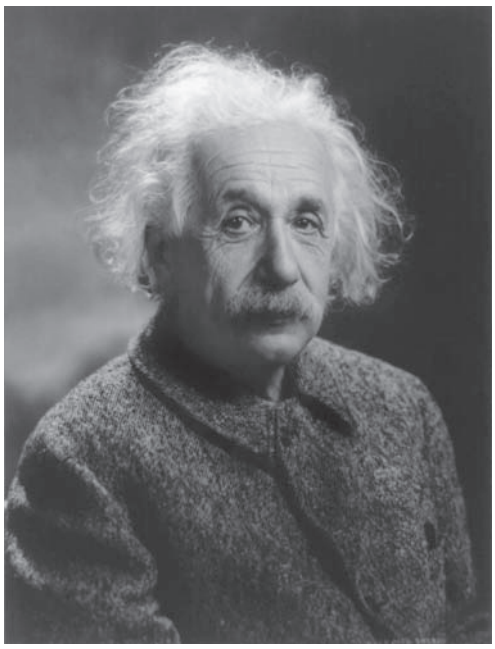


FIGURE 2.6 Albert Einstein (1879–1955). This German theoretical physicist, the most famous scientist of the 20th century, is widely known for his Theory of Relativity. Nonetheless, he was an epistemological realist. (Source: Library of Congress, Washington, DC.)

science extracts knowledge.[10] *Albert Einstein* (Figure 2.6), perhaps the best known scientist of the 20th century, was also a realist. Although he became famous for his work on *relativity*, he had no penchant for *relativism*. For example, in enunciating the "principle of special relativity," which deals with the relative movements of "inertial systems," he emphasized not that different inertial systems provide alternative worldviews, but that "the laws of nature are in concordance for all inertial systems."[11] That is, he didn't emphasize the *relative*; he emphasized the *absolute*. A panel of late-20th-century scientists assembled by the U.S. National Academy of Sciences expressed its adoption of the realist perspective in a similar fashion, making statements 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."[12]

Many philosophers, Comte among them, were also realists, but relativists are found most often among philosophers. Relativism existed as far back as 500 B.C.: Heraclitus' famous verses asserted that no man can bathe in the same river twice (because the water keeps flowing, and the river is thus never the same).[13] 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. Many relativist philosophies have been expressed over the centuries, but a major resurgence of these ideas occurred in the 1960s. One of the main characters in this philosophical revival 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

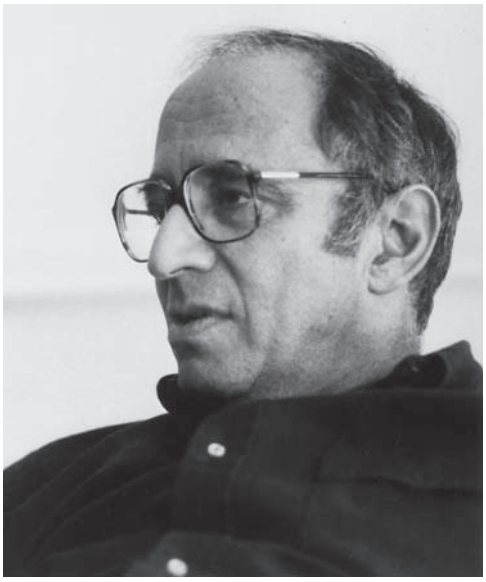


FIGURE 2.7 Thomas Kuhn (1922–1996). This American philosopher of science was influential in the resurgence of epistemological relativism in the late 20th century. (Source: MIT Museum, Massachusetts Institute of Technology, Cambridge, MA.)

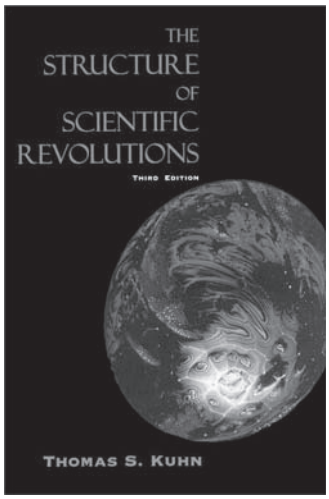


FIGURE 2.8 Cover of the 3rd edition of Kuhn's *The Structure of Scientific Revolutions* (University of Chicago Press, 1996). This book by Thomas Kuhn, which first appeared in 1962, is probably the best known philosophy of science book ever published.

paradigm that, by necessity, must eventually be discarded for scientific progress to take place.[14] 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. Because 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." He reluctantly retained the epistemological perspective that an empirical world that can be effectively known lies beyond the "incommensurability" of scientific paradigms.[14] That is, there were two Kuhns: the Kuhn who was a Kuhnian and the Kuhn who was a realist.[15] Across the Atlantic, several French philosophers of science were much bolder. 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) created the term *postmodernism,*[16] by which 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). Lyotard brought relativism to cultural values like Kuhn brought it to scientific paradigms.

Jacques Derrida (1930–2004) developed the notion of *deconstruction*,[17] 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.[18,19] 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,[20] even though Piaget felt that Foucault missed the main point of structuralism.[18] Foucault's connection with postmodernism derives from his

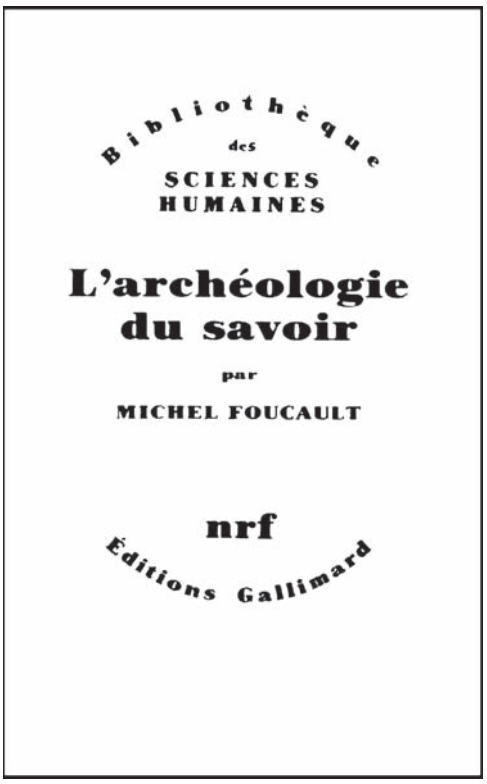


FIGURE 2.9 Cover of the original edition of Michel Foucault's *The Archeology of Knowledge* (Éditions Gallimard, 1969). This book by French philosopher Michel Foucault delineated the method that leads to relativism through structuralism.

identification of structures called *epistemes*[21] that resemble Kuhn's paradigms and Lyotard's "language games." One episteme succeeds another, but there is no actual progress.

When American authors in "science studies" fields such as 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 provided by Kuhn's writings as well as the work of newcomer Austrian psychologist *Ernst von Glasersfeld* (1917–), 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.[22]

Scientists were infuriated by the constructivists' attacks on realism because the erosion of public trust in science could lead to reduced federal funding of scientific research and, consequently, to a derailment of the scientific enterprise.[23–27] The constructivist philosophy is quite sensible, however, and cannot be blamed for its misuse by "science studies" authors.[28] As noted by serious philosophers and scientists, the criticism of realism does not imply the criticism of science.[29–31] After all, *absolute* realism is a metaphysical principle that has very little to do

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. (Source: The North American Fichte Society, University of Pennsylvania.)

with science. Although most people 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. Look at Figure 2.3 again. The top two panels 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 element is central to 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*,"[32] 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 nothing 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. This is the essence of constructivism — and, as a matter of fact, of philosophy in general.[33] As a scientist, you have nothing to fear from philosophers. Science describes the world in which we live; what lies beyond this world is as meaningful to scientists as the hypothetical knowledge of the genome of angels. According to the American Association for the Advancement of Science,[34] 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."

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.[35,36] Of course, books have been published on the scientific method,[37,38,216] 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 they are discussed in Sections 2.2, 2.3, and 2.4.

FIGURE 2.11 Looking for the truth. This comic strip is a pun on Hegel's dialectical conception of absolute knowledge.

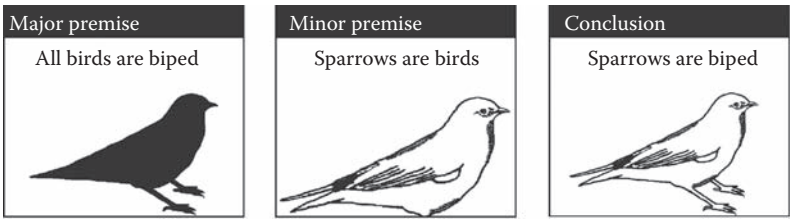


FIGURE 2.12 Syllogism. A basic syllogism consists of deriving a conclusion from a major premise and a minor premise.

So, the scientific method consists of applying common sense to scientific problems. But common sense is rather broad. Can't scientists offer some advice on how to optimize the use of common sense? In fact, many researchers have provided written advice to beginners. In the 17th century, René Descartes, in his *Discourse on Method*,[39] 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; and
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:[40]

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, and
4. Repeat the steps above to refine the possibilities that remain.

David Paydarfar and William Schwartz, professors at the University of Massachusetts Medical School, offer five principles for the conduct of successful scientific research:[41]

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; and
5. Cultivate smart friends.

In my opinion, one commonsense principle 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, and so on). 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, and so on). The principle of determinism may not apply in full to the most fundamental level of reality involved in quantum mechanics,[42,43] but it is a very safe guiding principle in all other areas of scientific inquiry. As statistician Bradley Efron states, "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."[44]

Naturally, an effect may have more than one cause. Serious research always involves a *control group* for this reason. 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 gets pregnant, 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 the paired ones.

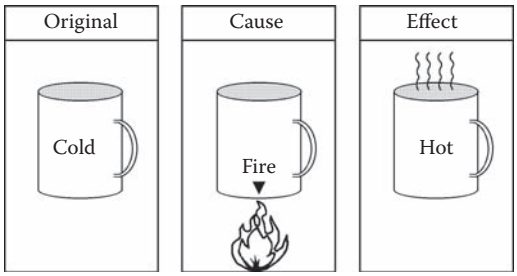


FIGURE 2.13 The principle of determinism. The principle of determinism asserts that every change in nature requires a cause. To warm up some coffee, heat is needed. No heat, no hot coffee.

Of course, the pairing with a male (without actual sex) could be the stimulus for the fall of the spirit. If you suspect this possibility, your control group should 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 you are studying. Because the control group is not exposed to the cause, it does not display the effect. No cause, no effect. No heat, no hot coffee.

In clinical studies, experimental control involves one additional element: the *placebo* control. Placebo refers to fake medication — sugar pills intended to please the patient without having any real pharmacological effect. There is much more to it, though.[45,46] Placebo medication is known to actually improve the condition of a small but significant number of patients. This finding 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 researchers want to know what the effects of the actual drug are, their control group must be a placebo group. Any changes observed in the placebo group will be due to "psychological" processes, while 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).

Believing in the principle of determinism is of no help if one does not look for the opportunity to apply it. Take the case of horoscopes. 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. If, by chance (if not by suggestion), some of the horoscope predictions turn out to be true, credulous readers may be satisfied with that. However, if readers write down each day's predictions and then count the number that turn out to be correct and incorrect, they may realize that the predictions are no more accurate than random guessing. Suppose that all that an astrologer has to do is predict whether the horoscope reader will have a good day or a bad day. The odds that the astrologer will guess correctly 100 times out of 100 are very small — but even credulous readers do 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 will predict correctly perhaps 7 out of 10 times. Credulous readers will consider this outcome very good and may not even bother to consider the odds of this outcome happening by chance (that is, by pure guessing). In actuality, standard statistical procedures allow one to calculate that the odds are higher than 1 in 20. (I doubt that the same credulous readers would ever drive on a highway if the odds of having a fatal accident were 1 in 20.) Thus, if horoscope readers seriously tested the hypothesis of a causal link between planet alignment and human predisposition, they would refute the hypothesis.

To use the terminology of the renowned logician Karl Popper,[47] 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. Because angels are immaterial, scientists cannot test the hypothesis. Now consider another hypothesis: "All sparrows are grey" (Figure 2.14).

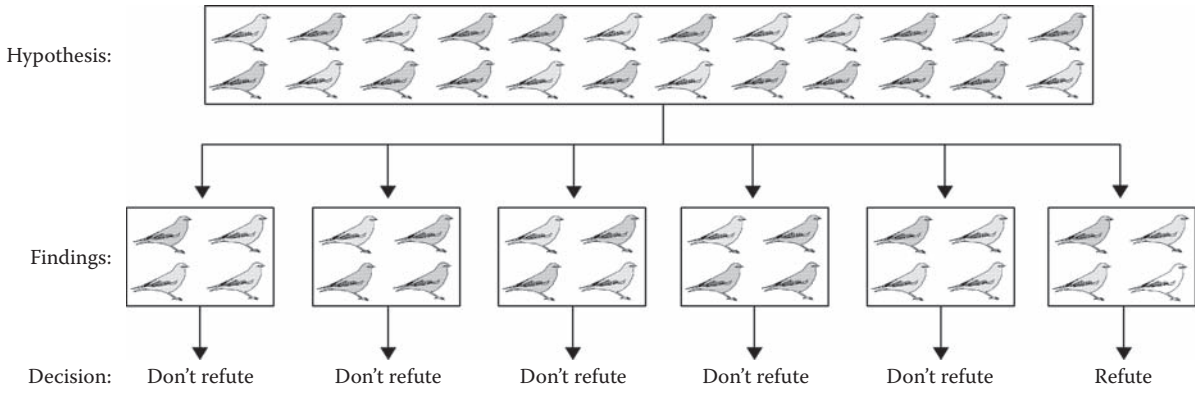


FIGURE 2.14 The refutability of hypotheses. If one has the hypothesis that all sparrows are grey, one can hold on to the hypothesis so long as one sees only grey sparrows. However, the sight of a single white sparrow is enough to refute the hypothesis.

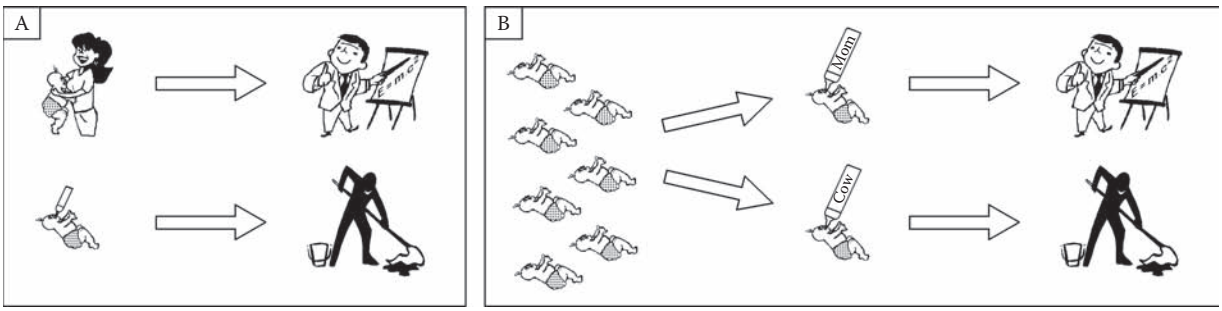


FIGURE 2.15 Correlation and causation. Although a correlation is often suggestive of causation (A), only controlled experiments allow reliable inferences about causality (B). See text for details.

Researchers can test this hypothesis by going outside and looking for sparrows. If they see only grey sparrows, they may accept the hypothesis, although they will not really be certain about how many grey sparrows they need to see before accepting the hypothesis. However, if they see just one white sparrow (see the rightmost rectangle in Figure 2.14), they 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 logic*), but the important conclusion is that the principle of determinism provides no help if scientists don't bother to test their hypotheses. To conduct science, refutable hypotheses are needed.

Disregard for hypothesis testing is not restricted to the realm of superstition. It is common even among scientists. Many scientists seem to inadvertently disregard Platt's advice mentioned earlier (that is, "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. Some reports indicate that children who are breast-fed as infants are more intelligent when they become adults (Panel A); that is, a correlation exists 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 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 (implying that the stimulation, not the milk, is the reason for greater intelligence). The existence of the correlation says nothing about cause and effect. To determine whether the mother's milk contains some substance that enhances intelligence, an appropriate experiment would need to be devised. For example, a sample of babies could be assigned randomly to two groups: one that receives mother's milk and one that receives cow's milk (or infant formula). Both groups would be bottle-fed, however. (See Panel B in Figure 2.15.) If the babies who are fed mother's milk grow up to be more intelligent than the babies who are fed cow's milk, then the researcher will 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,"[48–51] but many students seem to forget this fact by the time they finish graduate school and become research scientists.

2.2 RESEARCH ON POPULATIONS AND ORGANISMS

Research in circadian physiology usually involves one or more of four major categories of experimental procedures: recording, stimulation, lesioning, and transplantation (Figure 2.16). Recording a physiological variable is the sim-

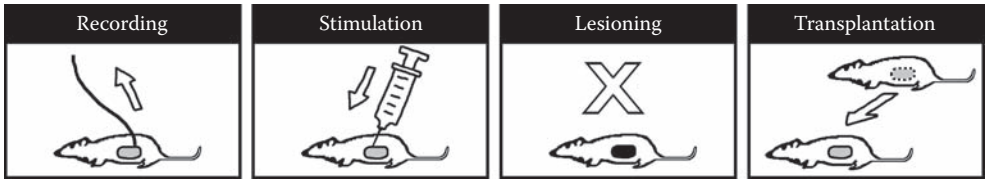


FIGURE 2.16 Research methods in physiology. Research on the vital processes of living organisms always involves one or more of four basic methods: recording, stimulation, lesioning, and transplantation.

FIGURE 2.17 Horses in the wild. The study of behavior and physiology of populations of organisms is one of many facets of circadian physiology. (Source: Photograph by Tim McCabe, U.S. Department of Agriculture Photography Center.)

plest 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 used for recording are generally the same as those used in traditional biological research; however, some adjustments are needed to ensure long-term monitoring of the processes under investigation. To study circadian rhythms, data must be recorded for at least several consecutive days. The required 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 2.17). When they do study populations, they may make use of *satellite telemetry* (Figure 2.18). 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.[52] More commonly, individual subjects are studied via land-based *radio telemetry*[53] (Figure 2.19) or by the satellite-assisted Global Positioning System.[54] Manufacturers of equipment for satellite or radio telemetry for use in the wild include North Star Science and Technology (Baltimore, Maryland); Telonics, Inc. (Mesa, Arizona); Advanced Telemetry Systems, Inc. (Isanti, Minnesota); Lotek Wireless Co. (Newmarket, Canada); Sirtrack Ltd. (Havelock North, New Zealand); and Titley Electronics Ltd. (Ballina, Australia).

In the laboratory, locomotor activity is most often monitored by *infrared motion detectors* or *running wheels*.

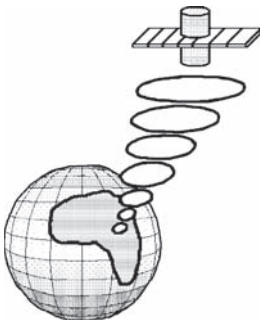


FIGURE 2.18 Satellite telemetry. Signals traced by satellite can be used to study populational rhythms in natural settings.

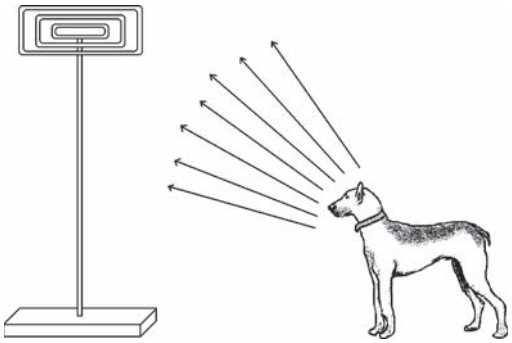


FIGURE 2.19 Radio telemetry. Radio signals emitted by a transmitter attached to an organism (such as a collar worn by a dog) allow for the monitoring of rhythms over relatively wide areas.

For practical reasons, miniature motion detectors are normally used for very small animals, such as insects,[55–57] while running wheels are used for rodents[58–60] (Figure 2.20). Commercial firms sell systems to monitor activity in insects (TriKinetics, Inc., Waltham, Massachusetts) and rodents (Actimetrics, Wilmette, Illinois; Mini-Mitter Co., Bend, Oregon), although the technical simplicity of the methods usually does not justify their high purchase price. Investigators can easily assemble their own data acquisition system. For example, a running-wheel system can be assembled using running wheels sold at pet stores (Figure 2.21), magnetic switches sold at home-improvement stores, and a personal computer. Personal computers have many interface ports that can be used to monitor the closure status of the switches (Figure 2.22). Several simple applications have been described,[61–63] and Exercise 2.4 at the end of this chapter describes an additional one. An infrared motion detector with a switch output, which can be used instead of a running wheel to monitor locomotor activity of mid- to large-sized animals, is available at RadioShack® stores (Invisible Beam Entry Alert, Product No. 49-312). Special circuits to monitor drinking and feeding are available commercially (for example,

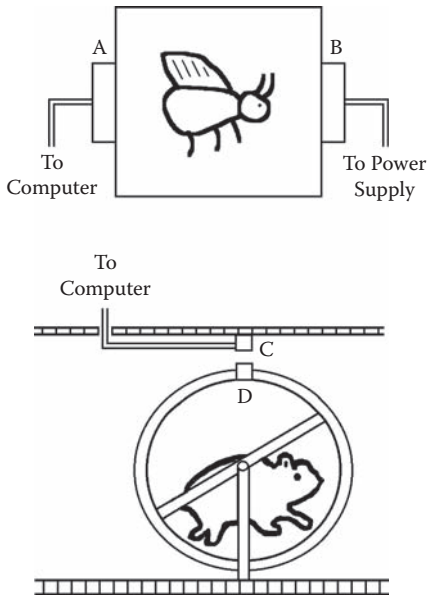


FIGURE 2.20 Monitoring activity rhythms in the laboratory. In laboratory settings, locomotor activity of individual organisms is often monitored by infrared movement detectors (A: sensor, B: emitter) or by mechanic or magnetic switches attached to running wheels (C: magnetic switch, D: magnet).

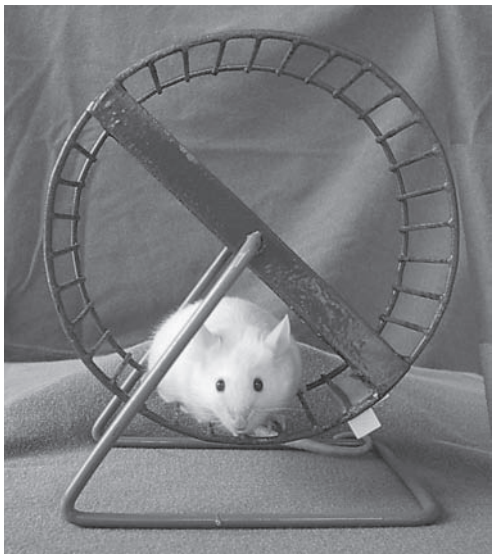


FIGURE 2.21 Mouse on wheels. An albino mouse stares at you from his running wheel.

Mini-Mitter Co., Bend, Oregon; Columbus Instruments, Columbus, Ohio).

Laboratory monitoring of physiological variables such as body temperature, heart rate, blood pressure, and sleep stages requires either a *tether* system[64–67] or a short-range *radio telemetry* system[68–71] (Figure 2.23). Tethering is less expensive than telemetry, but restricts the animal's movement in its cage. Telemetry, however, requires surgery to

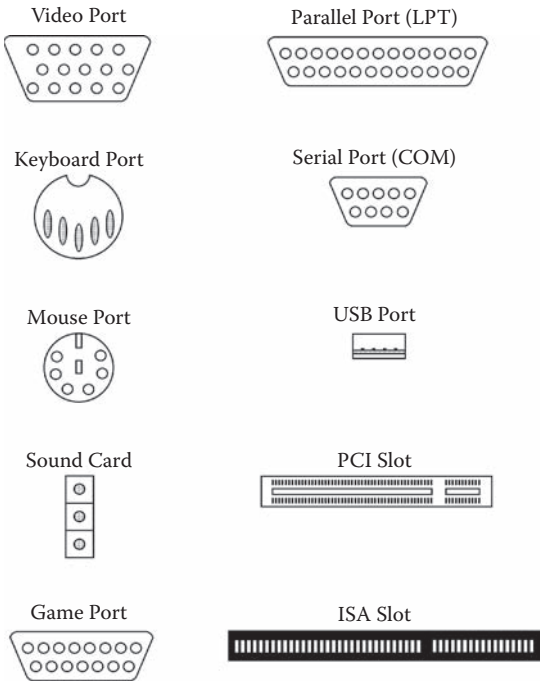


FIGURE 2.22 Standard computer interfaces. These diagrams identify the ten interface connectors available in most personal computers. Access to PCI slots and ISA slots usually requires opening of the computer cover. In recent years, directly accessible interfaces have been replaced with USB ports.

implant the radio transmitter, which, in some species, can blunt daily rhythmicity for up to a week.[72–75] In studies using human subjects and limited to temperature measurements, the transmitter may be swallowed instead of surgically implanted,[76] although it stays in the digestive system for only a few days.

Tethering equipment is marketed by most specialized suppliers of equipment for animal laboratory research, including Harvard Apparatus (Holliston, Massachusetts), Stoelting (Wood Dale, Illinois), and Kent Scientific (Torrington, Connecticut). The major manufacturers of biotelemetry equipment in the United States include the Mini-Mitter Co. (Bend, Oregon); Data Sciences, Inc. (St. Paul, Minnesota); and Biotelemetrics, Inc. (Boca Raton, Florida). The latter two companies offer radio transmitters capable of measuring body temperature, blood pressure, heart activity (electrocardiogram), brain activity (electroencephalogram), and muscle activity (electromyogram), as well as locomotor activity. Mini-Mitter's transmitters measure only body temperature, heart rate, and locomotor activity. However, Mini-Mitter manufactures traditional transmitters powered by batteries as well as *transponder* transmitters (that is, transmitters that are tele-energized by a radio receiver). The latter are especially convenient in long-term studies in which traditional transmitters run out of battery power. The disadvantage is that the transmitter (and, therefore, the animal) must remain close to the

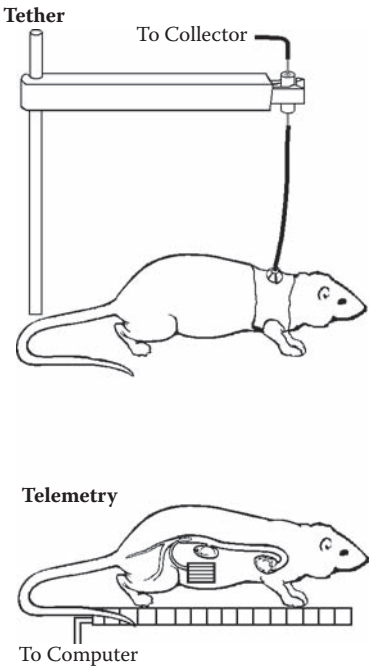


FIGURE 2.23 Monitoring physiological variables: tether and telemetry. Monitoring of physiological variables such as body temperature, heart rate, and concentration of hormones in the blood requires tether or short-range telemetry devices.

receiver to obtain power, although this is usually not a problem in laboratory studies.

The extremely small LTM transmitters manufactured by Titley Electronics (Ballina, Australia) are also worthy of mention. These transmitters, which were designed for temperature measurements in very small animals in field studies, weigh as little as 350 milligrams, including the battery. (A typical radio transmitter weighs over 2 grams without the battery.) When only temperature measurements are needed, a telemetry system can be assembled at a fraction of the cost of commercially available ones.[77–83] However, considerable knowledge of electronics and substantial debugging time are usually required.

In larger animals, an alternative to telemetry is the *data logger*[84–87] (Figure 2.24), a device that can record and *store* data. Data loggers allow experimental subjects to move freely over large distances without a loss of signal (because the "receiver" moves along with them). The experimenter cannot access the data until the logger is retrieved, however. Manufacturers of data loggers include SpaceLabs, Inc. (Issaquah, Washington); Onset Computer Corp. (Bourne, Massachusetts); Pico Technology Ltd. (St. Neots, United Kingdom); DataTaker Ltd. (Rowville, Australia); and Mini-Mitter Co. (Bend, Oregon). The Thermochron iButton (Dallas Semiconductor Corp., Dallas, Texas) is a convenient data logger for research in rodents when only temperature measurements are needed. These

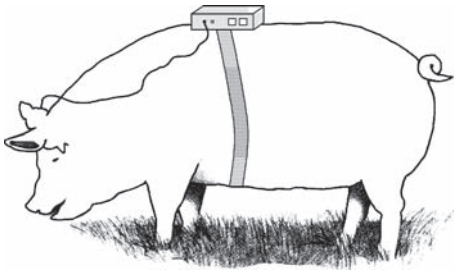


FIGURE 2.24 Monitoring physiological variables: data loggers. Data loggers provide an alternative to tether and telemetry devices in the monitoring of physiological variables.

miniature loggers (16-mm diameter) can be surgically implanted like radio transmitters. iButtons do not require separate receivers; however, they do not allow online access to the data they collect. As of mid-2004, iButtons were 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. However, if a study calls for data collection only at 1-hour intervals, data can be stored for up to 3 months. In addition, the price of an iButton is less than 10% of the price of a transponder radio-transmitter. A similar miniature data logger is marketed by SubCue Dataloggers (Calgary, Canada), although its price is less competitive.

Traditional methods of *indirect calorimetry*[88,89] can be used to monitor energy metabolism, as long as a computer is used to activate the air-switch valve and to collect the data (Figure 2.25). 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 oxygen gas (or "dephlogisticated air," as he called it), the legendary chemist Antoine Lavoisier observed that oxygen is equally necessary for combustion and respiration, and that both processes release carbon dioxide and heat.[90,91] As scientists learned more about the stoichiometric properties of oxidative processes, they were able to calculate the amount of nutrient used by an organism, and the amount of heat released by that organism, by measuring only the amount of oxygen the organism consumed.

As illustrated in Figure 2.26, 6 moles of oxygen are necessary to fully oxidize 1 mole of glucose, which results in 6 moles of water, 6 moles 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. Of course, most organisms also use other sources of energy, so the equation for glucose cannot be relied on solely. However, an "average" equation for the three main nutrients (carbohydrates, lipids, and proteins) in an organism's diet can be used as an approximation, and even more precise results can be obtained if the ratio of oxygen consumed to carbon

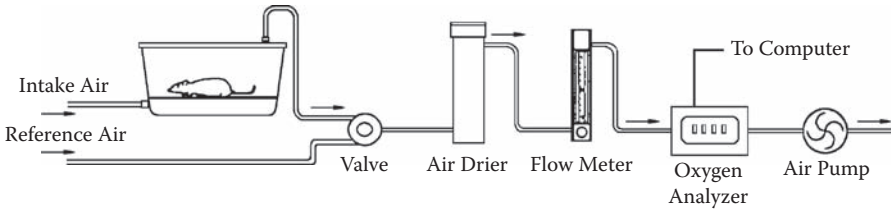


FIGURE 2.25 Indirect calorimetry. This diagram shows a typical setup for "open system" indirect calorimetry. The concentration of oxygen in the air that passes through the organism is compared with the concentration in the air that does not pass through the organism. The difference between the two concentrations reflects the fraction of oxygen consumed by the organism.

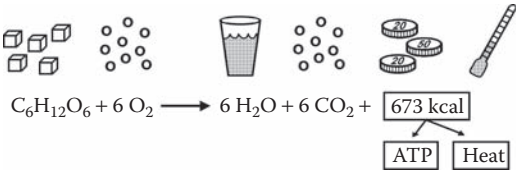


FIGURE 2.26 Why indirect calorimetry works. Measurement of oxygen consumption allows the computation of metabolic heat production because the stoichiometry of chemical reactions is independent of the steps taken to achieve the final result. As depicted here, the oxidation of one mole of glucose releases 673 kcal, and this result is true whether the glucose is metabolized by an organism or burned in a chemist's calorimeter.

dioxide produced (which is called the *respiratory quotient*, or RQ) is employed. The RQ value varies with nutritional parameters and ambient temperature.[92–100]

To measure the concentration of oxygen (and carbon dioxide, if needed) in the air used by the organism, gas analyzers are employed (see Figure 2.25). Companies that supply gas analyzers for biomedical research include Servomex (Crowborough, England), Columbus Instruments (Columbus, Ohio), 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 the actual amount of oxygen if the exact flow of air through the chamber is known.[101,102]

Another instrument that can be easily adapted for circadian physiology research is the *temperature gradient device*. This device, used for invertebrates[103,104] as well as for rodents,[105,106] 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 2.27, 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 make this diagram easier to understand visually, 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 for 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.[107,108])

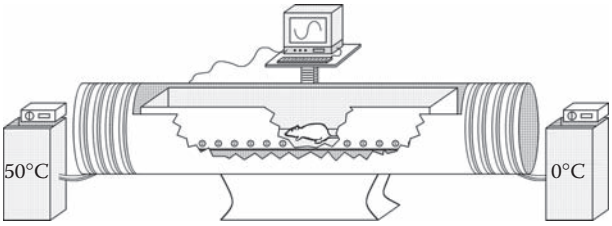


FIGURE 2.27 A temperature gradient device. By heating one end of a copper pipe and cooling the other end, a researcher can generate a temperature gradient in the animal's cage, thus allowing it to choose its preferred temperature.

In simpler life forms, such as fungi and bacteria, *race tubes* (Figure 2.28) are standard equipment in circadian research.[109–111] Colonies are placed at one end of a glass tube with plenty of nutritive medium, and the organisms 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.

Paper-and-pencil tests are often used in research with human subjects. One test employed widely 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. Individuals are classified along a scale of early risers to late risers based on their answers to various questions. In 1976 Horne and Östberg developed a popular morningness–eveningness inventory[112] (Table 2.1), and similar inventories have been developed since then.[113–116] Morningness–eveningness typology is covered in Chapter 14.

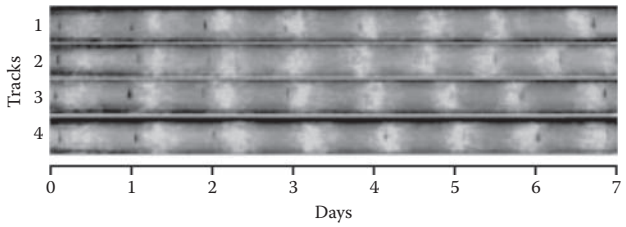


FIGURE 2.28 Race tubes. In microorganisms, growth rhythms are studied in "race tubes." The colony grows along a small glass tube, and new bands can be seen each day. This figure shows the growth of four colonies of bread mold over 7 days. (Source: Adapted from Cheng, P. et al. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 98: 7408–7413.)

2.3 RESEARCH ON ORGANS, CELLS, AND MOLECULES

Research at the level of whole organisms is necessary but not sufficient to understand the mechanisms of circadian rhythmicity. Researchers conduct a great deal of research at the level of individual organs, cells, and even molecules inside a cell. Before I discuss some of the techniques used to study circadian physiology at the level of organs and cells, I want to 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.

Figure 2.29 illustrates the three main planes of slicing (coronal, horizontal, and sagittal). Knowledge of these names will be very helpful when I discuss actual experiments in later chapters. Also important will be knowledge of the names of positions along the three axes. Just as you need to know north, south, east, and west when looking at a map, you need to know the anatomical terms shown in Figure 2.30. For example, the head of a pigeon is located in the anterior-medial-dorsal region of its body, just as Alaska is located in the northwest region of the world.

It is often convenient to use terms that denote relative, rather than absolute, position. Thus, in reference to a given location (say, point A in the left drawing in Figure 2.31), locations on the same side of the body (point B) are said to be *ipsilateral* to the reference location, while locations on the opposite side of the body (point C) are said to be *contralateral* to the reference location. Similarly, when a reference location in the body (point A in the right drawing) is selected, areas in the proximity of this location

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 to you go to bed compared with your usual bedtime?	[ ] Seldom or never later [ ] Less than one 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. & Östberg, O. (1976). A self-assessment questionnaire to determine morningness–eveningness in human circadian rhythms *International Journal of Chronobiology* 7: 97–110. © Taylor & Francis (www.tandf.co.uk). Reproduced with permission.

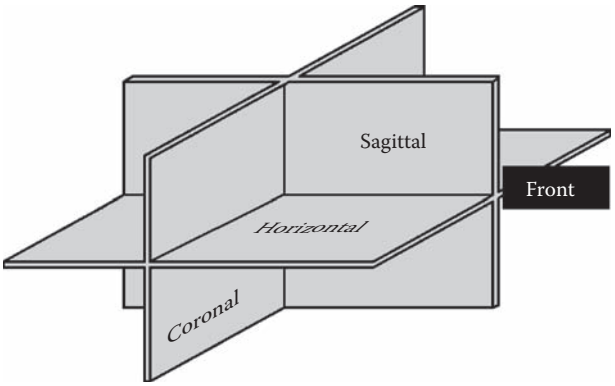


FIGURE 2.29 Anatomical planes. Research dealing with specific body organs often requires knowledge of basic anatomical terms, such as the three spatial planes.

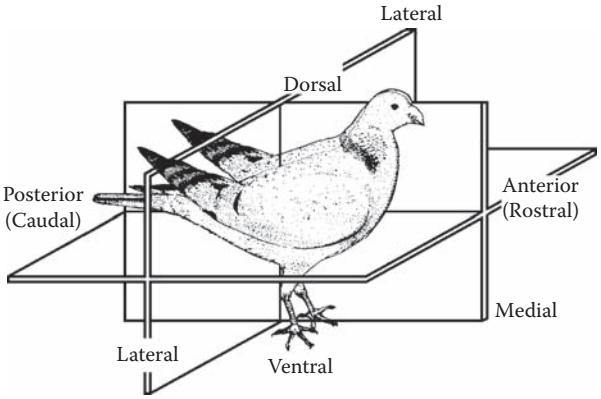


FIGURE 2.30 Anatomical directions. Directions along the three anatomical planes have special names that must be learned by beginners in physiological research.

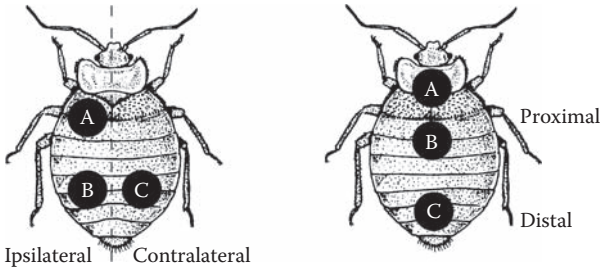


FIGURE 2.31 More anatomical terms. Relational anatomical terms are used to specify a location (B or C) not in reference to the whole body but in reference to another location within the body (A).

(point B) are called *proximal*, while 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 (including birds and mammals) usually concentrate their efforts in one of various "systems," as shown in Figure 2.32. 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 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 use 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 monitoring secretions in the blood, digestive tract, or other body compartments. The activity of excitable tissue (muscles and nerve cells) is investigated by observing changes in voltage or electric current. The activity of any organ can also be studied by measuring how much nutrient or oxygen the organ consumes, and — because nutrients and oxygen are carried to the organ in the blood — by monitoring how much blood flows to the organ.

When secretions in the blood are monitored, it is important to know what is meant by the term *blood*. As shown in Figure 2.33, 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

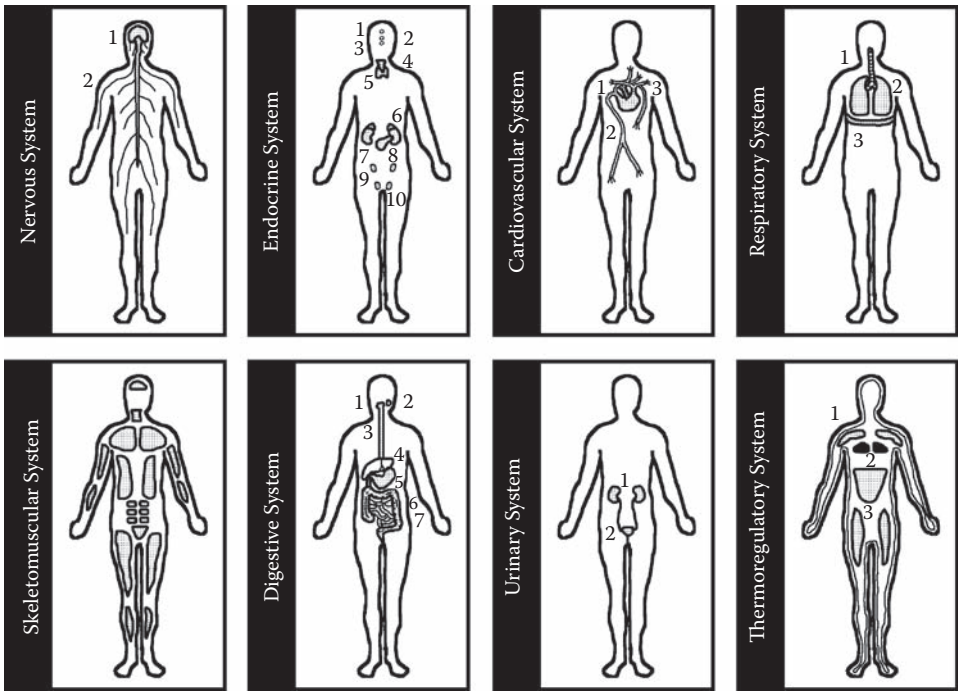


FIGURE 2.32 The major physiological systems. Those who conduct research at the level of body organs often specialize in one of the eight major physiological systems. See text for details.

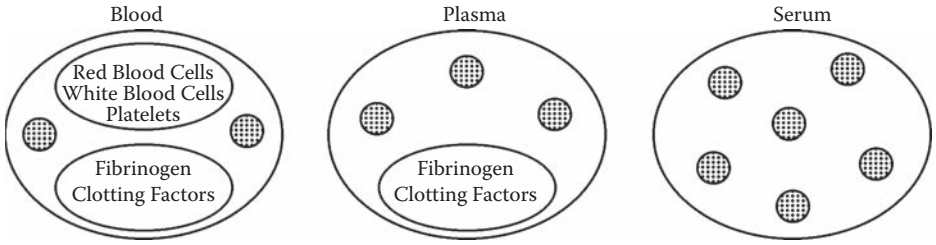


FIGURE 2.33 What's in your blood? Blood, plasma, and serum refer to different levels of integrity of the fluid that circulates through the heart, arteries, capillaries, and veins.

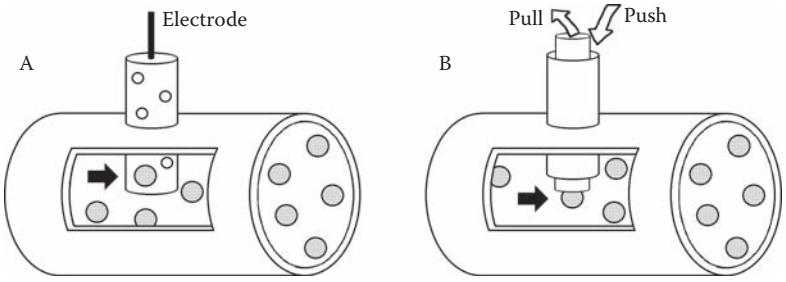


FIGURE 2.34 Dialysis. Dialysis has many applications in physiological research, such as in the operation of membrane electrodes (A) and push-pull cannulae (B). Dark arrows depict the movement of a substance into the electrode or cannula.

concentration of hormones and drugs using standard laboratory techniques.[117–124] In some cases, the less invasive procedures of urine or saliva collection can be used.[125–131] Special invasive techniques are required when measurements must be made *in vivo* (that is, inside the live animal). These measurements are usually obtained through the process of *dialysis* (Figure 2.34). Dialysis is the diffusion of liquid solutions across a semipermeable membrane. The diagram on the right (B) in Figure 2.34 shows a push-pull cannula inserted into an organ, blood vessel, or other body

compartment. Saline (or some 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.[132–135] The diagram on the left (A) 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.[136] Although this type of electrode could be used to measure oxygen consumption of individual organs, other techniques are used more frequently to monitor activity of nonsecretory organs.

Techniques that monitor tissue blood flow (as a measure of metabolic activity) include *radioactive microspheres*[137] and the high-budget procedures of *positron emission tomography (PET)* and *functional magnetic resonance imaging (fMRI)*.[138] The microsphere technique requires euthanasia of the experimental subjects and, therefore, can be used only in cross-sectional studies. PET and fMRI 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 recent finding that the responses of nerve cells and adjacent glial cells cannot be adequately differentiated.[139]

The 2-deoxy-glucose (2-DG) methodology can be used to monitor glucose utilization. Louis Sokoloff developed this methodology in the 1970s.[140,141] It involves the use of radioactively labeled 2-DG, which is an analog of glucose. Brain cells use glucose as their main substrate for energy metabolism; cells that are more active metabolize more glucose. Although brain cells use 2-DG similarly to glucose, the cells do not fully metabolize 2-DG and it is trapped in the tissues. Therefore, more active cells accumulate more 2-DG (and more radioactive molecules). Using radiographic techniques, researchers can determine what parts of the brain were more active following a 2-DG injection. This technique has been used successfully in circadian research,[142–144] 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.[145]

An alternative technique is that of *Fos imunnocytochemistry*. Fos is the protein produced by the c-*fos* (or just *fos*) gene, a gene widely used in cells in the process of gene activation. The amount of Fos produced is a reliable marker of overall gene activation and, therefore, of cell and organ function.[146,147] This technique has been used widely in circadian research;[148–154] 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 used successfully in circadian research. 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.[155–158] Although the luminescence is strong enough for *in vitro* studies, or *in vivo* studies of small organisms,[159,160] the signal is too weak to allow *in vivo* measurements in vertebrates.

Optical imaging can also be used for *phosphorescence* instead of luminescence, eliminating the need to develop transgenic animals. In this method, 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 the depletion of oxygen in the fine vasculature and, consequently, the greater the phosphorescence when the tissue is illuminated.[161] 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 yet been used in circadian research. 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, allowing optical imaging of cells and organs deep inside live animals.[162–164] 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 2.35). 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 recently been developed.[165]

2.3.2 Research on Cells

Research on cells of excitable tissue (muscle and nerve) normally relies on the recording of electrical activity (*electrophysiology*). Before discussing this technique, however, a brief review of neurobiology is needed. 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 2.36). The main difference between the two classes of chemicals is that neurotransmitters are released at nerve terminals exactly where they are meant to go, while hormones are released

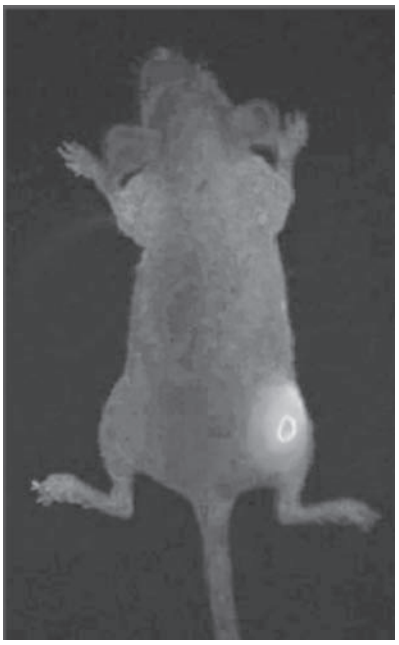


FIGURE 2.35 A glowing mouse. In this example of the use of quantum dot methodology, alloyed semiconductor quantum dots injected into a live mouse mark the location of a tumor. (Source: Photograph courtesy of Shuming Nie, Department of Biomedical Engineering, Emory University, Atlanta, GA.)

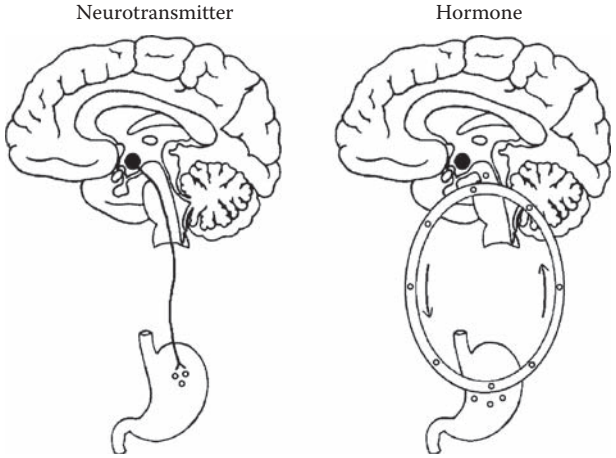


FIGURE 2.36 Two major information highways. Information transmitted in the body flows through two main highways: the nervous system and the endocrine system. The nervous system often uses neurotransmitters released at nerve terminals, while the endocrine system uses hormones released into the bloodstream.

in the circulating blood and go everywhere in the body. Nevertheless, 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.

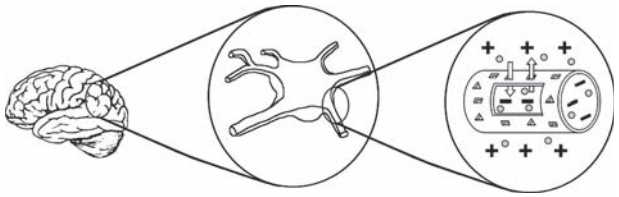


FIGURE 2.37 All about nerve cells. The main constitutive cells of the nervous system are neurons, specialized cells that are polarized like a battery because of selective permeability of the cell membrane.

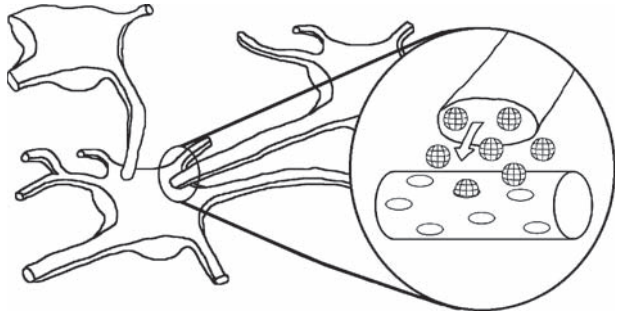


FIGURE 2.38 A chemical synapse. Neurons communicate with each other, and with muscles and glands, through synapses. Many synapses rely on the release of neurotransmitters from the presynaptic terminal and the binding of the transmitters to the postsynaptic terminal.

Neurons are the critical cells in the nervous system (Figure 2.37). 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 1/20th 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, which is why the activity of a nerve cell can be monitored by recording 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 2.38). 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 acetylcholine (used in all synapses between nerves and skeletal muscles), norepinephrine (used in synapses between nerves of the sympathetic nervous system and its target

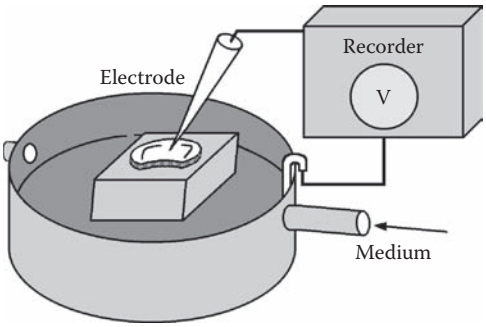


FIGURE 2.39 Electrophysiological recording. This diagram shows the main elements of an electrophysiological setup for recording the activity of nerve cells *in vitro*.

organs), dopamine (used in numerous circuits in the brain), and nitric oxide (a peculiar neurotransmitter, as it is found as a gas, not 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 example, 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.

Although phosphorescence techniques can be used for the study of individual cells,[166] electrophysiological recording is by far the most commonly used technique for the study of muscle and nerve cells. It has been used for 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).[167] In 1914 Edgar Adrian showed that the same principle applies to nerve cells,[168] and 25 years later Alan Hodgkin and Andrew Huxley performed the first intracellular recording of an action potential.[169] A typical experimental setup for electrophysiological recording from nerve cells *in vitro* is shown in Figure 2.39. 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 multi-unit activity, both *in vitro* and *in vivo*.[170–175]

2.3.3 Research on Molecules

Before examining techniques used to study circadian physiology at the molecular level, some basic principles of molecular biology should be reviewed. As summarized in Figure 2.40, 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 2.41).

When a gene is activated, it *transcribes* its double-stranded DNA sequence into a single-stranded RNA (ribonucleic acid) sequence, which is called the messenger RNA, or 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 phosphorilation, methylation, acetylation, and other processes.[176] 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 1 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 can be artificially induced to use them.[177] Ten of the 20 amino acids are called *essential* because they cannot be synthesized by the human body and must be obtained in the diet.

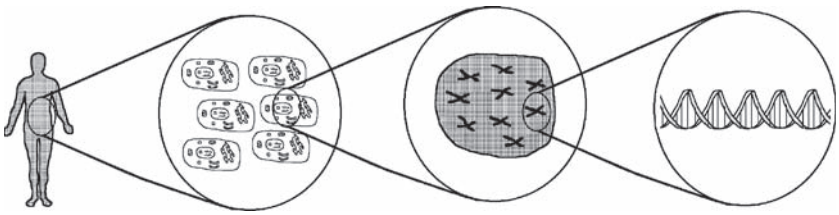


FIGURE 2.40 DNA: the blueprint of life. The body is made up of billions of cells. In the nucleus of each cell, chromosomes contain genetic information in the form of DNA molecules, segments of which constitute genes.

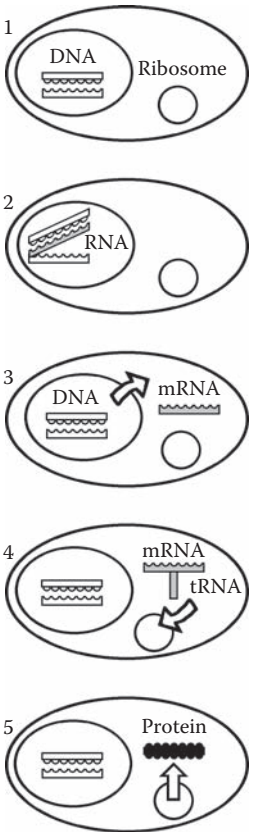


FIGURE 2.41 Gene expression. In eukaryotes (organisms whose cells have nuclei), genes are located in chromosomes in the nucleus of the cell and are normally inactive. When a gene is activated, it transcribes its double-stranded DNA sequence into a single-stranded RNA sequence (the messenger RNA, or mRNA). 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.

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 using 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 2.42). *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 few years, *mass spectrometry* has become a viable alternative for the identification and characterization of proteins.[178]

Because animals have several tens of thousands of genes, which may produce more than a million proteins, a researcher must be very lucky to identify a few genes or proteins of interest. 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.[179] 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. Because only active

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
Lysene	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. & Russell, L. D. (1997). *Molecular Biology.* Vienna, IL: Cache River Press; Nelson, D. L. & Cox, M. M. (2000). *Lehninger Principles of Biochemistry*, 3rd Edition. New York: Worth Publishers.

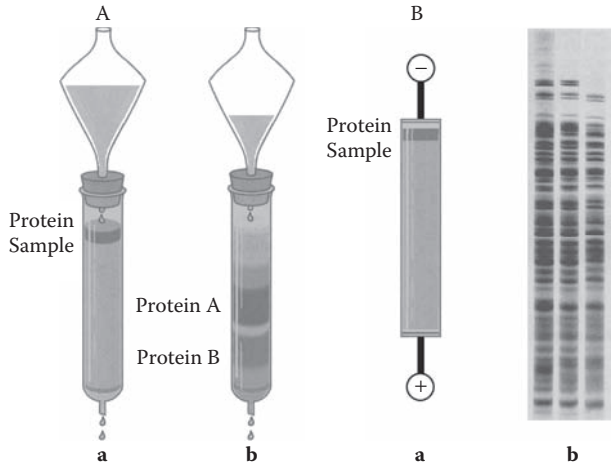


FIGURE 2.42 The two traditional methods of protein identification. Proteins can be separated by column chromatography (A) or gel electrophoresis (B). See text for details. (Source: Adapted from Nelson, D. L. & Cox, M. M. (2000). *Lehninger Principles of Biochemistry*, 3rd Edition. New York: Worth.)

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 (Figure 2.43) in studies on fungi,[180] insects,[181–184] and mammals.[185–189] Protein microarrays are currently in development,[190–192] and their use in circadian research can be expected to follow soon.

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. Two very important environmental variables 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 illumination intensity: radiant flux, radiance, and irradiance[193–195] (Figure 2.44). *Radiant flux* refers to the power of the emitted light source (radiant power) and, accordingly, is expressed in watts (W). When you buy a light bulb for your house, the bulb is rated according to its consumption of energy, not by the emitted light. Typically, radiant power is about one-quarter of the consumed power, so that a 100 W bulb puts out only 25 W (the rest is lost as heat). *Radiance*, which is more commonly used by vision researchers, measures the fraction of radiant power that can reach you. It indicates the radiant power per unit area per unit solid angle (see Figure 2.44) and, therefore, is expressed in watts per

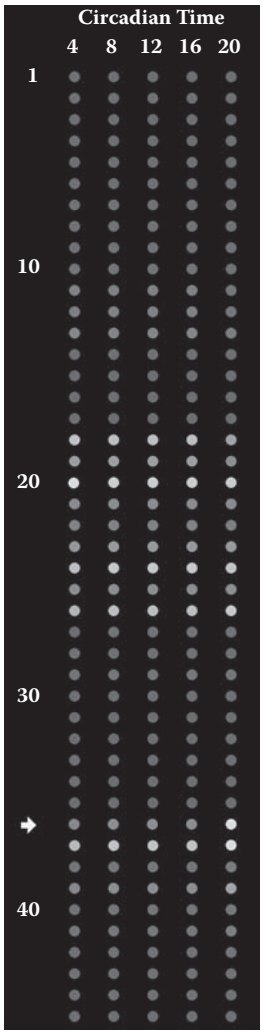


FIGURE 2.43 Tracking thousands of genes at the same time. DNA microarrays make it possible to track the expression of thousands of genes at the same time. In this short segment of a much larger array, expression of 45 genes in cells of the fruit fly (one gene per line) is tracked over five circadian times. Greater expression levels are indicated by whiter shades of grey. The arrow points to a gene that showed greater expression at circadian time (CT) 20 than at other times of the daily cycle. (Source: *Drosophila* Microarray Database at Washington University School of Medicine, St. Louis, MO.)

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. 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 humans call light is a very narrow range of electromagnetic radiation. As shown

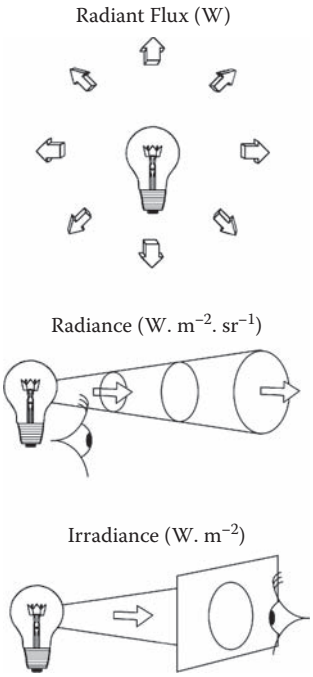


FIGURE 2.44 Measuring illumination. Three concepts are particularly important in the measurement of illumination intensity: radiant flux, radiance, and irradiance.

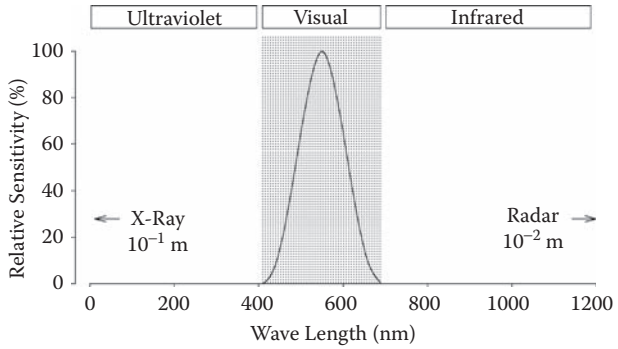


FIGURE 2.45 The narrow range of visual sensitivity. Vision is elicited by electromagnetic radiation. However, humans are blind to all radiation outside the narrow range of wavelengths from 400 to 700 nm.

in Figure 2.45, people are sensitive only to wavelengths between approximately 400 and 700 nm (1 nm = 10^{-9} m). Regardless of the intensity of illumination, they see no light below 400 nm or above 700 nm. Therefore, a set of *photometric* units has been created to supplement the *radiometric* units discussed earlier.[194,196] 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. At peak sensitivity (555 nm), an irradiance of 1 W · m^{-2} corresponds to an illuminance of 683 lux (sometimes abbreviated as lx). At shorter and longer wavelengths, 1 W · m^{-2} corresponds to less than 683 lux — in the ultraviolet and infrared ranges, it corresponds to 0 lux. Figure 2.46 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 nonhuman subjects. Photometric units are obviously biased

TABLE 2.3
Radiometric and Photometric Units

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

Note: For more details on units of measurement, see the last section of the *Dictionary of Circadian Physiology* at the end of the book. This table uses the following abbreviations: W (watt), m (meter), sr (steradian), lm (lumen), and lx (lux).

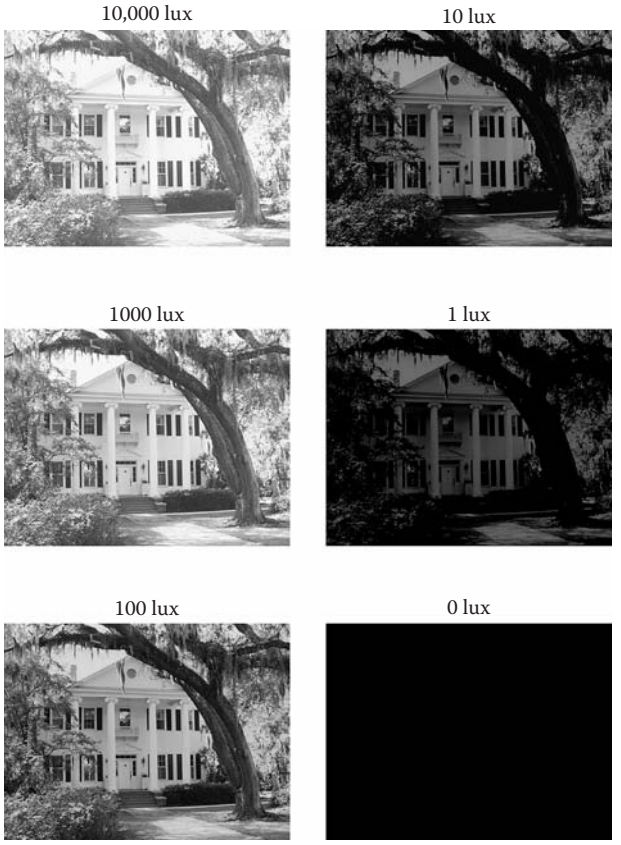


FIGURE 2.46 Quantifying the intensity of visual light. The intensity (brightness) of visual light is measured in lux. This series of photographs serves as a guide to the lux scale. Our perception of brightness follows a logarithmic function (e.g., 1000 lux is twice as bright as 100 lux).

FIGURE 2.47 A photometer. Photometers are instruments used to measure illuminance (light intensity in lux). (Source: Image courtesy of the Cooke Corporation, Auburn Hills, MI.)

towards 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 discussed in Chapter 11), it is prudent to use radiometric units most of the time.

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.[195] At 555 nm, 1 $W \cdot m^{-2}$ corresponds to approximately 2.79×10^{18} photons $\cdot s^{-1} \cdot m^{-2}$.

Instruments used to measure light intensity provide either photometric measurements (*photometers*) or radiometric measurements (*radiometers*), or both (*photometer–radiometers*). Because photometers are used widely in nonscientific applications (such as photography and civil engineering), they are easily available at moderate prices. Figure 2.47 shows a precise but inexpensive photometer manufactured by the Cooke Corporation (Auburn Hills, Michigan). A variety of companies manufacture photometers of varying precision and cost, and scientific equipment suppliers as well as optical and photographic stores sell these instruments. Especially useful in circadian research are the small photometers that are optionally included in wrist data loggers. Figure 2.48 shows the Actiwatch (manufactured by the Mini-Mitter Company, Bend, Oregon), which records arm movements and illumination level. Competitors include the Actigraph (Ambulatory Monitoring, Ardsley, New York) and the Actitrac (IM Systems, Baltimore, Maryland).

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 Laboratories (Newport, Rhode Island), United Detector Technology (Baltimore, Maryland), and Macam Photometrics (Livingston, Scotland). Photometers and radiometers

FIGURE 2.48 Wristwatch photo-sensitive data logger. Wristwatch data loggers are convenient instruments for monitoring locomotor activity in human subjects. Units with photocells also allow the monitoring of light-exposure patterns. (Source: Image courtesy of the Mini-Mitter Company, Bend, OR.)

measure the incident light regardless of its composition — the manufacturers assume you know the source of your light. 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), GretagMacbeth (Regensdorf, Switzerland), Glen Spectra (Stanmore, England), and Instrument Systems (Munich, Germany).

Another important environmental variable is *temperature*, which is a measure of the average kinetic energy of the molecules of a substance.[193,197,198] Temperature is measured with a *thermometer*, which was invented in the 1600s and has evolved considerably since then.[199–201] To simply monitor the temperature of the environment over extended periods of time, a standard *thermograph* (temperature chart recorder) can be used. Figure 2.49 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 provide digital output, so that the temperature data can be simultaneously transferred to a computer.

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,

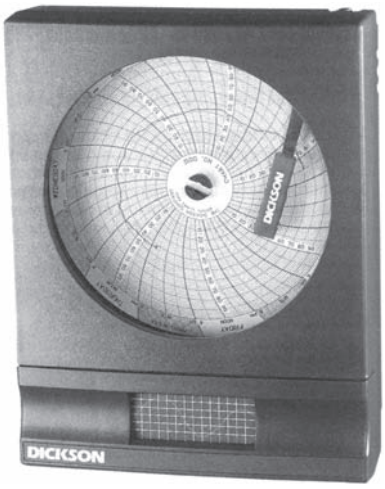


FIGURE 2.49 A thermograph. Thermographs provide a convenient way to record ambient temperature continuously for many days. (Source: Image courtesy of Dickson Instruments, Addison, IL.)

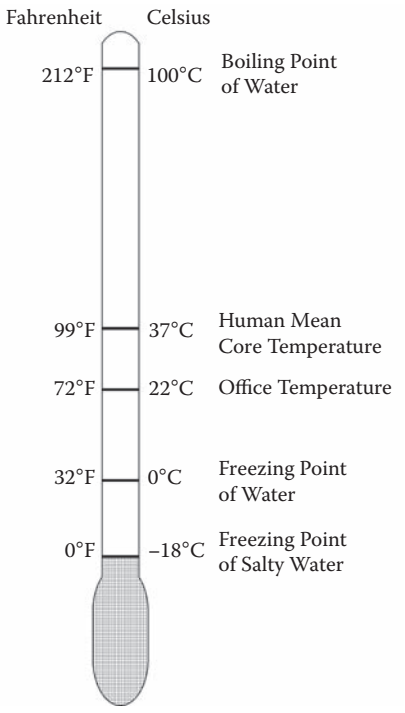


FIGURE 2.50 Temperature scales: Celsius and Fahrenheit. In most of the world, temperature is measured in degrees Celsius (°C). In the United States, the Fahrenheit scale (°F) is used. The diagram shows the correspondence of the two scales.

except among scientists, temperature is measured in degrees Fahrenheit (°F). The relationship between the Fahrenheit scale and the Celsius scale can be expressed as: $°F = 1.8 \times °C + 32$. Figure 2.50 shows the temperatures of important phenomena in the two scales.

FIGURE 2.51 Controlling the light–dark cycle. An electronic timer such as this one provides a simple way to turn lights on and off at desired times in a laboratory setting.

The circadian physiologist often needs to *manipulate* environmental variables in a research project. Three important variables are illumination, temperature, and food availability. Illumination sources include sunlight, incandescent light bulbs (using tungsten or tungsten-halogen filaments), fluorescent bulbs, xenon arc lamps, light-emitting diodes (LED), and lasers.[195,202] LEDs and lasers can provide radiation confined to a very narrow spectral region, which allows the generation of monochromatic stimuli without the need for special filters. In circadian research, the goal is usually to simulate sunlight, which means 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 the goals of the research. For general animal housing during experiments, values usually range from 10 to 1000 lux. (For comparison, the illuminance level of the full moon is about 0.1 lux, the average human indoor working space is 200 lux, and full daylight is 15,000 lux.[202])

In circadian research, the timing of light is just as important as its brightness. Therefore, some sort of device is needed to automate the on–off cycle of the lights. Figure 2.51 shows an inexpensive controller timer. These devices, 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 a researcher wishes to vary the period of the light–dark cycle (that is, to make 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 achieved 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 supposed to be *dark*. However, *dark* is a relative term. As 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. In laboratory situations that require human visual inspection of experimental subjects in darkness, illumination for human vision can be provided without introducing photic stimulation to the subjects. Some researchers use infrared viewers ("night-vision goggles"),[203–208] although dim red illumination in the normal human visual range (1 lux, $\lambda > 600$ nm) is often used in studies involving rodents.[209–214] Illumination bright enough to allow good human visual resolution (for example, for reading or performing surgery) cannot be attained by these means. A recent report indicates that sodium lamps can produce adequate illumination for human vision without disturbing the normal behavior of mice,[215] although thorough tests have not been conducted to ascertain that the murine circadian system is indeed unaffected by the illumination.

Control of ambient temperature typically is not a concern in circadian physiology. The standard heating and air-conditioning systems used in modern buildings are capable of maintaining a stable thermal environment with daily oscillations not exceeding 1 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 2.52 shows a refrigerated incubator with 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.

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. Automated fish feeders, available at most aquatic pet stores (Figure 2.53), provide a simple way to automate daily feeding. The very popular NutraMatic feeder (marketed by Role C. Hagen Corp., Mansfield, Massachusetts) can hold about 120 grams 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. These situations may require a custom-built feeder with a solenoid-activated access door. An alternative 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 programmed easily to deliver rewards only to lever-presses performed during a predefined interval of time each day. Sweeney Enterprises

FIGURE 2.52 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.

FIGURE 2.53 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.

(Boerne, Texas) manufactures large stand-alone industrial feeders that are helpful in field research and in some laboratory applications involving large animals.

SUMMARY

1. Research in circadian physiology is conducted according to the scientific method, which consists of the systematic application of common-sense principles, particularly the principle of determinism. Although absolute truths belong only in 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 monitoring secretion in the blood or other body fluid while the activity of muscles and nerve tissue can be studied by monitoring changes in voltage or electric current. Organ activity can also be studied by monitoring how much nutrient or oxygen the organ consumes and 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 important environmental variables are illumination and temperature. Circadian physiologists sometimes also need to *manipulate* environmental variables. Three important variables are illumination, temperature, and food availability.

EXERCISES

Exercise 2.1 Pronunciation of technical terms

If you have not yet installed the software package that accompanies this book, now is a good time to do it. Follow the instructions in the Software Installation section. Once the package is installed, double-click on the Circadian icon to open the program banner. Then select the SayIt program (the second icon from the right, just to the left of the music icon). This program provides the pronunciation of the various technical terms introduced in Chapter 2. Click on the down-arrow in the second or third drop-down menu (Anatomy or Physiology), then choose the term that you want to hear. Repeat the procedure for each term you want to hear. The pronunciations are guided by the rules of American English and by peculiarities of international usage. *Note:* The first drop-down menu (People) contains the names of the various circadian physiologists introduced in Chapter 1. If you have not listened to them yet, you may do so now.

Exercise 2.2 Plotting data (equally spaced data points)

The first step in any data-analysis procedure is to visually inspect the data. This exercise uses the program Plot to inspect a number of data files with equally spaced data points. Exercise 2.3 focuses on files that contain unequally spaced data points, including files that contain data collected at regular intervals but missing several points.

1. Double-click on the Circadian icon to open the program banner, then click on Plot (the first icon on the left).
2. The program window contains three main panels: a Source panel, a Data panel, and a display panel. Leave all values in the Data panel at their default values (the Unequally Spaced checkbox should not be checked).
3. In the Source panel, double-click on the Data subfolder, then click on the file A01. This file contains body temperature measurements of a squirrel, which were collected by telemetry every 6 minutes for 7 days.
4. Click on the Cartesian plot button (the purple button). A nice daily oscillation is displayed. Use the horizontal scroll bar (under the display panel) to view the next 6 days.
5. 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), which were 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 entire file (using the horizontal scroll bar). Note that any daily rhythmicity is masked by the recording noise.
7. The next chapter looks at ways to filter out noise, but you 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 entire file. Note that you can easily distinguish genuine data points from bad data points.
8. Note that the range of oscillation of the data points appears above the display panel (in this case, the range is 30.06 to 39.06°C). Because

most noise seems to be below 35.5°C, you can discard these points to see the genuine points better. The bottom of the Data panel contains 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. Note that, although the plot is still not very good, you now have a better view of the data.
10. Finally, 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, set the Low Filter back to 0. In the Data panel, adjust 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 modes. 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.

Exercise 2.3 Plotting data (unequally spaced data points)

1. Start the program Plot.
2. In the Data panel, select the checkbox Unequally spaced by clicking on it.
3. Select the sample data file A19. This file contains oral temperature measurements 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 did not follow step 2 above, you will receive an error message; otherwise, you will see a clear daily rhythm with lower values during the early morning.
5. To see the individual data points, click on the Dots option button at the bottom right of the program window.
6. 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.
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. This file contains measurements of air relative humidity taken at irregular intervals over 2 days in Walterboro, South Carolina. Because the data were collected infrequently, the graph does not look good in 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 2 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 zero 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.)

Exercise 2.4 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 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. Before you start, you should have a cage in which to keep the test animals.

1. 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 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. RadioShack sells a magnetic switch (Product No. 49-497) for about $6. To avoid further trips later, you may want to buy 4 or 5 switches right away.
3. The only other piece of equipment needed is a basic desktop personal computer. 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 2.22 (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.usbgear.com) sells a USB to game-port adapter for $26.
4. 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 attach to the female connector in the computer (or in the USB adapter). You can purchase one at RadioShack or any other electronics store.
5. Now look at Figure 2.54. 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 animal's 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 through 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 wires from the magnetic switches 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 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-ROM that comes with the book contains a short DOS program (Collect.exe) 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 in the folder where the other programs are stored (\Program Files\Circadian). 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 use all eight 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-ROM 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

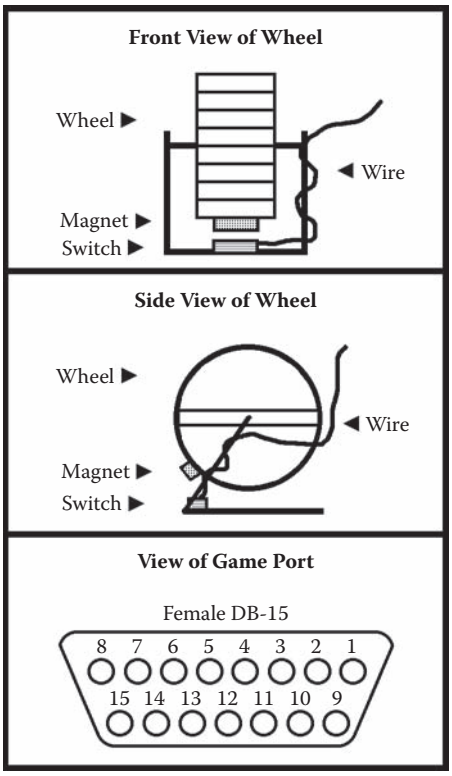


FIGURE 2.54 Running-wheel setup. These diagrams will help you set up your running-wheel data-acquisition system. See Exercise 2.4 for details.

Java) do not provide direct access to the game port. In these languages, you will need to make use of API calls.

9. When the system is working properly and recording data, put some animals in the cage (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 as Collect.exe 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. A data file for channel A starting on 25 December 2009 would be named A091225.txt.

SUGGESTIONS FOR FURTHER READING

There is only one book dedicated specifically to research methods in circadian physiology. Other books listed here deal with the fundamentals of the various disciplines pertinent to the study of circadian rhythms.

Young, M. (Ed.) (2005). *Methods in Enzymology, Vol. 393: Circadian Rhythms*. Academic Press, San Diego, CA. An edited book with chapters written by experts in behavioral, genetic, cellular, and molecular research methods in circadian physiology.

Schmidt-Nielsen, K. (1997). *Animal Physiology* (5th Edition). New York: Cambridge University Press. An excellent introductory physiology textbook. Schmidt-Nielsen knows how to keep the reader interested. I used an earlier edition when I was a student and loved it.

Kalat, J. W. (2004). *Biological Psychology* (8th Edition). 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 well written, and the production is impeccable.

Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. (2002). *Molecular Biology of the Cell* (4th Edition). New York: Garland Science. A thorough introductory textbook on cell biology, including molecular genetics. With over 1500 pages, the book provides the fundamentals essential for understanding current developments in molecular biology.

Kandel, E. R., Schwartz, J. H., and Jessell, T. M. (2000). *Principles of Neural Science* (4th Edition). Norwalk, CT: Appleton & Lange. 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. and Cover, J. A. (1998). *Philosophy of Science: The Central Issues*. New York: W. W. Norton. A wonderful introduction to philosophy of science in the 20th century. The book is actually an anthology, but Curd and Cover tie the various articles together with introductory essays. Among the various authors included in the anthology are Karl Popper, Thomas Kuhn,W. V. Quine, Carl Hempel, and Larry Laudan. (Important French authors such as Gaston Bachelard and Michel Foucault are not included.)

WEB SITES TO EXPLORE

Biological Clocks Program at Texas A&M University:
http://www.bio.tamu.edu/clocks/

Biological Clocks Program at the University of Houston:
http://www.bchs.uh.edu/research_clocks.htm

Center for Biological Timing at the University of Virginia:
http://www.cbt.virginia.edu

Center for Chronobiology at the University of Surrey:
http://www.surrey.ac.uk/SBMS/centre_for_chronobiology/

Center for Sleep & Circadian Biology, Northwestern Univ.:
http://www.northwestern.edu/cscb

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

Swiss Center for Chronobiology:
http://www.chronobiology.ch

LITERATURE CITED

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. & 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: J. B. Baillière.
9. Plato (1985). *The Republic* (Trans. by R. W. Sterling and W. C. Scott). New York: Norton.
10. Galilei, G. (1933). Il saggiatore. In: *Le Opere di Galileo Galilei, Volume 6*. Firenze: G. Barbera.
11. Einstein, A. (1955). *The Meaning of Relativity*, 5th Edition. Princeton, NJ: Princeton University Press.
12. Committee on the Conduct of Science (1989). *On Being a Scientist*. Washington, DC: National Academy Press.
13. Waterfield, R. (2000). *The First Philosophers: The Presocratics and Sophists*. New York: Oxford University Press.
14. Kuhn, T. S. (1962). *The Structure of Scientific Revolutions*. Chicago, IL: University of Chicago Press.

15. Bellone, E. (1978). Thomas Kuhn and the Book of Nature. *Scientia* 113: 683–688.
16. Lyotard, J. F. (1979). *La Condition Postmoderne: Rapport sur le Savoir.* Paris: Minuit.
17. Derrida, J. (1967). *La Voix et le Phénomène*. Paris: Presses Universitaires de France.
18. Piaget, J. (1968). *Le Structuralisme*. Paris: Presses Universitaires de France.
19. Auzias, J. M. (1971). *Clefs pour le Structuralisme*. Paris: Seghers.
20. Foucault, M. (1969). *L'Archéologie du Savoir.* Paris: Gallimard.
21. Foucault, M. (1966). *Les Mots et les Choses*. Paris: Gallimard.
22. von Glasersfeld, E. (1984). An introduction to radical constructivism. In: Watzlawick, P. (Ed.). *The Invented Reality.* New York: Norton, pp. 17–40.
23. Klotz, I. M. (1996). Postmodernist rhetoric does not change fundamental scientific facts. *Scientist* 10(15): 9.
24. Levitt, N. & Gross, P. R. (1996). Academic anti-science. *Academe* 82(6): 38–42.
25. Hodgkin, P. (1996). Medicine, postmodernism, and the end of certainty. *British Medical Journal* 313: 1568.
26. Horowitz, I. L. (1995). Searching for enemies. *Society* 33: 42–50.
27. 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.
28. Refinetti, R. (1997). Philosophy of science and physiology education. *American Journal of Physiology* 272: S31–S35.
29. Nickles, T. (1987). The reconstruction of scientific knowledge. *Philosophy and Social Action* 13: 91–104.
30. Miller, D. (1999). Being an absolute skeptic. *Science* 284: 1625–1626.
31. Gould, S. J. (2000). Deconstructing the "science wars" by reconstructing an old mold. *Science* 287: 253–261.
32. Hegel, G.W.F. (1964). *Phänomenologie des Geistes*. Stuttgart: F. Frommann.
33. Fougeyrollas, P. (1960). *La Philosophie en Question*. Paris: Denoël.
34. American Association for the Advancement of Science (1994). *Benchmarks for Science Literacy.* New York: Oxford University Press.
35. Medawar, P. B. (1979). *Advice to a Young Scientist*. New York: Basic Books.
36. Feyerabend, P. (1975). *Against Method: Outline of an Anarchistic Theory of Knowledge*. London: NLB.
37. Carey, S. S. (2004). A *Beginner's Guide to Scientific Method*, 3rd Edition. Belmont, CA.: Wadsworth.
38. Gower, B. (1997). *Scientific Method: A Historical and Philosophical Introduction*. London: Routledge.
39. Descartes, R. (2001). *Discourse on Method* (Trans. by J. Veitch). London: J. M. Dent.
40. Platt, J. R. (1964). Strong inference. *Science* 146: 347–353.
41. Paydarfar, D. & Schwartz, W. J. (2001). An algorithm for discovery. *Science* 292: 13.
42. D'Espagnat, B. (1979). The quantum theory and reality. *Scientific American* 241(5): 128–140.
43. Hilborn, R. C. (2000). *Chaos and Nonlinear Dynamics*, 2nd Edition. New York: Oxford University Press.
44. Efron, B. (2004). Statistics and the rules of science. *American Statistical Association News* 325: 2–3.
45. Bunk, S. (1998). Ethical debate on placebo use may prompt new trial designs. *Scientist* 12(8): 1, 7, 14.
46. Brown, W. A. (1998). Alternative medicine: it's time to get smart. *Scientist* 12(24): 13.
47. Popper, K. (1959). *The Logic of Scientific Discovery.* New York: Harper & Row.
48. Hurlburt, R. T. (2003). *Comprehending Behavioral Statistics*, 3rd Edition. Belmont, CA: Wadsworth.
49. Sprinthall, R. C. (2003). *Basic Statistical Analysis*, 7th Edition. Boston, MA: Allyn and Bacon.
50. Moore, D. S. (2004). *The Basic Practice of Statistics*, 3rd Edition. New York: Freeman.
51. Weiss, N. A. (2005). *Elementary Statistics*, 6th Edition. Reading, MA: Addison-Wesley.
52. Bennett, K. A., McConnell, B. J. & 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.
53. Berger, A., Scheibe, K. M., Michaelis, S. & 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.
54. Merrill, S. B. & Mech, L. D. (2003). The usefulness of GPS telemetry to study wolf circadian and social activity. *Wildlife Society Bulletin* 31: 947–960.
55. Meyer-Peters, H. (1993). Seasonality of circadian locomotor activity in an insect. *Journal of Comparative Physiology A* 171: 713–724.
56. Shimizu, I., Kawai, Y., Taniguchi, M. & Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
57. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T. & 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.
58. Albers, H. E., Gerall, A. A. & Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
59. Boulos, Z., Macchi, M., Houpt, T. A. & Terman, M. (1996). Photic entrainment in hamsters: effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
60. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
61. Antinuchi, C. D., Luna, F. & Busch, C. (1999). Automatic data recording of circadian rhythms. *Journal of Biological Education* 33: 220–222.

62. Oshima, I. & 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.
63. Sharma, V. K. (2003). A simple computer-aided device for monitoring activity of small mammals and insects. *Biological Rhythm Research* 34: 3–12.
64. Smith, T. L., Coleman, T. G., Stanek, K. A. & Murphy, W. R. (1987). Hemodynamic monitoring for 24 h in unanesthetized rats. *American Journal of Physiology* 253: H1335-H1341.
65. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M. & 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.
66. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D. & Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
67. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P. & Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
68. Refinetti, R. & Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
69. Kramer, K., Grimbergen, J., Brockway, B., Brockway, B. & Voss, H. P. (1999). Circadian respiratory rate rhythms in freely moving small laboratory animals using radio telemetry. *Lab Animal* 28(4): 38–41.
70. Ishii, K., Uchino, M., Kuwahara, M., Tsubone, H. & 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.
71. Ray, B., Mallick, H. N. & 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.
72. Refinetti, R. (1990). Experimentally induced disruption of the diurnal rhythm of body temperature of the rat. *Biotemas* 3(2): 47–58.
73. Tornatzky, W. & Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior* 53: 983–993.
74. Weinert, D., Uhlemann, S. & 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.
75. Leon, L. R., Walker, L. D., DuBose, D. A. & Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
76. Kolka, M. A., Levine, L. & Stephenson, L. A. (1997). Use of ingestible telemetry sensor to measure core temperature under chemical protective clothing. *Journal of Thermal Biology* 22: 343–349.
77. Rawson, R. O., Stolwijk, J. A. J., Graichen, H. & Abrams, R. (1965). Continuous radio telemetry of hypothalamic temperatures from unrestrained animals. *Journal of Applied Physiology* 20: 321–325.
78. Spencer, H. (1968). Thermally stable telemeter for thermoregulation studies. *Science* 161: 574–575.
79. 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.
80. Thornhill, J. A., Hirst, M. & 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.
81. Cunningham, C. L. & Peris, J. (1983). A microcomputer system for temperature biotelemetry. *Behavior Research Methods and Instrumentation* 15: 598–603.
82. Varosi, S. M., Brigmon, R. L. & Besch, E. L. (1990). A simplified telemetry system for monitoring body temperature in small animals. *Laboratory Animal Science* 40: 299–302.
83. Chesmore, E. D., Yoon, C. S., Talling, J. C., van Driel, K. S. & 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: Wageningen Academic Publishers, pp. 33–37.
84. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A. & 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.
85. Moore, R. J., Watts, J. T., Hood, J. A. & Burritt, D. J. (1999). Intra-oral temperature variation over 24 hours. *European Journal of Orthodontics* 21: 249–261.
86. Nocek, J. E., Allman, J. G. & Kautz, W. P. (2002). Evaluation of an indewelling ruminal probe methodology and effect of grain level on diurnal pH variation in dairy cattle. *Journal of Dairy Science* 85: 422–428.
87. Hermida, R. C., Fernández, J. R., Ayala, D. E., Mojón, A., Alonso, I. & Calvo, C. (2004). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiology International* 21: 147–160.
88. Adolph, E. F. (1950). Oxygen consumption of hypothermic rats and acclimatization to cold. *American Journal of Physiology* 161: 359–373.
89. Lindmark, L., Rudholm, L. & 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.

90. 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: Imprimerie Impériale (1862), pp. 174–183.
91. Lavoisier, A. L. & Laplace, P. S. de (1780). Mémoire sur la chaleur (*Mémoires de l'Academie des Sciences*, 1780, p. 355). In: *Oeuvres de Lavoisier, Tome II*. Paris: Imprimerie Impériale (1862), pp. 283–333.
92. 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.
93. Horst, K., Mendel, L. B. & Benedict, F. G. (1930). The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. *Journal of Nutrition* 3: 177–200.
94. 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.
95. Swift, R. W. & Forbes, R. M. (1939). The heat production of the fasting rat in relation to the environmental temperature. *Journal of Nutrition* 18: 307–318.
96. Pagé, E. & 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.
97. Nakatsuka, H., Shoji, Y. & Tsuda, T. (1983). Effects of cold exposure on gaseous metabolism and body composition in the rat. *Comparative Biochemistry and Physiology A* 75: 21–25.
98. Diamond, P. & LeBlanc, J. (1987). Hormonal control of postprandial thermogenesis in dogs. *American Journal of Physiology* 253: E521–E529.
99. Refinetti, R. (1989). Effect of ambient temperature on respiratory quotient of lean and obese Zucker rats. *American Journal of Physiology* 256: R236–R239.
100. McKechnie, A. E. & 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.
101. Livesey, G. & 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.
102. Mansell, P. I. & Macdonald, I. A. (1990). Reappraisal of the Weir equation for calculation of metabolic rate. *American Journal of Physiology* 258: R1347–R1354.
103. Barlow, C. A. & Kuenen, D. J. (1957). A new thermopreferendum apparatus for terrestrial isopods. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Ser. C* 60: 240–250.
104. Krafft, B. (1967). Thermopreferendum de l'araignée sociale *Agelena consociata* Denis. *Insectes Sociaux* 14: 161–182.
105. Laughter, J. S. & Blatteis, C. M. (1985). A system for the study of behavioral thermoregulation of small animals. *Physiology and Behavior* 35: 993–997.
106. Gordon, C. J., Lee, K. A., Chen, T. A., Killough, P. & Ali, J. S. (1991). Dynamics of behavioral thermoregulation in the rat. *American Journal of Physiology* 261: R705–R711.
107. 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.
108. Refinetti, R. (1995). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behavioral Neuroscience* 109: 523–527.
109. Cheng, P., Yang, Y. & Liu, Y. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 98: 7408–7413.
110. Froehlich, A. C., Liu, Y., Loros, J. J. & Dunlap, J. C. (2002). White collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. *Science* 297: 815–819.
111. Vitalini, M. W., Morgan, L. W., March, I. J. & Bell-Pedersen, D. (2004). A genetic selection for circadian output pathway mutations in *Neurospora crassa*. *Genetics* 167: 119–129.
112. Horne, J. A. & Östberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology* 4: 97–110.
113. Koscec, A., Radosevic-Vidacek, B. & Kostovic, M. (2001). Morningness-eveningness across two student generations: would two decades make a difference? *Personality and Individual Differences* 31: 627–638.
114. Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M. & Tisak, J. (2002). Investigation of morning-evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.
115. Roenneberg, T., Wirz-Justice, A. & Merrow, M. (2003). Life between clocks: daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
116. Di Milia, L., Smith, P. A. & Folkard, S. (2004). Refining the psychometric properties of the circadian type inventory. *Personality and Individual Differences* 36: 1953–1964.
117. Shanahan, T. L. & 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.
118. Kennaway, D. J. & 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.
119. Guerin, M. V., Deed, J. R., Kennaway, D. J. & 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.

120. Irvine, C. H. G. & Alexander, S. L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology* 11: 227–238.
121. Thrun, L. A., Moenter, S. M., O'Callaghan, D., Woodfill, C. J. I. & Karsch, F. J. (1995). Circannual alterations in the circadian rhythm of melatonin secretion. *Journal of Biological Rhythms* 10: 42–54.
122. Piccione, G., Caola, G. & 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.
123. Wright, K. P., Hughes, R. J., Kronauer, R. E., Dijk, D. J. & 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 U.S.A.* 98: 14027–14032.
124. Ruiter, M., La Fleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A. & 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.
125. Aschoff, J., Gerecke, U. & Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
126. Hack, L. M., Lockley, S. W., Arendt, J. & 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.
127. Moore-Ede, M. C., Kass, D. A. & Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
128. Roelfsema, F., van der Heide, D. & Smeenk, D. (1980). Circadian rhythms of urinary electrolyte excretion in freely moving rats. *Life Sciences* 27: 2303–2309.
129. Voultsios, A., Kennaway, D. J. & Dawson, D. (1997). Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. *Journal of Biological Rhythms* 12: 457–466.
130. Jelínková-Vondrasová, D., Hájek, I. & 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.
131. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C. & 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.
132. Hasegawa, M. & Ebihara, S. (1992). Circadian rhythms of pineal melatonin release in the pigeon measured by *in vivo* microdialysis. *Neuroscience Letters* 148: 89–92.
133. Nakahara, D., Nakamura, M., Iigo, M. & Okamura, H. (2003). Bimodal circadian secretion of melatonin from the pineal gland in a living CBA mouse. *Proceedings of the National Academy of Sciences U.S.A.* 100: 9584–9589.
134. Maywood, E. S., Hastings, M. H., Max, M., Ampleford, E., Menaker, M. & 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.
135. Sun, X., Liu, T., Deng, J. & Borjigin, J. (2003). Long-term *in vivo* pineal microdialysis. *Journal of Pineal Research* 35: 118–124.
136. Schneider, B. H., Hill, M. R. S. & Prohaska, O. J. (1990). Microelectrode probes for biomedical applications. *American Biotechnology Laboratory* 8(2): 17–23.
137. Foster, D. O. & 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.
138. Klaunberg, B. A. & Lizak, M. J. (2004). Considerations for setting up a small-animal imaging facility. *Lab Animal* 33(3): 28–34.
139. Kasischke, K. A., Vishwasrao, H. D., Fisher, P. J., Zipfel, W. R. & Webb, W. W. (2004). Neural activity triggers neuronal oxidative metabolism followed by astrocytic glycolysis. *Science* 305: 99–103.
140. 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.
141. Borowsky, I. W. & 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.
142. Schwartz, W. J., Davidsen, L. C. & Smith, C. B. (1980). *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *Journal of Comparative Neurology* 189: 157–167.
143. Room, P., Tielemans, A. J. P. C., De Boer, T., Tonnaer, J. A. D. M., Wester, J., Van den Broek, J. H. M. & 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.
144. Servière, J., Gendrot, G., LeSauter, J. & Silver, R. (1994). Host resets phase of grafted suprachiasmatic nucleus: a 2-DG study of time course of entrainment. *Brain Research* 655: 168–176.
145. Pellerin, L. & Magistretti, P. J. (2004). Let there be (NADH) light. *Science* 305: 50–52.
146. Morgan, J. I. & Curran, T. (1989). Stimulus-transcription coupling in neurons: role of cellular immediate-early genes. *Trends in Neural Science* 12: 459–462.
147. Dragunow, M. & Faull, R. (1989). The use of c-*fos* as a metabolic marker in neuronal pathway tracing. *Journal of Neuroscience Methods* 29: 261–265.
148. Prosser, R. A., Macdonald, E. S. & 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.

149. Krajnak, K., Dickenson, L. & 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.
150. Novak, C. M. & 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.
151. Sumová, A., Trávnícková, Z., Mikkelsen, J. D. & Illnerová, H. (1998). Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research* 801: 254–258.
152. Caldelas, I., Salazar-Juárez, A., Granados-Fuentes, D., Escobar, C. & 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.
153. Novak, C. M., Smale, L. & 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.
154. Guido, M. E., de Guido, L. B., Goguen, D., Robertson, H. A. & Rusak, B. (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *Journal of Biological Rhythms* 14: 275–280.
155. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M. & Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685.
156. Asai, M., Yamaguchi, S., Isejima, H., Janouchi, M., Moriya, T., Shibata, S., Kobayashi, M. & 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.
157. Izumo, M., Johnson, C. H. & 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 U.S.A.* 100: 16089–16094.
158. Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M., Hong, H. K., Oh, W. J., Yoo, O. J., Menaker, M. & Takahashi, J. S. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences U.S.A.* 101: 5339–5346.
159. Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S. & 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 U.S.A.* 90: 5672–5676.
160. Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-ike, N. & 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.
161. Rumsey, W. L., Vanderkooi, J. M. & Wilson, D. F. (1988). Imaging of phosphorescence: a novel method for measuring oxygen distribution in perfused tissue. *Science* 241: 1649–1651.
162. Seydel, C. (2003). Quantum dots get wet. *Science* 300: 80–81.
163. Larson, D. R., Zipfel, W. R., Williams, R. M., Clark, S. W., Bruchez, M. P., Wise, F. W. & Webb, W. W. (2003). Water-soluble quantum dots for multiphoton fluorescence imaging *in vivo*. *Science* 300: 1434–1436.
164. Bailey, R. E. & 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.
165. Bozza, T., McGann, J. P., Mombaerts, P. & Wachowiak, M. (2004). *In vivo* imaging of neuronal activity by targeted expression of a genetically encoded probe in the mouse. *Neuron* 42: 9–21.
166. Stephens, D. J. & Allan, V. J. (2003). Light microscopy techniques for live cell imaging. *Science* 300: 82–86.
167. Lucas, K. (1905). On the gradation of activity in a skeletal muscle-fibre. *Journal of Physiology* 33: 125–137.
168. Adrian, E. D. (1914). The all-or-none principle in nerve. *Journal of Physiology* 47: 460–474.
169. Hodgkin, A. L. & Huxley, A. F. (1939). Action potentials recorded from inside a nerve fibre. *Nature* 144: 710–711.
170. Meijer, J. H., Schaap, J., Watanabe, K. & Albus, H. (1997). Multi-unit activity recordings in the suprachiasmatic nuclei: *in vivo* versus *in vitro* models. *Brain Research* 753: 322–327.
171. 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.
172. Mrugala, M., Zlomanczuk, P., Jagota, A. & Schwartz, W. J. (2000). Rhythmic multi-unit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *American Journal of Physiology* 278: R987–R994.
173. Colwell, C. S. (2000). Rhythmic coupling among cells in the suprachiasmatic nucleus. *Journal of Neurobiology* 43: 379–388.
174. Saeb-Parsy, K. & 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.
175. Burgoon, P. W., Lindberg, P. T. & 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.
176. Silverman, P. H. (2004). Rethinking genetic determinism. *Scientist* 18(10): 32–33.
177. Chin, J. W., Cropp, T. A., Anderson, J. C., Mukherji, M., Zhang, Z. & Schultz, P. G. (2003). An expanded eukaryotic genetic code. *Science* 301: 964–967.

178. DePalma, A. (2003). Mass spectrometry and proteomics. *Scientist* 17(16): 44–46.
179. Grigorenko, E. V. (2002). *DNA Arrays: Technologies and Experimental Strategies*. Boca Raton, FL: CRC Press.
180. Correa, A., Lewis, Z. A., Greene, A. V., March, I. J., Gomer, R. H. & Bell-Pedersen, D. (2003). Multiple oscillators regulate circadian gene expression in *Neurospora*. *Proceedings of the National Academy of Sciences U.S.A.* 100: 13597–13602.
181. McDonald, M. J. & Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* 107: 567–578.
182. Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N. & Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32: 657–671.
183. Ceriani, M. F., Hogenesch, J. B., Yanovsky, M., Panda, S., Straume, M. & Kay, S. A. (2002). Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *Journal of Neuroscience* 22: 9305–9319.
184. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N. & 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 U.S.A.* 99: 9562–9567.
185. Kita, Y., Shiozawa, M., Jin, W., Majewski, R. R., Besharse, J. C., Greene, A. S. & Jacob, H. J. (2002). Implications of circadian gene expression in kidney, liver and the effects of fasting on pharmacogenomic studies. *Pharmacogenetics* 12: 55–65.
186. Grundschober, C., Delaunay, F., Pühlhofer, A., Triqueneaux, G., Laudet, V., Bartfai, T. & Nef, P. (2001). Circadian regulation of diverse gene products revealed by mRNA expression profiling of synchronized fibroblasts. *Journal of Biological Chemistry* 276: 46751–46758.
187. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H. & 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.
188. Duffield, G. E., Best, J. D., Meurers, B. H., Bittner, A., Loros, J. J. & 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.
189. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H. & Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.
190. Lane, L. (2003). Protein microarrays at the cusp. *Scientist* 17(14): 42–44.
191. Melton, L. (2004). Proteomics in multiplex. *Nature* 429: 101–107.
192. Ramachandran, N., Hainsworth, E., Bhullar, B., Eisenstein, S., Rosen, B., Lau, A. Y., Walter, J. C. & LaBaer, J. (2004). Self-assembling protein microarrays. *Science* 305: 86–90.
193. Uttal, W. R. (1973). *The Psychobiology of Sensory Coding*. New York: Harper & Row.
194. Zaha, M. A. (1972). Shedding some needed light on optical measurements. *Electronics* Nov 6: 91–96.
195. Wyszecki, G. & Stiles, W. S. (1982). *Color Science: Concepts and Methods*, 2nd Edition. New York: Wiley.
196. Johnston, S. F. (2001). *A History of Light and Colour Measurement: Science in the Shadows*. Bristol: Institute of Physics.
197. Cossins, A. R. & Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman and Hall.
198. Lide, D. R. (Ed.) (2002). *Handbook of Chemistry and Physics*, 83rd Edition. Boca Raton, FL: CRC Press.
199. Middleton, W. E. K. (1966). *A History of the Thermometer and Its Use in Meteorology.* Baltimore, MD: Johns Hopkins Press.
200. Christian, K. A. & Tracy, C. R. (1985). Measuring air temperature in field studies. *Journal of Thermal Biology* 10: 55–56.
201. Walsberg, G. E. & Weathers, W. W. (1986). A simple technique for estimating operative environmental temperature. *Journal of Thermal Biology* 11: 67–72.
202. Rea, M. S. (Ed.) (2000). *The IESNA Lighting Handbook*, 9th Edition. New York: Illuminating Engineering Society of North America.
203. Nelson, D. E. & 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.
204. Ralph, M. R. & Mrosovsky, N. (1992). Behavioral inhibition of circadian responses to light. *Journal of Biological Rhythms* 7: 353–359.
205. Shimomura, K. & Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
206. Challet, E., Losee-Olson, S. & Turek, F. W. (1999). Reduced glucose availability attenuates circadian responses to light in mice. *American Journal of Physiology* 276: R1063–R1070.
207. Boulos, Z., Macchi, M. M. & Terman, M. (2002). Twilights widen the range of photic entrainment in hamsters. *Journal of Biological Rhythms* 17: 353–363.
208. Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Lelièvre, V., Hu, Z. & Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology* 287: R1194–R1201.
209. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & 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.
210. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M. & Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.

211. Best, J. D., Maywood, E. S., Smith, K. L. & Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *Journal of Neuroscience* 19: 828–835.
212. Canal-Corretger, M. M., Vilaplana, J., Cambras, T. & 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.
213. Evans, J. A., Elliott, J. A. & Gorman, M. R. (2004). Photoperiod differentially modulates photic and nonphotic phase response curves of hamsters. *American Journal of Physiology* 286: R539–R546.
214. Schwartz, M. D., Nunez, A. A. & Smale, L. (2004). Differences in the suprachiasmatic nucleus and lower subparaventricular zone of diurnal and nocturnal rodents. *Neuroscience* 127: 13–23.
215. McLennan, I. S. & Taylor-Jeffs, J. (2004). The use of sodium lamps to brightly illuminate mouse houses during their dark phases. *Laboratory Animals* 38: 384–392.
216. Gauch, H. G. (2003). *Scientific Method in Practice*. Cambridge, UK: Cambridge University Press.

3 Analysis of Circadian Rhythmicity

CHAPTER OUTLINE

3.1 Data Analysis
3.2 Mean Level, Amplitude, and Phase
3.3 Period, Waveform, and Robustness
3.4 Statistical Significance

3.1 DATA ANALYSIS

Data analysis in circadian physiology mostly consists of identifying circadian rhythmicity in data sets that naturally contain many rhythmic and nonrhythmic components. Because the data points in a data set refer to successive observations made over time, the set is often called a *time series*. Several books on time series analysis are available[1–5] although most of them deal with economic issues, such as fluctuations in stock-market prices, and none of them focuses in detail on circadian rhythms. These books make a distinction between analysis in the "time domain" and analysis in the "frequency domain." The distinction concerns the methods used for analysis: methods in the *time domain* look for regularities in a time series itself, while methods in the *frequency domain* treat the time series as a composite of underlying oscillatory processes. Both classes of methods are used to analyze circadian rhythms.

The top panel of Figure 3.1 shows the body temperature data of a female golden hamster. Using a computer, I generated these "idealized" data for didactical purposes. Note 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. This pattern repeats itself day after day, except that a higher midday peak is reached every 4 days. If this data set were a natural data set, collected from a real hamster, I might have to limit myself to this description of the time series (that is, analysis in the *time domain*). I could probably calculate an equation that described body temperature as a function of time, but I would not be able to go any further. However, I *can* go further because I created the data set artificially, and I know exactly how it was generated.

The four lower panels in Figure 3.1 show the four components used to build the data set in the top panel. These components include 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"). These four components correspond to, and simulate, four processes known to affect the body temperature of a female hamster: A) circadian rhythmicity, B) ultradian rhythmicity, C) estrous rhythmicity, and D) biological noise. Even if I had not created the data set artificially, I could still find out that it includes the four components if I conducted *spectral analysis* (that is, analysis in the *frequency domain*). This example should remind you that, when researchers analyze circadian rhythms, they concentrate 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.[6,7]

Data analysis in general consists of two basic types: graphical or numerical. Graphical analysis relies on the observation of a graphical display of the data, while numerical analysis involves the computation of one or more *statistics* derived from raw data. For example, I asked the students in my Human Sexuality class to record the times at which they had sex over several weeks. Eleven students (ranging in age from 18 to 51 years) provided usable data sheets, which documented 71 sexual encounters in 2 weeks. A small sample of these sheets is shown in Figure 3.2. A simple way to analyze these 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.

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 example, 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; the daily feeding time is also

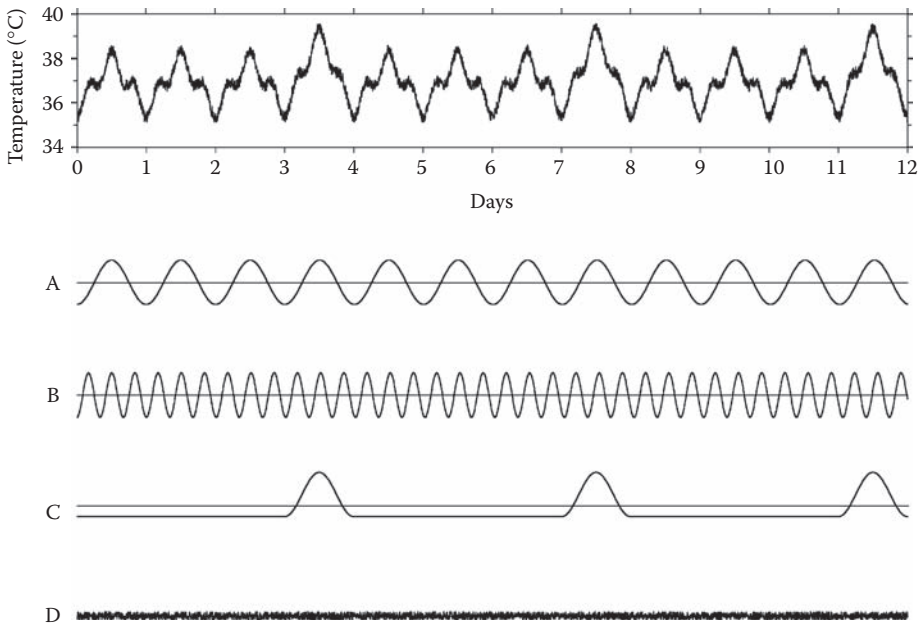


FIGURE 3.1 Composite rhythmicity. This 12-day-long data set with 6-minute resolution depicts an "idealized" record of the body temperature of a female golden hamster. The composite oscillatory pattern includes periodicities of 8, 24, and 96 hours, as well as high-frequency noise.

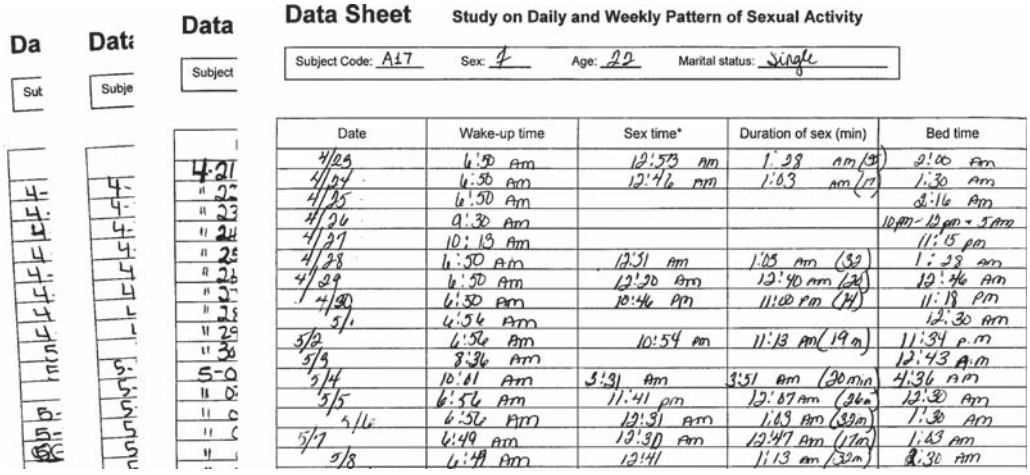


FIGURE 3.2 Have you had sex today? This figure shows parts of four representative data sheets from a study on the daily and weekly patterns of sexual activity of university students.

provided. The graphs 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 a procedure described in Section 3.2), while the length of the vector indicates the strength of rhythmicity (its "robustness," as defined in Section 3.3). It can be seen easily, for example, that the rhythm of blood cholesterol (C) is weaker and occurs later in the day than the rhythm of rectal temperature (T). In this case, numerical analysis provides more useful information about the data than graphical analysis alone. When numerical analysis is used, however, basic graphical analysis should be performed first. As Chris Chatfield — a statistics professor at the University of Bath (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

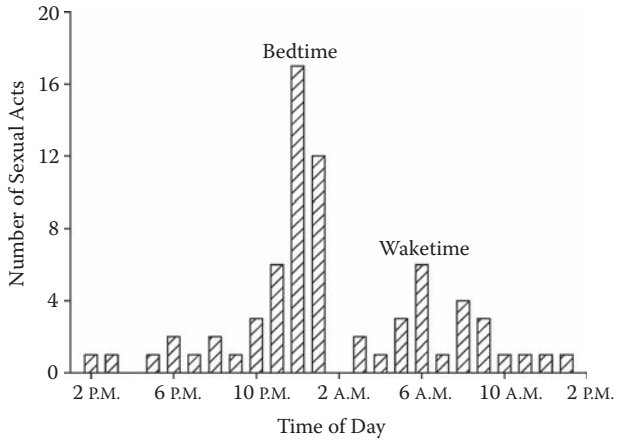


FIGURE 3.3 Time for sex. According to the results of a simple study conducted by the author, bedtime is the most popular time for sex.

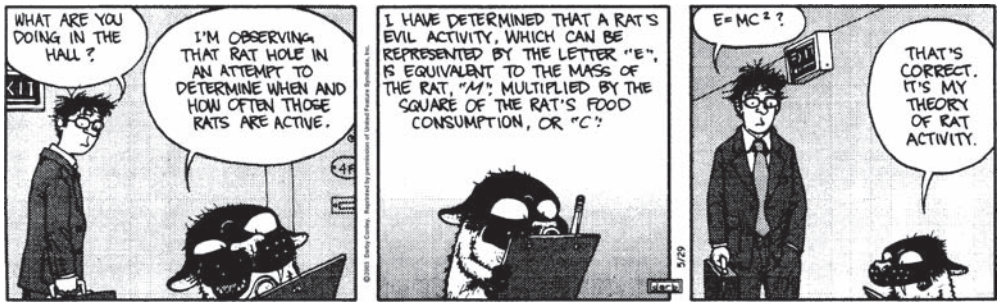


FIGURE 3.4 The importance of quantitative data analysis. As suggested by this comic strip from the cartoon *Get Fuzzy*, by Darby Conley, rigorous data collection can lead to detailed numerical analysis. (Source: © 2003 Darby Conley. Get Fuzzy reprinted by permission of United Feature Syndicate, Inc.)

and simple inspection of data sets as Cartesian plots (that is, as values plotted on the Y-axis against time in the X-axis). You may have used this program if you completed Exercise 2.2 in the preceding chapter.

When circadian physiologists analyze a time series to detect the presence of circadian rhythmicity, they 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 the top panel of Figure 3.7. *Oscillation* is commonly used to refer to the swinging of a pendulum (second panel), while *cycle* is associated with a circular pattern (third panel), and *wave* refers either to a swell moving along the surface of a body of water or to a regular repetitive process (bottom panel). 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), while *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). Circadian physiologists do not generally use *wave*, although it can be helpful in the formal analysis of rhythmic processes. For example, if you throw a rock in a pond, you generate a wave pattern (Figure 3.8). If you submerge yourself in the water, leaving only your eyes above the surface, you can 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 is 2^{-1} Hz (or 0.5 Hz) — which means that half a wave occurs each second, or one wave occurs every 2 seconds. *Mean level* is the water level

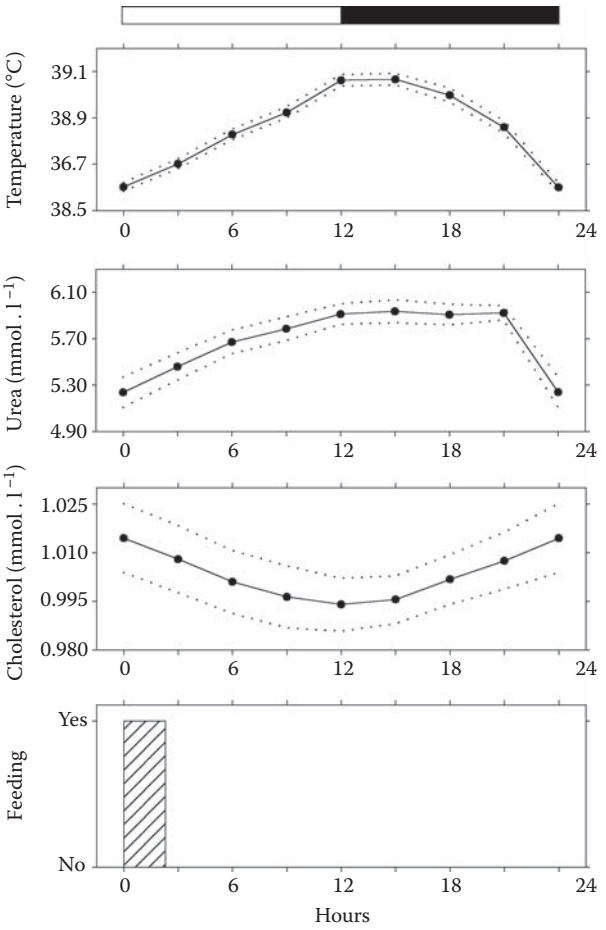


FIGURE 3.5 Daily rhythms in goats. This figure shows the mean values of rectal temperature, plasma concentration of urea, and plasma concentration of cholesterol measured at 3-hour intervals in goats; the daily feeding time is also provided. The data points represent the mean values for five goats, each 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. (Source: Piccione, G., Caola, G., & 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.)

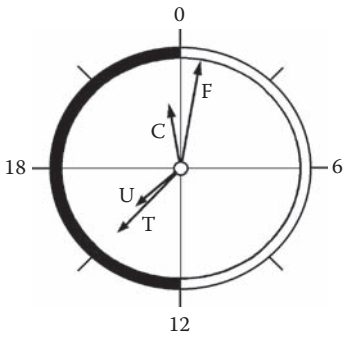


FIGURE 3.6 Vectors in a circle. This figure presents the results of numerical data analysis (time and magnitude of the daily peaks) of the four rhythms shown in Figure 3.5. (Note: F = feeding, C = cholesterol, T = temperature, U = urea.)

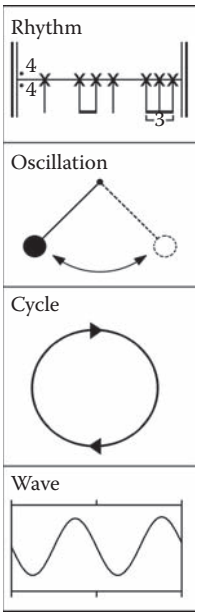


FIGURE 3.7 Periodic events. At least four words in English refer to periodic events: *rhythm*, *oscillation*, *cycle*, and *wave*.

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. When analyzing circadian rhythms, two additional parameters must be considered: 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, I assumed that you would not stay submerged in water for a long period of time 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, I assumed that you took it for granted that the waves were *stationary* (which is a technical term in time series analysis). When I say that waves are stationary (or that they exhibit *stationarity*), I 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 I analyze the first wave, the tenth wave, or the millionth wave. In other words, stationary waves are stationary because they always remain the same. As Section 3.3 demonstrates, circadian rhythms are *not* stationary. The closer a rhythm is to stationarity, the greater is its *robustness*.

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

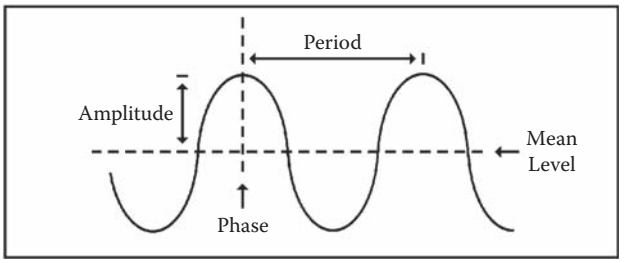


FIGURE 3.9 Rhythmic parameters. A stationary sinusoidal wave can be fully characterized by four parameters: mean level, amplitude, period, and phase.

3.2 MEAN LEVEL, AMPLITUDE, AND PHASE

Before examining the six parameters that characterize circadian rhythms, I first 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 the top panel of Figure 3.10. It consists of body temperature measurements taken from a male golden hamster every 6 minutes (0.1 hour) by telemetry. Although daily rhythmicity is evident, a great deal of noise (high-frequency oscillations) also seems to be present. To observe the daily rhythmicity more clearly, you may want to filter out the high-frequency oscillations. Two very simple and popular procedures for data filtering include the moving-averages procedure and the plain averaging of two or more contiguous data points. In plain averaging, one uses the arithmetic means of groups of contiguous data points instead of using all data points. For example, the bottom panel in Figure 3.10 shows 4-hour means. Because the original data set contained 10 values per hour, the 4-hour means were based on 40 data points. Note 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: now there is only one data point each 4 hours.

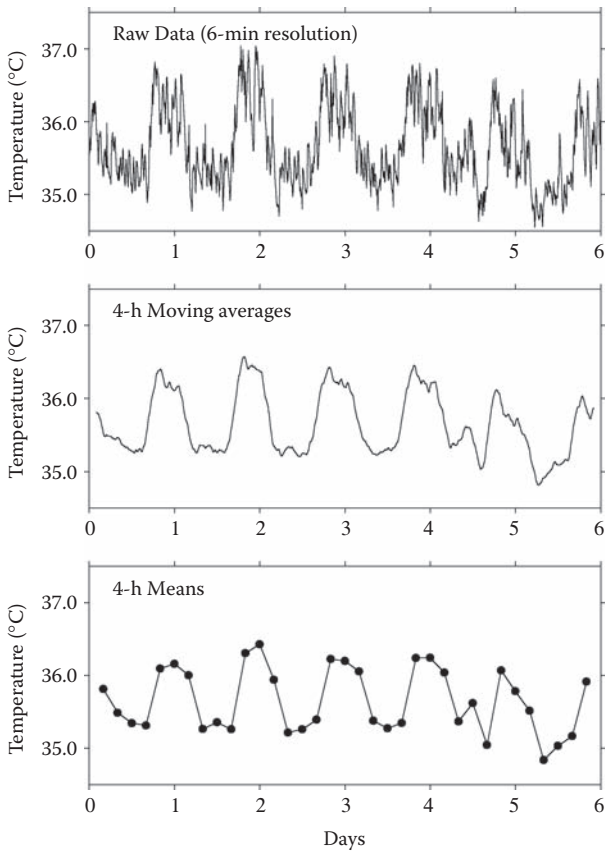


FIGURE 3.10 Smoothing things out. The data set shown in this figure contains the body temperature records of a golden hamster; temperature readings were taken by telemetry every 6 minutes for 6 consecutive days. This figure demonstrates that raw data can be smoothed by a moving averages procedure (in this case, with a 4-hour window size) or by the plain averaging of adjacent data points (also with a 4-hour window size). (Source: Refinetti, R. (1994). Circadian modulation of ultradian oscillation in the body temperature of the golden hamster. *Journal of Thermal Biology* 19: 269–275.)

To avoid this problem, a *moving averages* procedure can be used (middle panel in Figure 3.10). In this case, each data point is replaced by the mean of the 40 data points around it (that is, the 20 data points that precede it and the 20 data points that follow it). 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, the recording equipment may malfunction and generate spurious data points. If few spurious points are produced, 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 to 39°C range), the single spurious value may skew the mean.

TABLE 3.1
The Data Analysis Programs in the Circadian Physiology Software Package

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
Calculation of phase shifts (actogram)	Plot	Regression of onsets	7.1
Analysis of ultradian rhythms	Fourier	Fourier analysis	4.5

Note: This chapter describes the operating principle for each program. Tutorials on using these programs are provided in the exercises at the end of certain 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.

In this case, it may be necessary to accept the fact that errors do occur and, accordingly, 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, and so on. If the data set is *very* bad (with many spurious values, missing values, baseline drifts, and so on), 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.[8,9]

I will now discuss the analysis of the *mean level* of a rhythm (Figure 3.11). The mean of a time series, like the mean of any set of data, measures the "central tendency" of the data — that is, it measures 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, while that in Panel C "tends" to the middle. Three traditional indexes of central tendency include the *mode*, the *median*, and the *mean*, as shown in the figure and as described in introductory statistics textbooks.[10–13] 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 example, the mean of 2, 3, and 7 is 4 — that is: $(2 + 3 + 7) \div 3 = 4$.

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

Return to the pond where you threw a rock earlier, and this time count the number of ducks that you see in the morning and in the evening. Using the diagrams in Figure 3.13, you can see 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, while the mean number in the evening is 3. The mean number for all ten observations (that is, the *mean level* of your time series) is 4.5. Of course, the mean is slightly misleading, since you never observed 4.5 ducks in the pond (to start with, you never saw a half-duck floating around), but you understand that 4.5 is an average value. Your 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) equaled 3 ducks.

Instead of the range, statisticians normally use another index of variability: the *standard deviation* (abbreviated as SD). I will not discuss the concept of standard deviation at this time, but the importance of having some index of variability for the mean (whether it is the range or the standard deviation) must be emphasized. For example, consider the case of two men who lived in a far-away land (Figure 3.14). For 1 month, a whole chicken was fed every day to one of the men and nothing was fed 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, while the other man died of starvation (Panel D)!

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, called *cosinor rhythmometry*.[14]

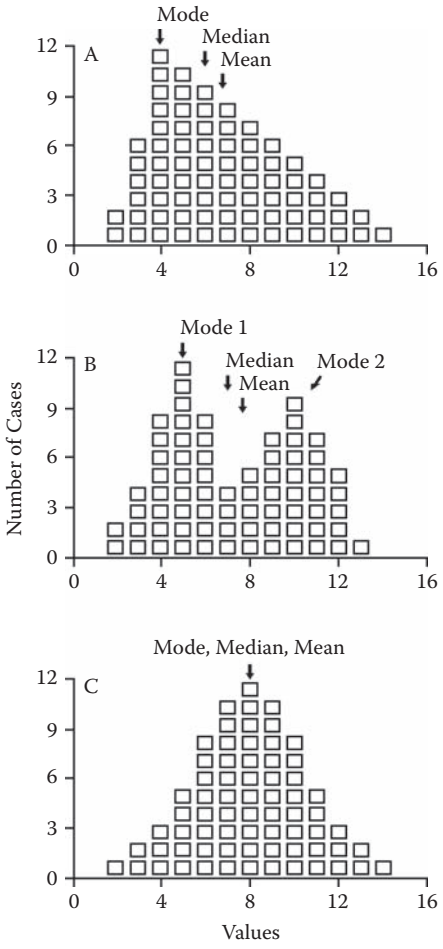


FIGURE 3.12 Measures of central tendency. Mode, median, and mean are three common measures of the central tendency of a distribution. They may be numerically distinct (A and B) or identical (C), depending on the shape of the distribution.

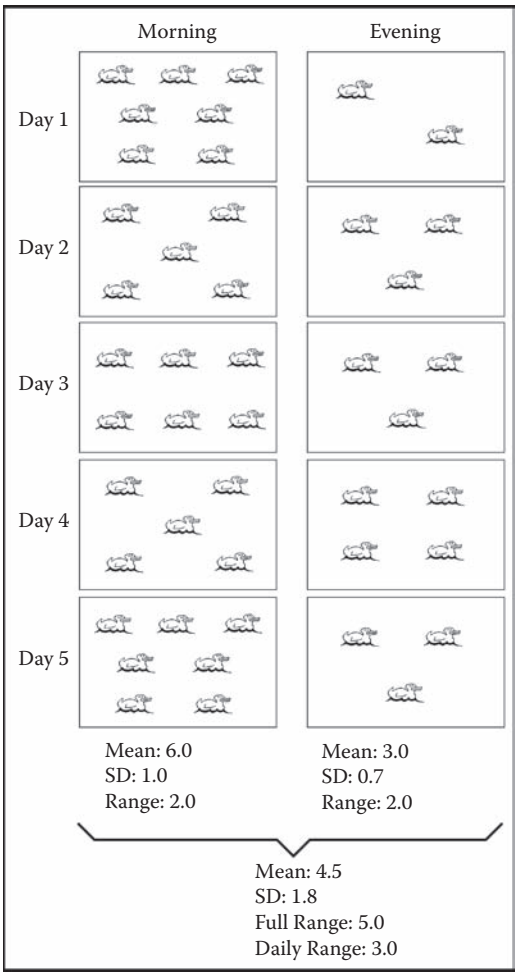


FIGURE 3.13 Mean, standard deviation, range, and amplitude. This simple example of counting the number of ducks in a pond at two times during the day allows for easy computation of measures of central tendency and variability.

Halberg reasoned that, because circadian rhythms can be thought of as smooth rhythms with added noise, a cosine wave could be fitted to the data to estimate the pattern of the smooth rhythm. Figure 3.15 shows two examples of cosine waves fitted to actual rhythms. Note that the fit is very good for the squirrel data (top) but not as good for the tree shrew data (bottom). Of course, more complex mathematical procedures could be used to improve the fit, and some circadian physiologists have followed this route.[15–19] However, the physiological meaning of the procedures is not clear at all. The addition of harmonics to the single cosinor improves the fit, but it also is closer to a spectral analysis of the time series. This analysis identifies multiple oscillatory processes, but one cannot determine whether these oscillatory processes are mere mathematical descriptions of noise inherent in the time series or a reflection of actual biological oscillations. The "single cosinor" method may have its limitations, but it is intuitive and has the advantage of simplicity. If "modeling" is to be used, I favor the single cosinor model. 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).[20]

I now turn to the computation of the *amplitude* of a rhythm (Fig 3.16). Technically, the amplitude of a smooth function equals the distance between the mean value and the peak (or between the mean value and the trough, as it is assumed that peak and trough are equidistant from the mean level). Therefore, the *amplitude* is half the *range of oscillation*. The range of oscillation, however, is more meaningful than the amplitude for circadian physiologists, primarily because it identifies the boundaries of the oscillation, while the amplitude is a concept that may facilitate computations in engineering but often serves only to confuse physiologists and clinicians. In addition, the range of oscillation is more meaningful than the amplitude because real circadian rhythms are not necessarily symmetrical, so

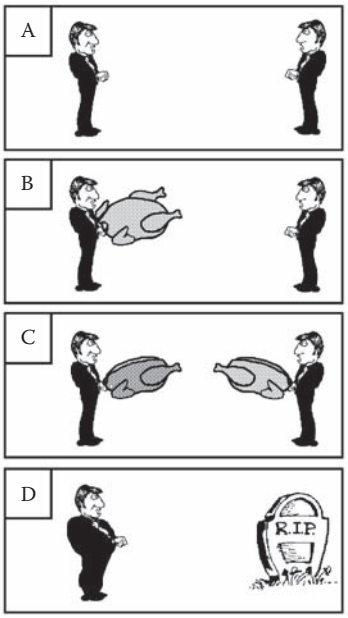


FIGURE 3.14 Means can be deceiving. If two men (A) are fed a chicken each day, they will, on average, be fed half a chicken a day. This average food intake may indicate either that one man eats a whole chicken and the other starves (B) or that each man eats half a chicken (C). Unfortunately, if situation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

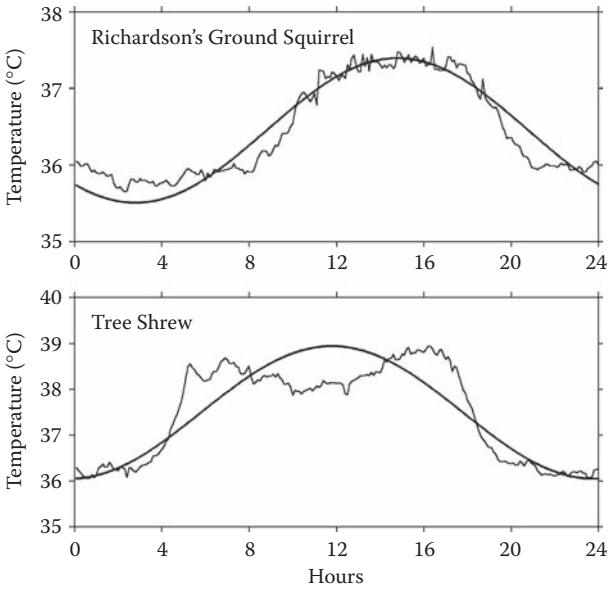


FIGURE 3.15 Cosinor. The cosinor method relies on fitting a cosine wave to the raw data set. This figure shows cosine waves fitted to the body temperature rhythms of a Richardson's ground squirrel and a tree shrew. The mean level of a rhythm calculated by the cosinor method is a rhythm-adjusted mean called *mesor*. (Source: 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.)

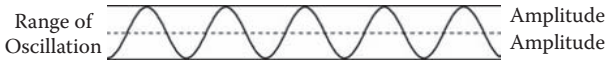


FIGURE 3.16 Amplitude. Amplitude is one of the parameters that characterize a circadian rhythm. For a sinusoidal wave, the amplitude is half the range of oscillation.

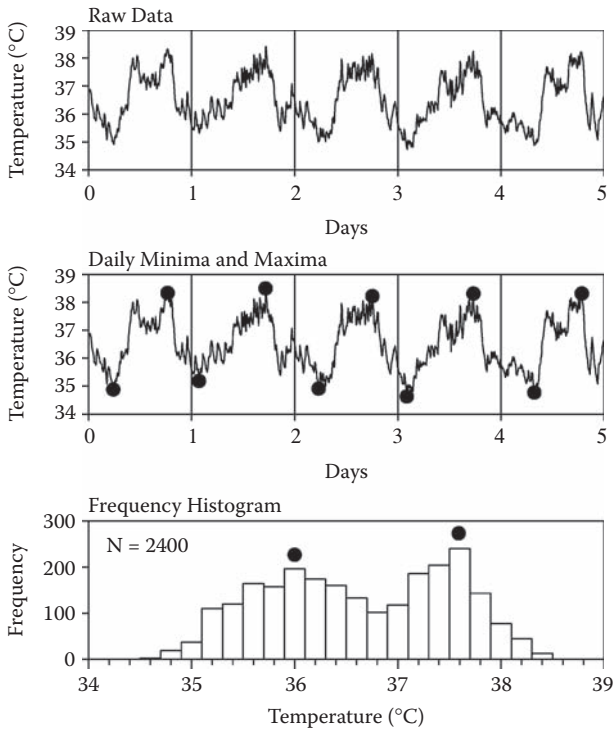


FIGURE 3.17 Standard methods for the calculation of rhythm amplitude. The top panel shows raw data depicting the body temperature rhythm of a fat-tailed gerbil. The middle panel illustrates the maxima-minus-minima method of computing rhythm amplitude, while the bottom panel illustrates the frequency histogram method. (Source: Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology* 119A: 295–300.)

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 is the amplitude (or the range of oscillation) of a rhythm calculated? As you did for the mean level, you can use the actual data or a cosine function fitted to the data. Figure 3.17 shows a 5-day segment of the body temperature records of a fat-tailed gerbil (a small rodent). The top panel shows the raw data, collected in 6-minute intervals. With 240 data points per day, you do not want to search for the daily peaks and troughs manually. A computer could be programmed to search for the peaks and troughs, as shown in the middle panel. If the rhythm is noisy, you can instruct the computer to filter the data prior to computing the daily minima and maxima. To

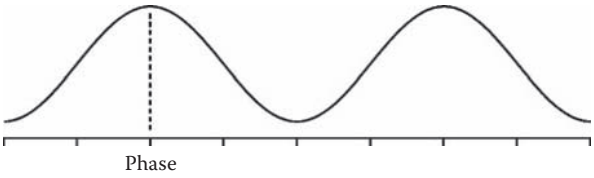


FIGURE 3.18 Phase. Phase is one of the parameters that characterize a circadian rhythm.

calculate the *range of oscillation* for the 5-day interval, you can simply average the five daily ranges of oscillation. To obtain the *amplitude*, 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*,[21] as shown in the bottom panel 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 or the range of oscillation (which is sometimes called "double-amplitude") that are computed by the cosinor method as described by Halberg[14] are always smaller than the values computed by the other two methods discussed here.

The *phase* of a rhythm (Figure 3.18) can also be calculated using several methods. The most traditional method in circadian physiology is based on a graphical display called an *actogram*. Maynard Johnson drew the first actogram in 1926,[22] many decades before the "computer revolution." Today, actograms can be generated easily from data automatically collected by a computer. Start with records of locomotor activity plotted in the familiar Cartesian style (top panel 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 (middle panel). The vertical alignment of the data provides instant information about the duration of the circadian cycle (that is, its period): drifts to the left indicate that the cycle is shorter than 24 hours (the onset of activity is earlier each day) while drifts to the right indicate that the cycle is longer than 24 hours (the onset is later each day). The drift to the left in Figure 3.19 indicates that the cycle is shorter than 24 hours.

Note that a very compact actogram (bottom panel) can be obtained by digitizing the activity values so that data points are plotted on a yes-or-no basis. This arrangement is especially helpful when inspecting many days worth of data or when a *double plot* is desirable. A *double plot* is simply an actogram double-plotted to facilitate inspection of records in which the activity pattern would otherwise run off the page because of drifts in 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 are shown instead of the single band displayed in a single-plotted actogram. The program Plot (see Table 3.1) can be used to construct simple actograms. More sophisticated programs, available commercially, include Chronobiology Kit Analysis (Stanford Software Systems, Santa Cruz, California), ClockLab Analysis (Actimetrics Software, Wilmette, Illinois), and Actiview Actogram Software (Mini-Mitter Company, Bend, Oregon).

Actograms provide an easy way to determine the phase of the rhythm. Traditionally, the daily onsets of activity are used as phase markers,[23–25] although the "offsets" can also be used.[26–28] Actograms can be used to determine several parameters of circadian rhythms, as shown in Figure 3.21 and discussed in detail later in this chapter. Note 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 of the cycle (its *period*) is called τ (*tau*). Phase shifts caused by endogenous or exogenous factors are designated as $\Delta\phi$ (*delta phi*). Not shown in the figure is the designation ϕ (without the Δ preceding it), which refers to the phase of a rhythm in relation to an *internal* reference point. If you need assistance with the pronunciation of Greek letters, consult the *Dictionary of Circadian Physiology* at the end of this book, or use the program SayIt (see Exercise 2.1 in Chapter 2).

Two other methods commonly used to determine the phase of circadian rhythms include the cosinor method and the method of identification of the peak of smoothed rhythms. Consider Figure 3.22. The top panel shows raw data concerning the locomotor activity of a golden hamster

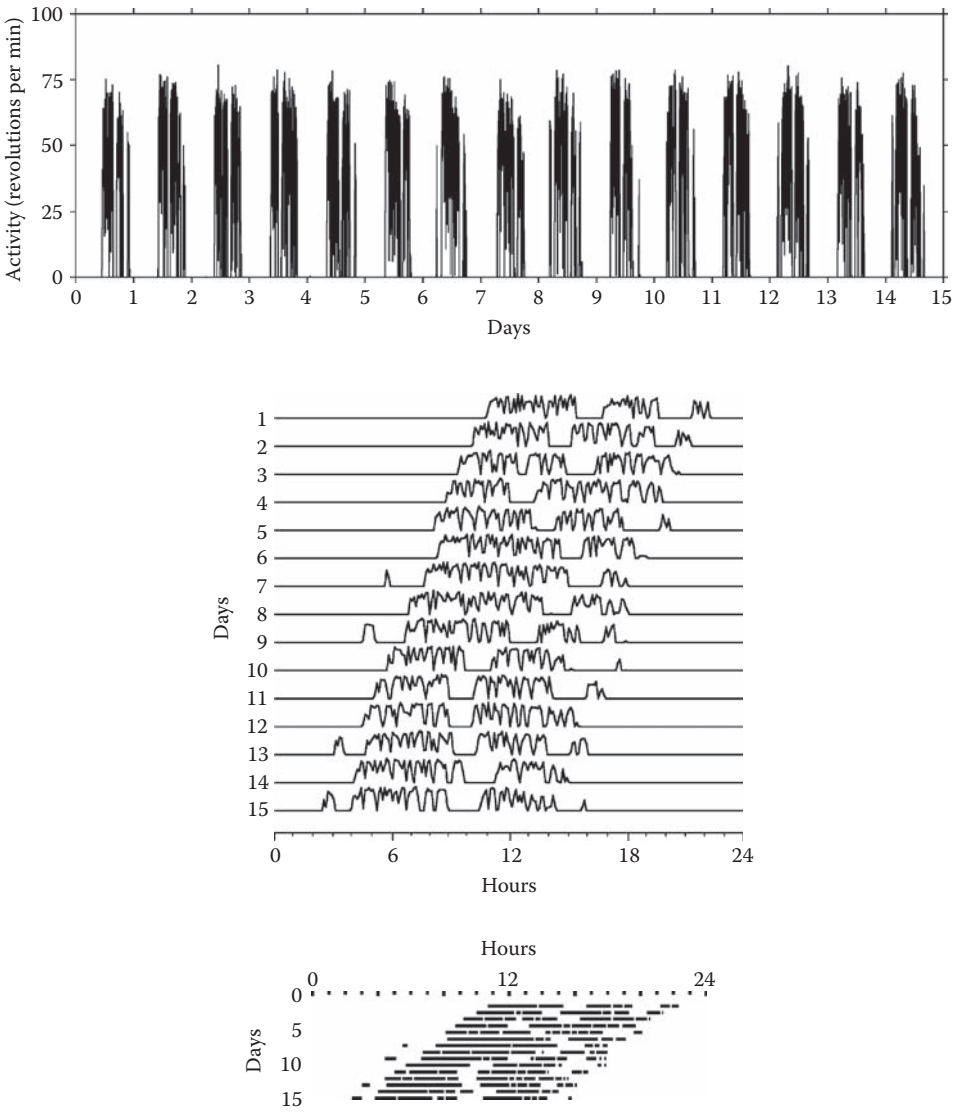


FIGURE 3.19 How an actogram is built. Construction of an actogram can be thought of as a process that starts with a regular Cartesian plot of the running-wheel activity of a mouse (top). Each successive 24-hour segment of the data set is then cut out, "stretched," and pasted below the preceding one (middle). A very compact actogram (bottom) can be obtained by digitizing the activity values (i.e., by plotting each point on a yes-or-no basis). (Source: Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.)

as determined every 6 minutes by telemetry. The middle panel 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*.[14] As an alternative, the raw data may be smoothed by a moving-averages procedure (bottom panel). 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 an actogram) and the peak (which is similar to the acrophase determined by the cosinor method).[29]

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 (that is, 360°/24 if the cycle is 24-hours long and t is measured in hours), and ϕ is the acrophase (in degrees). A system of three equations with three unknowns can be derived and solved in algebraic form.[14,30]

The program Acro (see Table 3.1) also calculates the mean level, amplitude, and acrophase of rhythms.

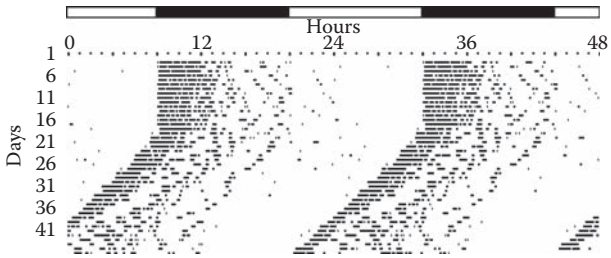


FIGURE 3.20 A double-plot. Actograms are often double-plotted to facilitate the inspection of activity records that drift over many days. In a double-plot, the second day of data is plotted not only under the first day but also to its right (so that there are 2 days per line). Similarly, the third day is plotted not only under the second day but also to its right. Each day of data is plotted in this manner. 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. This figure shows the data for a mouse that was under a light–dark cycle for the first 17 days and in constant darkness afterwards. (Source: Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.)

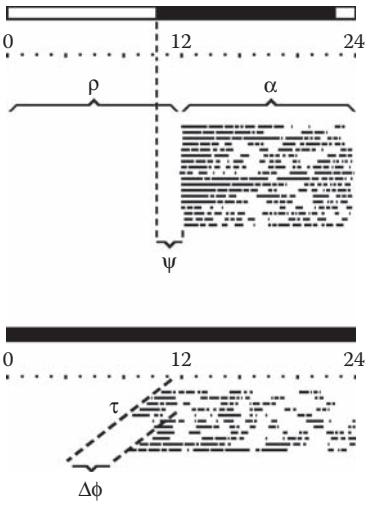


FIGURE 3.21 A Greek alphabet soup. Several letters of the Greek alphabet are used to denote properties of circadian rhythms: the duration of the daily rest interval (ρ), the duration of the active interval (α), the phase angle of entrainment (ψ), the period (τ), and a phase shift of the rhythm (Δϕ).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

However, 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 in 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

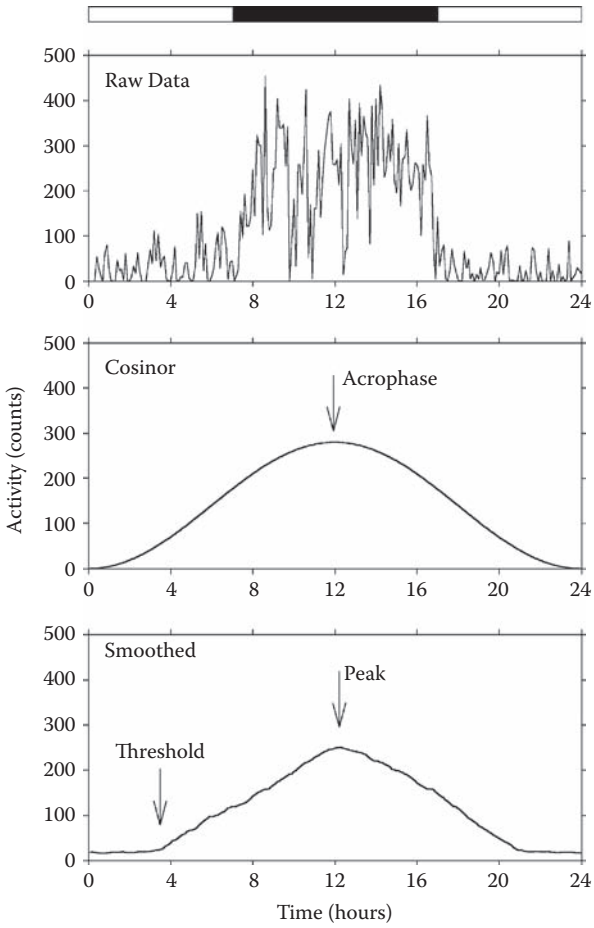


FIGURE 3.22 Other methods to determine the phase of a rhythm. The top panel shows raw data depicting the locomotor activity of a golden hamster monitored by telemetry. The middle panel illustrates how the acrophase of the rhythm is calculated using the cosinor method (the acrophase is the peak time of the cosine wave fitted to the data). The bottom panel illustrates how phase is calculated by identifying the peak of a smoothed curve derived from the raw data, using an 8-hour moving-averages filter. A threshold of the nocturnal elevation in the level of activity can also be identified. (Source: 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.)

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, several commercially available programs perform the single cosinor by solving the formal system of equations. These programs include Cosifit (Circesoft, Waltham, Massachusetts) and Time Series Analysis

Serial Cosinor (Expert Soft Technology, Esvres, France). 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.

Phase may also be computed using other procedures. The three procedures covered in this section (inspection of actograms, computation of the peak of the smoothed rhythm, and acrophase of the fitted cosine curve), however, are used most commonly. Different experimental conditions may require different computational procedures.[31–33]

3.3 PERIOD, WAVEFORM, AND ROBUSTNESS

So far, this chapter has discussed the computation of three of the six parameters that characterize circadian rhythms: mean level, amplitude, and phase. This section discusses 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 movement to the left tells you 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? This question can be answered in three different ways. An intuitive method is to note that the onsets advance 5 hours in 10 days. This means that, on average, an advance of 0.5 hour occurs each day. Therefore, the period must be: 24 – 0.5 = 23.5 hours. If the procedure is repeated for Panel B, the period in this case is calculated to be 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. You may also remember that 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, the slope can be calculated easily as 0.5 hour per day (and then added to 24 to obtain the period of 24.5 hours). The third solution requires a background in basic statistics but is also simple: linear regression can be used to find the equation that describes the change in onset times as a function of elapsed days. The slope of the regression line

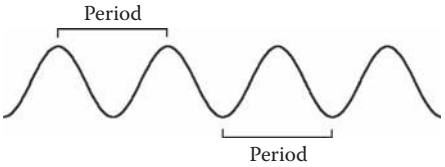


FIGURE 3.23 Period. Period is one of the parameters that characterize a circadian rhythm.

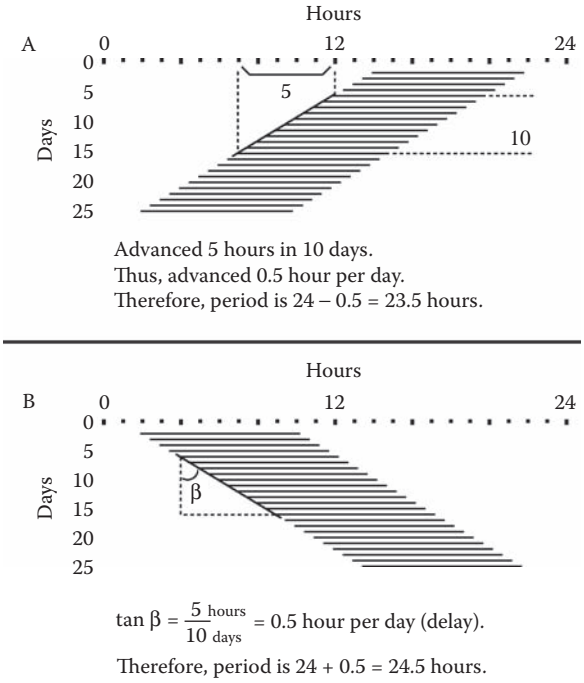


FIGURE 3.24 Calculation of circadian period using actograms. The slope of an imaginary line connecting the daily onsets of activity provides a measure of circadian period. The slope may be determined in an intuitive manner (A) or by using basic geometrical principles (B).

will indicate how much the period deviates from 24 hours (which, for the data in Panel B, should be +0.5). Thus, the period is now found to be 24.5 hours. It is not surprising that the three methods yield the same answer. Each method is just a different elaboration of the same basic procedure.

Inspection of actograms can still be used today to compute circadian period. However, other possibilities also exist. The method of *modulo adjustment* involves the inspection of actograms, but it takes advantage of a computer to force the actograms to display data in more convenient ways. This method allows you to determine the period of a time series by adjusting the time scale used to construct the actogram. As shown in Figure 3.25, you can start with activity records that are clearly shorter than 24 hours when plotted on a standard 24-hour scale (top panel). You then shorten the time scale (i.e., reduce the *modulus*) until the onsets are vertically aligned. At this point (that is, the panel indicated by the asterisk), you 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). *Note*: The *modulo* operator requires two integers. In computer programs, 22.4 hours are internally processed as 224 integer units.

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

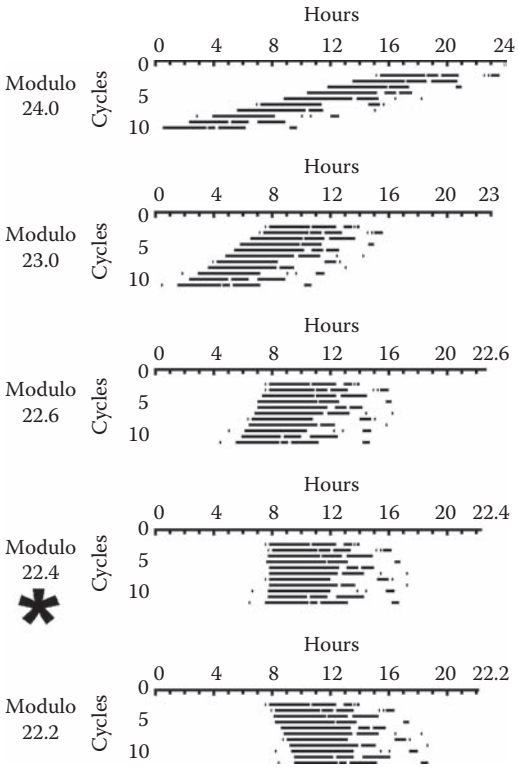


FIGURE 3.25 Calculation of circadian period by adjustment of plot modulo. The period of a rhythm, such as this rhythm of the running-wheel activity of a golden hamster, can be determined by successive adjustments of the plot scale (plot modulo). When the correct modulus is reached (in this case, at 22.4 hours, as indicated by the asterisk), the activity onsets align on a straight vertical line. (Source: Adapted from Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.)

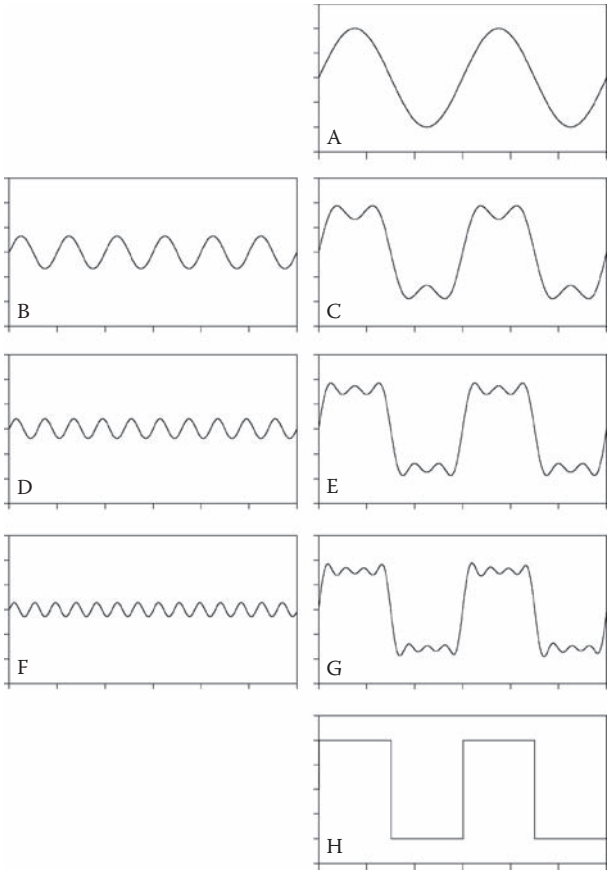


FIGURE 3.26 How Fourier analysis works. This "reverse-engineering" figure shows how a square wave (H) can be built out of the sum of many sine waves (A + B + D + F). Fourier analysis breaks down a complex wave into a set of simple sine waves.

easily be corrupted by the inspector's subjective biases. Therefore, automated data analysis procedures independent of human observers are much more reliable. One automated method for the computation of circadian period is *spectral analysis* (also called *Fourier analysis*, after Jean Baptiste Fourier, the 19th century French mathematician who developed it). Fourier determined 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 example, suppose you want to build a time series that looks like a string of square waves (Figure 3.26). You could start with a single sine wave that has the same period as that of the square wave that you want to build (Panel A). You would then add a sine wave with shorter period (Panel B) to obtain a slightly modified waveform (Panel C). If you continued this procedure through many steps, you would eventually reach a waveform that is essentially square (Panel H).

Of course, the goal in the analysis of circadian rhythms is the very opposite: you want to go from the complex wave to the series of simple waves, in the hope that you can identify the components of the rhythm. Fourier and others calculated the mathematical formulas required for this process,[34,35] so you 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), while a square wave (E) yields a series of peaks corresponding to the various rhythmic components (F).

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. Note 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 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.

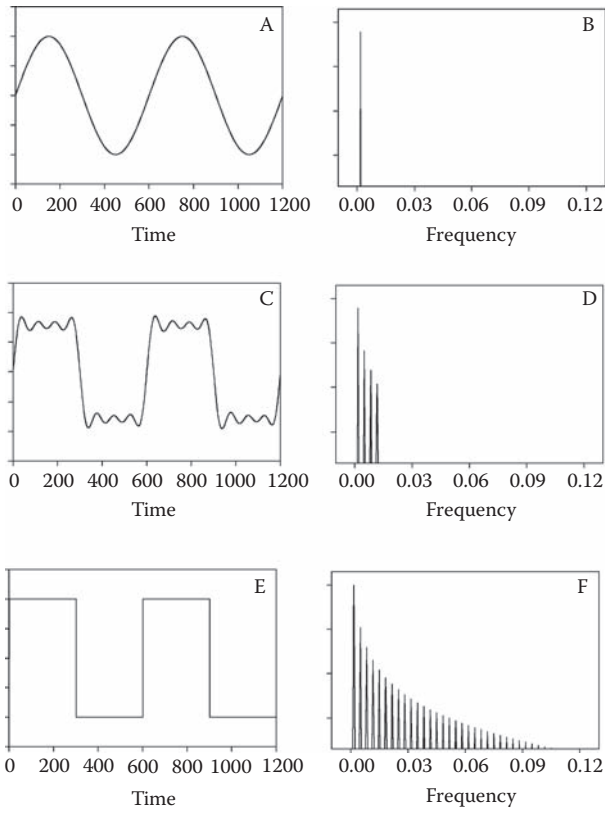


FIGURE 3.27 Fourier analysis. Fourier analysis yields a periodogram that indicates the magnitude of each component of a wave. The wave in (A) is a pure sine wave, so that its periodogram has a single peak (B). The wave in (C) can be decomposed into four sine waves with different frequencies and magnitudes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

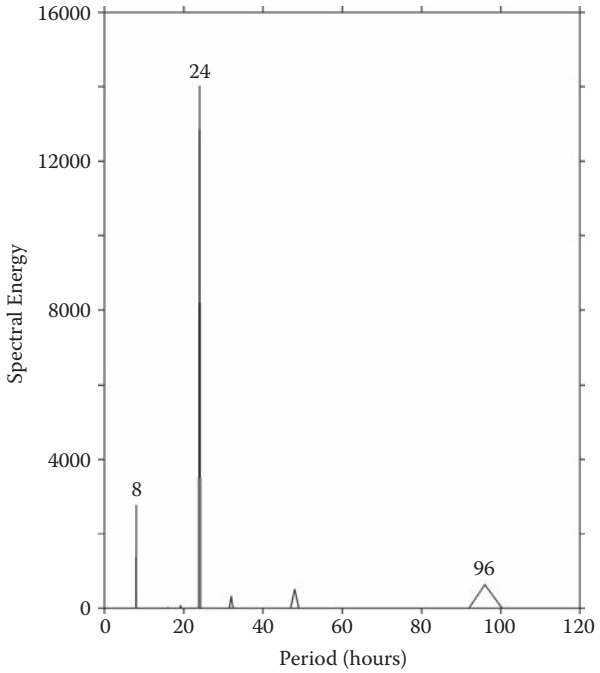


FIGURE 3.28 Fourier analysis in action. This periodogram is the result of Fourier analysis of the idealized body temperature rhythm of a female golden hamster (previously shown in Figure 3.1). A large 24-hour component, as well as several smaller components (including 8 and 96 hours), can be seen. The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

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 in Chapter 4), but it is not ideal for analyzing 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-min intervals for 10 consecutive days, Fourier analysis evaluates periods of 2400/1, 2400/2, 2400/3, and so on, 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.[36] This resolution is unacceptable; one would expect at least a 6-min (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.[37]

Numerous other methods for determining circadian period have been developed or adapted, including serial autocorrelation,[38–41] inter-onset averaging,[42–44] iterative harmonics,[45] acrophase counting,[46] singular value decomposition,[47] and nonlinear multiple components analysis.[18] Some of these methods are more reliable than others. The Enright periodogram[37] and the Lomb–Scargle periodogram[48] are particularly suitable for analyzing circadian period.

The *Enright periodogram*, proposed by James Enright,[49] is based on the same principle of temporal alignment used by the method of modulo adjustment discussed earlier. As shown in Figure 3.29, the period of a rhythmic time series can be determined by inspecting the alignment of segments plotted on various time scales. However, instead of relying directly on the alignment of the segments, the average waveform of the segments (bottom row in Figure 3.29) can be used. If a time scale (modulo) differs from the period of the rhythm, the average waveform tends to be flat, while a waveform with a distinct

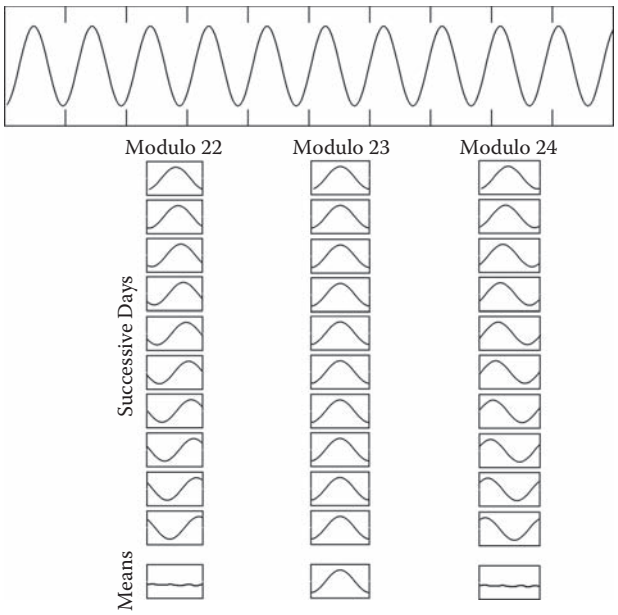


FIGURE 3.29 Calculation of circadian period by the Enright periodogram. The Enright periodogram procedure and the calculation of circadian period using the modulo adjustment of actograms procedure operate on the same principle. The difference between the two methods is that the Enright periodogram procedure provides a numerical value for the strength of each potential period (bottom row).

peak is seen when the modulo matches the period of the rhythm.

To avoid human subjectivity in determining what is and 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. Dörrscheidt and Beck[50] and Sokolove and Bushell[51] published two distinct but similar implementations of the procedure. The *chi square periodogram*, published by Sokolove and Bushell, has become particularly popular in the analysis of circadian periodicity.[52–68] Both the program Tau and the program Rhythm (see Table 3.1) can be used to analyze data with the chi square periodogram procedure. Tau is meant for the specific computation of circadian period, while Rhythm is intended for exploratory analysis of rhythmicity in general.

The *Lomb–Scargle periodogram* works just as well as the Enright periodogram, but it also allows the analysis of data collected at irregular intervals rather than under a rigorous protocol that conducts measurements at a regular interval, such as every 6 minutes or every hour. In addition, it permits the analysis of data sets intended to have equally spaced observations but that are missing one or more values because of equipment failure or other adversity. The procedure is based on N. R. Lomb's adaptation of Fourier analysis to unequally spaced time series.[69] The Lomb–Scargle periodogram is susceptible to artifacts,[70,71] as is the Enright periodogram. The Lomb–Scargle periodogram has been shown to match or surpass the Sokolove and Bushell implementation of the Enright periodogram in the analysis of circadian rhythms,[48] although it has not been widely adopted by circadian physiologists. The program LSP (see Table 3.1) can be used for the analysis of data with 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. Consider Figure 3.30. The left column shows 3-day segments of four 10-day-long data sets. A computer created the top three data sets as, respectively, sine waves with period of 24.0 hours, sine waves with period of 12.0 hours, and square waves with 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. The second and third columns in Figure 3.30 show the Enright (chi square) and Lomb–Scargle periodograms for the corresponding data sets. Note 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 also incorrectly detected a 24.0-hour component. 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.[37,50]

When analyzing circadian rhythms by the Enright periodogram procedure, one must make sure that the data set does not contain submultiple oscillations that might cause a peak to appear in the circadian range. This can be simply accomplished by filtering out high-frequency oscillations prior to periodogram analysis. 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 legitimate. This is the case in the periodograms for the square wave (third row in Figure 3.30). Small peaks at 12.0 hours appear alongside the large peaks at 24.0 hours, and they are legitimate peaks equivalent to the peaks that you saw in the Fourier periodogram for a square wave in Panel F of Figure 3.27. Note, however, that the Enright periodogram handled the square wave better than the Lomb–Scargle periodogram handled it, as demonstrated by the height of the 24.0-hour peaks. Both periodograms identified 24.0-hour rhythmicity in the data set of running-wheel activity (bottom row).

The fifth parameter of circadian rhythms is *waveform*. The four data sets shown in Figure 3.31 have the same

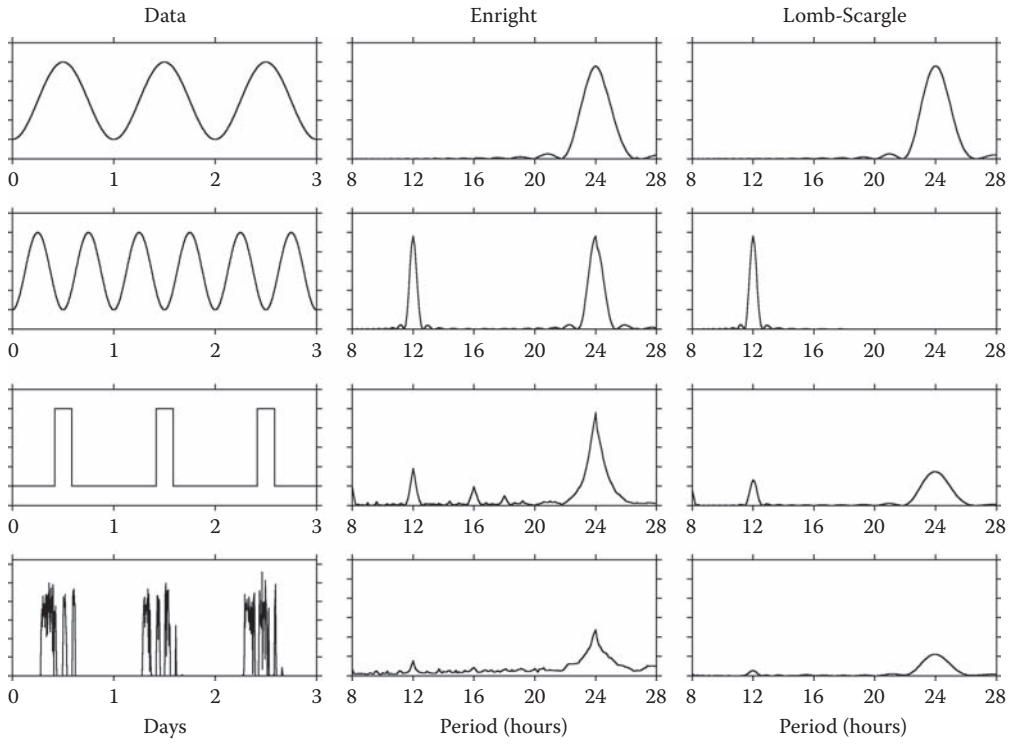


FIGURE 3.30 Enright and Lomb–Scargle periodograms in action. The left panels show 3-day segments of three artificial data sets and one data set containing the running-wheel activity of a golden hamster. The middle panels show Enright (chi square) periodograms for the corresponding data sets on the left panels. The right panels show Lomb–Scargle periodograms for the same data sets. All data sets were 10 days long, but only 3 days are shown. See text for details about interpretation of the periodograms. (Source: Adapted from Refinetti, R. (2004). Non-stationary time series and the robustness of circadian rhythms. *Journal of Theoretical Biology* 227: 571–581.)

mean level, amplitude, phase (on average), and period. Yet, they clearly differ from each other. Their *waveforms* are not the same: some are regular, some are irregular, some are smooth, and some are rather complex. It is unfortunate that circadian physiologists have given very little attention to this issue. In principle, one could obtain a quantitative description of waveforms by comparing the various rhythmic components revealed by spectral analysis of the rhythms. Figure 3.32 attempts this comparison using the body temperature rhythms of four mammalian species maintained under a 24-hour light–dark cycle. To ensure that the waveforms are representative, the analysis used average values from seven animals per species. The waves for 7 consecutive days were evaluated by Fourier analysis (although only 2 days are shown in the left panels to facilitate visual inspection). To eliminate high-frequency oscillations, all data sets were reduced to a 2-hour resolution (i.e., one value for each 2 hours). The use of 7 days with 12 values per day ensures that Fourier analysis can detect 24-hour rhythmicity properly. The left panels indicate that the rhythms of different species have different waveforms, and the right panels are 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." 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). It remains to be determined whether other laboratories can reproduce these interspecies differences in spectral profile and whether the differences are consistent in other species.

The sixth parameter that characterizes circadian rhythms is *robustness*. In the past, circadian physiologists used this term informally to denote the strength of a rhythm (how "clean" a rhythm is),[72–75] but the term was not formally defined until recently. The robustness of a rhythm is distinct from its amplitude, as well as from its other four parameters. Robustness measures the *stationarity* of a rhythm.[76] Look at Figure 3.32 again and compare the

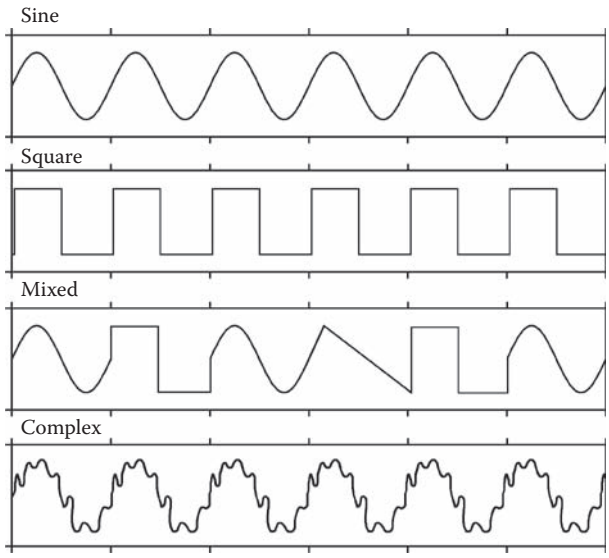


FIGURE 3.31 Waveforms. The four graphs show four different rhythms with clearly distinct waveforms, even though the rhythms have the same period, phase, mean level, and amplitude.

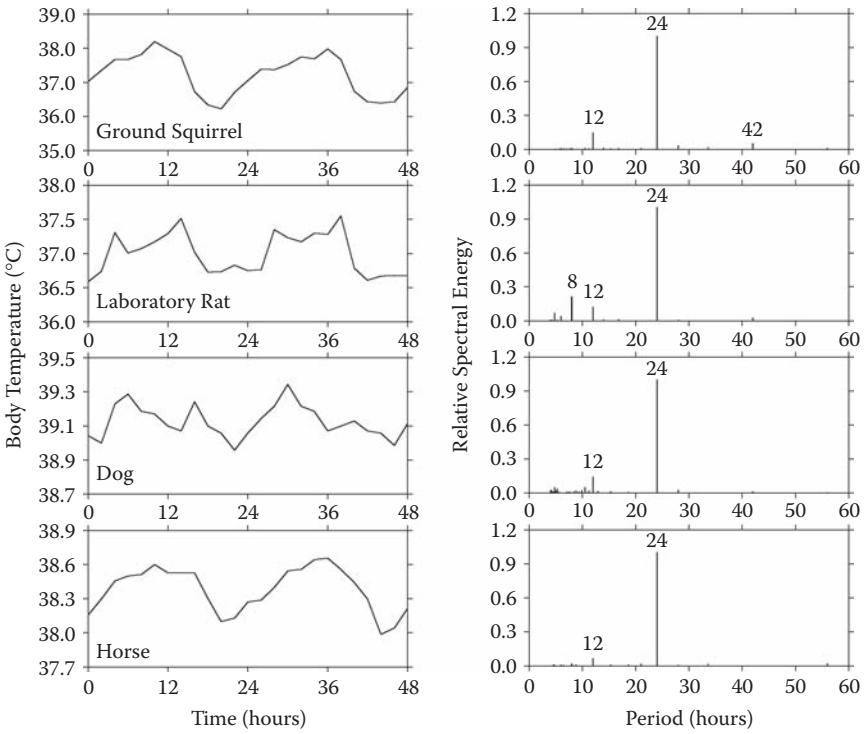


FIGURE 3.32 Quantifying waveforms by Fourier analysis. The waveforms of the body temperature rhythms of four mammalian species were quantified by Fourier analysis. The data sets were 7 days long (although only 2 days are shown) and contained mean body temperature values of 7 individuals with 2-hour resolution. A resolution of 2 hours was used to eliminate high frequency oscillations. 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. (Source: Archives of the Refinetti lab. Periodogram analyses previously unpublished.)

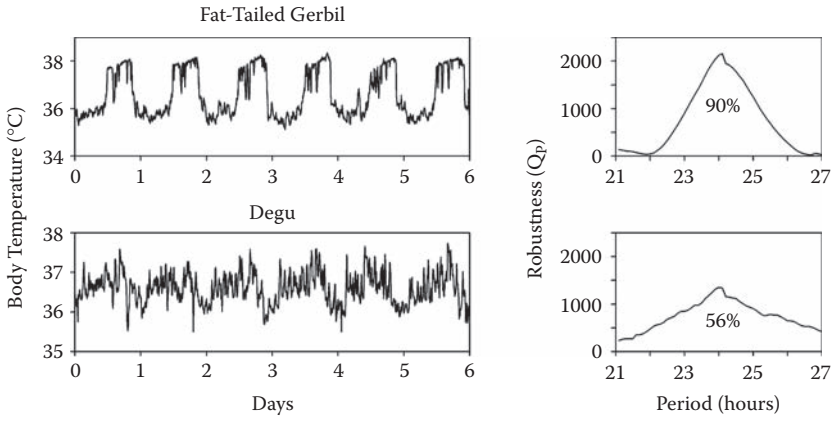


FIGURE 3.33 Calculation of rhythm robustness. This figure shows 6-day segments of 10-day long records of body temperature (with 6-minute resolution) of a fat-tailed gerbil and a degu, along with the Enright (chi square) periodograms that describe them. Rhythm robustness is calculated as the peak Q_P value expressed as a percentage of the maximal possible Q_P (in this case, 2400). The rhythm of the fat-tailed gerbil is much more robust than that of the degu. (Source: Adapted from Refinetti, R. (2004). Non-stationary time series and the robustness of circadian rhythms. *Journal of Theoretical Biology* 227: 571–581.)

waveforms of dogs and horses (left panel). 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*), while the rhythm of dogs is rather variable (hardly stationary).

The idea of quantifying rhythm robustness by means of the Q_P statistic of the chi square periodogram was introduced informally more than 20 years ago.[77] The Q_P statistic is 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 comprehended 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), while 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,[76] as exemplified in Figure 3.34. One can always question whether biological noise is true noise (i.e., stochastic variation) or some form of deterministic *chaos*,[78,79] but resolution of this issue is not necessary to analyze rhythm robustness in the circadian range.

A final note on the use of the Q_P statistic as an index of rhythm robustness is necessary: when Q_P values are computed for a data set, the more days (or circadian cycles) available for analysis, 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,

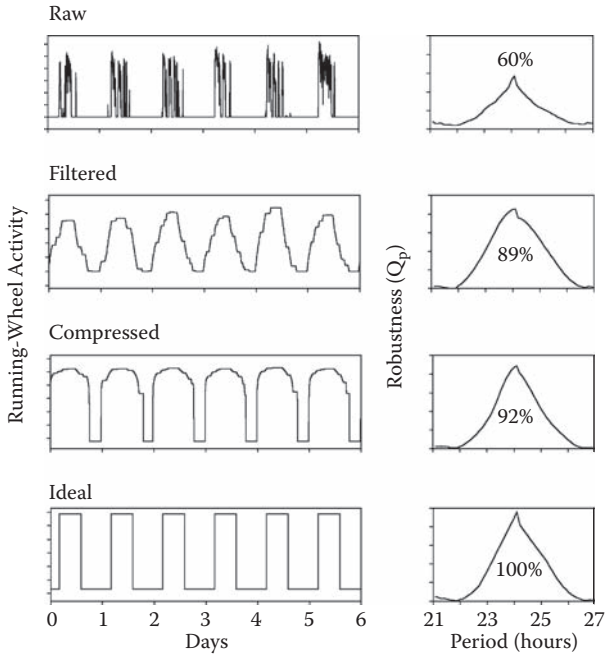


FIGURE 3.34 Exposing rhythm robustness. Filtering, and sometimes compression, of natural data sets (such as the records of running-wheel activity of a golden hamster) helps expose rhythm robustness. A clean square wave is shown as the "ideal" stationary wave (for these data) with robustness of 100%. (Source: Adapted from Refinetti, R. (2004). Non-stationary time series and the robustness of circadian rhythms. *Journal of Theoretical Biology* 227: 571–581.)

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 in

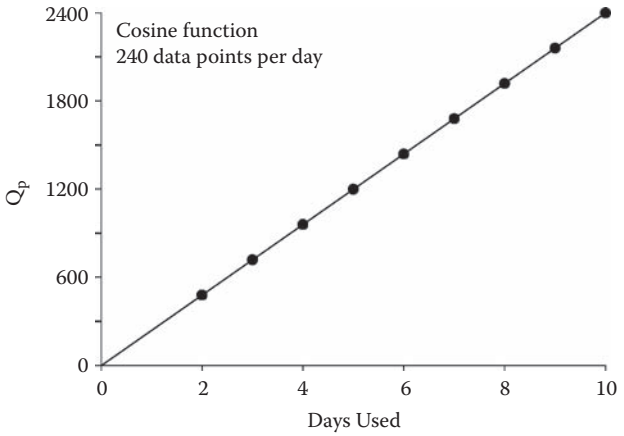


FIGURE 3.35 Maximal Q_P as a function of the length of a data set. The value of peak Q_P obtained by the chi square periodogram procedure in the analysis of a perfectly rhythmic data set is a linear function of the number of days used in the analysis. For this reason, it is important to express rhythm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

terms of percentage of maximum possible values (as in Figure 3.34) rather than in terms of absolute Q_P values.

3.4 STATISTICAL SIGNIFICANCE

The evaluation of statistical significance is an essential element of data analysis because it allows researchers to discount, with reasonable confidence, the possibility that they detected rhythmicity in their data by pure luck. Suppose that you collect six data points over time — every 4 hours for 24 hours (Figure 3.36). If all six readings are identical (Panel A), it seems reasonable to conclude that no daily rhythmicity exists. But does the pattern in Panel B indicate the presence of rhythmicity? Elementary algebra tells you that three distinct values arranged in groups of six, with repetition allowed, yield a total of 3^6 combinations. Thus, the sequence of values in Panel B is one out of 729 possible sequences — a rather unlikely event. Because it is so unlikely, you can be reasonably confident that you did not obtain it by chance. That is, you can be reasonably confident that real rhythmicity caused the particular arrangement of the values over time. Now, what about the other two panels? You might want to say that the arrangement in Panel C is also rhythmic, while that in Panel D is not — but, would it be reasonable to say so? When statistical significance is discussed, researchers are talking precisely about objective criteria for this type of decision. Ronald Fisher, the "father" of the science of statistics, believed that this process can provide the basis for *inductive logic*.[80]

The issue of *inferential statistics* — the general process associated with the computation of statistical significance — is rooted in the limitations of the ability to make

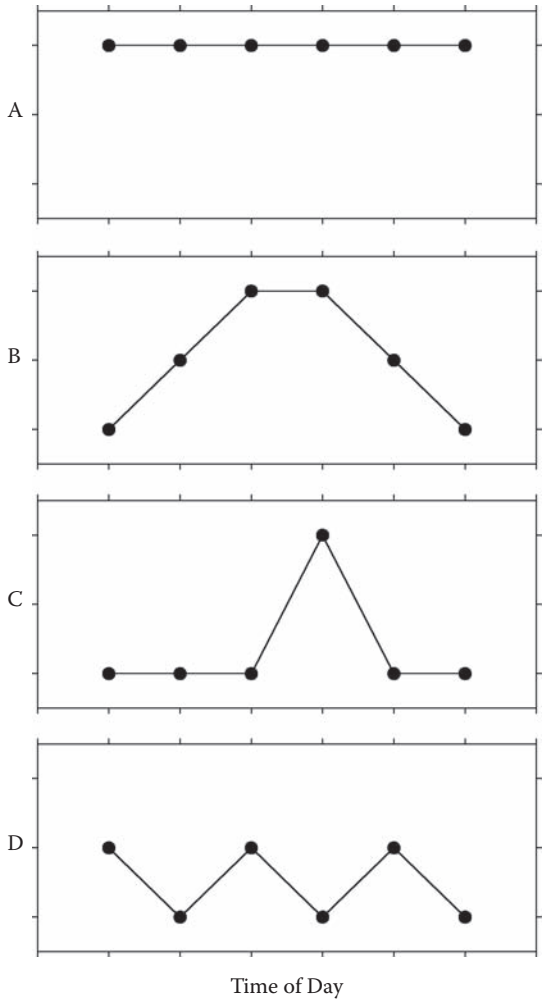


FIGURE 3.36 The importance of statistical testing. This figure shows four hypothetical data sets, some of which seem to exhibit daily rhythmicity and some of which do not. Which ones are truly rhythmic? Statistical testing is needed to quantify the degree of certainty of the inferences made about rhythmicity.

scientific observations. Suppose you want to test if aspirin relieves headaches. You could take 2000 people who are experiencing headaches, give aspirin to 1000 of them and placebo pills to the other 1000, and then see what happens. If every person who received aspirin got better, while no one who received placebo improved, it would be reasonable to conclude that aspirin does indeed relieve headache. Note, however, that you would be making an *inference*, namely, that everyone else in the world will react to the drug the same way that your 1000 "guinea pigs" did — even though you did not, and would not be able to, test them all. You would be inferring that any human being, including those who have not been born yet, will react in the same way as your experimental group. This is a *big* inference that requires more than just a casual hint.

In circadian physiology, scientists cannot observe every circadian rhythm that exists, that has ever existed,

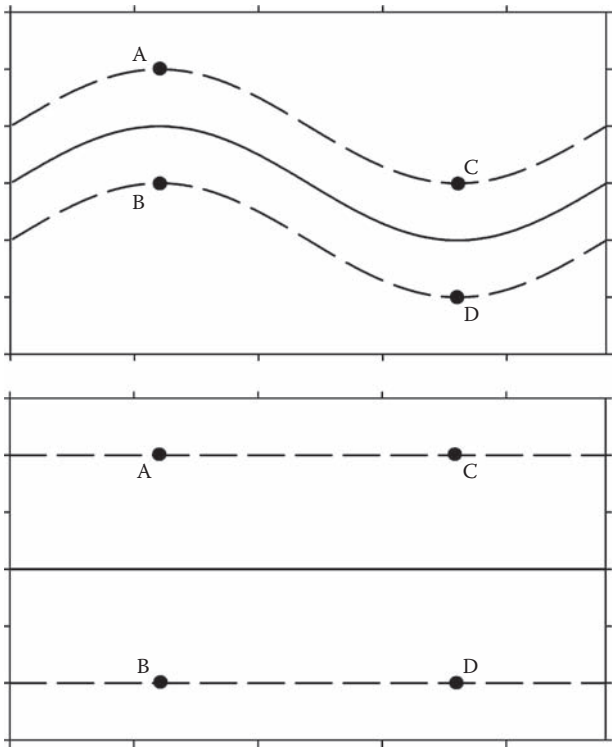


FIGURE 3.37 The problem of inferential statistics. Scientific knowledge is attained by observing samples from a much larger population of data. In both panels in this figure, the mean value of a hypothetical population is indicated by the solid line, and the variability around the mean is indicated by the dashed lines. If, for simplicity, you sample only two data points (say, A and D), you may conclude that the population is rhythmic regardless of whether it is actually rhythmic (top panel) or not (bottom panel).

and that will ever exist. They must resort to limited *samples* of the larger (virtually infinite) population of circadian rhythms. Suppose that the real phenomenon you want to measure is described by the solid line in the top panel of Figure 3.37. Suppose also that the variations due to normal biological noise are contained between the dashed lines. Now sample two data points at random. If you take points A and D, you will think — correctly — that there is a variation between morning and evening. If you take points A and C, you will be less impressed by the results but will reach the same conclusion. However, if you happen to take points B and C, you will think that there is no daily variation (or even that there is a very small variation in the opposite direction). The key question, of course, is what points did you actually take? Because you don't know what the real curve looks like (because you sampled only two points), you cannot know if you selected the "correct" points. What can you do? You could guess at what the curve looks like and then calculate the probability of getting each pair of points. But again, how can you guess correctly? Millions of scenarios are possible! Perhaps you should give up.

All hope is not lost. A sensible solution exists — if you are willing to lose a battle in order to win the war. The solution is to guess that *there is no real phenomenon*. That is, assume that there is no daily rhythmicity, as diagrammed in the lower panel of Figure 3.37. This assumption is called the *null hypothesis* (because the hypothesis states that no real phenomenon exists). You now can calculate the probability of obtaining each pair of data points. If the pair that you actually obtain has a low probability of coming out by chance, then you can infer that your null hypothesis is wrong — that is, you can *reject* the hypothesis. Because the null hypothesis states that no rhythmicity exists, its rejection implies that there is, in fact, rhythmicity. Victory!

Well, do not celebrate too early. The reasoning behind statistical inference is very good, but how are the probabilities actually calculated? Start with a simple case. Suppose that you are simply taking a sample from the population shown in Panel A in Figure 3.38. Let's say that you picked the values marked with an X. This gives you the sample distribution shown in Panel B. The question now is: does the sample distribution look similar enough to the populational distribution? If the sample were drawn randomly, its distribution should look like the populational distribution, and its mean should be similar to the population mean. Of course, you don't know what the population's parameters are — if you knew them, you wouldn't need to draw samples. However, you can use the trick of the null hypothesis again: if you compare two means (the famous *t test*), you assume that the difference between the means is zero, so that the population of differences between means has a mean of zero; if you compare several means (the popular *Analysis of Variance*, or ANOVA), you assume that the quotient of variances is 1; and likewise for other statistical tests.[81,82]

Once the population's parameters are estimated, you can draw thousands of random samples from the population, calculate the probabilities associated with all possible sample means, and check where your sample mean falls. Although you can do this for each experiment you conduct (the so-called *bootstrap* method),[83–87] the traditional approach is to mathematically derive a generic "probability density curve," such as that shown in Panel C of Figure 3.38. Values below point *a* in the probability density curve occur by chance less than 1% of the time (that is, point *a* delimits the cutoff for $p < 0.01$). If your sample mean is smaller than *a*, you know that your sample is a rare occurrence. But how rare is rare enough?

In the social sciences, $p < 0.05$ (point *b*) is considered a good breakpoint. This means that the *level of significance* of your decision is 5 in 100, or that you 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 α). The biological sciences often require that $p < 0.01$. The natural sciences may demand

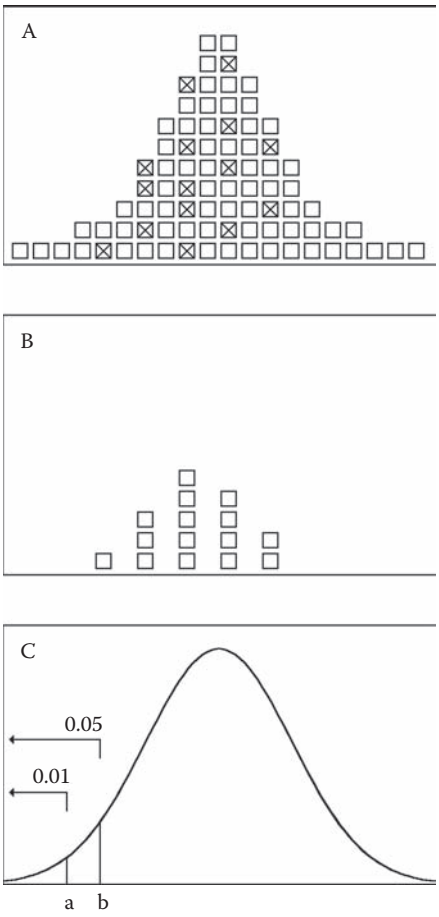


FIGURE 3.38 The rationale of inferential statistics. Because you have to make an inference about a population based on a sample, you need to estimate what other samples would look like. If the population that you are studying looks like the one in Panel A, and you take a sample that looks like the one in Panel B, you need to estimate whether this sample could be reasonably expected to come from the population. (If it cannot, then you have something special, something that cannot be explained simply by chance.) By drawing many random samples from the population, you can build a "probability density curve" such as that in Panel C. Then you can estimate how likely it is that your experimental results were due to chance alone.

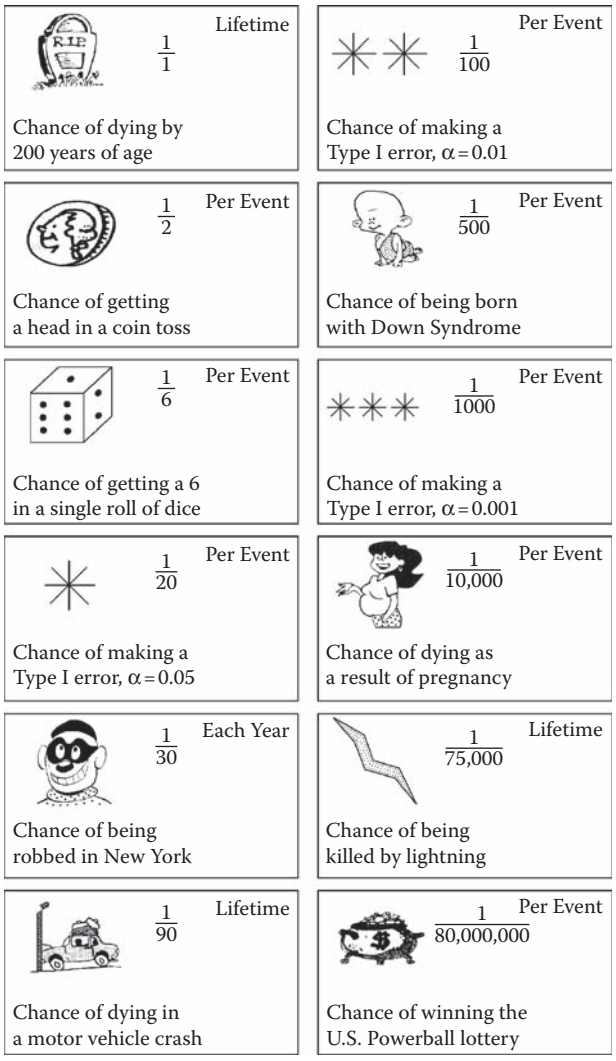


FIGURE 3.39 What are the odds? Although death is a certainty for all mortals (1/1 chance), most events in life take place with a lower probability. In scientific research, a Type I error refers to a situation in which you conclude that you found something interesting when your results were actually due to random variation. The probabilities of Type I error usually accepted by researchers ($\alpha = 0.05$, $\alpha = 0.01$, and $\alpha = 0.001$) span a reasonable range but are not as low as one might think.

$p < 0.001$ or lower. I do not know why these breakpoints were chosen. Decisions about statistical significance are often arbitrary. Figure 3.39 shows that, when $\alpha = 0.05$, one is more likely to commit a Type I error than to be robbed in New York City. That is, the probability of concluding that you found something interesting when the results are actually due to chance is very high if you 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 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 million), do they feel comfortable embracing the findings as scientific truths. Some researchers — particularly psychologists who must deal with weak experimental results — have presented arguments *against* the use of significance tests.[88,89] I and many statisticians believe that problems in significance tests derive not from the tests themselves but from a lack of appreciation for the intricacies of the procedure.[90–93]

3.4.1 Regular Time Series

This section addresses the statistical significance of procedures used in circadian physiology for the analysis of full time series. For parameters such as mean level, amplitude, and phase, tests of significance usually are not needed. Circadian physiologists often just want to describe these parameters. To provide an index of variability, you can use standard descriptive statistics. For example, if you calculate the amplitude of a rhythm by averaging the values (half the range of oscillation) over 10 days, you can compute the *standard deviation* of this averaged value (as mentioned in Figure 3.13). The standard deviation 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. If you designate each of n values as X_i, the mean as M, and the summation over all values (X_i, from i = 1 to i = n) as $\Sigma\, f(X_i)$, then the standard deviation is the square root of $\Sigma\,(X_i - M)^2 / n$. In a similar fashion, if you calculate the average mean level of a rhythm by averaging the mean levels of 10 individuals of a species, you can compute the standard deviation of this averaged value. This would result in an ugly sounding sentence: the standard deviation would be the square root of the *mean* of the squared deviations of each *mean* level from the *mean* of *mean* levels.

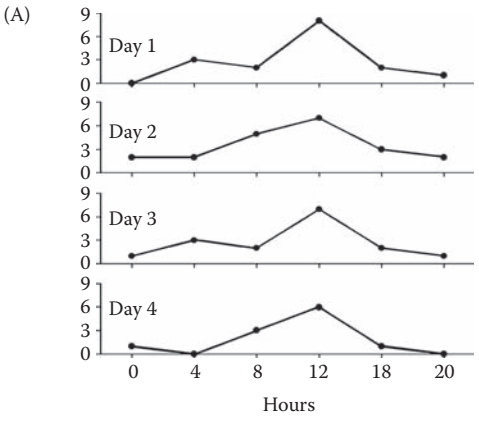
A small dose of inferential statistics may be desirable even when just the mean level, amplitude, or phase of a rhythm are being described. Because the standard deviation (SD) refers to the distribution of values in your own sample, you may want to replace it with an index of variability that estimates the variability in any sample. As explained in introductory statistics books,[10–13] your computation of SD should use (n –1) instead of n as the denominator: $SD = SQR[\Sigma\,(X_i - M)^2 / (n - 1)]$. If you divide the SD by the square root of the sample size ($\sqrt{n}$), you obtain the *standard error* of the mean (SE), a commonly used statistic. If you can assume that the population of values is normally distributed (a reasonable assumption in many cases), or if you have 30 or more values regardless of the distribution, you can build a *confidence interval* to indicate how confident you are that your sample mean reflects the populational mean. The 95% confidence interval goes from (M – SE · 1.96) to (M + SE · 1.96). The 99% confidence interval goes from (M – SE · 2.58) to (M + SE · 2.58). For example, when you counted the ducks swimming in a pond (Figure 3.13), you found a mean of 4.5 ducks with an SD of 1.8 ducks. Because you made 10 observations, your *n* is 10, and your SE is 0.57. Consequently, you can be 95% confident that the real number of ducks in the pond is between 3.4 and 5.6. If you consider only the 5 morning measurements, your 99% confidence interval extends from 4.8 to 7.2.

To compare the mean levels, amplitudes, or phases of two or more groups of observations, you can use standard statistical tests such as *t* tests and ANOVA.[81,82] If you want to determine the acrophase of a rhythm by the fitting of a cosine wave, however, you first must verify whether the fit is a good one. The fitted cosine wave will always have a peak — and the time of the peak will be considered to be the acrophase — but the acrophase will be meaningless if the cosine wave does not fit the data properly. The program Acro (see Table 3.1) assesses the goodness of the fit using an index computed as the ratio of the sum of squares of the best fit and the worst fit: the smaller the index, the better 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.

Techniques that detect circadian rhythmicity and test the reliability of estimates of period are particularly important in circadian physiology. Start by examining 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 3.40. You can use the time series shown in Panel A to 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 6 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. The quotient of the deviations from the means (SD_B/SD_W), however, is a very meaningful ratio. In this case, the quotient equals 3.47, which indicates that SD_B is larger than SD_W. In contrast, if no consistent rhythmicity existed in the data (Panel C), the data points would be distributed randomly over time, leading to an SD_B/SD_W ratio close to 1 (Panel D). To conduct a significance test, you would only need to determine if 3.47 (or 0.80) is significantly different from 1 — and you could do it by consulting the appropriate probability density curve (Fisher's *F* statistic).

Figure 3.41 shows the probability density curves for the *F* statistic and two other commonly used statistics. Note 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 powerful tool widely used in significance testing. Unfortunately, the ANOVA can tell you only if the distribution of your data differs from a flat



(B)

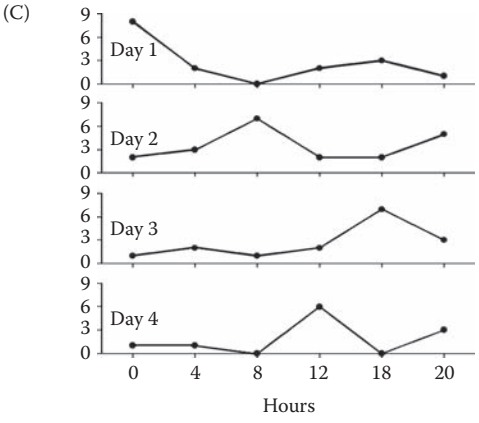
Time	0	4	8	12	16	20	Means
Day 1	0	3	2	8	2	1	2.7
Day 2	2	2	5	7	3	2	3.5
Day 3	1	3	2	7	2	1	2.7
Day 4	1	0	3	6	1	0	1.8
Means	1.0	2.0	3.0	7.0	2.0	1.0	

$M_w = 2.7$

$SD_w = 0.59$

$M_B = 2.7$ $SD_B = 2.05$

$\frac{M_B}{M_w} = 1$ $\frac{SD_B}{SD_w} = 3.47$



D

Time	0	4	8	12	16	20	Means
Day 1	8	2	0	2	3	1	2.7
Day 2	2	3	7	2	2	5	3.5
Day 3	1	2	1	2	7	3	2.7
Day 4	1	1	0	6	0	3	1.8
Means	3.0	2.0	2.0	3.0	3.0	3.0	

$M_w = 2.7$

$SD_w = 0.59$

$M_B = 2.7$ $SD_B = 0.47$

$\frac{M_B}{M_w} = 1$ $\frac{SD_B}{SD_w} = 0.80$

FIGURE 3.40 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, you have a significant finding). See text for details. (Note: M = mean, SD = standard deviation)

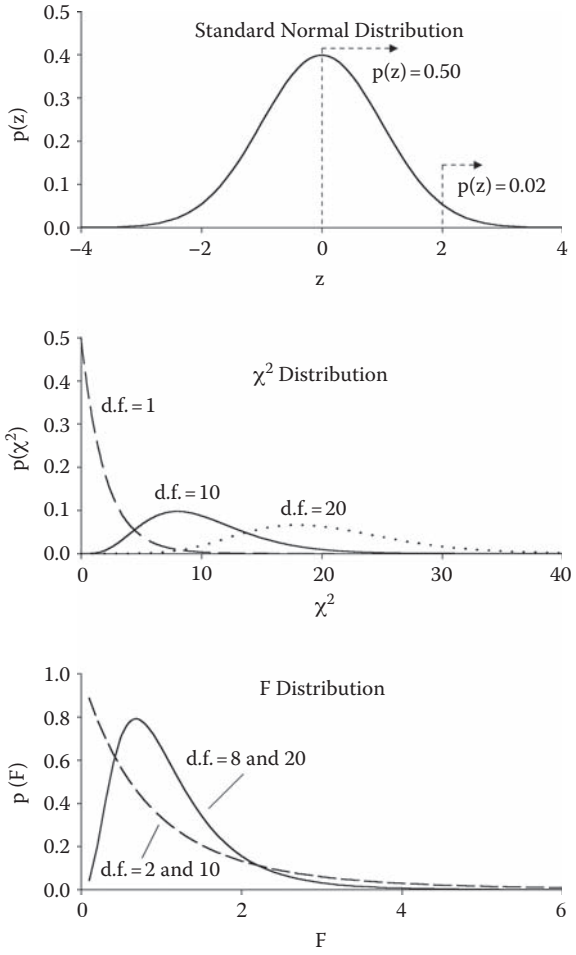


FIGURE 3.41 What do probability density curves look like? These figures show the probability density curves of three commonly used statistics. Multiple probability density curves exist for the χ^2 statistic and the F statistic. Only a few of these curves are shown.

distribution. It does not care whether there is a single daily peak (as is the case for the data set in Panel A of Figure 3.40) or many daily peaks. With an ANOVA, you cannot determine whether the rhythmicity is circadian or ultradian. The Enright periodogram solves this problem by 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.[50] Sokolove and Bushell used the χ^2 statistic instead,[51] which is why their implementation is called the chi square periodogram (chi square is the spelled-out form of χ^2). Note that Sokolove and 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 3.42. This does not mean that the computed period is incorrect if fewer than 10 days are used,

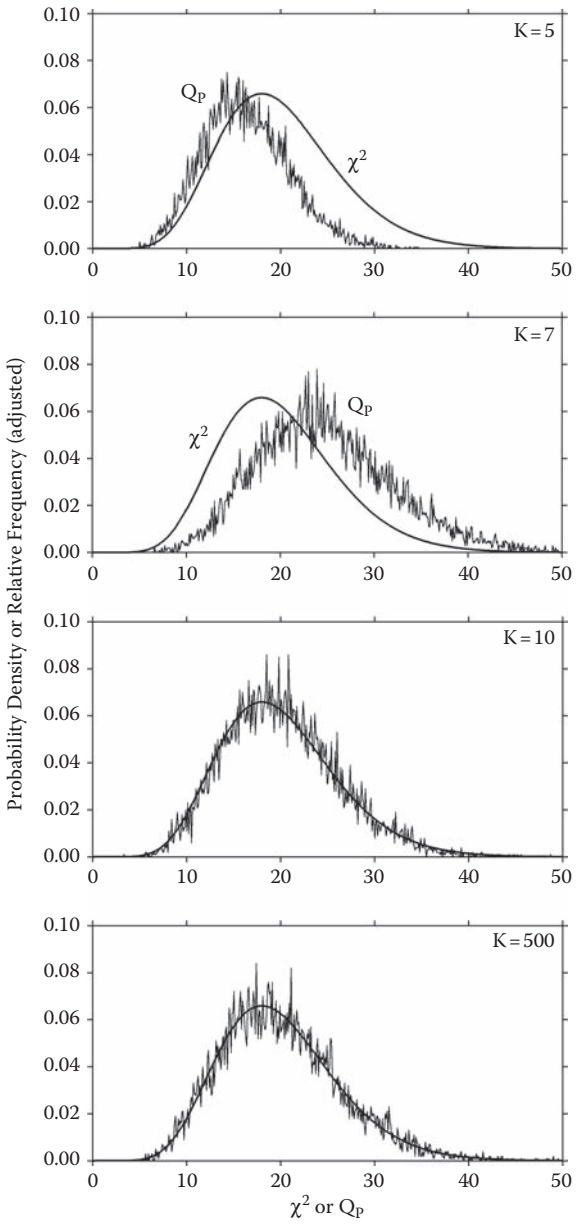


FIGURE 3.42 Just like a χ^2. The Q_P statistic of the chi square periodogram is distributed as χ^2 when the data set contains 10 or more days ($K \geq 10$). In all four panels, the curve for χ^2 is the probability density curve for 20 degrees of freedom, and the curve for Q_P is the relative frequency curve based on computations for the period of 21 data points on 10,000 sets of random numbers.

but it does indicate that the statistical significance cannot be reliably determined. For this reason, the program Tau (see Table 3.1) displays 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 tolerates noisy data better than Fourier analysis does. This does not mean, however, that it is insensitive to noise. The proportion of noise is a relevant property of a rhythm. Figure 3.43 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 no peak appears above the significance line when the time series contains 100% noise (bottom panels). If you are a keen observer, you also 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 example, compare this periodogram with that in the top panel of Figure 3.27). The chi square periodogram experiences much more "leakage" of spectral energy than does the Fourier periodogram. If one starts with a "clean" time series and is willing to repeat the analysis many times with slightly different segments of the time series, a much finer temporal resolution can be obtained with the Fourier periodogram than with the chi square periodogram. To test significance in the Fourier periodogram, Fisher developed the necessary statistic many years ago,[94] and an adaptation for the detection of multiple peaks was developed later.[95] The program Fourier (see Table 3.1) uses both methods.

Significance tests 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 hours to 25.0 hours in steps of 0.1 hour), the computation of the desired level of significance is not straightforward. Consider a race with five horses (Figure 3.44). Suppose that you are placing a bet on the horse that you think will win 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 the bet is 1 in 5 (1/5). Thus, your "level of significance" is 20%. Your odds will remain 1/5 no matter how many races you bet on; however, if you keep betting for five races with five horses, 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. In Figure 3.45, the curves on the left are sine waves (that is, 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 obviously different, but the difference gradually disappears as noise is added to them. It appears to my eyes that 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?

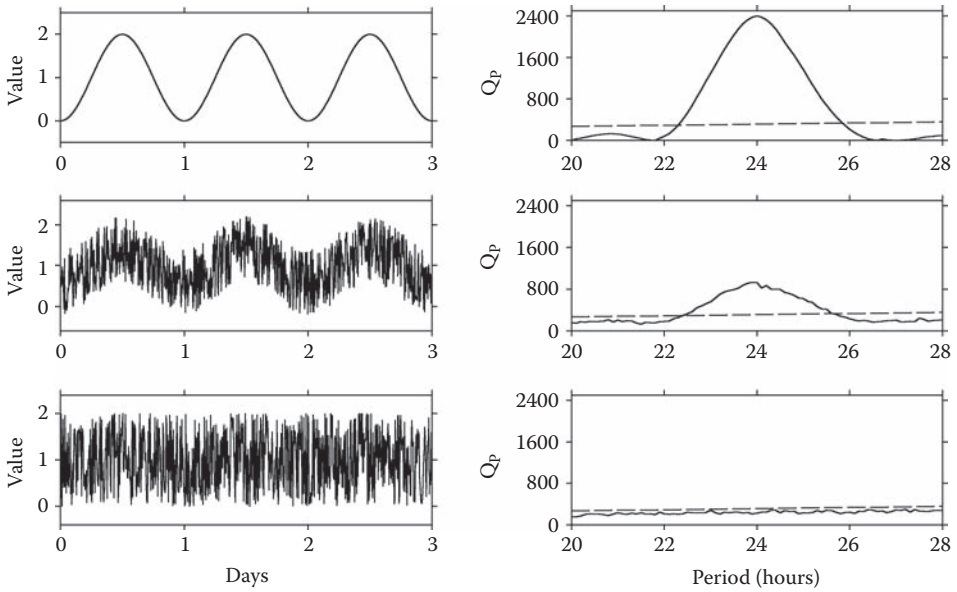


FIGURE 3.43 Determining the statistical significance of peaks in the chi square periodogram. The panels on the left show data sets that are rhythmic but contain variable amounts of noise (random variability). The panels on the right are the corresponding chi square periodograms. The dashed lines in the 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.

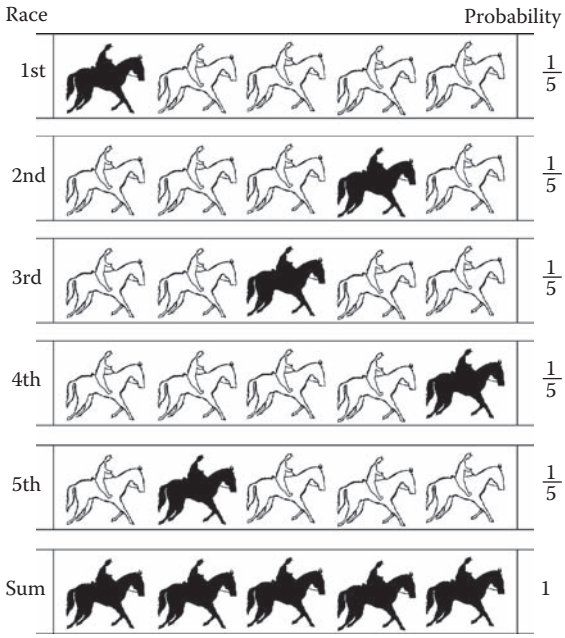


FIGURE 3.44 Inflating the level of significance. The probability of a Type I error in a statistical test is calculated under the assumption that the test will be performed only once. If multiple tests are performed, the computed probability must be corrected. Consider this figure. Assuming that all horses are equally likely to win a race, your probability of picking the winner in any individual race is 1 in 5. However, if you place bets on five races with five horses each, your probability of picking the winner in at least one race is 1.

Decisions about the breakpoint between significance and nonsignificance are subjective, as pointed out earlier (see Figure 3.39). The situation is complicated because hypothesis testing is a "Catch-22." As diagrammed in Figure 3.46, four outcomes are possible when a decision is made to adopt or dismiss an experimental hypothesis. Two of the outcomes present no problem: it is fine to adopt a good hypothesis (that is, to reject the null hypothesis when the null hypothesis is false), and it is fine to dismiss a bad hypothesis (that is, to accept the null hypothesis when the null hypothesis is true). However, the other two outcomes constitute errors. You commit a Type I error when you adopt a bad hypothesis (that is, reject the null hypothesis when the null hypothesis is true), and you commit a Type II error when you discard a good hypothesis (that is, 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 be perceived as charlatans trying to sell snake oil. Clinical practitioners, however, worry quite a bit about Type II errors because they do not want to be blamed for negligent treatment of a patient.

It is impossible to reduce the probabilities of both errors. If you decrease the probability of a Type I error (α), you simultaneously increase the probability of a Type II error (β), and vice versa. Procedures exist that increase the *power* of a test ($1 - \beta$) without greatly affecting α, but the possibility of an error can never be eliminated.

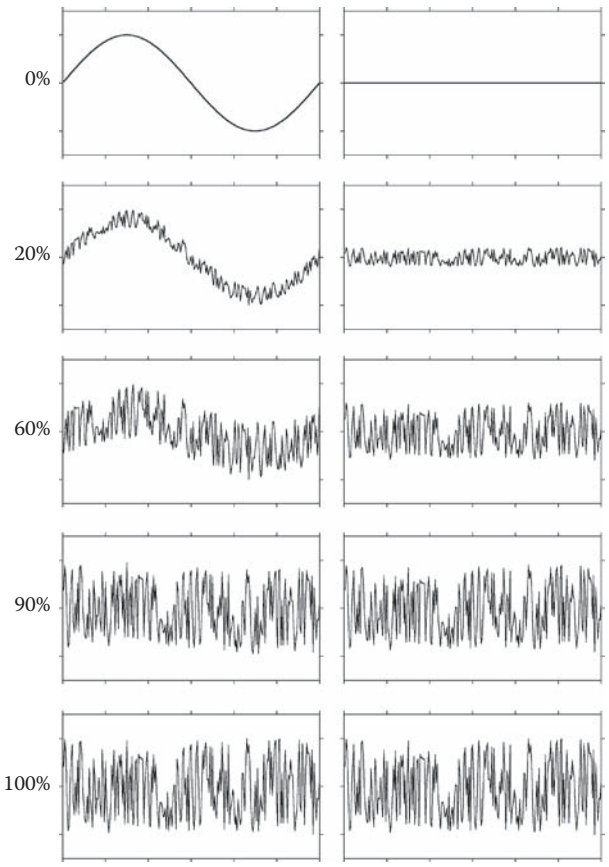


FIGURE 3.45 Statistical significance and statistical power. The left panels show artificial data sets containing sine waves contaminated with variable amounts of noise. The right panels show data sets containing only noise. For 0, 20, and 60% noise, the pairs of data sets are clearly distinct. For 90% noise, the two sets look identical, even though the one on the left has 10% signal. Our eyes cannot see the difference.

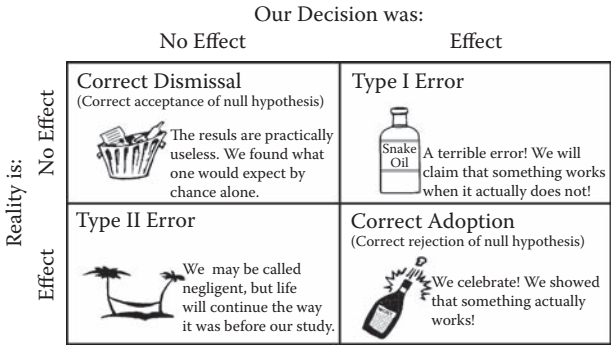


FIGURE 3.46 You can't win! One cannot increase power indefinitely (i.e., eliminate the chances of making a Type II error) without increasing the chances of making a Type I error. In scientific research, Type I errors are considered to be more abominable than Type II errors.

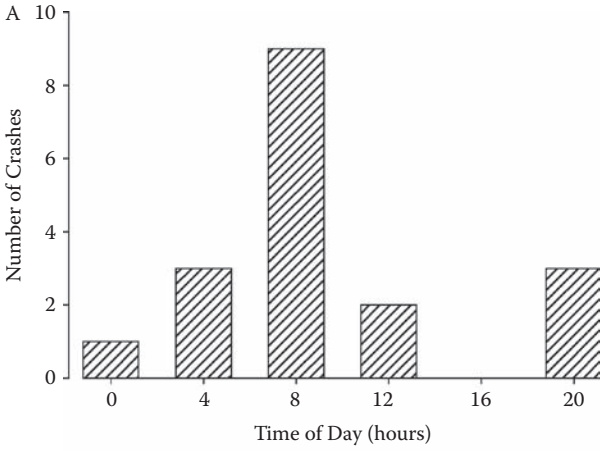
Any researcher would agree that an α of 1.00 is useless — if something is sure to happen by chance, it makes no sense to work for it. Thus, the level of significance must be stopped from escalating. A simple solution for the periodogram is to apply a Bonferroni correction[96] to the level of significance. If you want to keep the *familywise* probability of a Type I error at a level θ as you perform n tests, you must set α at a lower level, so that: $\theta = 1 - (1 - \alpha)^n$. The program Tau automatically makes the correction. The LSP program (see Table 3.1) does not perform the correction because the computations involved in the Lomb–Scargle periodogram already incorporate the ensemble of periods. Consequently, LSP is more powerful than Tau in the detection of significant rhythmicity when the periodogram is computed over a wide range of periods.[48]

3.4.2 Educed Time Series

In some types of studies, the nature of the data prevents the construction of quantitative time series. For example, if you are interested in the daily distribution of the occurrence of heart attacks, your time series may consist of many days with no events and some days with isolated single events. In these situations, it is sensible to give up 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 a 1-day duration and will, for example, show one heart attack at midnight, three heart attacks at 1 A.M., two heart attacks at 2 P.M., and so on. You must then ask whether daily rhythmicity is present in the educed rhythm — or, more precisely, whether the distribution of events along the day differs significantly from a flat distribution. The *Kolmogorov-Smirnov test*[97] provides a simple way to answer this question. The test makes no assumptions about the nature of the data.

Consider Figure 3.47, which addresses whether a daily pattern is present in the occurrence of airplane crashes. There are not enough plane crashes each day to allow the construction of a longitudinal time series. Researchers can, however, collect data over several years, which show the times of day when planes crash. They can then use these data to obtain an accumulated 1-day cycle, as shown in the top panel of Figure 3.47. The graph suggests that more crashes occur around 8 A.M. than at other times of the day. Is this occurrence due to chance alone? You can compare the observed distribution of crashes with a flat distribution (a flat distribution being what you would expect if no daily pattern existed). 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 equals 12 in this case. In contrast, the sum is 0 if no temporal pattern occurs

A



B

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

C

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

FIGURE 3.47 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 you 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.

(Panel C). So, if you had a probability density curve for the sum of absolute differences between observed and expected values, you could easily determine whether your score of 12 is unlikely to occur by chance alone. In actuality, the Kolmogorov-Smirnov test uses a slightly different statistic (that is, the largest difference for accumulated frequencies),[97] 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.

Note that the Kolmogorov-Smirnov test determines only whether the distribution of values differs from a flat distribution. As previously discussed with 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 provide any proof of circadian rhythmicity. A better alternative to the Kolmogorov-Smirnov test, if you expect the rhythmicity to take a sinusoidal form, is the *Rayleigh test*.[98] The Rayleigh test provides a correlation vector, nR^2, which is distributed as χ^2 and, consequently, can easily be 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[99] and to construct confidence intervals.[100]

A third alternative for the analysis of educed rhythms is to use the cosinor method 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,[101] 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.

When one-cycle data sets are analyzed, you must consider whether the cycle is a one-time event or a true rhythm. For example, suppose you go to a high school reunion held on a remote tropical island rarely visited by tourists. While enjoying the island, 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 3.48). This is your one-cycle data set. Now, can you conclude that the number of people at this beach follows a daily rhythm? 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 an entire year (Panel B). The daily cycle that you observed on that single day is an aberration, 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 collecting data for only 1 year, or about weekly rhythms after collecting data for only 1 week, or even about daily rhythms after collecting data for only 1 day. In some cases, you could assume that other cycles would follow the one that was actually observed, but the assumption is often 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; the subjects are killed for sample collection at intervals of several hours.[102] The fact that only one cycle is used may raise 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 — that is, 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 impossible to set a reasonable level of significance for the test of each gene. If you used the Bonferroni correction, you would need to set α at about 0.000005 to keep θ 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

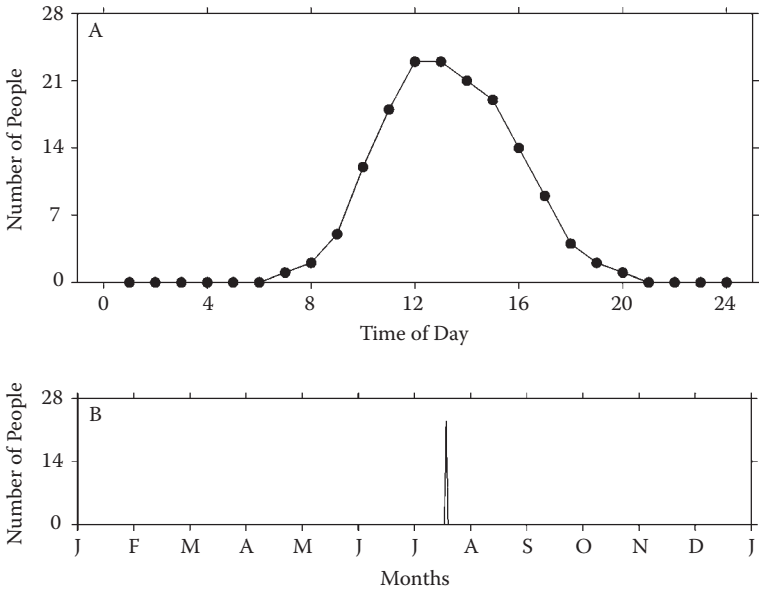


FIGURE 3.48 The issue of representativeness. The data in Panel A suggest that there is a daily pattern in the number of people that can be found at the beach. Any statistical test applied to this data set would reveal a significant effect of time of day. However, it is possible that the real pattern 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.

calculated how many genes could show rhythmic expression by chance alone, they found the figure to be 298.[103] Thus, the detection of rhythmicity was an artifact in more than half (66%) of the genes initially believed to exhibit rhythmicity. The authors of the cited study chose to apply a more stringent criterion that resulted in 22 "legitimately" rhythmic genes. However, they acknowledged that their final count was probably an underestimation.[103] Lowering α results in an elevation of β and, consequently, reduces the statistical power. The solution, if there is one, 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.[104,105]

This section concludes the discussion of procedures used to analyze 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. In Part II of the book, I describe rhythmic phenomena in living organisms — that is, 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.
3. The period of a rhythm is most commonly determined by various methods for inspecting actograms, by Fourier analysis, by the Enright (chi square) periodogram, or by the Lomb–Scargle periodogram. No standard procedure exists 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.
4. 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

Exercise 3.1 Parameters of rhythmic processes

This simple exercise uses the program Wave. 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. To start the program, double-click on the Circadian icon to open the program banner, then click on Wave (the eighth icon from the right).

Exercise 3.2 Smoothing a data set

This exercise uses the procedure of moving averages to smooth a data set (i.e., to filter out high-frequency oscillations). Section 3.2 explains the rationale for this procedure.

1. Double-click on the Circadian icon to open the program banner, then click on Plot (the first icon on the left). 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) in 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 click on the Cartesian plot button (the purple button) now. Browse through the entire data set (6 days). Note that there is a clear daily rhythm with higher values during the first part of each day. However, considerable "noise" (i.e., ultradian oscillations that do not seem to be regular and that obscure the daily oscillation) is also present.
3. To filter the data set, start the program Moving (the second icon from the left in the Circadian banner). If necessary, 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, don't use the default file name (MAV-A07) provided; instead, delete MAV-A07.txt and type B07.txt. Accept the default values for Data Points or Pre-filters.
4. Because ultradian oscillations in A07 are particularly conspicuous in the range of a few minutes, set the Averaging Window size to 24 minutes. Type in 4 (i.e., 4 bins or 24 minutes) and click on Execute.
5. To eliminate the need to switch between programs, create a second filtered file before returning 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, look at the results. Go back to Plot and load the first file that you created (B07, if you followed my suggestion). Note that the temporal pattern is very similar to that of the original file (A07), but the high-frequency oscillations have been filtered out. You may want to alternate between A07 and B07 a few times to observe the difference.
7. Next, look at the second file you created (C07). Open it now. The first thing you may notice is that the wave pattern is phase-advanced by 3 hours in comparison with A07. This is an artifact of the moving-averages procedure, which you can easily correct by starting the procedure 3 hours later. The important thing to note, 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.
8. You may also have noticed 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, while C07 is 3 hours shorter than A07. Because Plot plots only full days, an entire day seems to be missing in both B07 and C07.
9. Next, you can practice with other source files. Note that you have the option of telling the program to discard data points at the beginning or end of the file (using the Data Points panel). You may also use the Pre-filter panels to eliminate outliers. You can set the Averaging Window size to 1 (and avoid the moving-averages filter) if you only want to extract a section from a long data set and eliminate outliers.

Exercise 3.3 Constructing an actogram

As explained in Section 3.2, the actogram is a classic graphic in circadian physiology. Originally used only for records of running-wheel activity, it now is used for practically any type of variable that is recorded over an extended period of time. Data sets must have equally spaced data points 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 the running-wheel activity of a golden hamster maintained in constant darkness for 36 consecutive days. The number of wheel revolutions is accumulated in 6-minute bins (for a total of 8640 data points in the file). Because the data points are equally spaced, time tags are not needed.
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. Also note 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. You will learn how to determine the exact period in future exercises. Note that you can switch to black-and-white display 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 body temperature records 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, you end up plotting every single data point. Thus, in order to have a useful actogram, you must "clip off" the lower values. An arbitrary but convenient clipping level is the mean level of the rhythm. You will learn how to calculate the mean level later (Exercise 5.2). For now, click on the Clip box and type 36.2.
9. Click on the Actogram button. What a difference! You have created 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 (the data will automatically be replotted). The resulting actogram is not as clear as the black-and-white version, but it is readable.
11. Finally, select the data set A06. This file contains the locomotor activity records of a pill bug (a small terrestrial crustacean), measured with an infrared photocell for 19 days in constant darkness. 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 hamster or rat. You can still see, however, that the animal had a free-running period much shorter than 24.0 hours.

Exercise 3.4 Determining circadian period by modulo changes in an actogram

This exercise demonstrates a simple graphical procedure for determining the period of a rhythm.

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 locomotor activity records of a pill bug, measured with an infrared photocell for 19 days in constant darkness. The data resolution is 6 minutes, and the file contains no time tags.
4. Click on the Actogram button (the green button). The records are noisy, but you can still see that the circadian period is shorter than 24.0 hours. (You know that the period is shorter than 24.0 hours because the daily onsets do not align along a vertical line.) What if a day were shorter than 24.0 hours? For example, if the day were as short as the circadian period, the onsets would align along a vertical line. Thus, if you can find out the day length that causes the onsets to align along a vertical line, you will know the length of the circadian period.
5. Click several times on the down arrow by the text Modulo 24 h. 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.

6. Now, select the data file A17. This data set presents a greater 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 (a common occurrence when studying mammals with partial suprachiasmatic lesions). It would also 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 Chapter 4 and Chapter 5 deal with this issue.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Hurlburt, R. T. (2003). *Comprehending Behavioral Statistics* (3rd Edition). Belmont, CA: Wadsworth. An excellent introductory statistics textbook covering descriptive and inferential statistics, up to standard analysis of variance. Hurlburt takes an intuitive approach that is missing in most other books. Although the text is nominally targeted at behavioral scientists, the contents are actually general enough to apply to any field of research.

Hays, W. L. (1994). *Statistics* (5th Edition). Belmont, CA: Wadsworth. A wonderful upper-level statistics textbook covering the basics of inferential statistics in regression and analysis of variance.

Chatfield, C. (2004). *The Analysis of Time Series: An Introduction* (6th Edition). Boca Raton, FL: CRC Press. A good introduction to the analysis of time series, now in its sixth edition. This textbook is intended for undergraduate use 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. & Stoffer, D. S. (2000). *Time Series Analysis and Its Applications*. New York: Springer. An advanced textbook on time series analysis concentrating on autoregression and spectral analysis. This book does not specifically address the analysis of circadian rhythms, but it provides a solid background in time series analysis for readers well-trained in statistics and calculus.

Grinnell, F. (1992). *The Scientific Attitude* (2nd Edition). New York: Guilford Press. This eye-opening book is not about mathematical data analysis per se but 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.

WEB SITES TO EXPLORE

American Statistical Association:
http://www.amstat.org

SAS Institute Inc.:
http://www.sas.com

SPSS Inc.:
http://www.spss.com

StatSoft Inc.:
http://www.statsoftinc.com

Wavelet Tutorial:
http://engineering.rowan.edu/~polikar/WAVELETS/WTtutorial.html

LITERATURE CITED

1. Brockwell, P. J. & Davis, R. A. (2002). *Introduction to Time Series and Forecasting*, 2nd Edition. New York: Springer.
2. Chatfield, C. (2004). *The Analysis of Time Series: An Introduction,* 6th Edition. Boca Raton, FL: Chapman and Hall.

3. Hamilton, J. D. (1994). *Time Series Analysis*. Princeton, NJ: Princeton University Press.
4. Shumway, R. H. & Stoffer, D. S. (2000). *Time Series Analysis and Its Implications*. New York: Springer.
5. Percival, D. B. & Walden, A. T. (2000). *Wavelet Methods for Time Series Analysis*. New York: Cambridge University Press.
6. Halberg, F. (1992). Glossary and abbreviations. In: Halberg, F. & Watanabe, H. (Eds.). *Chronobiology and Chronomedicine*. Tokyo: Medical Review, pp. 5–8.
7. Cornélissen, G., Halberg, F., Schwartzkopff, O., Delmore, P., Katinas, G., Hunter, D., Tarquini, B., Tarquini, R., Perfetto, F., Watanabe, Y. & Otsuka, K. (1999). Chronomes, time structures, for chronobioengineering for "a full life." *Biomedical Instrumentation and Technology* 33: 152–187.
8. Levine, J. D., Funes, P., Dowse, H. B. & Hall, J. C. (2002). Signal analysis of behavioral and molecular cycles. *BMC Neuroscience* 3: art. 1.
9. Brown-Brandl, T. M., Yanagi, T., Xin, H., Gates, R. S., Bucklin, R. A. & Ross, G. S. (2003). A new telemetry system for measuring core body temperature in livestock and poultry. *Applied Engineering in Agriculture* 19: 583–589.
10. Hurlburt, R. T. (2003). *Comprehending Behavioral Statistics*, 3rd Edition. Belmont, CA: Wadsworth.
11. Sprinthall, R. C. (2003). *Basic Statistical Analysis*, 7th Edition. Boston, MA: Allyn and Bacon.
12. Moore, D. S. (2004). *The Basic Practice of Statistics*, 3rd Edition. New York: Freeman.
13. Weiss, N. A. (2005). *Elementary Statistics*, 6th Edition. Reading, MA: Addison-Wesley.
14. Nelson, W., Tong, Y. L., Lee, J. K. & Halberg, F. (1979). Methods for cosinor rhythmometry. *Chronobiologia* 6: 305–323.
15. Wang, Y. & Brown, M. B. (1996). A flexible model for human circadian rhythms. *Biometrics* 52: 588–596.
16. Ruf, T. (1996). The baseline cosinus function: a periodic regression model for biological rhythms. *Biological Rhythm Research* 27: 153–165.
17. Almirall, H. (1997). Modeling the body temperature throughout the day with a two-term function. *Behavior Research Methods, Instruments, and Computers* 29: 595–599.
18. Alonso, I. & Fernández, J. R. (2001). Nonlinear estimation and statistical testing of periods in nonsinusoidal longitudinal time series with unequidistant observations. *Chronobiology International* 18: 285–308.
19. Wang, Y., Ke, C. & Brown, M. B. (2003). Shape-invariant modeling of circadian rhythms with random effects and smoothing spline ANOVA decompositions. *Biometrics* 59: 804–812.
20. Halberg, F., Cornélissen, G., Katinas, G., Syutkina, E.V., Sothern, R.B., Zaslavskaya, R., Halberg, F., Watanabe, Y., Schwartzkopff, O., Otsuka, K., Tarquini, R., Frederico, P. & Siggelova, J. (2003). Transdisciplinary unifying implications of circadian findings in the 1950s. *Journal of Circadian Rhythms* 1: art. 2.
21. Refinetti, R. (1992). Analysis of the circadian rhythm of body temperature. *Behavior Research Methods, Instruments, and Computers* 24: 28–36.
22. Johnson, M. S. (1926). Activity and distribution of certain wild mice in relation to biotic communities. *Journal of Mammalogy* 7: 245–277.
23. Daan, S. & 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.
24. Nelson, D. E. & 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.
25. Grosse, J., Loudon, A. S. I. & 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.
26. Elliott, J. A. & Tarmarkin, L. (1994). Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. *Journal of Comparative Physiology A* 174: 469–484.
27. Meijer, J. H. & 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.
28. Vansteensel, M. J., Deboer, T., Dahan, A. & 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.
29. Refinetti, R. (1992). Non-parametric procedures for the determination of phase markers of circadian rhythms. *International Journal of Biomedical Computing* 30: 49–56.
30. Rosi, F., Greppi, G., Corino, C., Schoen, F. & Solca, F. (1981). An introduction to the study of biological rhythms. *Rivista di Biologia* 74: 155–190.
31. Klerman, E. B., Lee, Y., Czeisler, C. A. & 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.
32. Klerman, E. B., Gershengorn, H. B., Duffy, J. F. & Kronauer, R. E. (2002). Comparisons of the variability of three markers of the human circadian pacemaker. *Journal of Biological Rhythms* 17: 181–193.
33. Daan, S. & Oklejewicz, M. (2003). The precision of circadian clocks: assessment and analysis in Syrian hamsters. *Chronobiology International* 20: 209–221.
34. Walker, J. S. (1988). *Fourier Analysis*. New York: Oxford University Press.
35. Walker, J. S. (1996). *Fast Fourier Transforms*, 2nd Edition. Boca Raton, FL: CRC Press.
36. Enright, J. T. (1981). Data analysis. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology, Volume 4*). New York: Plenum, pp. 21–39.
37. Refinetti, R. (1993). Comparison of six methods for the determination of the period of circadian rhythms. *Physiology and Behavior* 54: 869–875.

38. Eijzenbach, V., Sneek, J. H. & 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.
39. Lumineau, S., Guyomarc'h, C., Boswell, T., Richard, J. P. & 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.
40. Mormont, M. C., Waterhouse, J., Bleuzen, P., Giacchetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset, J. L., Touitou, Y. & 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.
41. Hassnaoui, M., Pupier, R. & Rehailia, M. (2000). A concordance method for analyzing categorical time series: an application for the search of periodicities. *Biological Rhythm Research* 31: 177–201.
42. Albers, H. E., Gerall, A. A. & Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
43. Rao, A. V. & Sharma, V. K. (2002). A simple approach for the computation of multiple periodicities in biological time series. *Biological Rhythm Research* 33: 487–502.
44. Diambra, L., Lopes, J. R., Menna-Barreto, L. & Rigolino, R. (2002). Ciclograma: a tool for detection of rhythmicities in sleep/wake cycles. *Chronobiology International* 19: 793–803.
45. Klemfuss, H. & Clopton, P. L. (1993). Seeking tau: a comparison of six methods. *Journal of Interdisciplinary Cycle Research* 24: 1–16.
46. Refinetti, R. (1991). An extremely simple procedure for the analysis of circadian and estrous periodicity. *Physiology and Behavior* 50: 655–659.
47. Kanjilal, P. P., Bhattacharya, J. & 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.
48. 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.
49. Enright, J. T. (1965). The search for rhythmicity in biological time-series. *Journal of Theoretical Biology* 8: 426–468.
50. Dörrscheidt, G. L. & Beck, L. (1975). Advanced methods for evaluating characteristic parameters of circadian rhythms. *Journal of Mathematical Biology* 2: 107–121.
51. Sokolove, P. G. & Bushell, W. N. (1978). The chi square periodogram: its utility for analysis of circadian rhythms. *Journal of Theoretical Biology* 72: 131–160.
52. Vogelbaum, M. A. & Menaker, M. (1992). Temporal chimeras produced by hypothalamic transplants. *Journal of Neuroscience* 12: 3619–3627.
53. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
54. Lovegrove, B. G., Heldmaier, G. & Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
55. Wollnik, F. & 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.
56. Sollars, P. J., Kimble, D. P. & Pickard, G. E. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *Journal of Neuroscience* 15: 2109–2122.
57. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
58. Tosini, G. & 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.
59. Hut, R. A., Mrosovsky, N. & Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
60. Earnest, D. J., Liang, F. Q., Ratcliff, M. & Cassone, V. M. (1999). Immortal time: circadian clock properties of rat suprachiasmatic cell lines. *Science* 283: 693–695.
61. Bertolucci, C., Sovrano, V. A., Magnone, M. C. & Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
62. Shimizu, I., Kawai, Y., Taniguchi, M. & Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
63. Canal-Corretger, M. M., Vilaplana, J., Cambras, T. & 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.
64. Chen, W. M. & Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
65. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. & Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
66. Wee, R., Castrucci, A. M., Provencio, I., Gan, L. & Van Gelder, R. N. (2002). Loss of photic entrainment and altered free-running circadian rhythms in *math5*$^{-/-}$ mice. *Journal of Neuroscience* 22: 10427–10433.
67. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T. & 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.

68. Granados-Fuentes, D., Prolo, L. M., Abraham, U. & Herzog, E. D. (2004). The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *Journal of Neuroscience* 24: 615–619.
69. Lomb, N. R. (1976). Least-squares frequency analysis of unequally spaced data. *Astrophysics and Space Science* 39: 447–462.
70. Schimmel, M. (2001). Emphasizing difficulties in the detection of rhythms with Lomb–Scargle periodograms. *Biological Rhythm Research* 32: 341–345.
71. Van Dongen, H. P. A., Olofsen, E., Van Hartevelt, J. H. & Kruyt, E. W. (1999). Searching for biological rhythms: peak detection in the periodogram of unequally spaced data. *Journal of Biological Rhythms* 14: 617–620.
72. Refinetti, R., Kaufman, C. M. & 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.
73. Weinert, D., Fritzche, P. & Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
74. Cheng, P., Yang, Y. & Liu, Y. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 98: 7408–7413.
75. Gonze, D., Halloy, J. & Gldbeter, A. (2002). Robustness of circadian rhythms with respect to molecular noise. *Proceedings of the National Academy of Sciences U.S.A.* 99: 673–678.
76. Refinetti, R. (2004). Non-stationary time series and the robustness of circadian rhythms. *Journal of Theoretical Biology* 227: 571–581.
77. Eastman, C. & Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
78. Casdagli, M. (1991). Chaos and deterministic *versus* stochastic non-linear modelling. *Journal of the Royal Statistical Society B* 54: 303–328.
79. Stark, J. & Hardy, K. (2003). Chaos: useful at last? *Science* 301: 1192–1193.
80. Fisher, R. (1955). Statistical methods and scientific induction. *Journal of the Royal Statistical Society B* 17: 69–78.
81. McPherson, G. (1990). *Statistics in Scientific Investigation*. New York: Springer.
82. Kanji, G. K. (1993). *100 Statistical Tests*. London: Sage.
83. Efron, B. & Tibshirani, R. (1991). Statistical data analysis in the computer age. *Science* 253: 390–395.
84. Hall, P. (1992). *The Bootstrap and Edgeworth Expansion*. New York: Springer.
85. Efron, B. & Tibshirani, R. J. (1993). *An Introduction to the Bootstrap*. Boca Raton, FL: Chapman and Hall.
86. Simon, J. L. (1995). *Resampling: The New Statistics*. Arlington, VA: Resampling Stats.
87. Refinetti, R. (1996). Demonstrating the consequences of violations of assumptions in between-subjects analysis of variance. *Teaching of Psychology* 23: 51–54.
88. Bakan, D. (1966). The test of significance in psychological research. *Psychological Bulletin* 66: 423–437.
89. Fidler, F., Thomason, N., Cumming, G., Finch, S. & Leeman, J. (2004). Editors can lead researchers to confidence intervals, but can't make them think. *Psychological Science* 15: 119–126.
90. Rosnow, R. L. & Rosenthal, R. (1989). Statistical procedures and the justification of knowledge in psychological science. *American Psychologist* 44: 1276–1284.
91. Zuckerman, M., Hodgins, H. S., Zuckerman, A. & Rosenthal, R. (1993). Contemporary issues in the analysis of data: a survey of 551 psychologists. *Psychological Science* 4: 49–53.
92. Estes, W. K. (1997). Significance testing in psychological research: some persisting issues. *Psychological Science* 8: 18–20.
93. Altman, D. G. (2002). Poor-quality medical research: what can journals do? *Journal of the American Medical Association* 287: 2765–2767.
94. Fisher, R. A. (1929). Tests of significance in harmonic analysis. *Proceedings of the Royal Society of London A* 125: 54–59.
95. Siegel, A. F. (1980). Testing for periodicity in a time series. *Journal of the American Statistical Association* 75: 345–348.
96. Krauth, J. (1988). *Distribution-Free Statistics: An Application-Oriented Approach*. New York: Elsevier.
97. Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill.
98. Mardia, K. V. (1972). *Statistics of Directional Data*. New York: Academic Press.
99. Proschan, M. A. & Follmann, D. A. (1997). A restricted test of circadian rhythm. *Journal of the American Statistical Association* 92: 717–724.
100. Brazier, K. T. S. (1994). Confidence intervals from the Rayleigh test. *Monthly Notices of the Royal Astronomical Society* 268: 709–712.
101. Enright, J. T. (1989). The parallactic view, statistical testing, and circular reasoning. *Journal of Biological Rhythms* 4: 295–304.
102. Langmead, C. J., Yan, A. K., McClung, C. R. & Donald, B. R. (2003). Phase-independent rhythmic analysis of genome-wide expression patterns. *Journal of Computational Biology* 10: 521–536.
103. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N. & 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 U.S.A.* 99: 9562–9567.
104. Kirk, R. E. (1995). *Experimental Design: Procedures for the Behavioral Sciences*, 3rd Edition. Pacific Grove, CA: Brooks/Cole.
105. Maxwell, S. E. & Delaney, H. D. (2004). *Designing Experiments and Analyzing Data*, 2nd Edition. Mahwah, NJ: Lawrence Erlbaum Associates.

Part 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.)

4 Ultradian and Infradian Rhythms

CHAPTER OUTLINE

4.1 Environmental Rhythms
4.2 Ultradian Rhythms
4.3 Infradian Rhythm
4.4 Annual Rhythms

4.1 ENVIRONMENTAL RHYTHMS

Most people are not aware of the environmental rhythms that abound on Earth. As shown in Table 4.1, rhythmic oscillations in the environment range in period from a few femtoseconds (10^{-15} seconds) to tens of thousands of years. Some rhythms are human-made, such as 50 or 60 Hz alternated electric current and the 7-day week, while others are created by the Earth, the Moon, and the Sun. For example, 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's revolution around the Sun changes from circular to elliptical over thousands of years. This variation has exhibited a period of approximately 96,000 years during the last 5 million years (the *orbital eccentricity* rhythm). The Earth's axis of rotation is tilted, and the angle of inclination oscillates with a period of 41,000 years (the *obliquity* rhythm). There also 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 affect Earth's climate, as suggested in the 1940s by Croatian mathematician Milutin Milankovich.[3–5] Figure 4.2 provides an example of the effect of the orbital eccentricity rhythm on sea surface temperature. During the last 340,000 years, mean sea surface temperature of the Pacific

TABLE 4.1
Some Environmental Cycles on Earth

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 clock tower (day ÷ 24)
12.4 hours	Tides (attractive forces of Sun and Moon)
24 hours	Days (Earth's rotation)
1 week	Work–rest schedule 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)
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

Note: This list is illustrative, not exhaustive. Some of the environmental cycles, such as those of alternated current, bell rings, and the week, are human-made.

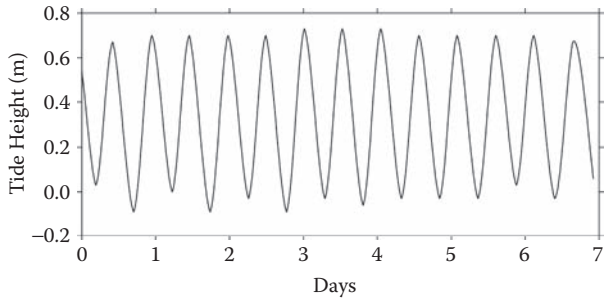


FIGURE 4.1 Going with the tide. The graph shows the variations of tide height at Biscayne Bay (Miami, FL) during the week of August 8, 2002. The mean time difference between successive high tides is 12.4 hours (12 hours and 25 minutes). (Source: Tide High and Low, Inc. www.saltwatertides.com).

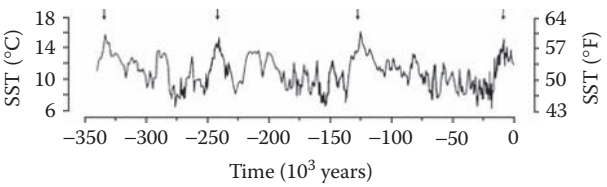


FIGURE 4.2 A long temperature record. The graph shows the sea surface temperature (SST) of the southwest Pacific Ocean during the last 340,000 years, as estimated by the magnesium-calcium ratio in foraminiferal shells. Celsius scale is on the left axis; Fahrenheit scale is on the right axis. Arrows point to temperature peaks with a period of approximately 96,000 years. (Source: Pahnke, K., Zahn, R., Elderfield, H. & Schulz, M. (2003). 340,000-year centennial-scale marine record of Southern Hemisphere climatic oscillation. *Science* 301: 948–952.)

Ocean has oscillated between 6 and 16°C (between 43 and 61°F), with veiled but noticeable peaks at intervals of approximately 96,000 years (indicated by the vertical arrows). Spectral analysis also identifies obliquity and precession components, which are invisible to human eyes, in these[6] and other records.[7–9]

If you observed Figure 4.2 carefully, you may have noticed that ocean temperature has been falling for the past 10,000 years. You were probably surprised by this observation because you have heard that *global warming* may become a major threat to humanity in the 21st 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* (that is, because of alterations to the atmosphere caused by humans, such as the increase of carbon dioxide emissions, which create a "greenhouse" effect).[11–18] Although a rise of 0.8°C is not very impressive in comparison with the expected fall of 6°C, the speeds of the two changes are dramatically different. A rate of 0.8°C per 50 years would result in a rise of 640°C in 40,000 years. Of course, all life forms on Earth would be dead much before that! Global temperature has decreased by 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 reverse the long-term cooling trend, and life on Earth may indeed be threatened by global warming before the end of the 21st century.[10] Migratory patterns of several species of birds have already been affected by global warming,[19] and much greater effects on all life forms can be predicted if the warming trend continues.[20]

Environmental rhythms have also been described with periods of a few thousand years,[21,22] a few decades,[23,24] or just a few years. Environmental cycles in the latter group include the El Niño Southern Oscillation, which recurs in mild form each year and more strongly in intervals of 2 to 10 years,[9,25,26] and the 10.5-year cycle of magnetic fields in sunspots.[27] Halberg recently claimed to have detected a 7-day cycle in geomagnetic disturbances,[28] although the data presented in the report were unconvincing. If such a cycle does exist, and it is not human-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 an arbitrary human convention without a physical correlate.[29]

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 4.3). As discussed later in this chapter, *tidal* cycles

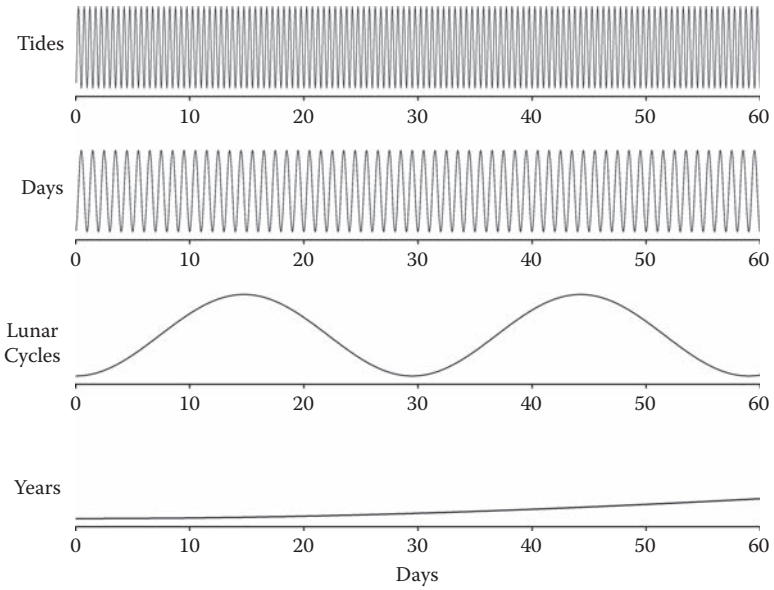


FIGURE 4.3 The clocks that time us. These diagrams provide a visual comparison of the four temporal domains of environmental cycles known to affect the internal clocks of living beings.

FIGURE 4.4 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. (Source: Photographs by R. Refinetti.)

(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.[30]

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 is formed on the opposite side of Earth. These bulges should result in 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 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).[31] *Daily cycles* are discussed in Chapter 5; *annual cycles* are the focus of the next three paragraphs.

The experience of annual environmental cycles is a normal part of life for most people, except those who live near the Equator. The difference between summer and winter, for example, is as clear as the difference between a green lawn and a snow blanket (Figure 4.4). In the northern hemisphere, the average daily temperature is low at the beginning of the year, rises for 6 months, and then falls again by the end of the year. This cycle is repeated year after year (Figure 4.5). The seasons alternate due to the Earth's revolution around the Sun (which takes 365.2 days), but the revolution itself is not the sole explanation. Seasons occur primarily because of the tilt of Earth's rotational axis (*obliquity*).[32–34] As the Earth moves around its orbit, it sometimes points toward the Sun and sometimes points away from the Sun (Figure 4.6). 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 this same time of year, the southern hemisphere experiences winter. When the Earth points away from the Sun, the northern hemisphere experiences winter and the southern hemisphere experiences summer. Days are longer in the summer because the Sun stays above the horizon longer. For example, at 40° North, the days provide 15 hours of sunlight in the summer but only 9 hours of sunlight in the winter.

The explanation for seasonal variation in temperature is more complicated, as illustrated in Figure 4.7. Because the Earth's axis is tilted, the same quantity of sunlight (solar radiation) is spread out over a wider area in the winter (*a* in Panel A) than 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. Regions close to the equator are not affected by the Earth's tilt, which is why very little seasonal variation occurs in tropical zones.

Latitude strongly affects climate because of Earth's spherical shape, not because of its tilt. As shown in Panel C of Figure 4.7, the northern and southern regions would receive less radiation per unit area than the equatorial region even if no tilt were present. This is why the North Pole is cold even in the middle of the summer. The Earth's tilt also 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 is so small as to be negligible. Even the much greater variation in distance from the Sun caused by the Earth's elliptic orbit has little effect on climate. When it is winter in the northern hemisphere, it is summer in the southern hemisphere, yet half the world cannot be far away from

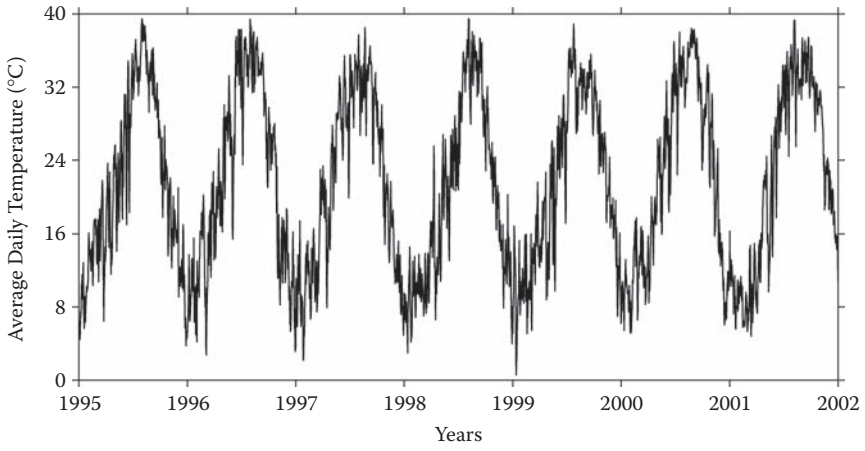


FIGURE 4.5 Hot and cold in Las Vegas. The graph shows the average daily temperature in Las Vegas, NV, for 7 consecutive years. Annual rhythmicity is evident. (Source: Average Daily Temperature Archive, University of Dayton, Dayton, OH.)

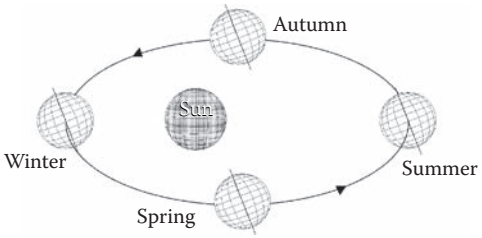


FIGURE 4.6 The Earth, the Sun, and the seasons. The primary cause of the seasons is the tilt of the Earth's rotational axis, which affects the flow of solar energy to each hemisphere as the Earth revolves around the Sun. The designations of summer and winter in this figure apply to the northern hemisphere. When it is summer in the northern hemisphere, it is winter in the southern hemisphere, and vice versa.

the Sun while the other half is close to it. If you look again at Figure 4.6, you will see that, in the northern hemisphere, the Earth is closest to the Sun during the winter and farthest from the Sun during the summer. This may seem counterintuitive, but keep in mind that the average distance between the Earth and the Sun is 150 million kilometers (93 million miles), so that variations of even several thousand kilometers are negligible.

Although most people 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 waveform of the precipitation cycle may be somewhat irregular, but day length and temperature have sinusoidal waveforms when averaged over many years (Figure 4.8). Note that the day-length curve is smoother than the temperature curve. In 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 example, Figure 4.9 shows the curves for average highs and record highs in New York City. Although both curves rise in the summer,

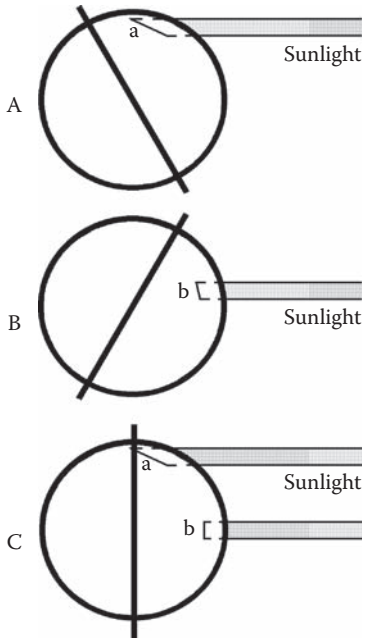


FIGURE 4.7 The deal about obliquity. Because the Earth's rotational axis is tilted (by 23.4°), the flux of solar energy is spread out over a wider surface during the winter (A) than during the summer (B). Latitudinal differences in irradiance, on the other hand, are due to Earth's sphericity and would exist regardless of obliquity (C).

the record highs are obvious deviations from the average highs. While 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 winter). Therefore, day length is more reliable than temperature as an indicator of time of year, even though temperature may have a stronger impact on the lives of organisms. Many organisms use day length, not temperature, as a seasonal clock to help them time their biological rhythms.

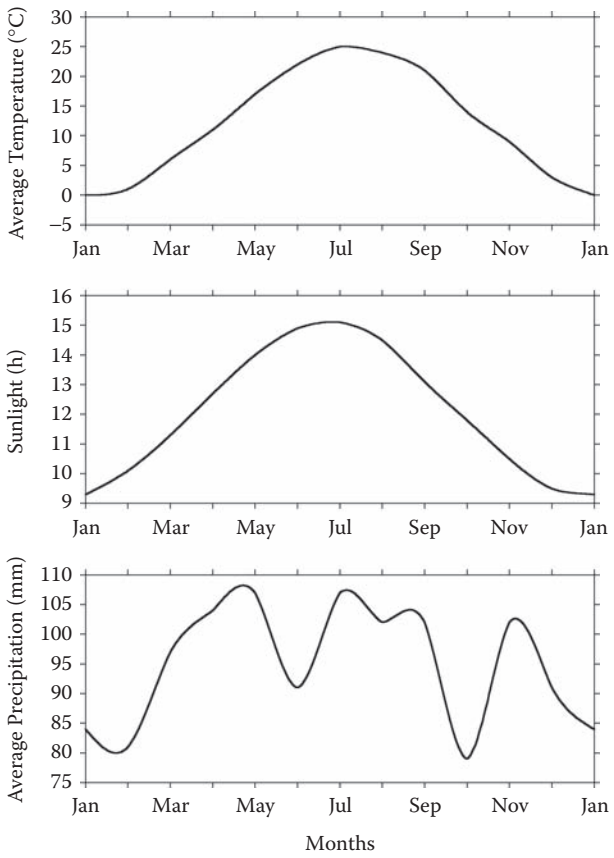


FIGURE 4.8 The weather in New York. The graphs show the seasonal variations in ambient temperature, day length, and precipitation in New York City averaged over the last 30 years. Temperature and day length exhibit a sinewave-like oscillatory pattern, while the oscillatory pattern of precipitation is more complex. (Sources: Weather Channel, www.weatherchannel.com and Tide High and Low, Inc., www.saltwatertides.com.)

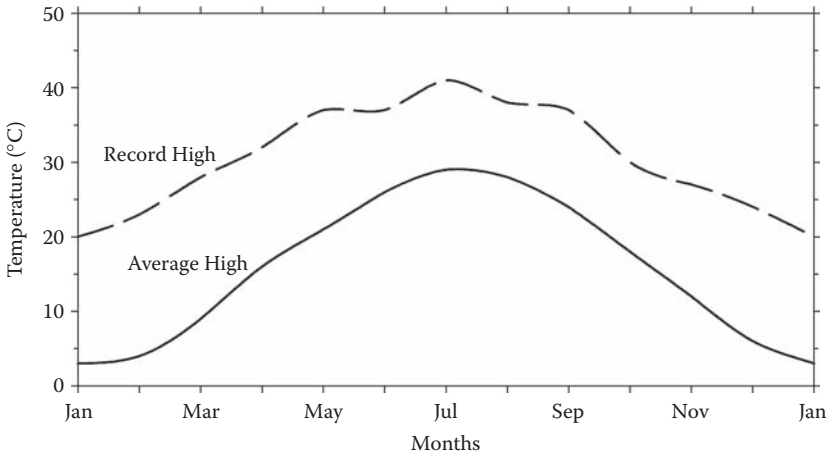


FIGURE 4.9 How hot is New York? Although the average high temperatures in New York City (averaged over 30 years) display a smooth seasonal pattern of oscillation, considerable day-to-day variation occurs. For example, although the average high in January is 3°C (i.e., barely above freezing), the record high was 20°C (in 1967). (Source: Weather Channel, www.weatherchannel.com.)

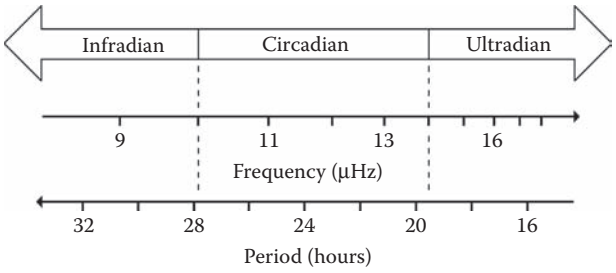


FIGURE 4.10 A self-centered worldview. For circadian physiologists, a biological rhythm is either circadian or "everything else." Oscillatory processes with frequencies below the circadian range are called *infradian*; those above the circadian range are called *ultradian*. Note that period is the inverse of frequency, so that infradian rhythms have periods longer than circadian rhythms, and ultradian rhythms have periods shorter than circadian rhythms.

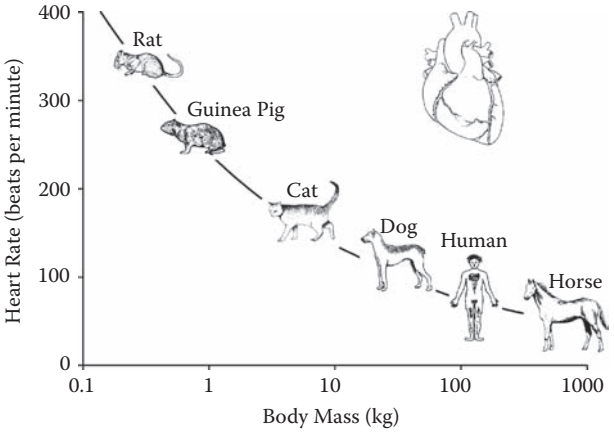


FIGURE 4.11 Heart rate and body size. In mammals, heart rate is a power function of body size. Smaller animals have higher heart rates than larger animals. (Source: Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *Journal of Applied Physiology* 22: 453–460.)

4.2 ULTRADIAN RHYTHMS

Circadian physiologists think of biological rhythms as either *circadian rhythms* or *everything else*. In terms of frequency of oscillation, all rhythms below circadian rhythms are called *infradian* rhythms, while all rhythms above circadian rhythms are called *ultradian* rhythms (Figure 4.10). Although no strictly defined boundaries exist, the designation *circadian* usually is reserved for biological oscillations between 10 and 14 μHz (that is, between 10×10^{-6} and 14×10^{-6} cycles per second). Most biological rhythms, however, are described not in terms of frequency of oscillation but in terms of its reciprocal, *period*. The designation *circadian* is usually reserved for biological rhythms with *periods* between 19 and 28 hours. Note, however, that the prefixes *infra* and *ultra* are counterintuitive when period is used: *infradian* rhythms have periods *longer* than circadian rhythms, while *ultradian* rhythms have periods *shorter* than circadian rhythms.

This section of the chapter discusses *ultradian* rhythms (that is, high-frequency biological oscillations). Infradian rhythms are discussed in Sections 4.3 and 4.4, and circadian rhythms are covered 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), they are an integral part of the temporal organization of physiological function.

4.2.1 Cardiac and Respiratory Rhythms

Many life forms have vascular systems that provide for 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.[35] 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,[36,37] so that a rat's heart at rest beats more than 300 times per minute, while a horse's heart beats fewer than 50 times per minute (Figure 4.11). The waveform of the cardiac rhythm depends on the species studied and on the arrangement of electrodes used to record the muscular activity. Figure 4.12 shows a diagram of a typical human *electrocardiogram*. A pattern of positive and negative deflections (called the P, Q, R, S, T, and U waves) can be seen.[38] The pattern repeats itself slightly more often than once per second, thus yielding a typical human heart rate of 70 beats per minute.

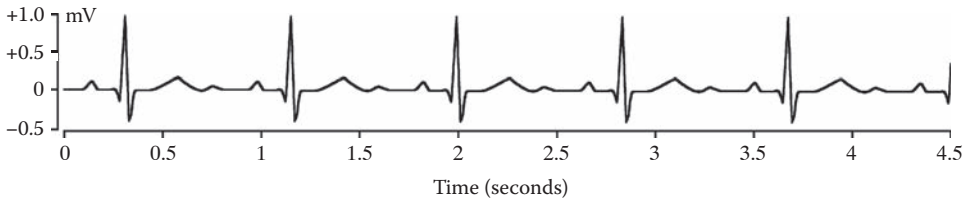


FIGURE 4.12 The cardiac rhythm. This diagram shows a typical human electrocardiogram. The same pattern repeats itself approximately every second (70 beats per minute at rest). The exact configuration of the waves depends on which of the standard electrode locations is used for the recording. (Source: Adapted from Ganong, W. F. (2001). *Review of Medical Physiology*, 20th Edition. New York: McGraw-Hill.)

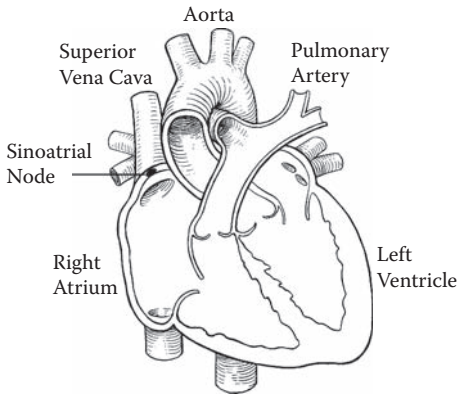


FIGURE 4.13 The cardiac clock. This diagram shows the location of the cardiac pacemaker in the sinoatrial node of the heart. (Source: Adapted from *Medical Illustration Library* (1994). Baltimore, MD: Williams & Wilkins.)

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.[39–41] Figure 4.13 indicates the location of the sinoatrial node. Individual cells in the node possess pacemaker function, and much has been learned about their operation in the last 20 years.[42] 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).[43–45]

While 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.[37] In vertebrates, breathing rate — like heart rate — is inversely proportional to body size,[36,37] so that a rat at rest breathes more than 70 times per minute, while a horse breathes fewer than 10 times per minute (Figure 4.14). 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.[46] 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 4.15). The pre-Bötzinger complex (preBötC) seems to be the site of the respiratory pacemaker,[47,48] but various groups of cells in the ventral medulla that exhibit pre-inspiratory activity (pre-I) may also play an essential role.[49] Although individual preBötC cells may have pacemaker properties — as individual sinoatrial cardiac cells do — this issue is still under debate.[50] The alternative is that rhythmicity

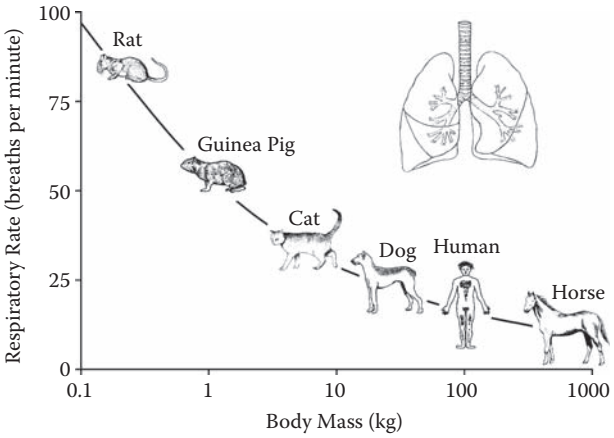


FIGURE 4.14 Respiratory rate and body size. In mammals, respiratory rate is a power function of body size. Smaller animals breathe more frequently than larger animals. Humans breathe approximately 18 times per minute at rest. (Source: Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *Journal of Applied Physiology* 22: 453–460.)

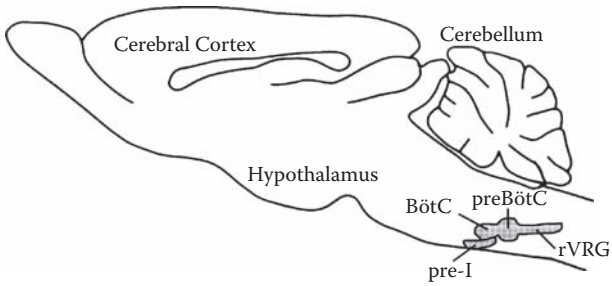


FIGURE 4.15 The respiratory clock. This diagram of the rodent brain shows the location of conglomerates of respiratory bulbospinal premotor neurons (i.e., neurons that control nerves responsible for breathing movements). Note: BötC = Bötzinger complex, preBötC = pre-Bötzinger complex, pre-I = neurons with preinspiratory discharge patterns, rVRG = rostral ventral respiratory group. (Source: Adapted from Feldman, J. L., Mitchell, G. S. & Nattie, E. E. (2003). Breathing: rhythmicity, plasticity, chemosensitivity. *Annual Review of Neuroscience* 26: 239–266.)

emerges from the interaction of many cells that are not individually rhythmic.

4.2.2 Neuroendocrine Rhythms

Nerve cells in the central nervous system exhibit "spontaneous activity," a fact that has been known since the first electrophysiological recordings of single-cell activity in the cerebral cortex.[51,52] 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.[53] Even before the activity of individual nerve cells could be measured,

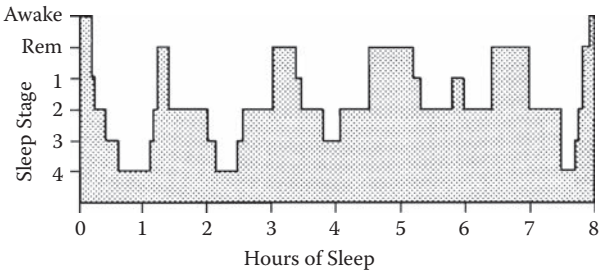


FIGURE 4.16 The cycles of sleep. This figure shows the variation of sleep stages (as determined by the pattern of brain waves in the electroencephalogram) of a typical human adult over the course of a night. Stages of light and deep sleep alternate with a period of approximately 90 minutes. (Source: Adapted from Shepherd, G. M. (1994). *Neurobiology*, 3rd Edition. New York: Oxford University Press.)

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.[54] 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.[55,56] Later studies on changes in brain waves during sleep revealed yet another dimension of ultradian oscillation, as shown in Figure 4.16. Note 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 at intervals of approximately 1 hour.[57–60] Because many endocrine glands are part of a loop that also involves the pituitary gland and the hypothalamus, it is often difficult to determine where the signal for pulsatile secretion is initiated. In the 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 then stimulate the gonads to secrete estrogens, progestins, and androgens.[38] 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.[61]

FIGURE 4.17 Living at the beach. Many organisms live by the sea and are affected by the alternation of the tides. (Source: © ArtToday, Tucson, AZ.)

4.2.3 Tidal Rhythms

Many organisms live in the interface between ocean and land and, therefore, are affected by the cycle of tides (Figure 4.17). 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. Debate continues about the existence of a specific circatidal clock distinct from the circadian clock (as opposed to a single clock that serves both functions), but it is well established that many intertidal organisms have the ability to generate circatidal rhythms endogenously.[62]

Figure 4.18 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 seen clearly that the crabs came out from their burrows to the surface only during 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.[63] This finding means that the tidal rhythm is *gated* by the daily rhythm of light and darkness. Laboratory tests conducted 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.[63]

Gating of tidal rhythms by the daily cycle of light and darkness has been shown in other intertidal organisms,[64,65] and lack of endogenous rhythmicity was observed in a different crab species.[66] More important, the existence of *endogenous* circatidal rhythms has been demonstrated in a variety of organisms, including algae, crustaceans, fishes, and reptiles.[64,67–71] An elegant laboratory study by Tadashi Akiyama (in Japan) demonstrated the synchronization of free-running circatidal rhythms by 12.5-hour

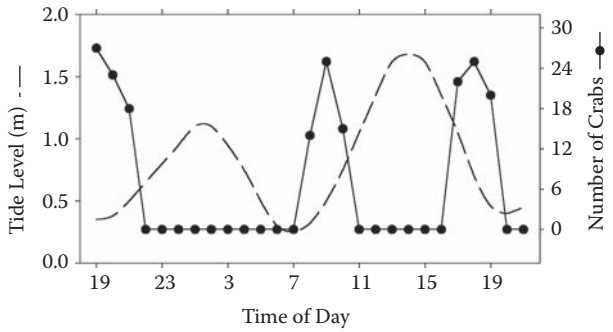


FIGURE 4.18 Showing up at low tide. The graph shows tide level and the number of fiddler crabs (*Uca uruguayensis*) found on the sand surface (that is, not in burrows) during a 24-hour interval. The crabs clearly come to the surface only at low tide. (Source: de la Iglesia, H. O., Rodríguez, E. M. & Dezi, R. E. (1994). Burrow plugging in the crab *Uca uruguayensis* and its synchronization with photoperiod and tides. *Physiology and Behavior* 55: 913–919.)

cycles of hydrostatic pressure in zooplankton.[65] Synchronization of endogenous rhythms by environmental cycles is a necessity for organisms to use their internal clocks to anticipate changes in the environment. An endogenous clock that cannot be reset by environmental stimuli will freerun indefinitely and will be of little use for an organism living in a natural environment.

4.2.4 Other Ultradian Rhythms

The smooth muscles of the gastrointestinal tract (stomach and intestines) rhythmically contract at the rate of 3 to 10 cycles per minute.[72] Several studies have identified ultradian oscillations in other organs and processes, but these findings need to be confirmed through replications in multiple laboratories. In one study, human subjects locked alone in a small room for 5 hours in the afternoon seemed to exhibit a rhythmic pattern of impatience that was reflected in bursts of locomotor activity (Figure 4.19). The period of this ultradian rhythm ranged from 0.5 to 2.5 hours in different subjects but was fairly consistent for each individual.[73] In fiddler crabs (*Uca pugilator*) foraging at the beach, a 21-minute cycle of feeding and retreating to the burrow was observed.[74] Short cycles of feeding (30 minutes) were also observed in golden hamsters (*Mesocricetus auratus*) and quail (*Coturnix coturnix*).[75,76] Other isolated observations include those of a 10 to 30 minute oscillation in human skin temperature,[77] a 1 to 4 hour oscillation in the core body temperature of sheep,[78] a 3 to 4 hour oscillation in the level of locomotor activity of root voles (*Microtus oeconomus*),[79] a 4 to 5 hour oscillation in the body temperature of lemmings,[80] and a 1.1 to 1.4 hour oscillation in carbon dioxide production of mice, rats, guinea pigs, monkeys, chickens, and quail.[81]

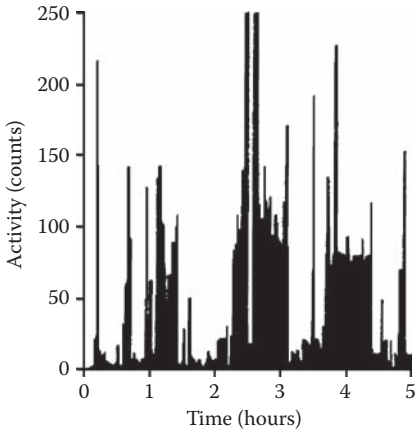


FIGURE 4.19 Killing time. The motor activity records of a young man locked in a small room for 5 hours show an ultradian pattern with a period of approximately 90 minutes. (Source: Grau, C. et al. (1995). Ultradian rhythms in gross motor activity of adult humans. *Physiology and Behavior* 57: 411–419.)

Many investigators conducting studies of circadian rhythms have noticed the presence of high-frequency oscillations that might constitute ultradian rhythms. Consider the 24-hour record of the body temperature of a golden hamster (Figure 4.20). Besides a large daily variation in temperature (which is 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.[82–84] In an attempt to characterize ultradian oscillations, spectral analyses of time series were conducted for a variety of 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.[85–97] Figure 4.21 shows the results of spectral analysis of the body temperature rhythm of golden hamsters. 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, top panel), the 8-hour component was the most common ultradian component. In the absence of a light–dark cycle (LL: constant light, DD: constant darkness), 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 waveform 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 the

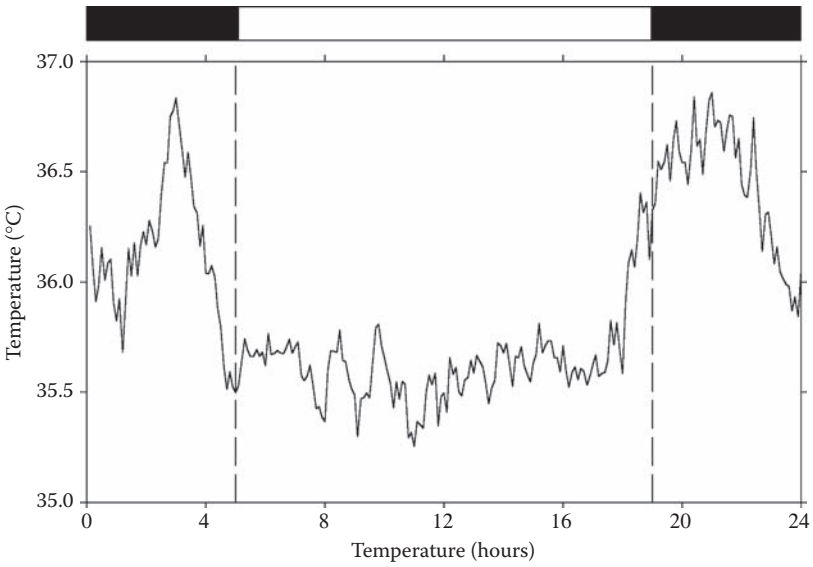


FIGURE 4.20 Small and large oscillations. This 24-hour record of the intra-abdominal temperature of a golden hamster shows a large circadian oscillation (discussed later) as well as many smaller high-frequency oscillations that may reflect ultradian rhythmicity. The horizontal white and dark bars at the top of the figure indicate the duration of the light and dark phases of the prevailing light–dark cycle. (Source: 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.)

analyses mentioned in the previous paragraph are *not* true 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.[98] 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 to 5 hours) in animals whose master circadian clock is surgically destroyed.[91,99,100] 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 a specific ultradian pacemaker is 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.

4.3 INFRADIAN 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 are discussed separately in Section 4.4.

4.3.1 Estrous Cycle

Animal reproduction 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.[101] Many humans restrict sexual activity to the infertile period of the woman's ovulatory cycle to prevent pregnancy (known as the "rhythm method" of contraception).[102,103] A woman's estrous cycle — like 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

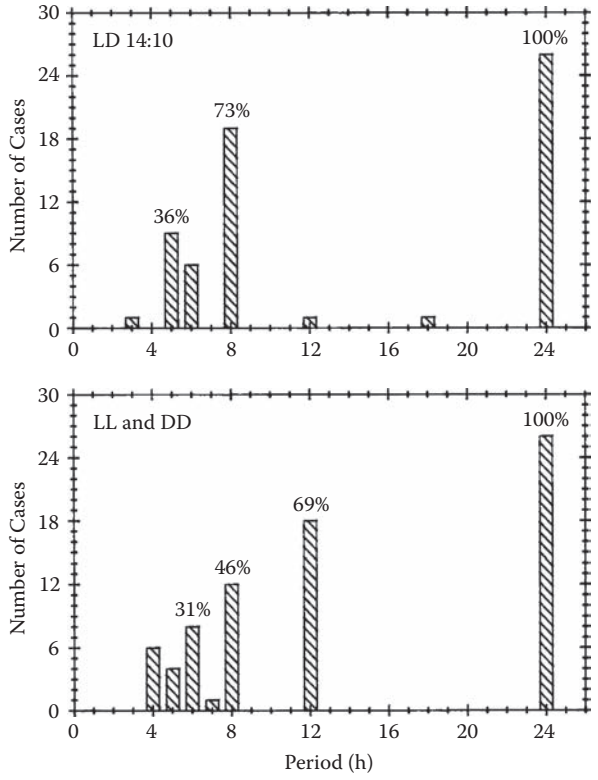


FIGURE 4.21 Relative consistency of ultradian rhythmicity. Time series of the body temperature of 52 golden hamsters were subjected to spectral analysis. Significant 24-hour periodicity was detected in all animals, but detection of ultradian periodicity was less consistent and depended on whether the animals were maintained under a light–dark cycle (LD 14:10, n = 26) or under constant conditions (constant light [LL] or constant darkness [DD], n = 26). (Source: Refinetti, R. (1994). Circadian modulation of ultradian oscillation in the body temperature of the golden hamster. *Journal of Thermal Biology* 19: 269–275.)

TABLE 4.2
Period of the Estrous Cycle in Some Vertebrate Species

Period (days)	Species	Sources
1	Chicken	152, 158, 159
1	Quail	154, 155, 177
1	Turkey	131, 441
4	Golden hamster	108, 113, 179
4	Rat	138, 141, 147
15	Owl monkey	150
15	Saki monkey	125
16	Antelope	132
16	Guinea pig	160
17	Sheep	130, 133, 142
21	Cattle	121, 162, 164
21	Degu	149
23	Horse	120, 127, 144
28	Human	104, 167, 220
28	Marmoset	124
31	Orangutan	126
40	Wombat	134, 137
46	Killer whale	123
110	Elephant	135
220	Dog	128, 139, 140

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

environmental cycles or with the body size of the species. Section 4.4 shows how reproduction is affected by annual rhythms, and Chapter 9 discusses 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 4.22. Because the average duration of the human menstrual cycle may vary from 23 to 36 days, and because most women experience a variation in the length of their own cycle (by a few days) from month to month, 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 waveform with a peak prior to ovulation, and progesterone peaks much later in the cycle.[101] Note also that 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.[104] The peak after menstruation may be due to sanitary considerations, while the peak at ovulation is an inherited evolutionary adaptation that favors fertilization and subsequent pregnancy. It has been shown that during the fertile phase of the menstrual cycle, women show preference for male body odor[105] and masculine facial features[106,107] — which, again, may favor sexual contact and subsequent pregnancy.

Although women are more likely to engage in sexual activity at certain times of the cycle, they can — and do — have intercourse on any day of the month. Females of most other species, however, are sexually receptive only around the time of ovulation (Figure 4.23).

Many variables besides hormonal secretion exhibit estrous rhythmicity. The female golden hamster, for example, shows 4-day rhythmicity in behavioral sexual receptivity,[108–110] in the pattern of vaginal discharges,[111–113] and in the amount and temporal organization of locomotor activity.[114–118] The estrous modulation of the daily amount of activity in female hamsters is evident in Figure 4.24. While male hamsters exhibit only small variations in activity from day to day, females rhythmically vary their activity levels several fold. Figure 4.25 provides a closer look

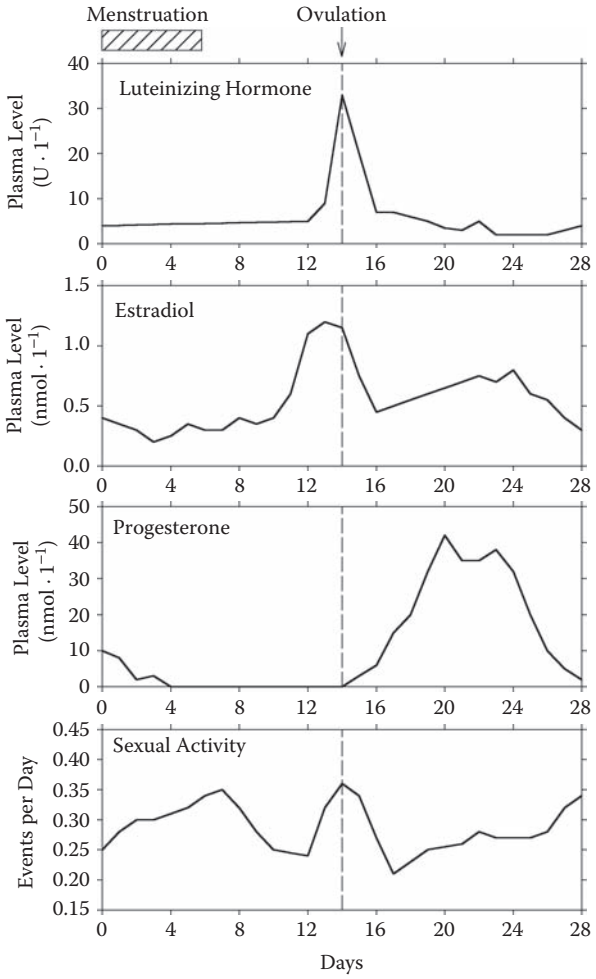


FIGURE 4.22 The menstrual cycle. The menstrual cycle involves not only the cyclic release of an egg (ovulation) throughout a woman's reproductive life but also rhythmic variations in hormone secretion and sexual activity. In women who do not take birth-control pills, self-initiated sexual activity with a partner peaks at the time of ovulation as well as right after menstruation. (Sources: Ganong, W. F. (2001). *Review of Medical Physiology*, 20th Edition. New York: Lange Medical; Adams, D. B. et al. (1978). Rise in female-initiated sexual activity at ovulation and its suppression by oral contraceptives. *New England Journal of Medicine* 299: 1145–1150.)

FIGURE 4.23 Wild sex. In many animals, including the lion, the frequency of sexual intercourse is modulated by the female's estrous cycle. (Source: Photograph by Michael Wain, © 2003. Reproduced with permission.)

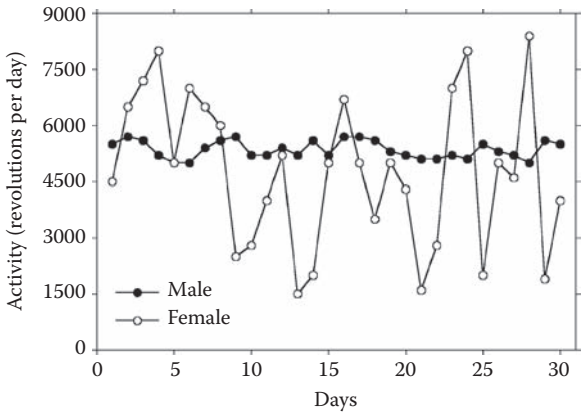


FIGURE 4.24 Run for sex. As exemplified by a pair of golden hamsters housed in separate cages, male hamsters exhibit moderate day-to-day variation in the amount of running-wheel activity, but female hamsters exhibit great variation associated with the 4-day estrous cycle. (Source: Refinetti, R. & Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.)

at the estrous variation in the activity pattern of a female hamster. The animal was maintained in constant darkness, and at first inspection you may perceive only a "messy" free-running circadian rhythm. Note, however, the consistent alternating pattern of 2 days with much activity and 2 days with little activity. The days with much activity are the day of ovulation and the day immediately preceding it (the days of *estrus* and *proestrus*, respectively), while the days with little activity are the remaining days of the estrous cycle (*diestrus* days).

Many other species also exhibit estrous rhythmicity in hormonal secretions,[119–136] vaginal discharges,[128,133,134,137–140] behavioral sexual receptivity,[129,130,136,139,141–144] and locomotor activity.[87,119,121,137,138,145–150] In birds, egg laying is a convenient marker of estrous rhythmicity.[151–155] In addition, many species of mammals and birds exhibit estrous rhythmicity in body temperature.[113,122,126,131,137,138,146–150,154,156–164] Figure 4.26 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 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 to 0.6°C (~ 1°F)

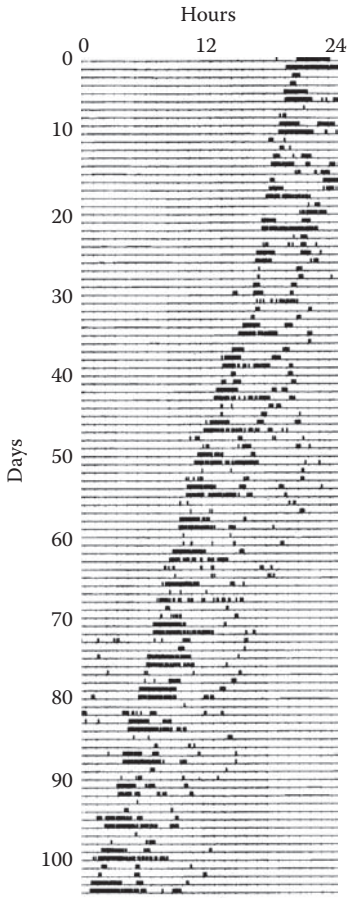


FIGURE 4.25 Estrous signature. The estrous cycle of a female golden hamster is evident in the actogram of her running-wheel activity records. The hamster was kept under constant environmental conditions (constant temperature and constant darkness). Despite occasional disruptions, you can clearly see a pattern of 2 days with heavy activity followed by 2 days with sparse activity. (If you are not familiar with actograms, refer to Figure 3.19 in Chapter 3.) (Source: Archives of the Refinetti lab.)

higher than during the follicular phase.[165–173] The relationship between ovulation and body temperature is consistent enough to be used as a tool for contraception or to help treat infertility.[174–176] The postovulatory elevation in body temperature may be due to the thermogenic (heat-producing) action of progesterone, but this is not the case 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.[113,147]

When animals (or humans) are maintained in an environment devoid of temporal cues, their estrous cycles free-run with periods shorter or longer than those observed under natural conditions.[108,109,150,152,177,178] Figure 4.27 provides an example of a free-running estrous cycle in a domestic hen (*Gallus domesticus*). I chose to use the hen

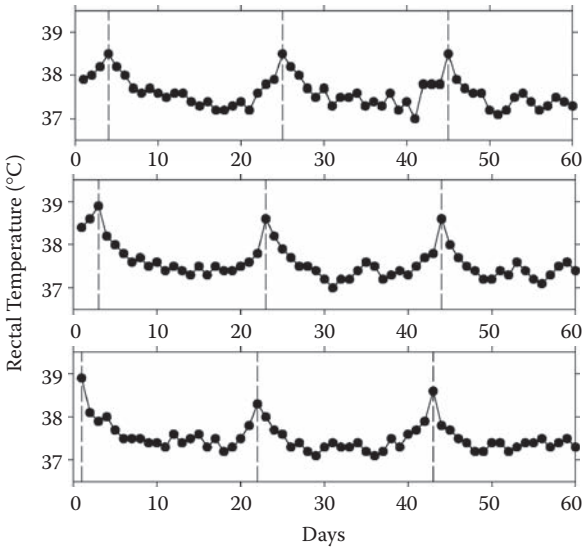


FIGURE 4.26 Bovine estrous cycle. These records of rectal temperature of three cows (*Bos taurus*) were obtained daily at dawn for 2 consecutive months. A sharp rise in temperature can be seen on the day of estrus (vertical dashed lines) every 21 days. Estrus was determined by observation of vaginal discharge, increased locomotor activity, and acquiescence to mounting by a bull. (Source: Piccione, G., Caola, G., & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.)

because its estrous cycle has a short period (~ 28 hours) that can be displayed easily 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 free run under constant environmental conditions indicates that they are not imposed by the environment but, instead, are endogenously generated. The endogenous nature of estrous rhythmicity could also 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 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.[141,179–181]

4.3.2 Weekly Rhythms

The grouping of 7 days into a unit called a *week* is common in most of the world.[29] It is also typical 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 going to church observed in people with religious habits

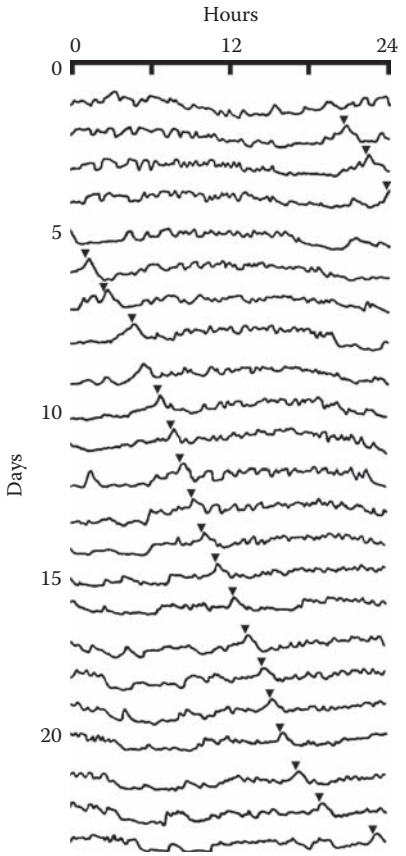


FIGURE 4.27 Laying eggs. The ovulatory cycle of the domestic chicken (*Gallus domesticus*) is so short that it can be plotted in actogram format just like a circadian rhythm. In this figure, the lines indicate body temperature (ranging from 39.5 to 41.5°C), and the small inverted triangles indicate the time of oviposition (egg laying). Note that body temperature rises daily at the time of oviposition. (If you are not familiar with actograms, refer to Figure 3.19 in Chapter 3.) (Source: Kadono, H. & 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: Springer, pp. 91–99.)

(Figure 4.28). 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 result, more traffic accidents take place on Fridays and Saturdays than on other days of the week (Figure 4.29). It is interesting that the number of deaths due to traffic accidents peaks on Saturdays, but the number of deaths due to suicide is uniform throughout the week.[182]

People tend to sleep 1 to 3 hours longer on Friday and Saturday nights than on weekday nights.[183–185] People also tend to eat more on weekends (2000 kcal per day) than during the workweek (1800 kcal per day).[186] Young married couples have sex almost twice as often on Sundays as on weekdays;[187] as a consequence, testosterone secretion in men is elevated on weekends.[188] In contrast, the

FIGURE 4.28 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. (Source: Photograph by R. Refinetti.)

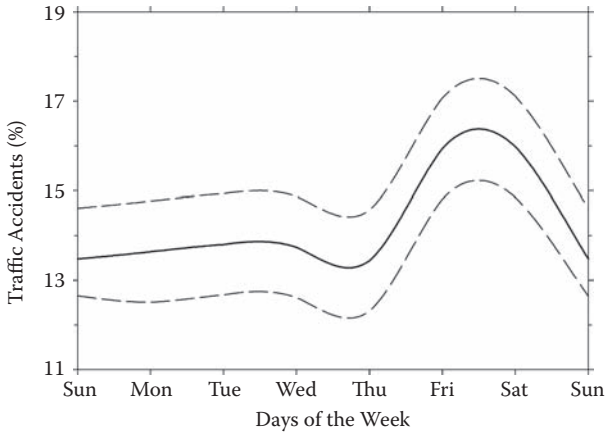


FIGURE 4.29 Watch the road! Highway traffic accidents in the United States show a weekly rhythm. Accidents are more common on Fridays and Saturdays than on other days of the week. The solid line shows the percentage of weekly accidents by day of the week as averaged for the years 1995 through 1999. The dashed lines indicate the 95% confidence intervals for the means. (Source: National Center for Statistics and Analysis, U.S. Department of Transportation.)

frequency of human births is *lower* on weekends than on weekdays,[189–191] 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.[192] The frequency of heart attacks (myocardial infarctions and cardiac arrests) in the general population also shows weekly rhythmicity, although the peak day varies from Friday to Monday in different studies.[193–196]

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. As discussed in Chapter 1, Halberg, who has been 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.[197] Later in his career, however, he coined the adjective *circaseptan* to refer to hypothetical endogenous rhythms with a period of approximately 7 days. The experimental evidence used to support the existence of circaseptan rhythms is far from compelling, however.

The existence of weekly rhythms in human bodily functions is poorly documented. In a recent article, Halberg and colleagues reported 7-day rhythms of blood pressure and heart rate in human subjects,[198] but the data they presented were unconvincing at best. The data shown in Figure 4.30 exemplify the results obtained by Halberg and his colleagues. High-frequency oscillations (apparently of random origin) are so great that even daily rhythmicity is difficult to see. Because data are shown for only a 1-week period, any evidence of weekly rhythmicity could only be suggestive, but no weekly pattern is evident. 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 about the reliability of this "microscopic" statistical analysis.

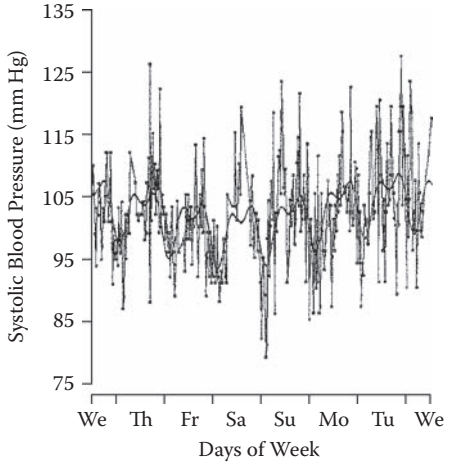


FIGURE 4.30 Weekly or weakly rhythm? It has been suggested that these records of systolic blood pressure of a human subject display weekly rhythmicity, but visual inspection reveals only daily rhythmicity, if any rhythmicity at all. (Source: Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K. & Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s–44s.)

In another study, Halberg's team claimed to have identified circaseptan rhythms of blood pressure in a set of 11 pairs of newborn twins.[28] Their data, which had a probability of Type I error of 0.08, showed that the within-twins variability was smaller than the inter-twins variability. They believed that this result indicated the existence of a genetic basis for 7-day rhythmicity. No evidence of a real rhythm was presented, however.

In one of very few rigorous studies of weekly rhythmicity in individual subjects, 12 horses were studied for 70 consecutive days, so that ten full 7-day cycles could be monitored.[199] Each day, in the morning and the evening, plasma concentrations of lactic acid, blood pressure, and rectal temperature were measured in six athletic horses (subjected to a weekly schedule of fitness training) and six sedentary horses (not subjected to the weekly schedule). Figure 4.31 shows data from one of the athletic horses. The plasma concentration of lactic acid showed a feeble 7-day rhythm (top left panel), which was barely significant as determined by chi square periodogram analysis (top middle panel) and Lomb–Scargle periodogram analysis (top right panel), 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 entire 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. These findings suggest 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 inspired the concept of the *month*, which today is defined independently of the lunar cycle.[200] Like the week, the month has effects on human behavior (Figure 4.32). 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. There also 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.[188]

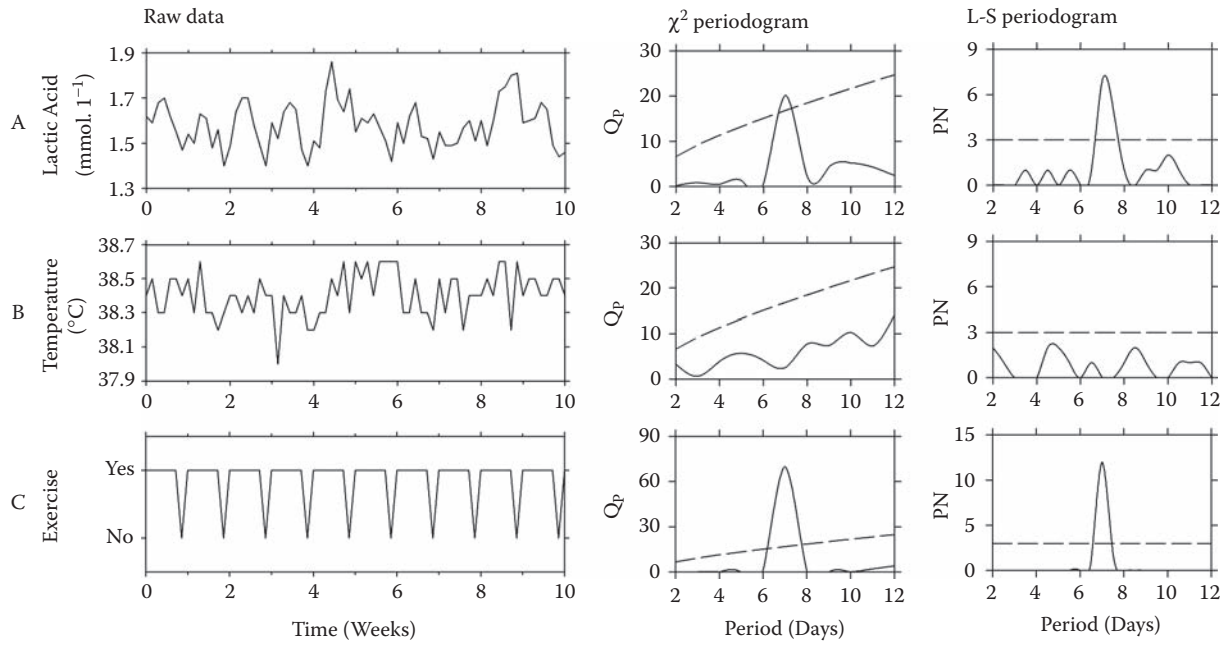


FIGURE 4.31 Feeble weekly rhythmicity in the horse. This figure shows raw data and their analyses by the chi square periodogram and Lomb–Scargle periodogram procedures for plasma concentration of lactic acid, rectal temperature, and exercise schedule of a horse (*Equus caballus*). There is significant 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, refer to Section 3.3 in Chapter 3.) (Source: Piccione, G., Caola, G. & Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.)

FIGURE 4.32 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. (Source: Photograph by R. Refinetti.)

The lunar cycle can affect organisms through the variation in nighttime luminosity and tide levels. This variation is modest and has modest effects on organisms, which probably explains why the literature on biological rhythms contains few studies on lunar rhythms as compared with 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).[31] Most of the variation in the tides 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.[201]

Figure 4.33 provides an example of a biological lunar rhythm. The number of sea flies (*Clunio marinus*) completing metamorphosis from the larval stage (eclosion) was recorded in the laboratory for 2 months while the larvae were kept under an artificial lunar cycle with 4 nights of "moonlight" each month (as denoted by the horizontal bar at the top of the figure).[202] Note that eclosion takes place immediately after the full moon. Lunar rhythms (and semilunar rhythms associated with spring

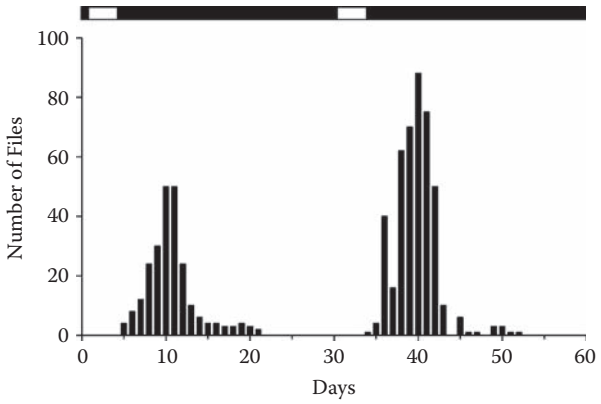


FIGURE 4.33 Lunar rhythm. The graph shows a lunar rhythm in the emergence of *Clunio marinus*, a small fly that inhabits tidal waters. Colonies were maintained under a daily light–dark cycle (12 hours of light and 12 hours of darkness) supplemented with a lunar cycle (artificial moonlight for 4 nights every 30 days, as shown by the horizontal bars at the top of the graph). (Source: Neumann, D. (1989). Circadian components of semilunar and lunar timing mechanisms. *Journal of Biological Rhythms* 4: 285–294.)

tides) have been shown to freerun in the absence of environmental lunar cycles[70,203] and, therefore, must be endogenously generated. Because 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. Before artificial lighting became a common feature of human homes, the light of the full moon may have been a significant source of sleep disruption, which is known to affect the mental state of psychiatric patients.[204] Although a few recent studies have found some evidence of lunar rhythmicity in the timing of human births,[191] the meal size of human adults,[205] and the frequency of injuries caused by animal bites,[206] many studies have failed to document any connection between the phases of the moon and these or other human functions.[187,207–212] The influence of the moon on humans is likely equivalent to that of other celestial bodies in the solar system — that is, it is meaningful to superstitious people who believe in horoscopes, but it is nonexistent for other people.

4.3.4 Other Infradian Rhythms

A curious infradian rhythm that does not fit into any of the categories discussed in the previous section involves the Canadian lynx (*Lynx canadensis*) and the Arctic hare (*Lepus arcticus*), both shown in Figure 4.34. 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 4.35, fur returns exhibit a remarkably regular 10-year rhythm. The cause of the rhythm is not fully known, but it is related to a rhythm in the size of the lynx population, which depends on variations in the population of the lynx's main prey, the hare.[213] Because 10 years is also the approximate period of the cycle of sunspots, the phenomena may be causally related. The cycle of sunspots has been suggested as the cause of a 10-year cycle of moth population in Norway.[214] 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.[214] Rhythms with approximately 10-year periods have also been described for population density of gerbils,[215] tree growth,[216] and urinary excretion of 17-ketosteroid in humans.[217] Unusual infradian rhythms with periods ranging from a few months to several years have been described in dormice,[218] lemmings,[219] and humans.[220]

One problem in the study of infradian rhythms is the endless number of possible rhythms. The examination of almost any time series covering a number of years reveals 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 4.36. The graphs show data from 1960 to 2001 about civilian passenger aircraft crashes. The top

FIGURE 4.34 Predator and prey. The lynx (*Lynx canadensis*) and the Arctic hare (*Lepus arcticus*) are closely connected as predator and prey in the tundra zone of Canada. (Source: National Image Library, U.S. Fish and Wildlife Service.)

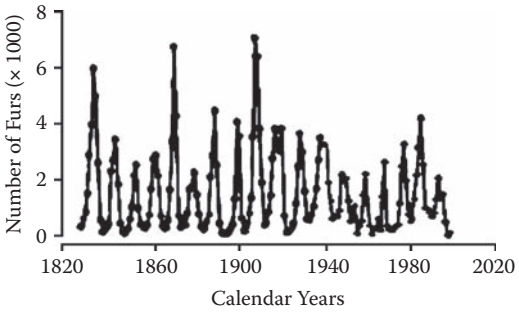


FIGURE 4.35 If you need a fur coat. The number of lynx furs procured each year in an area of approximately 270,000 km^2 in northwest Canada shows a 10-year cycle that results from the cycling of the lynx population. (Source: Stenseth, N. C., Falck, W., Chan, K. S., Bjørnstad, O. N., O'Donoghue, M., Tong, H., Boonstra, R., Boutin, S., Krebs, C. J. & Yoccoz, N. G. (1998). From patterns to processes: phase and density dependencies in the Canadian lynx cycle. *Proceedings of the National Academy of Sciences U.S.A.* 95: 15430–15435.)

graph shows the number of crashes, while the bottom graph shows the number of passenger deaths resulting from the crashes. In both cases, a 12-year rhythm appears to exist, 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.

The idea of an infradian rhythm of human reproduction much longer than the menstrual cycle is common in the popular literature. 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 expression implies that some sort of clock in the woman's body starts to tick 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.[221–224] Is this just a metaphor, or is there really some sort of *pregnancy predestination*?

In a world where every life form has a limited lifespan, 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 women stopped bearing children, and scientists did not immediately develop human cloning, human life would soon vanish. Nature, however, made 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

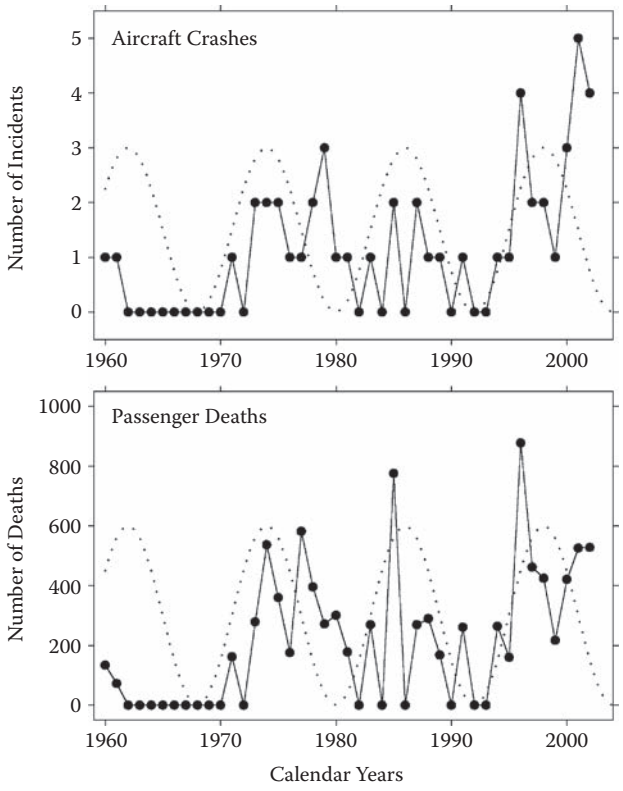


FIGURE 4.36 Is there a 12-year cycle of airplane accidents? The top graph shows the yearly number of civilian passenger aircraft crashes with more than 150 fatalities per incident over the course of four decades. The bottom graph shows the total number of passenger deaths resulting from the crashes. In both graphs, a 12-year rhythmic pattern seems to exist, as suggested by the dotted lines. However, numerical analysis of the data produces inconclusive results. (Source: *TIME Almanac 2003* (2002). Des Moines, IA: TIME Books.)

could speak of pregnancy predestination. Anyone who has experienced the strong emotions of parenthood can testify that missing out on parenthood would be missing out 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.

If there is no biological clock that tells women to have children early in life, is it okay for mature women to have children? This question has no straightforward answer. People age 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. Physical and social tensions also exist. Nonetheless, many women choose to postpone

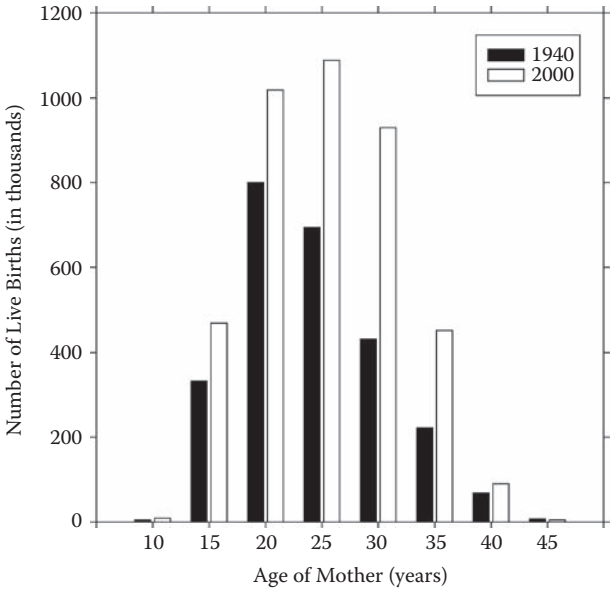


FIGURE 4.37 Are you ready for a baby? The graph shows the number of live births in the United States as a function of the mother's age in 1940 and 2000. More births occurred overall in 2000 than in 1940 (primarily because of population growth), but the proportion of women in the 25 to 35 year-old brackets that gave birth also increased substantially. (Source: National Center for Health Statistics, U.S. Centers for Disease Control and Prevention.)

motherhood until their 30s or 40s. Figure 4.37 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 that more births occurred in 2000 than in 1940 (because the population grew in that interval), and that the proportion of women giving birth at older ages also increased. In the year 2000, women 35 to 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.

Another example of an infradian clock involves mice. Adult mice often kill preweaning infants, or pups. They seem to have some sort of infradian clock, however, that reduces their infanticidal instinct at 18 to 20 days after mating, the time when the dam gives birth to the pups.[225] The existence of this "clock" protects the mouse's offspring.

4.4 ANNUAL RHYTHMS

Annual rhythms have a period of 1 year and are a subclass of infradian rhythms. They are so ubiquitous, however, 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 4.38).

FIGURE 4.38 Christmas in South Carolina. Christmas is celebrated once a year in late December, which institutes a robust annual rhythm of Christmas celebration. (Source: Photograph by R. Refinetti.)

Many physiological and behavioral processes exhibit annual rhythmicity. The following section covers annual rhythms studied under natural or seminatural conditions in the presence of annual environmental cycles. These *seasonal rhythms* are so named because they cycle with the seasons. *Circannual rhythms*, which are endogenously generated rhythms that normally cycle with the seasons but can persist in the absence of environmental cycles, are described in a later section. Chapter 9 discusses how annual rhythms interact with circadian rhythms.

4.4.1 Seasonal Rhythms

Some seasonal rhythms are rather obvious. For example, 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 4.39). Small animals (both vertebrates and invertebrates) show seasonal variation in burrowing and nest-building behavior.[226–230] Not obvious, however, is the fact that under the frozen tundra lives a large community of microbes (mostly fungi) that reaches maximal biomass in the late winter and dwindles in the summer.[231] It is also not *a priori* evident that people diagnosed as schizophrenics are born more often in the winter than in other seasons[232,233] or that human sensory processes exhibit seasonal variation.[234,235] Other curious seasonal rhythms include the lowering of the body temperature of prairie dogs (which are not hibernators) by 3°C in the winter,[236] a six-fold increase in the number of deer-vehicle crashes in late autumn,[237] a doubling of the duration of water dives by elephant seals in the winter,[238] and a 30% rise in the occurrence of human spouse-battering in the summer.[239]

FIGURE 4.39 Alaskan frozen tundra. Not seen in this picture of the frozen Alaskan tundra is the large community of fungi that thrives under the snow cover. (Source: National Image Library, U.S. Fish and Wildlife Service.)

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.[240] Chapter 16 discusses the more unusual case of individuals who become severely depressed in the winter (*seasonal affective disorder*). The winter is also a bad time of year for people with cardiovascular disorders, as the frequency of heart attacks (myocardial infarction, ventricular fibrillation, cardiac arrest, and so on) varies seasonally by about 3% and peaks in winter.[182,193–195,241] Other causes of death also exhibit seasonal variation, so that the total number of deaths per day shows a clear seasonal rhythm that peaks in winter (Figure 4.40). If you spend time outdoors, you may have observed the phenomenon of *animal migration* in late fall.[242–247] For example, if you live in the northern hemisphere, you may see birds flying south in the fall and north in the spring. 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 winter.

A number of physiological parameters exhibit seasonal rhythmicity: body mass, cold-induced thermogenesis, food intake, heterothermy, melatonin secretion, molting, and reproductive capacity. Figure 4.41 shows the records of average body mass of five European hamsters (*Cricetus cricetus*) housed in an outdoor enclosure in Germany for 2.5 years. Note 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,[248–252] rodents,[230,253–266] and other animals.[267–274]

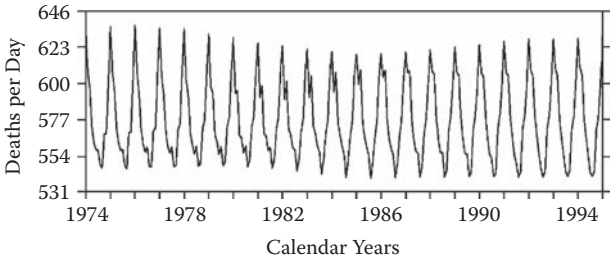


FIGURE 4.40 A season to die. As shown by these data from the Canadian Vital Statistics Database, there is a robust annual rhythm in human deaths. Most deaths occur in January. (Source: Trudeau, R. (1997). Monthly and daily patterns of death. *Statistics Canada Health Reports* 9(1): 43–50.)

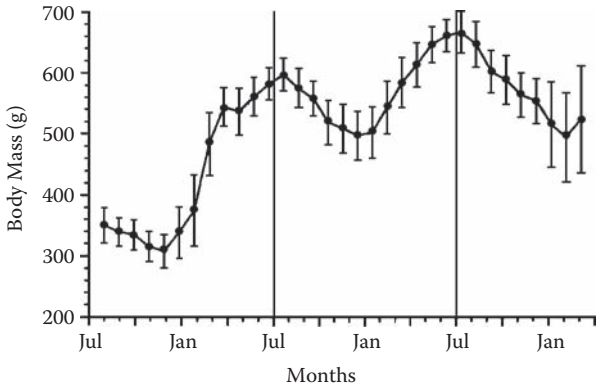


FIGURE 4.41 Gain and lose weight. Many animals gain weight in the summer and lose weight in the winter. This graph shows the mean variation in body mass (± SE) of five European hamsters (*Cricetus cricetus*) maintained in an outdoor enclosure in Germany for 3 years. (Source: Wollnik, F. & Schmidt, B. (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.)

The most obvious feature of winter is the cold weather, which transforms aqueous precipitation from rain to snow. The process of thermoregulation is not discussed in detail until Chapter 10; however, it should be obvious that warm-blooded animals (mainly birds and mammals) must be able to produce more body heat during the winter than in the summer, so that they can counteract the environmental cold (Figure 4.42). 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*.[275–277] Consider Figure 4.43, which shows the mean metabolic rates of wild rats (*Rattus 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. Note that the metabolic rates of the two groups of rats are very similar when the animals are tested between 0 and 20°C, but that winter-acclimatized rats have

FIGURE 4.42 A cold wolf. As the temperature of the environment falls in the winter, even well-insulated animals must increase their metabolic rate to maintain their body temperature at the normal level. (Source: Yellowstone National Park Wildlife Graphics, U.S. National Park Service.)

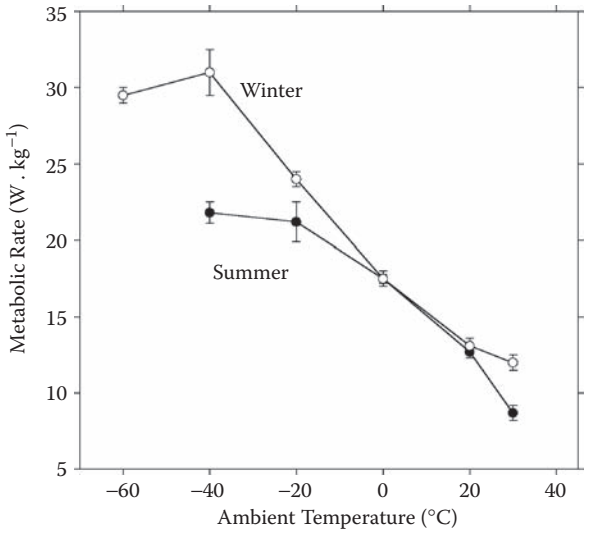


FIGURE 4.43 Compensating for the cold of winter. The graph shows the metabolic rates of wild rats (*Rattus norvegicus*) captured in Canada in the summer and in the winter and tested at different ambient temperatures. Winter-acclimatized rats show greater heat-producing capacity (cold-induced thermogenesis) at ambient temperatures below –10°C (14°F). Each data point corresponds to the mean (± SE) of approximately eight animals. (Source: Hart, J. S. & Heroux, O. (1963). Seasonal acclimatization in wild rats (*Ratt)us norvegicus*). *Canadian Journal of Zoology* 41: 711–716.)

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 winter-acclimatized rats is 40% higher than that of summer-acclimatized rats. Thus, the capacity for *cold-induced thermogenesis* oscillates with the seasons. The

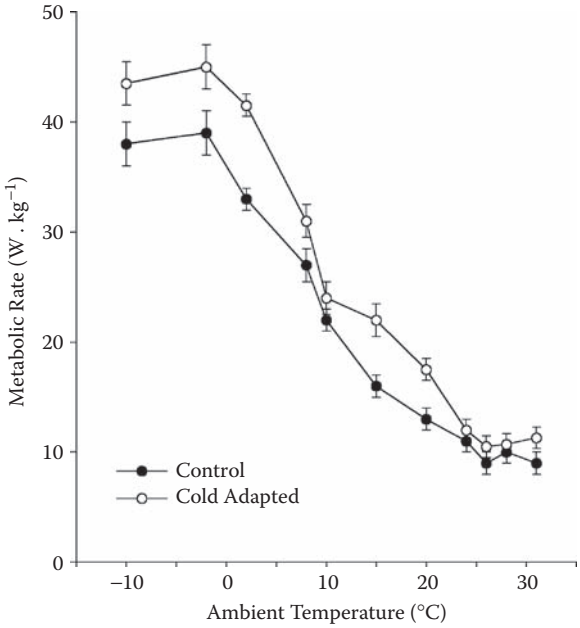


FIGURE 4.44 Isolating the effect of ambient temperature. The graph shows the metabolic rates of domestic mice (*Mus musculus*) maintained in the laboratory under cold (4°C) or neutral (26°C) conditions for 4 weeks and then tested at different ambient temperatures. Although cold-adapted (cold-acclimated) mice exhibit higher metabolic rates than control mice at most test temperatures, the difference is small when compared with the effect of test temperature itself. Each data point corresponds to the mean (± SE) of approximately eight animals. (Source: Oufara, S., Barré, H., Rouanet, J. L. & Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *American Journal of Physiology* 253: R39–R45.)

process of metabolic acclimatization seems to be less effective in birds than in mammals,[278] but it has been reported in both classes.[279–284]

As mentioned 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 has a short *photophase* (and a long *scotophase*). Therefore, it is natural to wonder whether winter acclimatization is caused by the low temperature or by the short photophase. One way to investigate the issue in laboratory animals is to maintain a constant photoperiod and to vary only the adaptation temperature (thus, producing *acclimation* but not full *acclimatization*). Figure 4.44 shows the mean metabolic rates of domestic mice (*Mus musculus*) acclimated to the cold (4°C) or to a neutral environment (26°C) for 4 weeks before being tested at various temperatures. Both groups show an elevation of metabolic rate at low test temperatures, but the elevation is greater in the cold-adapted group than in the control group. These data show that acclimation alone (i.e., without the effect of photoperiod) can produce an increase in cold-induced thermogenesis. Similar

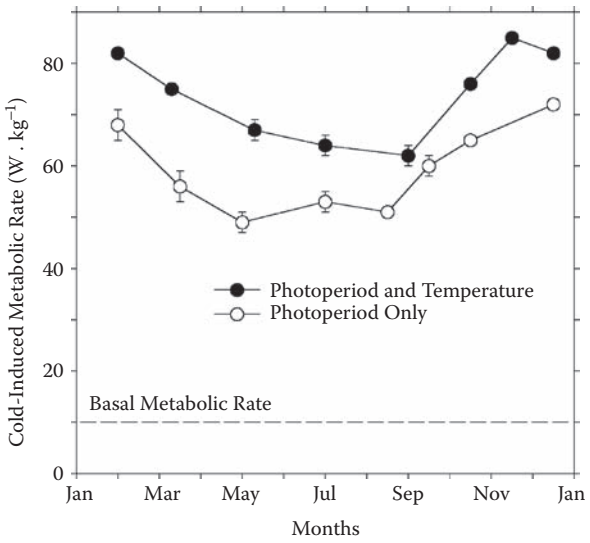


FIGURE 4.45 Isolating the effect of photoperiod. The graph shows the maximal cold-induced metabolic rate (the metabolic rate obtained at the lowest ambient temperature at which the animals could prevent a fall in body temperature) of Siberian hamsters (*Phodopus sungorus*) kept either outdoors in Germany or indoors at thermoneutrality (23°C) under a natural photoperiod matching the outdoor photoperiod. 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 to 18 animals. The horizontal dashed line indicates the mean metabolic rate at thermoneutrality. (Source: Heldmaier, G., Steinlechner, S. & Rafael, J. (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *Journal of Comparative Physiology* 149: 1–9.)

results have been obtained in various studies in rats,[285–290] birds,[291–294] and other animals.[295–299] However, notice in Figure 4.44 that the effect of cold acclimation is rather modest when compared with the effect of test temperature. For example, the metabolic rate of the cold-acclimated group is only 16% higher than that of the control group at –4°C, while 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 effect, animals may be kept under a constant ambient temperature and subjected to varying photoperiods. Although some studies based on this approach revealed an effect of photoperiod alone,[282,300,301] others did not.[258,302,303]

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 4.45.

FIGURE 4.46 A hungry prairie dog. As the temperature of the environment falls in winter, homeothermic animals must increase their intake of food to maintain the high metabolic rate needed to preserve normal body temperature. (Source: © ArtToday, Tucson, AZ.)

Note that there is a seasonal variation in cold-induced thermogenesis in animals that were adapted only to photoperiodic variation (open circles), but that 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 fully explained by the 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 can estimate that temperature adaptation accounts for 75% of the full seasonal variation in thermogenic capacity — and, therefore, that photoperiod accounts for only 25% of the response. This was the case in three studies on Siberian hamsters[304–306] and a study on rats.[307] However, other studies indicated a much greater role of photoperiod in deer mice,[308] equivalent roles of temperature and photoperiod in pouched mice,[309] and no role of either temperature or photoperiod in collard lemmings.[255] While it is quite possible that different species react differently to temperature and photoperiod, the conflicting results may simply reflect 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 4.46). Although food must be ingested year round, animals exposed to natural seasonal fluctuations in the environment often exhibit seasonal fluctuations in food

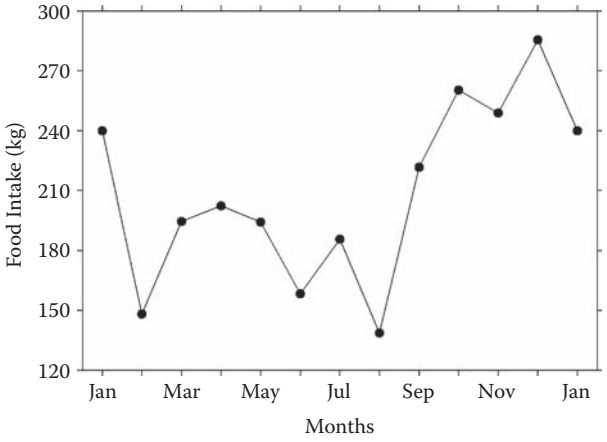


FIGURE 4.47 Burn your food. As shown by these records of food intake of a South African fur seal (*Arctocephalus pusillus*) kept outdoors throughout the year at the Toronto Zoo (in Canada), many animals consume more food during the winter than during the summer. (Source: Shearer, D. S., Valdes, E. V., Qyarzun, S. E., Steinsky, L. & Atkinson, J. L. (1995). Food intake patterns in captive South African fur seals (*Arctocephalus pusillus pusillus*). *Proceedings of the Nutrition Advisory Group (American Zoo and Aquarium Assoc.)* 1: 213–217.)

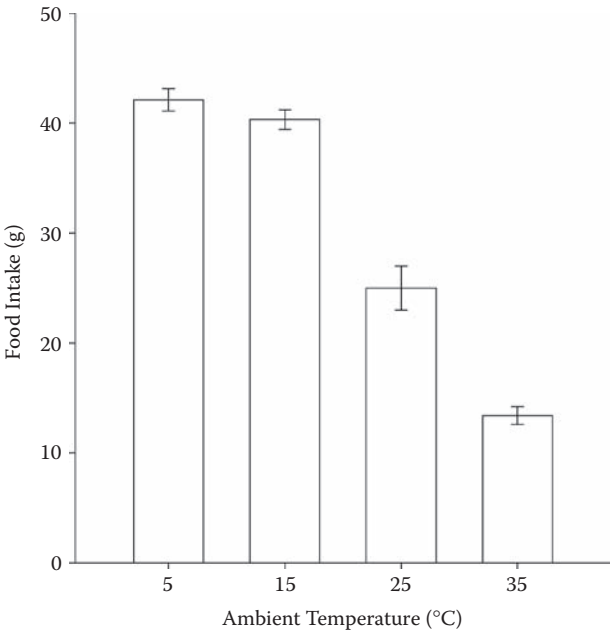


FIGURE 4.48 Isolating the effect of ambient temperature. This graph shows the mean food intake of groups of laboratory rats kept under identical photoperiods but at different ambient temperatures. Food intake is greater at lower temperatures. Each bar corresponds to the mean (± SE) of ten rats. (Source: Witty, R. T. & Long, J. F. (1970). Effect of ambient temperature on gastric secretion and food intake in the rat. *American Journal of Physiology* 219: 1359–1363.)

intake.[260,267,274,310–314] For example, Figure 4.47 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, the seal clearly 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. 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. Figure 4.48 illustrates this specific effect of ambient temperature. Groups of laboratory rats (*Rattus norvegicus*) were housed at different ambient temperatures under the same photoperiod, and their daily food intake was measured. The figure clearly shows that the rats consumed more food when the ambient temperature was lower. Increased food intake at lower ambient temperature has been documented in a large number of studies in rats[315–320] and other species.[321–326]

These data suggest that 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),[273,310] but in the majority of studies food intake was greater during the summer (or during the long photoperiod).[253,260,263,264,267,269,274,311,312,327] Of course, ambient temperature can be a stimulus for increased food intake only for animals that increase their food intake in the winter. 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 4.49, for example, 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. The reduction of food intake in the winter, then, must result from elaborate physiological adjustments made in response to environmental changes (or in response to an endogenous process that anticipates the environmental changes, as is discussed later). Food availability may be an *ultimate*

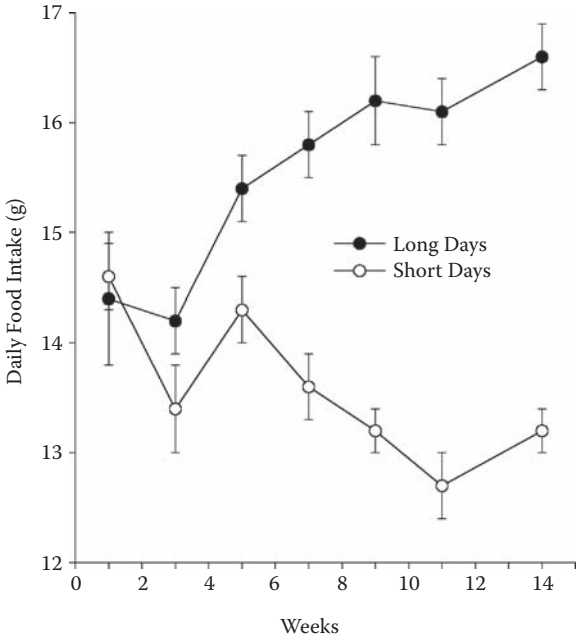


FIGURE 4.49 Isolating the effect of photoperiod. This graph shows the mean daily food intake of two groups of laboratory rats maintained under the same ambient temperature (23°C) but at different photoperiods (Short days: 8 hours of light per day; Long days: 16 hours of light per day) for 14 consecutive 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. (Source: Shoemaker, M. B. & 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.)

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 normally a *proximal* cause of the seasonal variation in food intake.

Another parameter of seasonal rhythmicity is *heterothermy*, the partial blockade of thermoregulatory processes during winter *hibernation* or, mostly in invertebrates and lower vertebrates, summer *estivation*.[328] Hibernators include squirrels[329–341] (Figure 4.50), hamsters,[257,342–348] chipmunks,[349,350] bats,[351,352] marmots,[263,353] hedgehogs,[354,355]

FIGURE 4.50 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. (Source: National Image Library, U.S. Fish and Wildlife Service.)

prairie dogs,[260] and some marsupials.[356] Bears experience prolonged sleep episodes in the winter but do not exhibit the drastic reduction in metabolic rate and body temperature of true hibernators.[357,358] As 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*. Arthropods (such as insects, crustaceans, and arachnids) have their own process of seasonal inactivity, although they are cold-blooded all year round. The period of inactivity in arthropods during which growth stops (*diapause*) is controlled by both ambient temperature and photoperiod.[359–364]

Figure 4.51 illustrates the hibernation pattern of a hedgehog (*Erinaceus europaeus*). The animal was housed under natural photoperiod and natural ambient temperature in France. Note 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 decreased to just a few degrees above freezing. Note also that the duration of the hypothermic bouts was longer during the colder months.

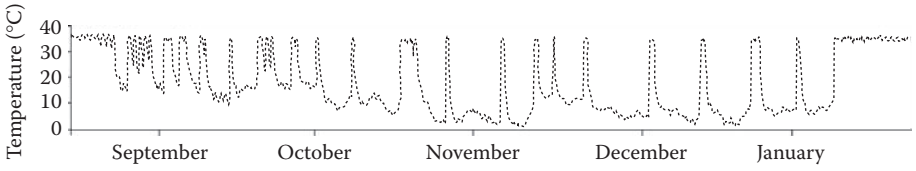


FIGURE 4.51 Hedgehog sleeps through the winter. The body temperature records of a hedgehog (*Erinaceus europaeus*) maintained under natural conditions of illumination and ambient temperature in France show a clear pattern of hibernation during the fall and early winter. Brief periods of normothermia are interspersed with longer periods of deep hypothermia. (Source: Saboureau, M., Vignault, M. P. & 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.)

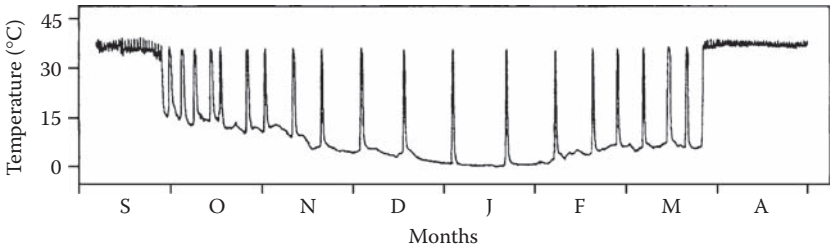


FIGURE 4.52 Squirrel sleeps through the winter. The body temperature records of a European ground squirrel (*Spermophilus citellus*) maintained under natural conditions of illumination and ambient temperature in Holland show a clear pattern of hibernation throughout the fall and winter. Brief periods of normothermia are interspersed with longer periods of deep hypothermia. (Source: Hut, R. A., Barnes, B. M. & 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.)

Except during the interbout intervals of euthermia, the animal remained still, as if sleeping. An atypical aspect of these data 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 4.52, 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 bout occurred in late March.

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 hibernation timing.[365,366] Most investigators agree that an animal entering hibernation is not simply a chilled warm-blooded animal. Instead, they believe that hibernation is a *regulated* state — that is, a state of intended low metabolism that allows body temperature to fall along with ambient temperature.[365–368] Chapter 10 explains that most of the metabolic reduction occurring during hibernation results from a passive, temperature-dependent process that regulates body temperature rather than metabolism.

Red deer (*Cervus elaphus*) do not hibernate but they exhibit a seasonal variation in subcutaneous temperature, as illustrated by the combined data for nine deer housed outside in the Slovak Republic (Figure 4.53). Subcutaneous temperature depends more on ambient temperature than does core temperature. The seasonal variation in subcutaneous temperature in this case, however, is not a mere reflex of the seasonal variation in ambient temperature. The lowest values of subcutaneous temperature occur in March, 2 months after the lowest values of ambient temperature.[369] Note that subcutaneous temperatures return to a higher level at approximately the same time that food becomes available (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.

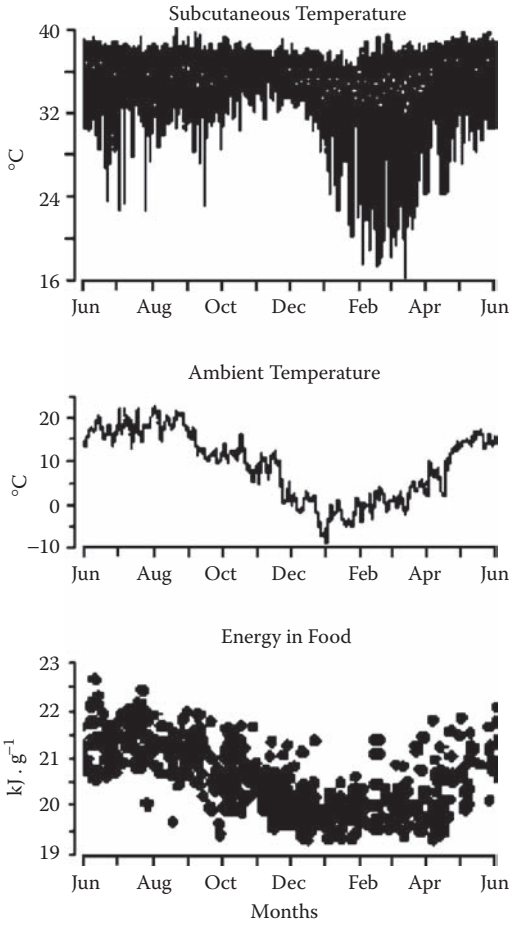


FIGURE 4.53 Deer get cold in the winter. The red deer (*Cervus elaphus*) does not hibernate, but its subcutaneous temperature is lower during the winter. Note that the lowest level of subcutaneous temperature in this animal, kept outdoors in the Slovak Republic, is achieved 2 months after the lowest ambient temperature. Thus, the fall in subcutaneous temperature is not merely a reflection of the fall in ambient temperature, but it is a regulated process due perhaps to the lower energy content of food. (Source: Arnold, W., Ruf, T., Reimoser, S., Tataruch, F., Onderscheka, K. & Schober, F. (2004). Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). *American Journal of Physiology* 286: R174–R181.)

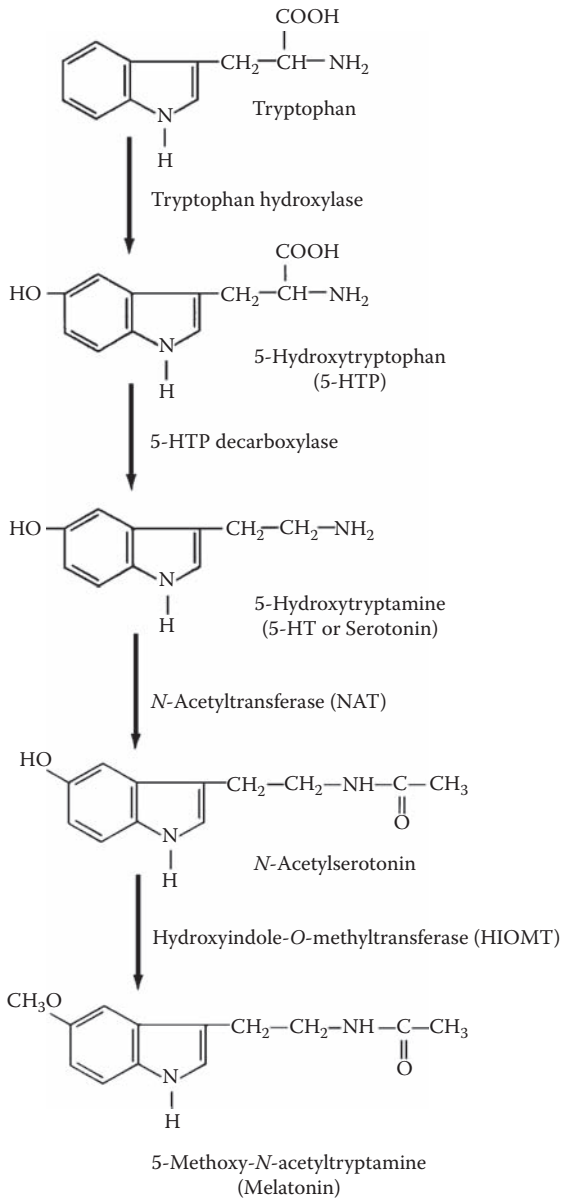


FIGURE 4.54 Making melatonin. This figure shows the steps needed to synthesize melatonin from dietary tryptophan. The enzymes involved in the reactions appear beside the arrows. Some reactions require cofactors that are not shown. (Source: Adapted from Feldman, R. S. & Quenzer, L. F. (1984). *Fundamentals of Neuropsychopharmacology.* Sunderland, MA: Sinauer.)

Hormone secretion is another physiological parameter that exhibits seasonal rhythmicity. Seasonal rhythmicity in the secretion of various hormones has been documented in many species,[263,269,271,311,370–372] but 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. Figure 4.54 shows the structural formula of melatonin (5-methoxy-*N*-acetyltryptamine). In

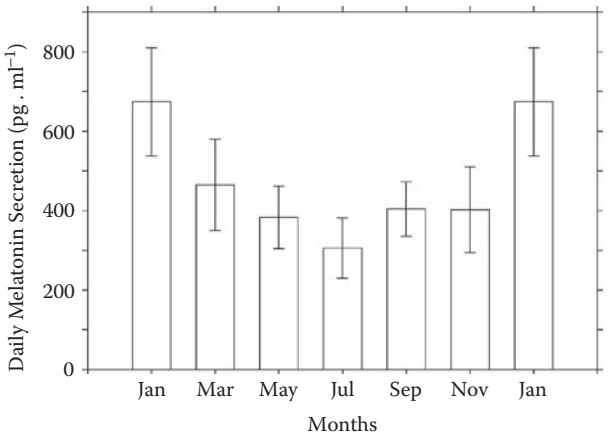


FIGURE 4.55 Finnish goats. The graph shows the mean daily melatonin secretion (measured in the serum) of goats housed indoors with a variable light–dark cycle matching the outdoor photoperiod in Finland throughout the year. An annual rhythm of melatonin secretion is clearly seen. Each bar corresponds to the mean (± SE) of seven goats. (Source: Alila-Johansson, A., Eriksson, L., Soveri, T. & 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.)

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,[373–379] as exemplified in Figure 4.55. The data refer to serum melatonin concentration in goats housed indoors under a light–dark cycle matching the outdoor photoperiod in Finland. Note 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 20th century.[380] In vertebrate animals, melatonin plays a central role in communicating photoperiodic information to the various organs in the body.[381] As 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. This topic is discussed later in this book.

Two other physiological parameters that exhibit photoperiodism are *molting* and *reproductive capacity.* Many species undergo a seasonal change in pelage (fur),[250,265,283,382–391] as exemplified by the change in fur color in Siberian hamsters (Figure 4.56). Seasonal changes

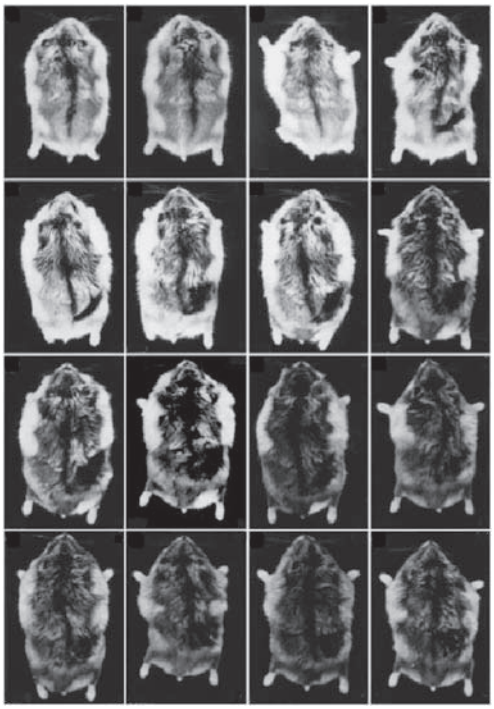


FIGURE 4.56 Winter white. The Siberian hamster (*Phodopus sungorus*) exhibits an annual rhythm of pelage coloration, as shown in this series of photographs. The sequence is left to right, then top to bottom. The pelage is light in the winter (top left) and dark in the summer (bottom right). (Source: Kuhlmann, M. T., Clemen, G. & Schlatt, S. (2003). Molting in the Djungarian hamster (*Phodopus sungorus* Pallas): seasonal or continuous process? *Journal of Experimental Zoology* 295A: 160–171; © 2003 Wiley-Liss, Inc. Reprinted by permission of Wiley-Liss, a subsidiary of John Wiley & Sons, Inc.)

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), while others reproduce only under short photoperiods, and still others only under long photoperiods. Photoperiodic reproduction has been described in plants,[392–395] invertebrates,[361,364,396] lower vertebrates,[397–399] birds,[250,400–402] and many mammalian species, including Siberian hamsters,[230,388,403–406] Syrian hamsters,[110,112,179,343,407–412] other rodents,[253,254,257,259,261,264,347,413–418] and other mammals.[120,132,271,371,390,419–422] Deer (Figure 4.57) are short-day breeders, while dormice (Figure 4.58) are long-day breeders. Note in Figure 4.58 that plasma testosterone levels in male dormice (a species of small nocturnal rodents) are low during much of the year but rise sharply in the summer.

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.[423]

FIGURE 4.57 White-tail deer. Several animal species, including the deer, undergo an annual cycle of reproductive capability. (Source: Photograph by Scott Bauer, Agricultural Research Service, U.S. Department of Agriculture.)

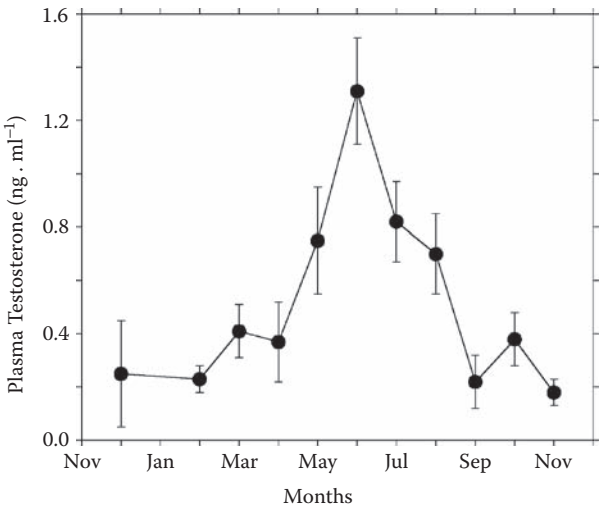


FIGURE 4.58 The season for sex. In males of many species, the production of testosterone varies with the seasons. In fat dormice (*Glis glis*) kept indoors but subjected to the natural variation in photoperiod and ambient temperature in France, testosterone secretion peaks in the early summer. Each data point corresponds to the mean (± SE) of seven dormice. (Source: Jallageas, M., Mas, N. & Nouguier-Soulé, J. (1991). Control of annual endocrine rhythms in the edible dormouse: nonprimary effect of photoperiod. *Journal of Biological Rhythms* 6: 343–352.)

Is 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 entire year (that is, humans are capable of reproducing year round). The frequency of human conception and birth, however, exhibits a small seasonal variation.[191,424–427]

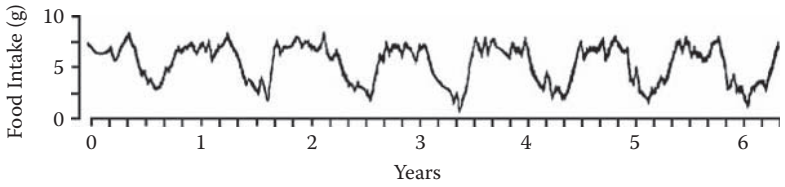


FIGURE 4.59 Circannual rhythm of food intake. In many animals, the annual rhythm of food intake is controlled by an internal clock. This graph shows the variation in food intake of a chipmunk (*Tamias striatus*) maintained in the laboratory for 6 years. Ambient temperature was maintained constant year 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 seven cycles in 6 years implies a free-running period of approximately 10 months. (Source: Richter, C. P. (1978). Evidence for existence of a yearly clock in surgically and self-blinded chipmunks. *Proceedings of the National Academy of Sciences U.S.A.* 75: 3517–3521.)

4.4.2 Circannual Rhythms

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.[428] Environmental cycles affect both types of rhythms, however. Type I rhythms are *evoked by* environmental cycles, while Type II rhythms are *synchronized to* environmental cycles. The anatomical location of the circannual pacemaker has yet to be identified,[366,429,430] although likely candidates include the pituitary gland and the hypothalamus.[431]

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. The organism must be studied for many consecutive years until enough data are available for appropriate analysis. 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[432–436] and mammals.[120,254,256,261,391,417,421,437–440] Figure 4.59 provides one example. 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). Note the clear annual rhythm of food intake. Seven cycles are present 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). Because 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 4.60 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 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. Because almost five full cycles are completed in 4 years, the free-running rhythm has a period of 9 to 10 months. A third example is shown in Figure 4.61. 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 a constant photoperiod, with 13 hours of light and 11 hours of darkness per day. Note the presence of seven peaks of testicular width in 6 years, which indicates a free-running period of 10 months.

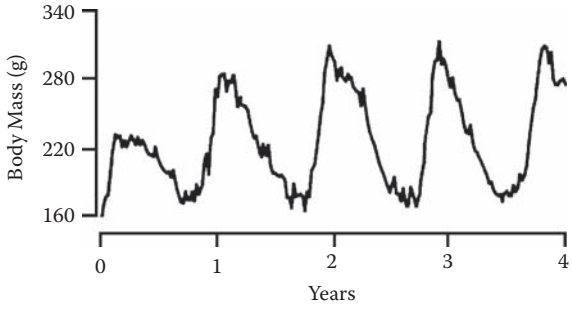


FIGURE 4.60 Circannual rhythm of body weight. In many animals, the annual rhythm of body mass is controlled by an internal clock. This graph shows the variation in body mass of a golden-mantled ground squirrel (*Spermophilus lateralis*) maintained in the laboratory for 4 years. Ambient temperature was maintained constant 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. (Source: Hiebert, S. M., Thomas, E. M., Lee, T. M., Pelz, K. M., Yellon, S. M. & 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.)

This concludes the discussion of infradian and ultradian rhythms. Chapter 5 examines the phenomenology of circadian rhythms, while the chapters in Part III discuss the endogenous and exogenous mechanisms responsible for overt rhythmicity.

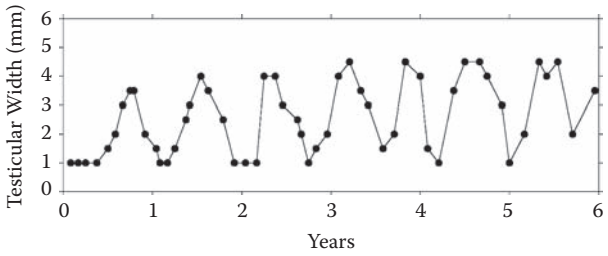


FIGURE 4.61 Circannual rhythm of reproductive capability. In many animals, the annual rhythm of reproductive capability is controlled by an internal clock. This graph shows the variation in testicular size of a tropical bird, the stonechat (*Saxicola torquata axillaris*), maintained in the laboratory for over 6 years. Ambient temperature (20°C) and photoperiod (13 hours of light and 11 hour of darkness per day) were kept constant over the years. Despite the absence of seasonal cues, a clear rhythm of testicular size can be observed. The occurrence of seven cycles in 6 years implies a free-running period of approximately 10 months. (Source: Gwinner, E. & Dittami, J. (1990). Endogenous reproductive rhythms in a tropical bird. *Science* 249: 906–908.)

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

Exercise 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 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. 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). You may need to apply gentle pressure around the vagina to expel the secretion. The table below will help you identify the stages of the estrous cycle. Don't be discouraged if you cannot always identify the stage. You will be conducting inspections every day, so missing a few data points will not prevent you from seeing the overall pattern. After you have collected data for about 3 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

Exercise 4.2 Detecting rhythmicity in a data set

This exercise uses the program Rhythm to detect rhythmicity in various data sets. As explained in Section 3.3, the program uses the chi square periodogram procedure to evaluate the presence of statistically significant rhythmicity.

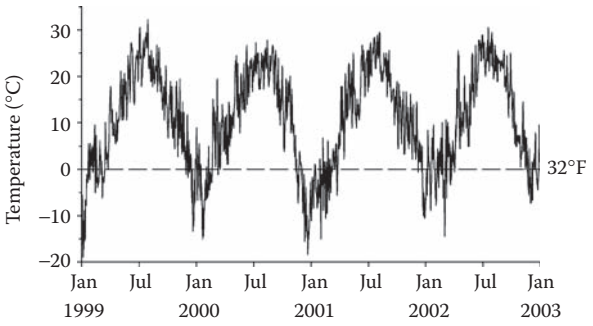


FIGURE 4.62 Ups and downs in Chicago. The graph shows the mean daily temperature in Chicago (Illinois) from January 1999 to January 2003. There is a clear annual rhythm with higher temperatures in the summer and lower temperatures in the winter. (Source: National Weather Service, U.S. National Oceanic and Atmospheric Administration.)

1. Double-click on the Circadian icon to open the program banner, then click on Rhythm (the fourth icon from the left).
2. Open 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. Refer to the graph provided in Figure 4.62.
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 4.62 clearly suggests the existence of 12-month rhythmicity).
6. Click on Execute. The Results panel reports that a statistically significant 12-month periodicity exists in the data, as 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). Note 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 a search for only yearly rhythmicity was requested.
7. Checking for lower harmonics is especially important if the wrong target periodicity is specified. Select the data file A04. As shown 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 the program Plot and inspect the data in the actogram format before proceeding.
8. In the Data Resolution panel, enter the correct information (Bin size is 6 and unit is Minutes). 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 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 (that is, it repeats itself exactly twice every 48 hours).
10. Now change the Target Periodicity to 24 hours and click on Execute again. This time, the program shows that there is significant periodicity but that no harmonics were detected. The true periodicity is 24 hours (as inspection of the actogram clearly suggested).
11. Keep in mind that the program looked only for broad periodicity. When Rhythm indicates that 24-hour periodicity exists in data set A04, it is actually indicating that periodicity occurs between about 23 and 25 hours. The exercises in Chapter 5 deal with programs designed to determine the exact period of a circadian rhythm.

Exercise 4.3 Detecting rhythmicity in a single cycle

A process that completes a cycle only once is not really rhythmic. Thus, determining that a single cycle is indeed cyclic is no assurance of rhythmicity. In the real world, however, sometimes only one cycle is available. As described in Section 3.3, the program Onecycle provides a simple means to determine the presence of periodicity in a single cycle.

1. Double-click on the Circadian icon to open the program banner, then click on Onecycle (the third icon from the left).
2. The program is very simple and does not require data files. Simply type in the values. The program assumes that the data points are equally spaced, but the units of time are irrelevant.
3. Suppose you measured the growth of a plant once a day for a week and you noticed that the plants grew more on Monday and Tuesday than on any other day. Is this evidence of 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. The program indicates that the distribution of growth over the days of the week is not significantly different from a random pattern (D =

0.0627, $p > 0.05$). In this case, there is no statistically significant one-cycle periodicity.

5. Click on Cancel to clear the data-entry panel and enter another data set. To collect these data, you stood by a public monument for 1 day and counted the number of people who were there. 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 people 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 entered the data in the program, do it now. Then click on OK.
7. In this case, the temporal distribution differs significantly from that of a random pattern ($D = 0.3043$, $p < 0.01$). The cycle might not occur again, but the increase in the number of visitors during business hours was real on the day that you were there.

Exercise 4.4 Detecting periodicity in a sequence of infrequent events

This exercise uses the program Rayleigh to detect periodicity in a sequence of infrequent events. As explained in Section 3.3, the program uses the Rayleigh test to determine whether events are significantly concentrated around a single time of day.

1. Double-click on the Circadian icon to open the program banner, then click on Rayleigh (the sixth program from the left).
2. The program is very simple and does not require data files. Simply type in the values.
3. Suppose you live by the beach and spend a lot of time just watching the ocean. For the last week, you wrote 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 P.M., 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 P.M., 5 P.M.	12, 17

4. Now you wonder whether the transit of ships in front of your house shows daily rhythmicity. To determine if a sinusoidal pattern is present in your data, type in the values from the rightmost column of the table. Make sure to type one value per line. After you enter 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.
6. Now suppose you work at the emergency room of a hospital and you write down the times when patients arrive. Your records for 3 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 no evidence of significant rhythmicity was found. The nR^2 of 2.601 has a probability greater than 0.05 ($p = 0.074$) under the null hypothesis.

Exercise 4.5 Analyzing ultradian rhythms

In Section 3.3 you learned that Fourier analysis is often a poor choice to evaluate circadian rhythmicity, but it is a powerful tool in the analysis of ultradian rhythmicity. This exercise uses the program Fourier to evaluate multiple rhythmicities in various data sets.

1. Double-click on the Circadian icon to open the program banner, then click on Fourier (the fifth program from the left).
2. Open 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 hours and 24 hours) are combined into a single data string. The resolution is 6 minutes.
3. Before proceeding, 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. Note that the time series is perfectly stationary (that is, the waveform 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 consists of pure cosine waves. Scroll down to the period of 11.99 (that is, 12 hours) and note 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 (that is, 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 four waves are combined (with periods of 6, 10, 12, and 24 hours). As before, 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 waveform 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).
8. Analyze the data file A04. This file was used 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 (24.1 hours) for at least the first half of the record. In Cartesian mode, you should notice that the time series is clearly not stationary (that is, the waveform varies quite a bit from day to day).
9. Now, use Fourier to analyze A04. Select A04 in the Source panel and click on OK. 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. Note also that the period of 24.1 hours is not listed (instead, the main period is listed as 23.99). As discussed in Section 3.3, Fourier analysis often lacks resolution in the circadian range. In contrast, note that the analysis in the ultradian range is too detailed. 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

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

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. A nice, short review of the literature on the neuroendocrine mechanisms that control annual rhythms in mammals.

Aschoff, J. (Ed.). (1981). *Biological Rhythms* (Volume 4 of *Handbook of Behavioral Neurobiology*). New York: Plenum. A classic in the biological rhythms literature. Although a quarter of a century 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 graduate-level textbook on biological rhythms, covering circadian and circannual rhythms.

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 20 years old, it remains a valuable review of early studies on hibernation and daily torpor in mammals and birds.

Saunders, D. S., Steel, C. G. H., Vafopoulou, X., and Lewis, R. D. (2002). *Insect Clocks* (3rd Edition). New York: Elsevier. This book covers circadian and annual rhythms in insects in great detail and is directed at active researchers in the field.

WEB SITES TO EXPLORE

Endocrine Society:
http://www.endo-society.org

PubMed (U.S. National Library of Medicine):
http://www4.ncbi.nlm.nih.gov/PubMed

Seasonal Rhythms (Howard Hughes Medical Institute):
http://www.hhmi.org/biointeractive/clocks/seasons.html

The Seasons (University of Tennessee):
http://csep10.phys.utk.edu/astr161/lect/time/seasons.html

LITERATURE CITED

1. Thurman, H. V. & Trujillo, A. P. (2005). *Essentials of Oceanography*, 8th Edition. Upper Saddle River, N.J.: Prentice Hall.
2. Bradley, R. S. (1999). *Paleoclimatology: Reconstructing Climates of the Quaternary*, 2nd Edition. San Diego: Academic Press.
3. Kerr, R. A. (1987). Milankovitch climate cycles through the ages. *Science* 235: 973–974.
4. Daintith, J., Mitchell, S., Tootill, E. & Gjertsen, D. (1994). *Biographical Encyclopedia of Scientists*, 2nd Edition. Bristol: Institute of Physics.
5. Woodward, F. I. (1987). *Climate and Plant Distribution*. Cambridge, UK: Cambridge University Press.
6. Pahnke, K., Zahn, R., Elderfield, H. & 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. & 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., de Garidel-Thoron, T., Mix, A. C. & 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. & Sachs, J. P. (2002). El Niño-like pattern in Ice Age tropical Pacific Sea surface temperature. *Science* 297: 226–230.
10. Karl, T. R. & 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. & 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. & 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. & Briffa, K. R. (2001). The evolution of climate over the last millennium. *Science* 292: 662–667.
16. Vinnikov, K. Y. & 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. & 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. & Wanner, H. (2004). European seasonal and annual temperature variability, trends, and extremes since 1500. *Science* 303: 1499–1503.
19. Cotton, P. A. (2003). Avian migration phenology and global climate change. *Proceedings of the National Academy of Sciences U.S.A.* 100: 12219–12222.
20. Roberts, L. (1988). Is there life after climate change? *Science* 242: 1010–1012.
21. Bond, G., Showers, W., Cheseby, M., Lotti, R., Almasi, P., de Menocal, P., Priore, P., Cullen, H., Hajdas, I. & Bonani, G. (1997). A pervasive millenial-scale cycle in North Atlantic Holocene and Glacial climates. *Science* 278: 1257–1266.
22. Oppo, D. W., McManus, J. F. & Cullen, J. L. (1998). Abrupt climate events 500,000 to 340,000 years ago: evidence from subpolar North Atlantic sediments. *Science* 279: 1335–1338.
23. Scuderi, L. A. (1993). A 2000-year tree ring record of annual temperatures in the Sierra Nevada mountains. *Science* 259: 1433–1436.
24. Chavez, F. P., Ryan, J., Lluch-Cota, S. E. & Ñiquen, M. (2003). From anchovies to sardines and back: multidecadal change in the Pacific Ocean. *Science* 299: 217–221.
25. Kerr, R. A. (1990). The climate system as a ticking clock. *Science* 249: 1246–1247.
26. Burdick, A. (1991). El Niño antiguo. *The Sciences* 31(3): 8–9.
27. Kondratyev, K. Y. & Nikolsky, G. A. (1970). Solar radiation and solar activity. *Quarterly Journal of the Royal Meteorological Society* 96: 509–522.
28. Cornélissen, G., Engebretson, M., Johnson, D., Otsuka, K., Burioka, N., Posch, J. & Halberg, F. (2001). The week, inherited in neonatal human twins, found also in geomagnetic pulsations in isolated Antarctica. *Biomedicine and Pharmacotherapy* 55: 32–50.
29. Zerubavel, E. (1985). *The Seven Day Circle: The History and Meaning of the Week*. New York: Free Press.
30. Aschoff, J. (1981). A survey on biological rhythms. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology Volume 4*). New York: Plenum, pp. 3–10.
31. Zaha, M. A. (1972). Shedding some needed light on optical measurements. *Electronics* Nov 6: 91–96.
32. Trewartha, G. T. & Horn, L. H. (1980). *An Introduction to Climate*, 5th Edition. New York: McGraw-Hill.
33. Miller, A. & Thompson, J. C. (1983). *Elements of Meteorology*, 4th Edition. Columbus: Charles E. Merrill.
34. Danielson, E. W., Levin, J. & Abrams, E. (2003). *Meteorology*, 2nd Edition. Boston: McGraw-Hill.
35. Barnes, R. S. K. (1998). *The Diversity of Living Organisms*. Malden, MA: Blackwell.
36. Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *Journal of Applied Physiology* 22: 453–460.
37. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th Edition. New York: Cambridge University Press.
38. Ganong, W. F. (2001). *Review of Medical Physiology,* 20th Edition. New York: McGraw-Hill.
39. Meek, W. J. & 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.

40. Bozler, E. (1943). The initiation of impulses in cardiac muscle. *American Journal of Physiology* 138: 273–282.
41. Hutter, O. F. & 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.
42. Accili, E. A., Proenza, C., Baruscotti, M. & DiFrancesco, D. (2002). From funny current to HCN channels: 20 years of excitation. *News in Physiological Sciences* 17: 32–37.
43. Miller, N. E. (1978). Biofeedback and visceral learning. *Annual Review of Psychology* 29: 373–404.
44. Bindu, P. N. & 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.
45. Hatch, J. P., Borcherding, S. & Norris, L. K. (1990). Cardiopulmonary adjustments during operant heart rate control. *Psychophysiology* 27: 641–648.
46. Hilaire, G. & Pásaro, R. (2003). Genesis and control of the respiratory rhythm in adult mammals. *News in Physiological Sciences* 18: 23–28.
47. Smith, J. C., Ellenberger, H. H., Ballanyi, K., Richter, D. W. & Feldman, J. L. (1991). Pre-Bötzinger complex: a brainstem region that may generate respiratory rhythm in mammals. *Science* 254: 726–729.
48. Koshiya, N. & Smith, J. C. (1999). Neuronal pacemaker for breathing visualized *in vitro*. *Nature* 400: 360–363.
49. Onimaru, H. & Homma, I. (2003). A novel functional neuron group for respiratory rhythm generation in the ventral medulla. *Journal of Neuroscience* 23: 1478–1486.
50. Feldman, J. L., Mitchell, G. S. & Nattie, E. E. (2003). Breathing: rhythmicity, plasticity, chemosensitivity. *Annual Review of Neuroscience* 26: 239–266.
51. Adrian, E. D. (1941). Afferent discharges to the cerebral cortex from peripheral sense organs. *Journal of Physiology* 100: 159–191.
52. Mountcastle, V. B. (1957). Modality and topographic properties of single neurons of cat's somatic sensory cortex. *Journal of Neurophysiology* 20: 408–434.
53. Lydic, R. (1989). Central pattern-generating neurons and the search for general principles. *FASEB Journal* 3: 2457–2468.
54. Berger, H. (1929). Über das Elektrenkephalogramm des Menschen. *Archiv für Psychiatrie und Nervenkrankheiten* 87: 527–570.
55. Shepherd, G. M. (1994). *Neurobiology*, 3rd Edition. New York: Oxford University Press.
56. Kandel, E. R., Schwartz, J. H. & Jessell, T. M. (2000). *Principles of Neural Science*, 4th Edition. New York: McGraw-Hill.
57. Knobil, E. (1987). A hypothalamic pulse generator governs mammalian reproduction. *News In Physiological Sciences* 2: 42–43.
58. Loudon, A. S. I., Wayne, N. L., Krieg, R., Iranmanesh, A., Veldhuis, J. D. & Menaker, M. (1994). Ultradian endocrine rhythms are altered by a circadian mutation in the Syrian hamster. *Endocrinology* 135: 712–718.
59. Weibel, L., Spiegel, K., Follenius, M., Ehrhart, J. & Branderberger, G. (1996). Internal dissociation of the circadian markers of the cortisol rhythm in night workers. *American Journal of Physiology* 270: E608–E613.
60. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L. & 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.
61. Matagne, V., Lebrethon, M. C., Gérard, A. & 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.
62. Palmer, J. D. (2000). The clocks controlling the tide-associated rhythms of intertidal animals. *BioEssays* 22: 32–37.
63. de la Iglesia, H. O., Rodríguez, E. M. & Dezi, R. E. (1994). Burrow plugging in the crab *Uca uruguayensis* and its synchronization with photoperiod and tides. *Physiology and Behavior* 55: 913–919.
64. Wikelski, M. & Hau, M. (1995). Is there an endogenous tidal foraging rhythm in marine iguanas? *Journal of Biological Rhythms* 10: 335–350.
65. 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.
66. 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.
67. Palmer, J. D. (1967). Daily and tidal components in the persistent rhythmic activity of the crab, *Sesarma*. *Nature* 215: 64–66.
68. Palmer, J. D. & 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.
69. 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.
70. Cummings, S. M. & Morgan, E. (2001). Time-keeping system of the eel pout, *Zoarces viviparus*. *Chronobiology International* 18: 27–46.
71. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
72. Sanders, K. M., Ördög, T., Koh, S. D. & Ward, S. M. (2000). A novel pacemaker mechanism drives gastrointestinal rhythmicity. *News in Physiological Sciences* 15: 291–298.
73. Grau, C., Escera, C., Cilveti, R., García, M., Mojón, A., Fernandez, J. R. & Hermida, R. C. (1995). Ultradian rhythms in gross motor activity of adult humans. *Physiology and Behavior* 57: 411–419.

74. Wilkens, J. L. & Fingerman, M. (1965). Heat tolerance and temperature relationships of the fiddler crab, *Uca pugilatur*, with reference to body coloration. *Biological Bulletin* 128: 133–141.
75. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A. & Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
76. Lumineau, S., Guyomarc'h, C. & Richard, J. P. (2000). Ontogeny of the ultradian rhythm of activity in Japanese quail. *Chronobiology International* 17: 767–776.
77. Doust, J. W. L. (1979). Periodic homeostatic fluctuations of skin temperature in the sleeping and waking state. *Neuropsychobiology* 5: 340–347.
78. Mohr, E. & Krzywanek, H. (1990). Variations of core-temperature rhythms in unrestrained sheep. *Physiology and Behavior* 48: 467–473.
79. Halle, S. (1995). Diel pattern of locomotor activity in populations of root voles, *Microtus oeconomus*. *Journal of Biological Rhythms* 10: 211–224.
80. Haim, A., Saarela, S., Hohtola, E. & 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.
81. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G. & Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
82. Goldberger, A. L., Rigney, D. R. & West, B. J. (1990). Chaos and fractals in human physiology. *Scientific American* 262(2): 42–49.
83. Goldberger, A. L. (1991). Is the normal heartbeat chaotic or homeostatic? *News in Physiological Sciences* 6: 87–91.
84. Stark, J. & Hardy, K. (2003). Chaos: useful at last? *Science* 301: 1192–1193.
85. Jilge, B. (1985). The rhythm of food and water ingestion, faeces excretion and locomtor activity in the guinea pig. *Zeitschrift für Versuchstierkunde* 27: 215–225.
86. Stupfel, M., Gourlet, V., Court, L., Mestries, J., Parramon, A. & 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.
87. Wollnik, F. & 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.
88. Kronauer, R. E. & Jewett, M. E. (1992). The relationship between circadian and hemicircadian components of human endogenous temperature rhythms. *Journal of Sleep Research* 1: 88–92.
89. Golombek, D. A., Ortega, G. & Cardinali, D. P. (1993). Wheel running raises body temperature and changes the daily cycle in golden hamsters. *Physiology and Behavior* 53: 1049–1054.
90. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
91. Refinetti, R. (1994). Circadian modulation of ultradian oscillation in the body temperature of the golden hamster. *Journal of Thermal Biology* 19: 269–275.
92. Ortega, G. J., Romanelli, L. & Golombek, D. A. (1994). Statistical and dynamical analysis of circadian rhythms. *Journal of Theoretical Biology* 169: 15–21.
93. Robinson, E. L. & Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
94. Scheibe, K. M., Berher, A., Langbein, J., Streich, W. J. & Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biological Rhythm Research* 30: 216–233.
95. Benstaali, C., Bogdan, A. & 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.
96. Hermida, R. C., Ayala, D. E., Fernández, J. R., Mojón, A., Alonso, I. & Calvo, C. (2002). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiology International* 19: 461–481.
97. Hadtstein, C., Wühl, E., Soergel, M., Witte, K. & Schaefer, F. (2004). Normative values for circadian and ultradian cardiovascular rhythms in childhood. *Hypertension* 43: 547–554.
98. Refinetti, R. (1996). Ultradian rhythms of body temperature and locomotor activity in wild-type and tau-mutant hamsters. *Animal Biology* 5: 111–115.
99. Gerkema, M. P., Groos, G. A. & 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.
100. Eastman, C. I., Mistlberger, R. E. & Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology and Behavior* 32: 357–368.
101. Johnson, M. H. & Everitt, B. J. (2000). *Essential Reproduction*, 5th Edition. Oxford, UK: Blackwell.
102. Allgeier, E. R. & Allgeier, A. R. (2000). *Sexual Interactions*, 5th Edition. Boston: Houghton Mifflin.
103. Crooks, R. & Baur, K. (2005). *Our Sexuality*, 9th Edition. Pacific Grove, CA: Brooks/Cole.
104. Adams, D. B., Gold, A. R. & 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.
105. Rikowski, A. & Grammer, K. (1999). Human body odour, symmetry and attractiveness. *Proceedings of the Royal Society of London B* 266: 869–874.
106. Penton-Voak, I. S., Perrett, D. I., Castles, D. L., Kobayashi, T., Burt, D. M., Murray, L. K. & Minamisawa, R. (1999). Menstrual cycle alters face preference. *Nature* 399: 741–742.

107. Macrae, C. N., Alnwick, K. A., Milne, A. B. & Schloerscheidt, A. M. (2002). Person perception across the menstrual cycle: hormonal influences on social-cognitive functioning. *Psychological Science* 13: 532–536.
108. Alleva, J. J., Waleski, M. V. & Alleva, F. R. (1971). A biological clock controlling the estrous cycle of the hamster. *Endocrinology* 88: 1368–1379.
109. Fitzgerald, K. M. & Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences U.S.A.* 73: 2923–2927.
110. Swann, J. & 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.
111. 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.
112. Seegal, R. F. & Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. *Biology of Reproduction* 12: 223–231.
113. Refinetti, R. & Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.
114. 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.
115. Morin, L. P., Fitzgerald, K. M. & Zucker, I. (1977). Estradiol shortens the period of hamster circadian rhythms. *Science* 196: 305–307.
116. Takahashi, J. S. & Menaker, M. (1980). Interaction of estradiol and progesterone: effects on circadian locomotor rhythm of female golden hamsters. *American Journal of Physiology* 239: R497–R504.
117. Davis, F. C., Stice, S. & 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.
118. Fritzsche, P., Riek, M. & Gattermann, R. (2000). Effects of social stress on behavior and corpus luteum in female golden hamsters (*Mesocricetus auratus*). *Physiology and Behavior* 68: 625–630.
119. Sridaran, R. & 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.
120. Palmer, E., Driancourt, M. A. & Ortavant, R. (1982). Photoperiodic stimulation of the mare during winter anoestrus. *Journal of Reproduction and Fertility* 32(S): 275–282.
121. Lewis, G. S. & Newman, S. K. (1984). Changes throughout estrous cycles of variables that might indicate estrus in dairy cows. *Journal of Dairy Science* 67: 146–152.
122. Rajamahendran, R., Robinson, J., Desbottes, S. & 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.
123. Walker, L. A., Cornell, L., Dahl, K. D., Czekala, N. M., Dargen, C. M., Joseph, B., Hsueh, A. J. W. & 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.
124. Häster, L. & 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.
125. Shideler, S. E., Savage, A., Ortuño, A. M., Moorman, E. A. & 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.
126. Asa, C. S., Fischer, F., Carrasco, E. & Puricelli, C. (1994). Correlation between urinary pregnanediol glucuronide and basal body temperature in female orangutans, *Pongo pygmaeus*. *American Journal of Primatology* 34: 275–281.
127. Nagamine, N., Nambo, Y., Nagata, S., Nagaoka, K., Tsunoda, N., Taniyama, H., Tanaka, Y., Tohei, A., Watanabe, G. & 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.
128. Kooistra, H. S., Okkens, A. C., Bevers, M. M., Popp-Snijders, C., van Haaften, B., Dieleman, S. J. & 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.
129. McMillan, H. J. & 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.
130. Gray, C. A., Bartol, F. F., Taylor, K. M., Wiley, A. A., Ramsey, W. S., Ott, T. L., Bazer, F. W. & Spencer, T. E. (2000). Ovine uterine gland knock-out model: effects of gland ablation on the estrous cycle. *Biology of Reproduction* 62: 448–456.
131. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W. & 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. Skinner, D. C., Richter, T. A., Malpaux, B. & Skinner, J. D. (2001). Annual ovulation cycles in an aseasonal breeder, the springbok (*Antidorcas marsupialis*). *Biology of Reproduction* 64: 1176–1182.

133. Duggavathi, R., Bartlewski, P. M., Barrett, D. M. W. & 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.
134. West, M., Galloway, D., Shaw, J., Trouson, A. & Paris, M. C. J. (2004). Oestrous cycle of the common wombat, *Vombatus ursinus*, in Victoria, Australia. *Reproduction, Fertility and Development* 16: 339–346.
135. Brown, J. L., Walker, S. L. & 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.
136. Atsalis, S., Margulis, S. W., Bellem, A. & Wielebnowski, N. (2004). Sexual behavior and hormonal estrus cycles in captive aged lowland gorillas (*Gorilla gorilla*). *American Journal of Primatology* 62: 123–132.
137. Peters, D. G. & 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.
138. Brobeck, J. R., Wheatland, M. & Strominger, J. L. (1947). Variations in regulation of energy exchange associated with estrus, diestrus and pseudopregnancy in rats. *Endocrinology* 40: 65–72.
139. Bouchard, G., Youngquist, R. S., Vaillancourt, D., Krause, G. F., Guay, P. & Paradis, M. (1991). Seasonality and variability of the interestrous interval in the bitch. *Theriogenology* 36: 41–50.
140. Beijerink, N. J., Dieleman, S. J., Kooistra, H. S. & Okkens, A. C. (2003). Low doses of bromocriptine shorten the interestrous interval in the bitch without lowering plasma prolactin concentration. *Theriogenology* 60: 1379–1386.
141. 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.
142. Johnson, G. A., Stewart, M. D., Gray, C. A., Choi, Y., Burghardt, R. C., Yu-Lee, L. Y., Bazer, F. W. & 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.
143. Zehr, J. L., Tannenbaum, P. L., Jones, B. & 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.
144. Handler, J., Wüstenhagen, A., Schams, D., Kindahl, H. & 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.
145. Francis, A. J. P. & 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.
146. Yang, Y. & 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.
147. Kent, S., Hurd, M. & Satinoff, E. (1991). Interactions between body temperature and wheel running over the estrous cycle in rats. *Physiology and Behavior* 49: 1079–1084.
148. Redden, K. D., Kennedy, A. D., Ingalls, J. R. & 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.
149. Labyak, S. E. & Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
150. Rauth-Widmann, B., Fuchs, E. & 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.
151. Bhatti, B. M. & Morris, T. R. (1978). Entrainment of oviposition in the fowl using light-dark cycles. *British Poultry Science* 19: 333–340.
152. Kadono, H. & 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: Springer, pp. 91–99.
153. Konishi, T. (1980). Circadian rhythm of ovipositional time in Japanese quail. In: Tanabe, Y. (Ed.). *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin: Springer, pp. 79–90.
154. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
155. Houdelier, C., Guyomarc'h, C. & Lumineau, S. (2002). Daily temporal organization of laying in Japanese quail: variability and heritability. *Chronobiology International* 19: 377–392.
156. Marrone, B. L., Gentry, R. T. & 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.
157. Krohmer, R. W. & 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.
158. Bobr, L. W. & 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.
159. Kadono, H., Besch, E. L. & Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.

160. Czaja, J. A. & 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.
161. Wrenn, T. R., Bitman, J. & Sykes, J. F. (1958). Body temperature variations in dairy cattle during the estrous cycle and pregnancy. *Journal of Dairy Sciences* 41: 1071–1076.
162. Fordham, D. P., Rowlinson, P. & McCarthy, T. T. (1988). Oestrus detection in dairy cows by milk temperature measurement. *Research in Veterinary Science* 44: 366–374.
163. Kyle, B. L., Kennedy, A. D. & Small, J. A. (1998). Measurement of vaginal temperature by radiotelemetry for the prediction of estrus in beef cows. *Theriogenology* 49: 1437–1449.
164. Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
165. Harvey, O. L. & Crockett, H. E. (1932). Individual differences in temperature changes of women during the course of the menstrual cycle. *Human Biology* 4: 453–468.
166. Rubenstein, B. B. & Lindsley, D. B. (1937). Relation between human vaginal smears and body temperatures. *Proceedings of the Society for Experimental Biology and Medicine* 35: 618–619.
167. 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.
168. Kleitman, N. & Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
169. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
170. Severino, S. K., Wagner, D. R., Moline, M. L., Hurt, S. W., Pollak, C. P. & Zendell, S. (1991). High nocturnal body temperature in premenstrual syndrome and late luteal phase dysphoric disorder. *American Journal of Psychiatry* 148: 1329–1335.
171. Nakayama, K., Yoshimuta, N., Sasaki, Y., Kadokura, M., Hiyama, T., Takeda, A., Sasaki, M. & Ushijima, S. (1992). Diurnal rhythm in body temperature in different phases of the menstrual cycle. *Japanese Journal of Psychiatry and Neurology* 46: 235–237.
172. Kattapong, K. R., Fogg, L. F. & Eastman, C. I. (1995). Effect of sex, menstrual phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International* 12: 257–266.
173. Baker, F. C., Driver, H. S., Paiker, J., Rogers, G. G. & 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.
174. Tompkins, P. (1944). The use of basal temperature graphs in determining the date of ovulation. *Journal of The American Medical Association* 124: 698–700.
175. Moghissi, K. S. (1980). Prediction and detection of ovulation. *Fertility and Sterility* 34: 89–98.
176. Martinez, A. R., van Hooff, M. H. A., Schoute, E., van der Meer, M., Broekmans, F. J. M. & 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.
177. Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.
178. Reinberg, A., Halberg, F., Ghata, J. & 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.
179. Kent, G. C., Ridgway, P. M. & Strobel, E. F. (1968). Continual light and constant estrus in hamsters. *Endocrinology* 82: 699–703.
180. Johns, M. A., Feder, H. H. & 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.
181. Albers, H. E., Gerall, A. A. & Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
182. Trudeau, R. (1997). Monthly and daily patterns of death. *Statistics Canada Health Reports* 9(1): 44–50.
183. Binkley, S., Tome, M. B., Crawford, D. & Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
184. Mauvieux, B., Gouthière, L., Sesboüe, B. & 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.
185. Roenneberg, T., Wirz-Justice, A. & Merrow, M. (2003). Life between clocks: daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
186. De Castro, J. M. (1991). Weekly rhythms of spontaneous nutrient intake and meal pattern of humans. *Physiology and Behavior* 50: 729–738.
187. Palmer, J. D., Udry, J. R. & Morris, N. M. (1982). Diurnal and weekly, but no lunar rhythm in human copulation. *Human Biology* 54: 111–121.
188. Hirschenhauser, K., Frigerio, D., Grammer, K. & Magnusson, M. S. (2002). Monthly patterns of testosterone and behavior in prospective fathers. *Hormones and Behavior* 42: 172–181.
189. Ruffieux, C., Marazzi, A. & Paccaud, F. (1992). The circadian rhythm of the perinatal mortality rate in Switzerland. *American Journal of Epidemiology* 135: 936–951.
190. Anderka, M., Declercq, E. R. & Smith, W. (2000). A time to be born. *American Journal of Public Health* 90: 124–126.

191. Mikulecky, M. & 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.
192. Bellamy, N., Sothern, R. B. & Campbell, J. (2004). Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. *Journal of Rheumatology* 31: 379–389.
193. Cornélissen, G., Breus, T. K., Bingham, C., Zaslavskaya, R., Varshitsky, M., Mirsky, B., Teibloom, M., Tarquini, B., Bakken, E. & Halberg, F. (1993). Beyond circadian chronorisk: worldwide circaseptan-circasemiseptan patterns of myocardial infarctions, other vascular events, and emergencies. *Chronobiologia* 20: 87–115.
194. Spielberg, C., Falkenhahn, D., Willich, S. N., Wegscheider, K. & 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.
195. Allegra, J. R., Cochrane, D. G., Allegra, E. M. & Cable, G. (2002). Calendar patterns in the occurrence of cardiac arrest. *American Journal of Emergency Medicine* 20: 513–517.
196. Kinjo, K., Sato, H., Shiotani, I., Kurotobi, T., Ohnishi, Y., Hishida, E., Nakatani, D., Mizuno, H., Yamada, Y., Fukui, S., Fukunami, M., Nanto, S., Matsu-ura, Y., Takeda, H. & Hori, M. (2003). Variation during the week in the incidence of acute myocardial infarction: increased risk for Japanese women on Saturdays. *Heart* 89: 398–403.
197. Kaiser, H., Cornélissen, G. & 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.
198. Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K. & Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s–44s.
199. Piccione, G., Caola, G. & Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
200. Couderc, P. (1971). Calendar. *Encyclopedia Americana* 5: 184–190.
201. Balling, R. C. & Cerveny, R. S. (1995). Influence of lunar phase on daily global temperatures. *Science* 267: 1481–1483.
202. Neumann, D. (1989). Circadian components of semilunar and lunar timing mechanisms. *Journal of Biological Rhythms* 4: 285–294.
203. Saigusa, M. (1980). Entrainment of a semilunar rhythm by a simulated moonlight cyclein the terrestrial crab, *Sesarma haematocheir*. *Oecologia* 46: 38–44.
204. Raison, C. L., Klein, H. M. & Steckler, M. (1999). The moon and madness reconsidered. *Journal of Affective Disorders* 53: 99–106.
205. De Castro, J. M. & Pearcey, S. M. (1995). Lunar rhythms of the meal and alcohol intake of humans. *Physiology and Behavior* 57: 439–444.
206. Bhattacharjee, C., Bradley, P., Smith, M., Scally, A. J. & Wilson, B. J. (2000). Do animals bite more during a full moon? Retrospective observational analysis. *British Medical Journal* 321: 1559–1561.
207. Gutiérrez-García, J. M. & Tusell, F. (1997). Suicides and the lunar cycle. *Psychological Reports* 80: 243–250.
208. Laverty, W. H. & Kelly, I. W. (1998). Cyclical calendar and lunar patterns in automobile property accidents and injury accidents. *Perceptual and Motor Skills* 86: 299–302.
209. Simón, A. (1998). Aggression in a prison setting as a function of lunar phases. *Psychological Reports* 82: 747–752.
210. Chapman, S. & Morrell, S. (2000). Barking mad? Another lunatic hypothesis bites the dust. *British Medical Journal* 321: 1561–1563.
211. Waldhoer, T., Haidinger, G. & Vutuc, C. (2002). The lunar cycle and the number of deliveries in Austria between 1970 and 1999. *Gynecologic and Obstetric Investigation* 53: 88–89.
212. Núñez, S., Méndez, L. P. & Aguirre-Jaime, A. (2002). Moon cycles and violent behaviours: myth or fact? *European Journal of Emergency Medicine* 9: 127–130.
213. Stenseth, N. C., Falck, W., Chan, K. S., Bjørnstad, O. N., O'Donoghue, M., Tong, H., Boonstra, R., Boutin, S., Krebs, C. J. & Yoccoz, N. G. (1998). From patterns to processes: phase and density dependencies in the Canadian lynx cycle. *Proceedings of the National Academy of Sciences U.S.A.* 95: 15430–15435.
214. Selås, V., Hogstad, O., Kobro, S. & 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.
215. Davis, S., Begon, M., De Bruyn, L., Ageyev, V. S., Klassovskiy, N. L., Pole, S. B., Viljugrein, H., Stenseth, N. C. & Leirs, H. (2004). Predictive thresholds for plague in Kazakhstan. *Science* 304: 736–738.
216. Nintcheu-Fata, S., Katinas, G., Halberg, F., Cornélissen, G., Tolstykh, V., Michael, H. N., Otsuka, K., Schwartzkopff, O. & Bakken, E. (2003). Chronomics of tree rings for chronoastrobiology and beyond. *Biomedicine and Pharmacotherapy* 57: 24s–30s.
217. Halberg, F., Cornélissen, G., Watanabe, Y., Otsuka, K., Fiser, B., Siegelova, J., Mazankova, V., Maggioni, C., Sothern, R. B., Katinas, G. S., Syutkina, E. V., Burioka, N. & Schwartzkopff, O. (2001). Near 10-year and longer periods modulate circadians: intersecting anti-aging and chronoastrobiological research. *Journal of Gerontology* 56A: M304–M324.
218. Mrosovsky, N., Melnyk, R. B., Lang, K., Hallonquist, J. D., Boshes, M. & Joy, J. E. (1980). Infradian cycles in dormice (*Glis glis*). *Journal of Comparative Physiology A* 137: 315–339.
219. Gilg, O., Hanski, I. & Sittler, B. (2003). Cyclic dynamics in a simple vertebrate predator-prey community. *Science* 302: 866–868.

220. Richter, C. P. (1965). *Biological Clocks in Medicine and Psychiatry.* Springfield, IL: Charles C Thomas.
221. McKaughan, M. (1987). *The Biological Clock: Reconciling Careers and Motherhood in the 1980's*. New York: Doubleday.
222. Armstrong, P. (1996). *Beating the Biological Clock: The Joys and Challenges of Late Motherhood*. London, UK: Headline.
223. Paulson, R. J. & Sachs, J. (2000). *Rewinding Your Biological Clock: Motherhood Late in Life*. San Francisco: W. H. Freeman.
224. Birrittieri, C. (2004). *What Every Woman Should Know About Fertility and Her Biological Clock*. Franklin Lakes, NJ: New Page Books.
225. Perrigo, G., Bryant, W. C. & vom Saal, F. S. (1990). A unique neural timing system prevents male mice from harming their own offspring. *Animal Behaviour* 39: 535–539.
226. Davis, R. C., Hassall, M. & Sutton, S. L. (1977). The vertical distribution of isopods and diplopods in a dune grassland. *Pedobiologia* 17: 320–329.
227. Perron, F. E. (1978). Seasonal burrowing behavior and ecology of *Aporrhais occidentalis* (Gastropoda: Strombacea). *Biological Bulletin* 154: 463–471.
228. Stark, H. E. (1963). Nesting habits of the California vole, *Microtus californicus*, and microclimatic factors affecting its nests. *Ecology* 44: 663–669.
229. 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.
230. Puchalski, W., Bulova, S. J., Lynch, C. B. & Lynch, G. R. (1988). Photoperiod, temperature and melatonin effects on thermoregulatory behavior in Djungarian hamsters. *Physiology and Behavior* 42: 173–177.
231. Schadt, C. W., Martin, A. P., Lipson, D. A. & Schmidt, S. K. (2003). Seasonal dynamics of previously unknown fungal lineages in tundra soils. *Science* 301: 1359–1361.
232. Templer, D. I., Ruff, C. F., Halcomb, P. H., Barthlow, V. L. & Ayers, J. L. (1978). Month of conception and birth of schizophrenics as related to the temperature. *Orthomolecular Psychiatry* 7: 231–235.
233. Kendell, R. E. & Adams, W. (1991). Unexplained fluctuations in the risk for schizophrenia by month and year of birth. *British Journal of Psychiatry* 158: 758–763.
234. Bassi, C. J. & Powers, M. K. (1986). Daily fluctuations in the detectability of dim lights by humans. *Physiology and Behavior* 38: 871–877.
235. Pöllmann, L. (1994). Circadian and circannual variations in the evaluation of thermal comfort in a constant climate. *Indoor Environment* 3: 145–148.
236. Bakko, E. B., Porter, W. P. & Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
237. Haikonen, H. & Summala, H. (2001). Deer-vehicle crashes: extensive peak at 1 hour after sunset. *American Journal of Preventive Medicine* 21: 209–213.
238. Bennett, K. A., McConnell, B. J. & 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.
239. Michael, R. P. & Zumpe, D. (1986). An annual rhythm in the battering of women. *American Journal of Psychiatry* 143: 637–640.
240. Harmatz, M. G., Well, A. D., Overtree, C. E., Kawamura, K. Y., Rosal, M. & Ockene, I. S. (2000). Seasonal variation of depression and other moods: a longitudinal approach. *Journal of Biological Rhythms* 15: 344–350.
241. Müller, D., Lampe, F., Wegscheider, K., Schultheiss, H. P. & Behrens, S. (2003). Annual distribution of ventricular tachycardias and ventricular fibrillation. *American Heart Journal* 146: 1061–1065.
242. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
243. Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A., Teo, S. L. H., Seitz, A., Walli, A. & Fudge, D. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293: 1310–1314.
244. Hernandez, J., Bonnedahl, J., Waldenström, J., Palmgren, H. & Olsen, B. (2003). *Salmonella* in birds migrating through Sweden. *Emerging Infectious Diseases* 9: 753–755.
245. Froy, O., Gotter, A. L., Casselman, A. L. & Reppert, S. M. (2003). Illuminating the circadian clock in monarch butterfly migration. *Science* 300: 1303–1305.
246. Hunter, E., Metcalfe, J. D. & 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.
247. Rattenborg, N. C., Mandt, B. H., Obermeyer, W. H., Winsauer, P. J., Huber, R., Wikelski, M. & Benca, R. M. (2004). Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLOS Biology* 2: 924–936.
248. Totzke, U., Hubinger, A. & 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.
249. Piersma, T. & 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.
250. Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M. & 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.

251. Totzke, U., Hübinger, A., Dittami, J. & 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.
252. Guglielmo, C. G. & 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.
253. Dark, J., Zucker, I. & Wade, G. N. (1983). Photoperiodic regulation of body mass, food intake, and reproduction in meadow voles. *American Journal of Physiology* 245: R334–R338.
254. 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.
255. Maier, H. A. & 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.
256. Lee, T. M. & Zucker, I. (1991). Suprachiasmatic nucleus and photic entrainment of circannual rhythms in ground squirrels. *Journal of Biological Rhythms* 6: 315–330.
257. Wollnik, F. & 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.
258. Haim, A., McDevitt, R. M. & 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.
259. Mrosovsky, N. (1990). Circannual cycles in golden-mantled ground squirrels: fall and spring cold pulses. *Journal of Comparative Physiology A* 167: 683–689.
260. 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.
261. Hiebert, S. M., Thomas, E. M., Lee, T. M., Pelz, K. M., Yellon, S. M. & 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.
262. Chevillard, L., Portet, R. & Cadot, M. (1963). Growth rate of rats born and reared at 5 and 30°C. *Federation Proceedings* 22: 699–702.
263. Concannon, P., Levac, K., Rawson, R., Tennant, B. & Bensadoun, A. (2001). Seasonal changes in serum leptin, food intake, and body weight in photoentrained woodchucks. *American Journal of Physiology* 281: R951-R959.
264. Shoemaker, M. B. & 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.
265. Kuhlmann, M. T., Clemen, G. & Schlatt, S. (2003). Molting in the Djungarian hamster (*Phodopus sungorus* Pallas): seasonal or continuous process? *Journal of Experimental Zoology* 295A: 160–171.
266. Peacock, W. L., Król, E., Moar, K. M., McLaren, J. S., Mercer, J. G. & 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.
267. Fuglei, E. & 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.
268. Körtner, G. & Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiology and Behavior* 58: 31–36.
269. Lincoln, G. A., Rhind, S. M., Pompolo, S. & 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.
270. Nieminen, P., Mustonen, A. M., Asikainen, J. & 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.
271. Fitzgerald, B. P. & McManus, C. J. (2000). Photoperiodic versus metabolic signals as determinants of seasonal anestrus in the mare. *Biology of Reproduction* 63: 335–340.
272. Kowalczyk, R., Jedrzejewska, B. & 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.
273. Génin, F., Schilling, A. & Claustrat, B. (2003). Melatonin and methimazole mimic short-day-induced fattening in gray mouse lemurs. *Physiology and Behavior* 79: 553–559.
274. Peltier, T. C., Barboza, P. S. & Blake, J. E. (2003). Seasonal hyperphagia does not reduce digestive efficiency in an Arctic grazer. *Physiological and Biochemical Zoology* 76: 471–483.
275. Cossins, A. R. & Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman and Hall.
276. Gordon, C. J. (1993). *Temperature Regulation in Laboratory Rodents*. New York: Cambridge University Press.
277. Roberts, J. C. (1996). Thermogenic responses to prolonged cold exposure: birds and mammals. In: Fregly, M. J. & Blatteis, C. M. (Eds.). *Handbook of Physiology, Section 4: Environmental Physiology.* New York: Oxford University Press.
278. Dawson, W. R. & Marsh, R. L. (1989). Metabolic acclimatization to cold and season in birds. In: Bech, C. & Reinertsen, R. E. (Eds.). *Physiology of Cold Adaptation in Birds*. New York: Plenum.

279. Hildwein, G. (1970). Capacités thermorégulatrices d'un mammifère insectivore primitif, le tenrec: leurs variations saisonnières. *Archives des Sciences Physiologiques* 24: 55–71.
280. Veghte, J. H. (1965). Thermal and metabolic responses of the gray jay to cold stress. *Physiological Zoology* 37: 316–328.
281. Hart, J. S. & Heroux, O. (1963). Seasonal acclimatization in wild rats (*Rattus norvegicus*). *Canadian Journal of Zoology* 41: 711–716.
282. Kronfeld-Schor, N., Haim, A., Dayan, T., Zisapel, N., Klingenspor, M. & Heldmaier, G. (2000). Seasonal thermogenic acclimation of diurnally and nocturnally active desert spiny mice. *Physiological and Biochemical Zoology* 73: 37–44.
283. Cooper, S. J. (2002). Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. *Physiological and Biochemical Zoology* 75: 386–395.
284. Jefimow, M., Wojciechowski, M. & 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.
285. Heroux, O., Hart, J. S. & 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.
286. 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.
287. Allard, M. & LeBlanc, J. (1988). Effects of cold acclimation, cold exposure, and palatability on postprandial thermogenesis in rats. *International Journal of Obesity* 12: 169–178.
288. Schmidek, W. R., Zachariassen, K. E. & 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.
289. Gwosdow, A. R. & Besch, E. L. (1985). Effect of thermal history on the rat's response to varying environmental temperature. *Journal of Applied Physiology* 59: 413–419.
290. Griggio, M. A. & 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.
291. Duchamp, C., Barré, H., Delage, D., Rouanet, J. L., Cohen-Adad, F. & Minaire, Y. (1989). Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. *American Journal of Physiology* 257: R744–R751.
292. Hohtola, E. & 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.
293. Barré, H., Cohen-Adad, F., Duchamp, C. & Rouanet, J. L. (1986). Multilocular adipocytes from muscovy ducklings differentiated in response to cold acclimation. *Journal of Physiology* 375: 27–38.
294. Marjoniemi, K. & 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.
295. Chevillard, L., Guntz, M. C., Petrovic, V. & 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.
296. Pohl, H. (1965). Temperature regulation and cold acclimation in the golden hamster. *Journal of Applied Physiology* 20: 405–410.
297. Oufara, S., Barré, H., Rouanet, J. L. & Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *American Journal of Physiology* 253: R39–R45.
298. Smith, B. K. & 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.
299. Pohl, H. & Hart, J. S. (1965). Thermoregulation and cold acclimation in a hibernator, *Citellus tridecemlineatus*. *Journal of Applied Physiology* 20: 398–404.
300. 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.
301. Saarela, S. & 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.
302. Haim, A. & Martinez, J. J. (1992). Seasonal acclimatization in the migratory hamster *Cricetulus migratorius*: the role of photoperiod. *Journal of Thermal Biology* 17: 347–351.
303. McDevitt, R. M. & 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.
304. Heldmaier, G., Steinlechner, S. & Rafael, J. (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *Journal of Comparative Physiology* 149: 1–9.
305. Heldmaier, G. & Jablonka, B. (1985). Seasonal differences in thermogenic adaptation evoked by daily injections of noradrenaline. *Journal of Thermal Biology* 10: 97–99.
306. Rafael, J. & Vsiansky, P. (1985). Photoperiodic control of the thermogenic capacity in brown adipose tissue of the Djungarian hamster. *Journal of Thermal Biology* 10: 167–170.
307. Hagelstein, K. A. & Folk, G. E. (1979). Effects of photoperiod, cold acclimation and melatonin on the white rat. *Comparative Biochemistry and Physiology C* 62: 225–229.
308. Blank, J. L. & Ruf, T. (1992). Effect of reproductive function on cold tolerance in deer mice. *American Journal of Physiology* 263: R820–R826.

309. Ellison, G. T. H., Skinner, J. D. & 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.
310. Shearer, D. S., Valdes, E. V., Qyarzun, S. E., Steinsky, L. & Atkinson, J. L. (1995). Food intake patterns in captive South African fur seals (*Arctocephalus pusillus pusillus*). *Proceedings of the Nutrition Advisory Group (American Zoo and Aquarium Assoc.)* 1: 213–217.
311. Rhind, S. M., McMillen, S. R., Pekas, J. C. & 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.
312. Storeheier, P. V., Sehested, J., Diernaes, L., Sundset, M. A. & 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.
313. De Castro, J. M. (1991). Seasonal rhythms of human nutrient intake and meal pattern. *Physiology and Behavior* 50: 243–248.
314. Moon, H. K. & Shim, J. E. (2004). Seasonal variation of food consumption patterns in Korea. *Asia Pacific Journal of Clinical Nutrition* 13: S167.
315. Brobeck, J. R. (1948). Food intake as a mechanism of temperature regulation. *Yale Journal of Biology and Medicine* 20: 545–549.
316. Witty, R. T. & Long, J. F. (1970). Effect of ambient temperature on gastric secretion and food intake in the rat. *American Journal of Physiology* 219: 1359–1363.
317. Yamauchi, C., Fujita, S., Obara, T. & 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.
318. 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.
319. Armitage, G., Harris, R. B. S., Hervey, G. R. & 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.
320. 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.
321. Gasnier, A. & 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.
322. Iampietro, P. F., Bass, D. E. & Buskirk, E. R. (1958). Caloric intake during prolonged cold exposure. *Metabolism* 7: 149–153.
323. Savory, C. J. (1986). Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowls. *Physiology and Behavior* 38: 353–357.
324. Manning, J. M. & Bronson, F. H. (1990). The effects of low temperature and food intake on ovulation in domestic mice. *Physiological Zoology* 63: 938–948.
325. Henderson, D., Fort, M. M., Rashotte, M. E. & Henderson, R. P. (1992). Ingestive behavior and body temperature of pigeons during long-term cold exposure. *Physiology and Behavior* 52: 455–469.
326. Agetsuma, N. (2000). Influence of temperature on energy intake and food selection by macaques. *International Journal of Primatology* 21: 103–111.
327. Kauffman, A. S., Cabrera, A. & Zucker, I. (2001). Energy intake and fur in summer- and winter-acclimated Siberian hamsters (*Phodopus sungorus*). *American Journal of Physiology* 281: R519–R527.
328. IUPS Thermal Commission (2001). Glossary of terms for thermal physiology. *Japanese Journal of Physiology* 51: 245–280.
329. Lyman, C. P. (1954). Activity, food consumption and hoarding in hibernators. *Journal of Mammalogy* 35: 545–552.
330. Dawe, A. R. & Spurrier, W. A. (1969). Hibernation induced in ground squirrels by blood transfusion. *Science* 163: 298–299.
331. Ruit, K. A., Bruce, D. S., Chiang, P. P., Oeltgen, P. R., Welborn, J. R., & 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.
332. Frerichs, K. U., Kennedy, C., Sokoloff, L. & 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.
333. Buck, M. J., Squire, T. L. & 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.
334. Twente, J. W. & Twente, J. A. (1965). Effects of core temperature upon the duration of hibernation of *Citellus lateralis*. *Journal of Applied Physiology* 20: 411–416.
335. Larkin, J. E., Franken, P. & Heller, H. C. (2002). Loss of circadian organization of sleep and wakefulness during hibernation. *American Journal of Physiology* 282: R1086–R1095.
336. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. & Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
337. Tattersall, G. J. & 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.
338. Kauffman, A. S., Paul, M. J. & Zucker, I. (2004). Increased heat loss affects hibernation in golden-mantled ground squirrels. *American Journal of Physiology* 287: R167–R173.

339. Millesi, E., Prossinger, H., Dittami, J. P. & Fieder, M. (2001). Hibernation effects on memory in European ground squirrels (*Spermophilus citellus*). *Journal of Biological Rhythms* 16: 264–271.
340. Hut, R. A., Barnes, B. M. & 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.
341. Barnes, B. M. (1989). Freeze avoidance in a mammal: body temperatures below 0°C in an Arctic hibernator. *Science* 244: 1593–1595.
342. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
343. Jansky, L., Haddad, G., Kahlerová, Z. & Nedoma, J. (1984). Effect of external factors on hibernation of golden hamsters. *Journal of Comparative Physiology B* 154: 427–433.
344. Oklejewicz, M., Daan, S. & Strijkstra, A. M. (2001). Temporal organisation of hibernation in wild-type and *tau* mutant Syrian hamsters. *Journal of Comparative Physiology B* 171: 431–439.
345. Lyman, C. P., O'Brien, R. C., Greene, G. C. & Papafrangos, E. D. (1981). Hibernation and longevity in the Turkish hamster *Mesocricetus brandti*. *Science* 212: 668–670.
346. Lyman, C. P., O'Brien, R. C., & Bossert, W. H. (1983). Differences in tendency to hibernate among groups of Turkish hamsters (*Mesocricetus brandti*). *Journal of Thermal Biology* 8: 255–257.
347. Jagiello, G. M., Fang, J. S., Sung, W. K., Hembree, W. C. & 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.
348. Waßmer, T. & Wollnik, F. (1997). Timing of torpor bouts during hibernation in European hamsters (*Cricetus cricetus* L.). *Journal of Comparative Physiology B* 167: 270–279.
349. 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.
350. 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.
351. Menaker, M. (1959). Endogenous rhythms of body temperature in hibernating bats. *Nature* 184: 1251–1252.
352. Menaker, M. (1964). Frequency of spontaneous arousal from hibernation in bats. *Nature* 203: 540–541.
353. Arnold, W., Heldmaier, G., Ortmann, S., Pohl, H., Ruf, T. & Steinlechner, S. (1991). Ambient temperatures in hibernacula and their energetic consequences for Alpine marmots (*Marmota marmota*). *Journal of Thermal Biology* 16: 223–226.
354. Fowler, P. A. & 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.
355. Saboureau, M., Vignault, M. P. & 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.
356. Körtner, G., Song, X. & 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.
357. Nelson, R. A., Wahner, H. W., Jones, J. D., Ellefson, R. D. & Zollman, P. E. (1973). Metabolism of bears before, during, and after winter sleep. *American Journal of Physiology* 224: 491–496.
358. Watts, P. D. (1989). Whole body thermal conductance of denning ursids. *Journal of Thermal Biology* 14: 67–70.
359. Stross, R. G. & Hill, J. C. (1965). Diapause induction in Daphnia requires two stimuli. *Science* 150: 1462–1464.
360. Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemistry and Physiology A* 77: 513–517.
361. Lair, K. P., Bradshaw, W. E. & Holzapfel, C. M. (1997). Evolutionary divergence of the genetic architecture underlying photoperiodism in the pitcher-plant mosquito, *Wyeomyia smithii*. *Genetics* 147: 1873–1883.
362. Wang, X., Ge, F., Xue, F. & You, L. (2004). Diapause induction and clock mechanism in the cabbage beetle, *Colaphellus bowringi* (Coleoptera: Chrysomelidae). *Journal of Insect Physiology* 50: 373–381.
363. Ogden, N. H., Lindsay, L. R., Beauchamp, G., Charron, D., Maarouf, A., O'Callaghan, C. J., Waltner-Toews, D. & 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.
364. Tachibana, S. I. & Numata, H. (2004). Effects of temperature and photoperiod on the termination of larval diapause in *Lucilia sericata* (Diptera: Calliphoridae). *Zoological Science* 21: 197–202.
365. Hudson, J. W. (1973). Torpidity in mammals. In: Whittow, G. C. (Ed.). *Comparative Physiology of Thermoregulation*. New York: Academic Press, vol. 3, pp. 97–165.
366. Wang, L. C. H. & Lee, T. F. (1996). Torpor and hibernation in mammals: metabolic, physiological, and biochemical adaptations. In: Fregly, M. J. & Blatteis, C. M. (Eds.). *Handbook of Physiology, Section 4: Environmental Physiology.* New York: Oxford University Press, vol. 1, pp. 507–532.
367. French, A. R. (1988). The patterns of mammalian hibernation. *American Scientist* 76: 568–575.
368. Lyman, C. P. (1982). Entering hibernation. In: Lyman, C. P., Willis, J. S., Malan, A. & Wang, L. C. H. (Eds.). *Hibernation and Torpor in Mammals and Birds*. New York: Academic Press, pp. 37–53.

369. Arnold, W. Ruf, T., Reimoser, S., Tataruch, F., Onderscheka, K. & Schober, F. (2004). Nocturnal hypometabolism as an overwintering strategy of red deer (*Cervus elaphus*). *American Journal of Physiology* 286: R174–R181.
370. Alila-Johansson, A., Eriksson, L., Soveri, T. & Laakso, M. L. (2003). Serum cortisol levels in goats exhibit seasonal but not daily rhythmicity. *Chronobiology International* 20: 65–79.
371. Guerin, M. V. & 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.
372. Souza, M. I. L., Bicudo, S. D., Uribe-Velásquez, L. F. & Ramos, A. A. (2002). Circadian and circannual rhythms of T3 and T4 secretions in Polwarth-Ideal rams. *Small Ruminant Research* 46: 1–5.
373. Thrun, L. A., Moenter, S. M., O'Callaghan, D., Woodfill, C. J. I. & Karsch, F. J. (1995). Circannual alterations in the circadian rhythm of melatonin secretion. *Journal of Biological Rhythms* 10: 42–54.
374. Guerin, M. V., Deed, J. R., Kennaway, D. J. & 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.
375. Alila-Johansson, A., Eriksson, L., Soveri, T. & 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.
376. Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K. & 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.
377. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J. & 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.
378. Bertolucci, C., Foà, A. & Van't Hof, T. J. (2002). Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. *Hormones and Behavior* 41: 414–419.
379. García, A., Landete-Castillejos, T., Zaragata, L., Garde, J. & Gallego, L. (2003). Seasonal changes in melatonin concentrations in female Iberian red deer (*Cervus elaphus hispanicus*). *Journal of Pineal Research* 34: 161–166.
380. Bünning, E. (1960). Circadian rhythms and the time measurement in photoperiodism. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 249–256.
381. Gorman, M. R., Borman, B. D. & Zucker, I. (2001). Mammalian photoperiodism. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 481–508.
382. Rust, C. C., Shackelford, R. M. & Meyer, R. K. (1965). Hormonal control of pelage cycles in the mink. *Journal of Mammalogy* 46: 549–565.
383. Rust, C. C. (1962). Temperature as a modifying factor in the spring pelage change of sort-tailed weasels. *Journal of Mammalogy* 43: 323–328.
384. Harris, G. D., Huppi, H. D., & Gessaman, J. A. (1985). The thermal conductance of winter and summer pelage of *Lepus californicus*. *Journal of Thermal Biology* 10: 79–81.
385. Walsberg, G. E. (1991). Thermal effects of seasonal coat change in three subarctic mammals. *Journal of Thermal Biology* 16: 291–296.
386. Al-Hilli, F. & Wright, E. A. (1988). The effects of environmental temperature on the hair coat of the mouse. *Journal of Thermal Biology* 13: 21–24.
387. Duncan, M. J. & 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.
388. Goldman, S. L., Dhandapani, K. & Goldman, B. D. (2000). Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 417–428.
389. Palchykova, S., Deboer, T. & Tobler, I. (2003). Seasonal aspects of sleep in the Djungarian hamster. *BMC Neuroscience* 4: art. 9.
390. Burkhardt, J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *Journal of Agricultural Science* 37: 64–68.
391. Lincoln, G. A., Andersson, H. & 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.
392. Oda, Y. (1969). The action of skeleton photoperiods on flowering in *Lemna perpusilla*. *Plant Cell Physiology* 10: 399–409.
393. Park, D. H., Somers, D. E., Kim, Y. S., Choy, Y. H., Lim, H. K., Soh, M. S., Kim, H. J., Kay, S. A. & Nam, H. G. (1999). Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285: 1579–1582.
394. Roden, L. C., Song, H. R., Jackson, S., Morris, K. & 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 U.S.A.* 99: 13313–13318.
395. Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A. & Coupland, G. (2004). Photoreceptor regulation of CONTANS protein in photoperiodic flowering. *Science* 303: 1003–1006.
396. Madhavan, K. & 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.
397. Razani, H., Hanyu, I. & Aida, K. (1987). Critical day-length and temperature level for photoperiodism in gonadal maturation of goldfish. *Experimental Biology* 47: 89–94.

398. Okuzawa, K., Furukawa, K., Aida, K. & 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.

399. Chang, C. F., Hu, H. J. & Sun, L. T. (1993). Effects of temperature and photoperiod on ovarian development in female ayu, *Plecoglossus altivelis*. *Journal of Thermal Biology* 18: 197–201.

400. King, V. M., Bentley, G. E. & 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.

401. Zivkovic, B. D., Underwood, H., Steele, C. T. & 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.

402. Bentley, G. E., Spar, B. D., MacDougall-Shackleton, S. A., Hahn, T. P. & Ball, G. F. (2000). Photoperiod regulation of the reproductive axis in male zebra finches, *Taeniopygia guttata*. *General and Comparative Endocrinology* 117: 449–455.

403. Puchalski, W. & Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.

404. Anchordoquy, H. C. & 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.

405. Larkin, J. E., Freeman, D. A. & 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.

406. Zhou, S., Cagampang, F. R. A., Stirland, J. A., Loudon, A. S. I. & 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.

407. Gaston, S. & Menaker, M. (1967). Photoperiodic control of hamster testis. *Science* 158: 925–928.

408. Pratt, B. L. & Goldman, B. D. (1986). Activity rhythms and photoperiodism of Syrian hamsters in a simulated burrow system. *Physiology and Behavior* 36: 83–89.

409. Maywood, E. S., Buttery, R. C., Vance, G. H. S., Herbert, J. & 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.

410. Heideman, P. D. & 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.

411. Shimomura, K., Nelson, D. E., Ihara, N. L. & Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.

412. Loudon, A. S. I., Ihara, N. & 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.

413. Jallageas, M., Mas, N. & Nouguier-Soulé, J. (1991). Control of annual endocrine rhythms in the edible dormouse: nonprimary effect of photoperiod. *Journal of Biological Rhythms* 6: 343–352.

414. Edmonds, K. E. & 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.

415. Edmonds, K. E., Riggs, L. & 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.

416. Sicard, B., Fuminier, F., Maurel, D. & 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.

417. Concannon, P. W., Parks, J. E., Roberts, P. J. & 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.

418. Concannon, P. W., Castracane, V. D., Rawson, R. E. & 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.

419. Holloway, J. C. & Geiser, F. (1996). Reproductive status and torpor of the marsupial *Sminthopsis crassicaudata*: effect of photoperiod. *Journal of Thermal Biology* 21: 373–380.

420. Génin, F. & 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.

421. Karsch, F. J., Robinson, J. E., Woodfill, C. J. & 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.

422. Ishikawa, A., Sakamoto, H., Katagiri, S. & 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.

423. Fowler, G. S., Wingfield, J. C., Boersma, P. D. & 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.
424. Roenneberg, T. & Aschoff, J. (1990). Annual rhythm of human reproduction: I. Biology, sociology, or both? *Journal of Biological Rhythms* 5: 195–216.
425. Randall, W. (1990). The solar wind and human birth rate: a possible relationship due to magnetic disturbances. *International Journal of Biometeorology* 34: 42–48.
426. Randall, W. (1995). The annual temporal pattern of human births in the U.S.A.. *Biological Rhythm Research* 26: 505–520.
427. Chandwani, K. D., Cech, I., Smolensky, M. H., Burau, K. & Hermida, R. C. (2004). Annual pattern of human conception in the state of Texas. *Chronobiology International* 21: 73–93.
428. Zucker, I., Lee, T. M. & Dark, J. (1991). The suprachiasmatic nucleus and annual rhythms of mammals. In: Klein, D. C., Moore, R. Y. & Reppert, S. M. (Eds.). *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press, pp. 246–259.
429. Zucker, I. (2001). Circannual rhythms: mammals. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 509–528.
430. Ruby, N. F. (2003). Hibernation: when good clocks go cold. *Journal of Biological Rhythms* 18: 275–286.
431. Lincoln, G. A., Andersson, H. & 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.
432. Gwinner, E. (1977). Photoperiodic synchronization of circannual rhythms in the European starling (*Sturnus vulgaris*). *Naturwissenschaften* 64: 44.
433. Gwinner, E. & Dittami, J. (1990). Endogenous reproductive rhythms in a tropical bird. *Science* 249: 906–908.
434. Piersma, T. (2002). When a year takes 18 months: evidence for a strong circannual clock in a shorebird. *Naturwissenschaften* 89: 278–279.
435. Lumineau, S. & 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.
436. Scheuerlein, A. & 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.
437. 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.
438. Richter, C. P. (1978). Evidence for existence of a yearly clock in surgically and self-blinded chipmunks. *Proceedings of the National Academy of Sciences U.S.A.* 75: 3517–3521.
439. Zucker, I., Boshes, M. & Dark, J. (1983). Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. *American Journal of Physiology* 244: R472–R480.
440. Anchordoquy, H. C. & 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.
441. Liu, H. K., Nestor, K. E., Long, D. W. & 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

CHAPTER OUTLINE

5.1 Environmental and Populational Rhythms
5.2 Behavioral Rhythms
5.3 Autonomic Rhythms

5.1 ENVIRONMENTAL AND POPULATIONAL RHYTHMS

You may be wondering why this chapter is titled "Daily and Circadian Rhythms." Daily rhythms are the same thing as circadian rhythms, aren't they? Yes and no. According to the *American Heritage Dictionary*, the adjective *daily* applies to something that happens or that is performed 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, one needs to look at their histories.

Daily derives from the Old English *dæglic* and dates back to the 12th century.[1] Halberg created the term *circadian* in the 1950s by combining the Latin terms *circa* (about) and *dies* (day).[2] Thus, *circadian* literally means "approximately daily." During the 1950s and most of the 1960s, biologists argued 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 he felt the need for a term that could emphasize the endogenous nature of the rhythms. He believed 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, Halberg recollected that he created the term *circadian* in 1950 or 1951, although he did not formally introduce it to the scientific community until 1959.[2] The new word became popular instantly. The U.S. National Library of Medicine's PubMed database lists 16 journal articles published in 1960 that include *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 1999.

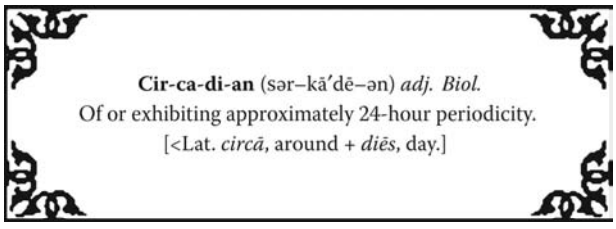

Cir-ca-di-an (sər–kā′dē–ən) *adj. Biol.*
Of or exhibiting approximately 24-hour periodicity.
[<Lat. *circā*, around + *diēs*, day.]

FIGURE 5.1 What does *circadian* mean? This is the definition of *circadian* given by a popular dictionary. (Source: *The American Heritage Dictionary of the English Language* (1994). New York: Houghton Mifflin.)

Halberg contributed to the confusion in using the new word by suggesting that *circadian* could apply to free-running rhythms *and* rhythms observed under a 24-hour environmental cycle.[2] Of course, an endogenous rhythm does not stop being endogenous when it is synchronized to an environmental cycle, but 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 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*"). *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* (or its French equivalent) 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

FIGURE 5.2 Sunrise over Pelican Island National Wildlife Refuge, Florida. The alternation of day and night constitutes a robust environmental daily rhythm. (Source: National Image Library, U.S. Fish and Wildlife Service.)

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 adjective *24-hour*, such as in "24-hour rhythm."[29–34]

This section examines *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 in single individuals). Daily and circadian rhythms in individual behavioral (voluntary) functions are examined in Section 5.2, and rhythms in autonomic (involuntary) functions are examined in Section 5.3.

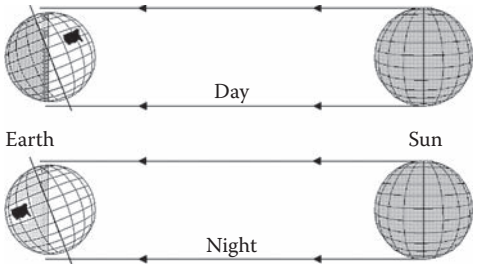


FIGURE 5.3 Day and night. The alternation of day and night results from the Earth's rotation around its axis. The side of the Earth exposed to the Sun experiences the day, while the opposite side experiences the night.

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, while the opposite side experiences the darkness of the night (Figure 5.3). 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*.

A day — in the sense of *nychthémeron* — corresponds to the interval of time required for a full rotation of the Earth. This interval depends on how a full rotation is defined, however. The time required for a full rotation with respect to the stars is called a *siderial 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 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 Section 4.1. For example, 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 always 24 hours.

The most conspicuous difference between day and night is the change in illumination. On a clear day, outdoors illuminance is in the order of 10^4 lux, while on a clear night, with a full moon, illuminance is in the order of 10^{-1} lux.[40] Although it is not as dramatic or as regular as the change in illumination, a daily variation in ambient

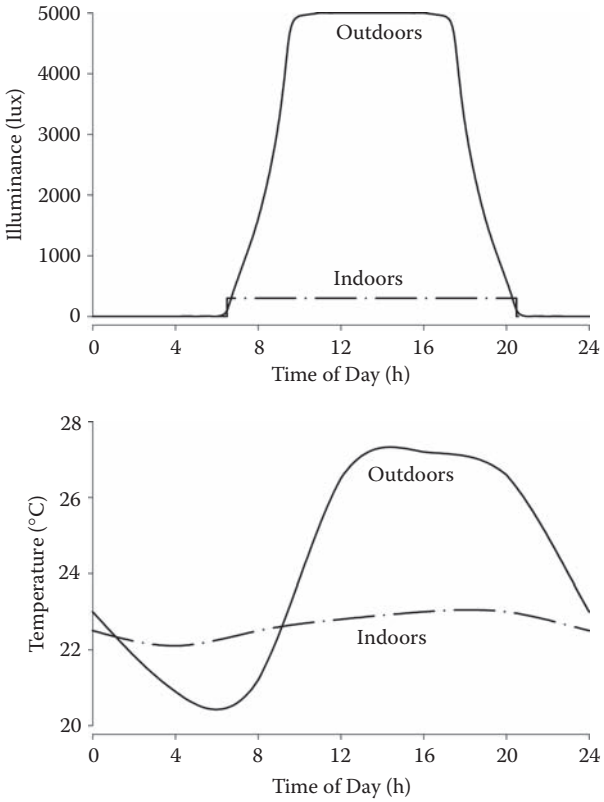


FIGURE 5.4 A typical mild summer day in Walterboro, South Carolina. The graphs show the daily variation of illuminance (luminosity) and ambient temperature during a mild summer day, as measured by the author. The indoor readings refer to an air-conditioned room with artificial lighting controlled by a timer.

temperature and relative humidity also occurs.[35–37] Figure 5.4 shows records of illuminance and air temperature for a typical summer day in the little town of Walterboro, South Carolina. On this 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 similar to the outdoor one. What is clearly different, however, is the much lower level attained after the transition (200 to 300 lux). The lower panel in Figure 5.4 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 demonstrated in Figure 5.5, which shows records of air temperature for the city of Miami. Day–night differences in temperature seem to be declining by approximately 1°C per 100 years in most of the world.[41]

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 example, aquatic organisms generally experience a much smaller daily variation in environmental temperature than 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 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 life events occur too infrequently to characterize a rhythmic process. Each person is born only once, may be married one or a few times, and occasionally has a major motor-vehicle accident or life-threatening health problem. However, if these events are considered

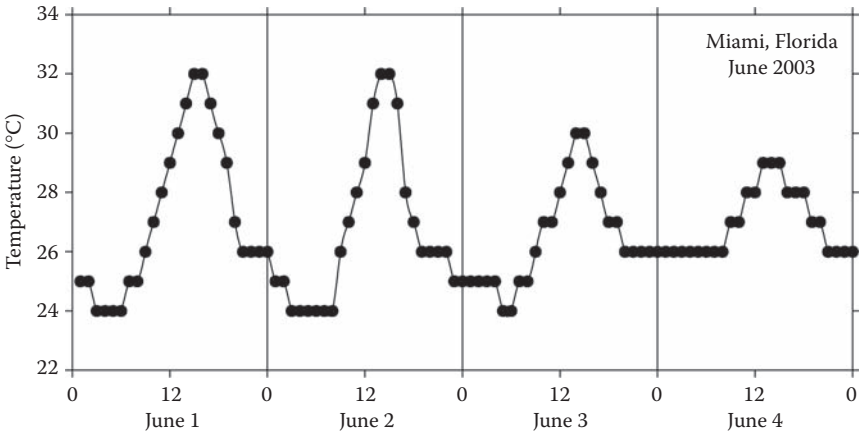


FIGURE 5.5 Late spring in Miami, Florida. The graph shows hourly measurements of ambient temperature in Miami during the first 4 days of June 2003. Although the temperature rose and fell each day, the range of oscillation was much greater during the first 2 days than during the last 2 days. (Source: Weather Channel, www.weatherchannel.com.)

at a populational level, they may exhibit daily rhythmicity. Take the example of *sexual activity*. The average American in his or her early adult years has sex two to three times a week.[42] 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. Figure 5.6 shows the results from a study of 78 young married couples who kept detailed records of their sexual activity for 3 months.[43] Although these couples had sex almost any time of the day, most episodes occurred around 11 P.M. (A similar but much smaller data set was examined in Chapter 3.)

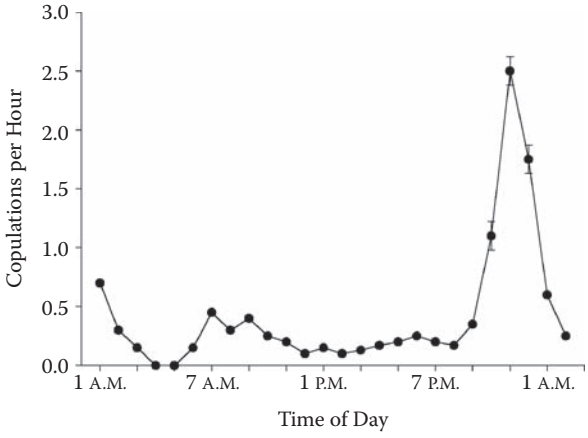


FIGURE 5.6 Time for sex. The sexual encounters of 78 young married couples were recorded for 3 consecutive months. This figure shows the mean copulation rates (± SE) across the day. A clear populational rhythm with a pronounced peak around 11 P.M. is present. (Source: Palmer, J. D., Udry, J. R. & Morris, N. M. (1982). Diurnal and weekly, but no lunar rhythm in human copulation. *Human Biology* 54: 111–121.)

Sexual activity is the route to reproduction, but it is unlikely that the time of day when intercourse occurs bears any relationship to the time of day when parturition (the act of giving birth) takes place 9 months later. The parturition time of day, however, does exhibit a populational rhythm (Figure 5.7). Several studies, based on thousands of births recorded over many years, have consistently found a daily rhythm of parturition that peaks around noon.[44–47] Figure 5.8 shows the hourly distribution of deliveries at the Rambam Medical Center in Israel. The top graph shows the distribution for 41,626 deliveries over an 8-year interval, while the lower graph shows the distribution for only 5289 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. 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.[44]

Road traffic is another populational phenomenon that exhibits daily rhythmicity. "Rush hour" traffic (Figure 5.9)

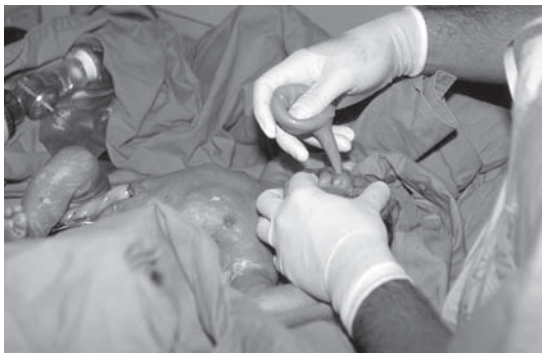


FIGURE 5.7 A newborn baby. There is a daily rhythm in the birth of human babies. (Source: © ArtToday, Tucson, AZ.)

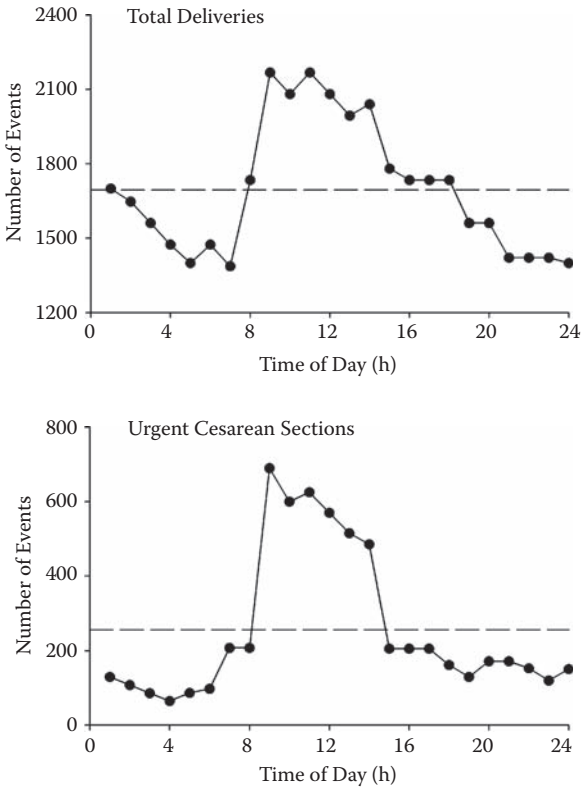


FIGURE 5.8 The time to be born. The graphs show the daily distributions of deliveries (total deliveries and urgent cesarean deliveries) over an 8-year interval in an Israeli maternity hospital (Rambam Medical Center). The distributions clearly deviate from a flat pattern corresponding to the 24-hour mean (dashed line). Most deliveries occur between 8 A.M. and 4 P.M. (Source: Goldstick, O., Weissman, A. & Drugan, A. (2003). The circadian rhythm of "urgent" operative deliveries. *Israel Medical Association Journal* 5: 564–566.)

is very common in large cities at the beginning and end of the work day. 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.).[48] Although this statistic is interesting in its own right, the authors of the study also computed the hourly distribution of accidents after correction for the daily variation in regular traffic volume. That is, more accidents are expected to occur when more vehicles are 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?

FIGURE 5.9 Stuck in traffic. Traffic congestion is a common occurrence in large cities. It follows a daily rhythm with greater congestion at the beginning and end of the business day. (Source: © ArtToday, Tucson, AZ.)

The answer is *yes*. Accidents are five times more likely to occur at 4 A.M. than at other times of the day.[48] Drivers are probably sleepier at 4 A.M. and more prone to fall asleep while driving or to lose concentration on the driving task. Poor visibility at night might also be a causative agent; however, in this case one would also expect to see a high frequency of accidents early in the night whereas high frequency in the early night is not actually observed. Poor driver visibility is also unlikely to cause an increase in animal–vehicle crashes that occurs shortly after sunset. As shown in Figure 5.10 — which is based on the records of 13,379 vehicle crashes with wild moose in Finland over a 9-year interval — many more crashes occur shortly after sunset than shortly before sunrise.[49] The temporal pattern in this case is probably not related to a human rhythm of attention, 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. probably results from a greater movement of moose at this time.

Several other populational rhythms have been described. For example, suicides tend to occur in the morning or early afternoon,[32,50,51] as do heart attacks.[52–54] Unexpected postoperative deaths are more common between 12 A.M. and 6 A.M.,[55] while perinatal mortality is higher in the evening.[45] Aggressive behavior of psychiatric patients occurs more commonly around 1 P.M.,[56] while accidents in children happen more commonly around 4 P.M.[57,58] Medical students and residents are more likely

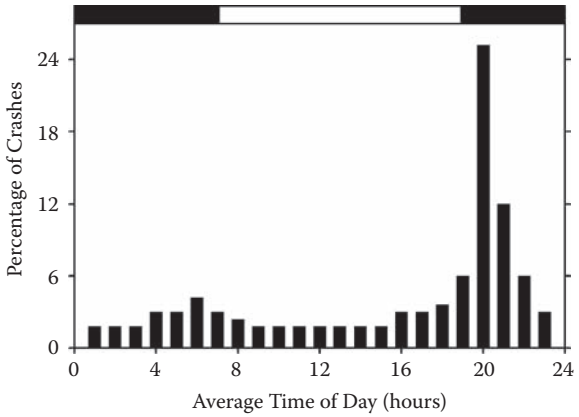


FIGURE 5.10 Road kill. The graph shows the daily distribution of vehicle crashes with moose (*Alces alces*) on Finnish highways from 1989 to 1997. The horizontal dark and white bars at the top indicate the duration of darkness and sunlight, respectively. Because the times of sunrise and sunset vary during 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. Most collisions occur shortly after sunset. (Source: Haikonen, H. & Summala, H. (2001). Deer-vehicle crashes: extensive peak at 1 hour after sunset. *American Journal of Preventive Medicine* 21: 209–213.)

to be exposed accidentally to pathogens during the day, when the hospital is busiest; however, if correction is made for the number of people on duty, accidental exposures occur much more commonly at night, with a peak at 11 P.M.[59]

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 freerun and, except under special circumstances, cannot be considered to be *circadian* rhythms. That is, populational rhythms can be considered only to be *daily* rhythms. Of course, these daily rhythms could be endogenously generated and only be modulated by environmental factors (rather than fully caused by environmental factors). 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 are reviewed in the next two sections.

5.2 BEHAVIORAL RHYTHMS

You may never have thought about it, but 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 a day, and the number of molecules in 1 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 accomplish things during the day and sleep at night — you are a *diurnal* organism. If mice live 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 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 with 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). In Table 5.1, you can see that the effect size of the daily rhythm of body temperature averages 0.82 (in a possible range of 0 to 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 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 is discussed in greater detail in Chapter 10.

5.2.1 Locomotor Activity

Animals commonly move from one place to another (Figure 5.11). 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,[60–76] reptiles,[77–81] fishes,[15,82–86] and birds.[87–102] In mammals, the majority of studies have been conducted on rodents, including laboratory rats,[9,30,103–135] domestic mice,[136–149] hamsters,[150–160] squirrels,[161–167] degus,[168–172] molerats,[173–176] voles,[17,177,178] guinea pigs,[179–181] Nile grass rats,[182–185] and other species.[19,186–197] Other mammals

TABLE 5.1
Comparison of the "Effect Size" of Circadian Rhythmicity with That of Other Biological and Physical Phenomena

Phenomenon	Effect Size	Comments
Twelve consecutive measurements of the height of 6 people (adults)	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.
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 rapidly during the first months of life, so that the effect of individual differences becomes almost negligible.
The performance of 12 students in 6 graded activities in a psychology class	0.40	Some students consistently perform better than others, but the different activities require different skills. Thus, the student effect size is modest.
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.
Twelve daily measurements of ambient temperature at 6 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 (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 (12 months per year) for 6 consecutive years (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 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.

Note: For consistency, all phenomena involve 12 treatment levels with 6 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).

whose daily activity patterns have been well described include rabbits,[198–200] cats,[201,202] dogs,[203,204] sheep,[205,206] horses,[207,208] tree shrews,[209,210] various marsupials,[211–214] and other species.[215–224] Many studies have been conducted on primates,[225–240] including humans.[241–250]

FIGURE 5.11 A tern on flight. Flying animals, like animals on land and water, exhibit a daily rhythm of activity. (Source: © ArtToday, Tucson, AZ.)

Figure 5.12 shows sample activity records for individuals of five species of small mammals. 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 (*Spermophilus 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. While 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 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 5.13, the actogram of locomotor activity of a mouse is exceptionally "clean," with all activity concentrated in a

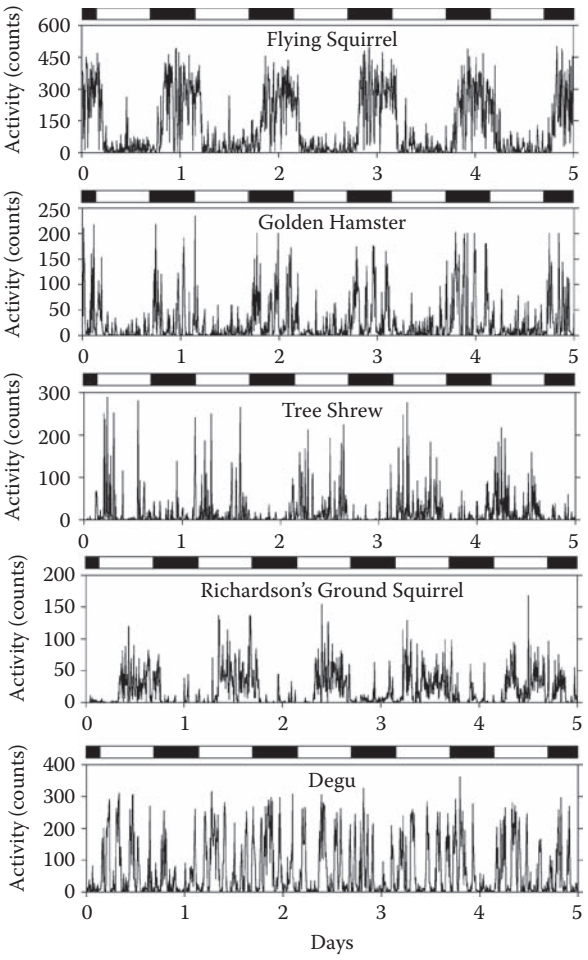


FIGURE 5.12 Rhythms of activity. This figure shows representative 5-day segments of the records of locomotor activity (measured by telemetry with 6-minute resolution) of individuals of 5 different species of small mammals. The horizontal white and dark bars at the top of each graph indicate the duration of the light and dark phases of the prevailing light–dark cycle, respectively. Most of the activity of the flying squirrel and golden hamster is restricted to the dark phase, while most of the activity of the tree shrew and Richardson's ground squirrel is restricted to the light phase. The activity rhythm of the degu is not as robust as that of the other four species. (Sources: 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; unpublished data from the archives of the Refinetti lab.)

short segment of the daily cycle, almost exclusively at night. Note, however, that the mouse does not run continuously through 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 mi) each night.[145] As mentioned in Chapter 3, the daily segment of activity is called α (*alpha*), while the segment of rest is called ρ (*rho*).

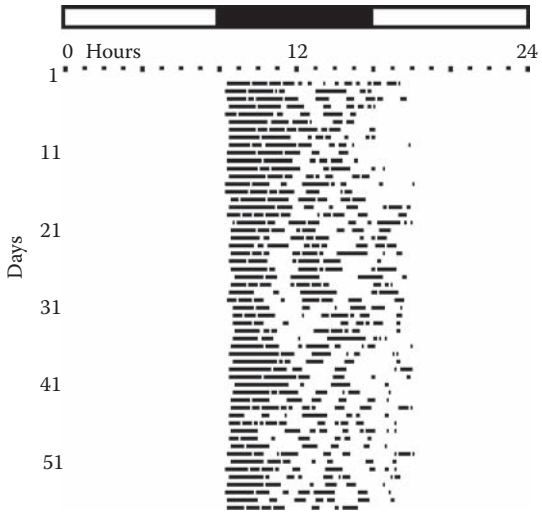


FIGURE 5.13 Clean data. This actogram of the running-wheel activity records of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle with 8 hours of darkness per day (LD 16:8) exemplifies the robustness of the rhythm of running-wheel activity. (If you are not familiar with actograms, refer to Figure 3.19 in Chapter 3.) (Source: 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. Figure 5.14 shows examples of activity patterns of individual Nile grass rats (*Arvicanthis niloticus*) averaged over 10 consecutive days. This species exhibits more inter-individual 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 more compact pattern of activity that is relatively constant throughout the light phase, while the records in Panels C and D show a pattern that is intermediary between the other two patterns. In one study, not all Nile grass rats were diurnal when housed with running wheels,[184] but the animals observed to be nocturnal may have been members of an idiosyncratic subgroup. In my laboratory, the activity rhythms of all 54 Nile grass rats housed with running wheels have been predominantly diurnal.[182,185,251] 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.[252] Observations in the wild in Kenya were consistent with the laboratory data in revealing a predominantly diurnal pattern of activity.[253] 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,[254] even though a predominance of activity usually occurs either during the day or during the night.

Interspecies comparisons of daily activity patterns are facilitated by the quantification of rhythm robustness, as previously described in Chapter 3. Figure 5.15 presents

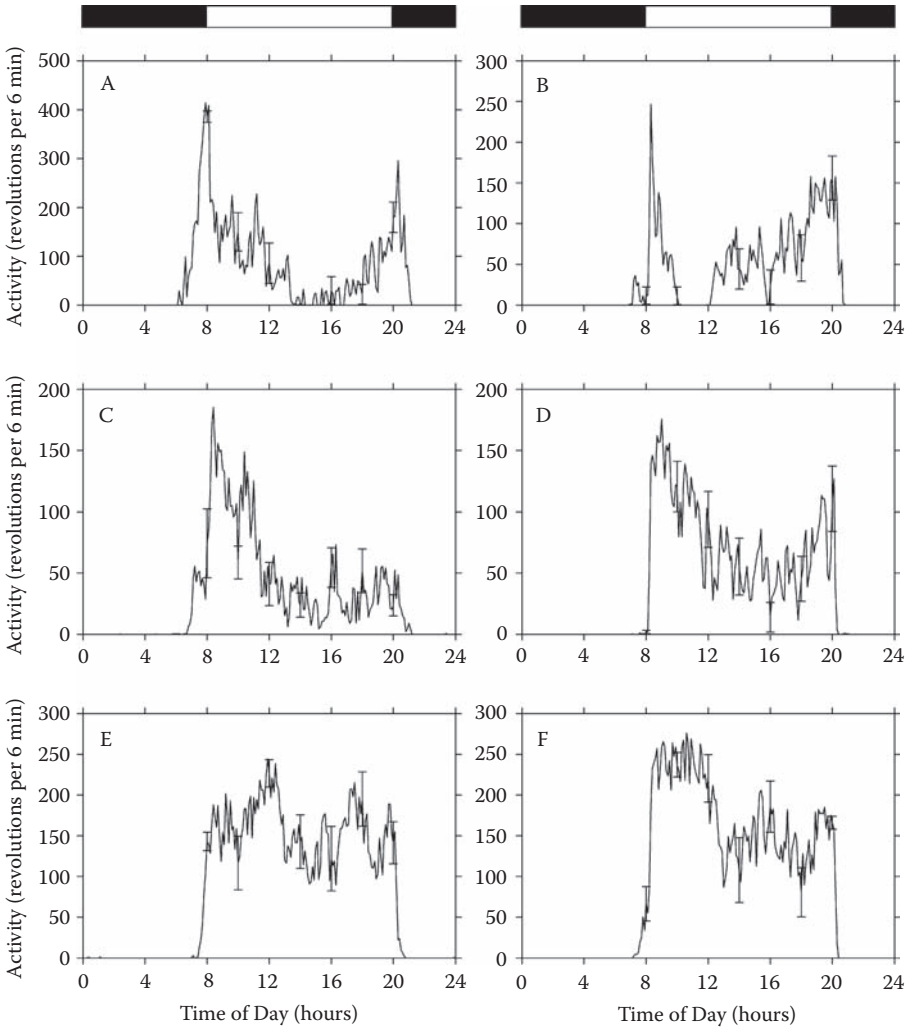


FIGURE 5.14 Intraspecies comparison of activity patterns. The graphs show the daily distributions of running-wheel activity of six Nile grass rats (*Arvicanthis niloticus*) maintained under a light–dark cycle with 12 hours of light per day (LD 12:12). In each graph, the data points represent the means of 10 consecutive days for each 6-minute interval. To avoid "cluttering" the graphs, the standard errors of the means are plotted only at 2-hour intervals. Although all six animals exhibit robust daily rhymicity, with activity predominantly during the light phase, the detailed pattern of activity varies from animal to animal. (Source: Archives of the Refinetti lab.)

the mean robustness of the running-wheel activity rhythms of six species of laboratory rodents. Laboratory rats (*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, *Mesocricetus auratus*) have the most robust rhythms of all laboratory rodents. Other aspects of the physiology and genetics of mice are much better known than those of golden hamsters; otherwise, 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 September 2004, restricted to articles containing the term *circadian*, yielded 12,647 articles of studies conducted on mice but only 985 articles of studies conducted on golden hamsters.

Daily rhythms of activity consistently have been found to persist in constant environmental conditions; this finding suggests that they are endogenously generated *circadian* rhythms. Persistence of rhythmicity in the absence of a light–dark cycle does not prove that the rhythm is endogenously generated, however. The rhythm might be controlled by any of millions of other geophysical variables that oscillate daily as the Earth rotates around its axis. These variables include ambient temperature, humidity, magnetic fields, and so on. How can you make sure that absolute constant conditions have been established in

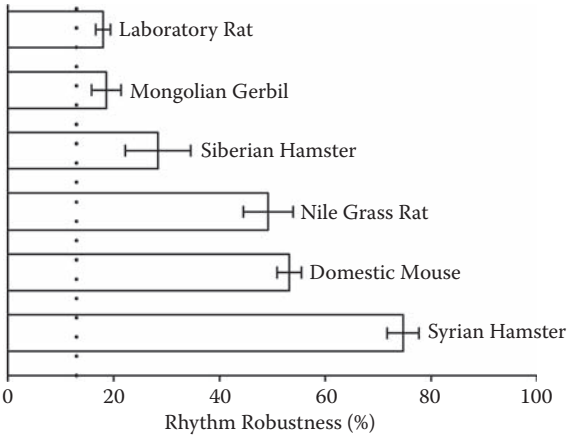


FIGURE 5.15 Interspecies comparison of rhythm robustness. The graph shows the mean robustness (± SE) of the running-wheel activity rhythm of several laboratory rodent species (based on samples of 5 to 15 animals per species). The dotted line indicates the lowest robustness for statistically significant rhythmicity. The existence of interspecies differences is evident. (Source: Archives of the Refinetti lab.)

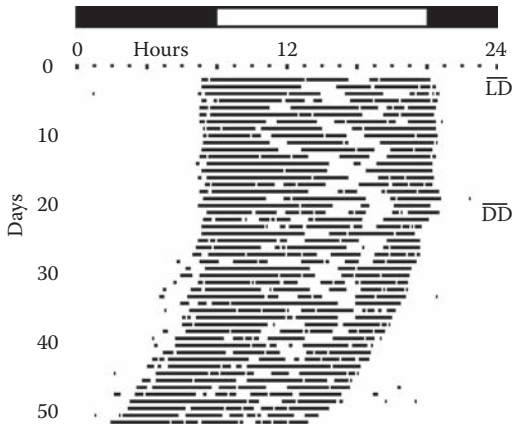


FIGURE 5.16 Circadian rhythms freerun under constant conditions. This actogram shows the rhythm of running-wheel activity of a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle (LD) for 20 days and in constant darkness (DD) for the following 30 days. Ambient temperature was 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. (Source: Archives of the Refinetti lab.)

a study? You cannot. Does this mean that you cannot truly tell whether there is real endogenous rhythmicity?

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. Therefore, they must be genuine *circadian rhythms*.

Figure 5.16 provides one example of a free-running circadian rhythm. 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 drift of activity onsets to the left. The freerun data strongly suggest that the rhythm is endogenously generated. However, you might argue that some unknown geophysical cycle with a 23.8-hour period could be causing the observed rhythmicity. Figure 5.17 presents data for three grass rats 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. Note 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 shown in Chapter 6, this finding 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,[60,63,64,67,71,73–76,255–258] reptiles,[78–81,259–262] fishes,[82,83,85,86] and birds.[88–92,94,95,97–102,263] In mammals, the majority of studies has been conducted on rodents, including laboratory rats,[23,104,110,111,114,115,117,124–126,128,129,133,264–268] domestic mice,[140–142,145,148,269–273] hamsters,[151,156,157,274–281] squirrels,[162,163,165,282,283] degus,[171,172,284] Nile grass rats,[182–185] chipmunks,[196,197,285] and other species.[173–178,180,195,286–288] Other mammals whose free-running activity patterns have been described include rabbits,[198,200] cats,[201,202] dogs,[203,204] tree shrews,[209,210] and other species.[214,215,220,223] Many studies have been conducted on primates,[225,232,235–237,239,289,290] including humans.[241,243,244,247,249,250,291–295]

When animals are free-running in constant darkness, without the influence of environmental light–dark cycles, it becomes relevant to compare the duration of the active phases (α) of different species. How long are the animals active in each circadian cycle? Figure 5.18 shows the records of representative individuals of three rodent species. The free-running periods of the three animals were

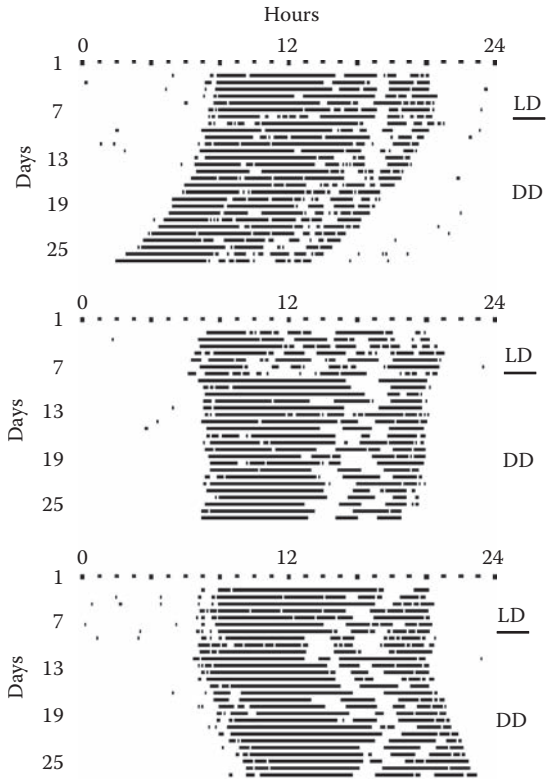


FIGURE 5.17 Excluding other environmental cues. These actograms show the records of running-wheel activity of three Nile grass rats kept under a light–dark cycle (LD) for 1 week and in constant darkness (DD) for 2 weeks in adjacent cages in the same laboratory. Even though all potential geophysical time 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 animal exhibited a period longer than 24.0 hours. Therefore, the rhythmicity must be endogenously generated. (Source: Archives of the Refinetti lab.)

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. Note that the hamster is active for only about 9 hours each day, while 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 — in Figure 5.18 the total durations of actual activity equal 6 hours for the hamster, 10.5 hours for the grass rat, and 9.5 hours for the mouse. Nevertheless, α can be used to compare interspecies activity rhythms. Table 5.2 shows mean values of α for 48 species. The species are listed in alphabetical order by Latin name. (Consult the *Organisms Used* appendix at the end of the book if you need help identifying the common names.) In Table 5.2, α ranges from as little as 8 hours in *Protophormia terraenovae* (an insect, the blow fly) to as much as 18 hours in *Tinca tinca* (the tench fish).

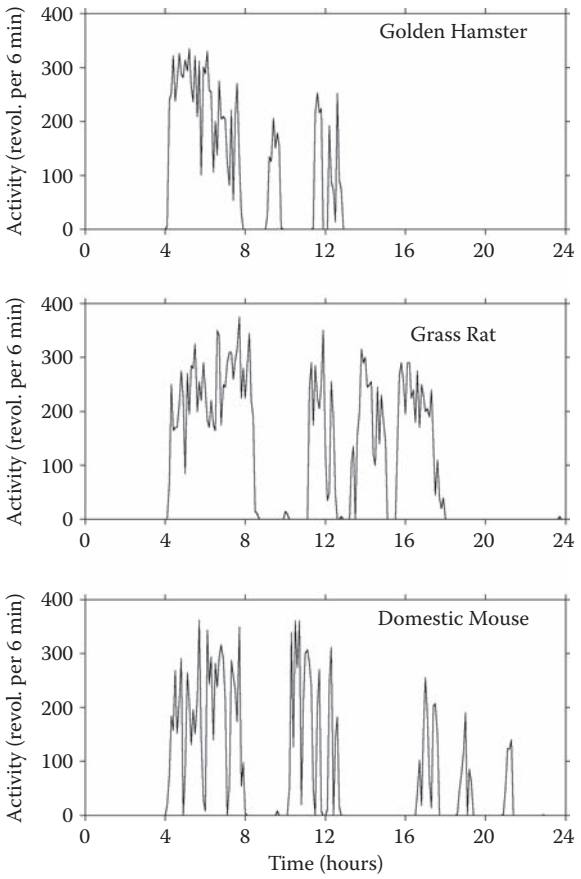


FIGURE 5.18 Activity patterns in constant darkness. The graphs show 1-day sections of the records of running-wheel activity of a golden hamster (*Mesocricetus auratus*), a Nile grass rat (*Arvicanthis niloticus*), and a domestic mouse (*Mus musculus*) maintained in constant darkness. The patterns of activity, which are representative of the patterns exhibited by other members of these species, clearly differ. The golden hamster concentrates its daily activity in an interval of about 8 hours, while the grass rat and the mouse distribute their activity over wider intervals. All three animals, especially the mouse, take several breaks during the active phase of their cycles. (Source: Archives of the Refinetti lab.)

The ability to track the times when events take place is an important consideration in the study of free-running rhythms. Under a light–dark cycle, one can easily say, for example, that an animal ate a meal 3 hours after lights-on. This observation is not possible, however, when the animal is kept 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 of 1 second.[296,297] This system of time measurement applies to all the civilized world 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 within an individual's life. Therefore, a separate system

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 (h)	Sources[c]
Aotus trivirgatus	15	326
Apis cerana	10	74
Apis mellifera	11	651
Arvicanthis ansorgei	14	286
Arvicanthis niloticus	17	736
Callithrix jacchus	13	737
Carassius auratus	13	82, 738
Cavia porcellus	15	180
Clethrionomys rutilus	10	177
Columba livia	14	91
Coturnix coturnix	11	317, 318
Danio rerio	10	83
Dasyuroides byrnei	12	214
Dimorphostylis asiatica	9	255
Dipodomys merriami	11	260
Drosophila melanogaster	17	71, 739
Eutamias sibiricus	12	285
Felis catus	17	202
Funambulus pennanti	14	740
Gekko gecko	16	80
Georychus capensis	12	174, 175
Glaucomys volans	10	162
Glyphiulus cavernicolus	12	76
Hipposideros speoris	11	215
Homo sapiens	16	241, 243, 244, 249, 250, 291, 292, 295, 329, 339, 390, 499, 622, 741–747
Iguana iguana	12	78, 79, 363
Leucophaea maderae	13	748
Lycosa tarentula	10	60
Macaca nemestrina	15	232
Mesocricetus auratus	9	156, 157, 749–755
Microcebus murinus	14	235–237
Microtus arvalis	9	178
Mus booduga	12	287, 288
Mus musculus	14	140, 142, 145, 269, 271, 273, 752, 754, 756–758
Octodon degus	13	169, 171, 284
Oryctolagus cuniculus	14	200
Passer domesticus	12	97, 98, 759
Phodopus sungorus	12	760
Podarcis sicula	10	362
Protophormia terraenovae	8	761
Rattus norvegicus	13	110, 115, 117, 129, 264, 266, 268, 309, 762–765
Saimiri sciureus	14	327
Sceloporus occidentalis	12	766
Spalacopus cyanus	12	195
Sturnus vulgaris	14	89, 90
Tamias striatus	9	196
Tinca tinca	18	85
Tupaia belangeri	12	209

[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 were obtained in constant dim light (< 1 lux). The values shown are the means for each species. Some species exhibit greater interindividual variability than others.

[b] For common English equivalents of scientific species names, refer to the *Organisms Used* appendix at the end of the book.

[c] Refer to *Literature Cited* section of this chapter.

FIGURE 5.19 Grazing antelopes. Animals in the wild, as well as in the laboratory, exhibit a daily rhythm of ingestive behavior. (Source: National Image Library, U.S. Fish and Wildlife Service.)

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 example, an organism has a circadian period of 26 hours, then each circadian hour lasts 65 solar minutes rather than 60 solar minutes. Because sunrise and sunset do not occur within an 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 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. Recently, some researchers proposed the use a single definition of circadian time for both nocturnal and diurnal organisms,[298] but so far no one has adopted the proposal.

5.2.2 Feeding and Excretion

Eating is an essential behavior for all animals (Figure 5.19). Drinking is also essential for many terrestrial animals, although some can extract water from wet food or even from dry food.[299] Daily and/or circadian rhythmicity of feeding has been documented in a large number of species, particularly laboratory rats,[9,25,103,104,106,113,115,117,118,122,130,300–311] other rodents,[178–180,269,271,273,312] birds,[89,93,95,98,99,102,313–319] and other animals.[10,198,199,320–325] The latter include nonhuman primates[225,239,240,289,290,326–328]

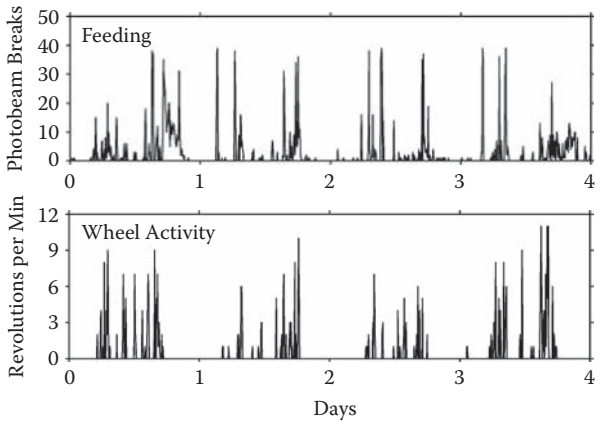


FIGURE 5.20 Feeding rhythm. The graphs show 4-day segments of the records of feeding and running-wheel activity of a laboratory rat (*Rattus norvegicus*) kept in constant darkness. Feeding was monitored by an infrared sensor attached to the food hopper. The records indicate that ingestive behavior is relatively spread out through subjective night with peaks of feeding activity occurring approximately at the same time as peaks of locomotor activity. (Source: Archives of the Refinetti lab.)

and humans.[329–331] Figure 5.20 provides an example of a free-running rhythm of feeding. A laboratory rat was maintained in constant darkness, and its feeding and wheel-running activities were monitored. Note that the feeding pattern differs significantly from that of humans. Rats nibble throughout the subjective night (when they are active) instead of eating 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.[332]

Later chapters will discuss the definition of circadian rhythmicity in detail. It must be emphasized here, however, 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 provides convincing evidence that *the organism* can generate circadian rhythms. However, when various rhythms are studied, there is no obvious reason to believe that *each rhythm* is independently generated. For example, if both locomotor activity and feeding exhibit circadian rhythmicity, the two rhythms may be independently generated. 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. Even though all free-running rhythms recorded in an organism can be called *circadian*, only empirical research enables researchers to determine whether each rhythm is autonomously generated or is "externally" driven by another rhythm. This issue is discussed in Chapter 9.

The goal of feeding is to extract energy from food. However, not everything in ingested food can be assimilated. The processing of assimilated products generates unusable metabolites, so that excretion of feces and urine is a necessity of life (Figure 5.21). Daily and/or circadian rhythms of excretion have been documented in various mammalian species,[25,179,198,199,203,240,304,333,334] including humans.[247,249,335–340] Figure 5.22 provides an example of a daily rhythm of urinary excretion. A tree shrew (*Tupaia belangeri*) was maintained under a light–dark cycle (LD 12:12) with food and water freely available, and its urinary output was monitored. Micturition occurs almost exclusively during the day in this diurnal species.

FIGURE 5.21 An old outhouse. Excretion (urination and defecation) exhibits daily rhythmicity. (Source: © ArtToday, Tucson, AZ.)

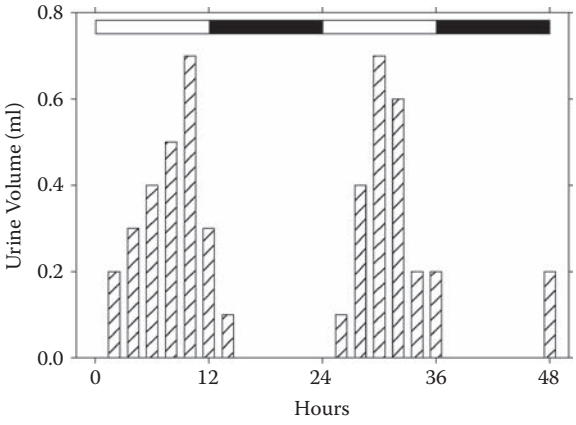


FIGURE 5.22 Daily rhythm of urinary excretion. The graph shows the pattern of urinary excretion of a tree shrew (*Tupaia belangeri*) maintained under a light–dark cycle with 12 hours of light per day (as indicated by the horizontal white and dark bars). The animal was housed in a metabolic chamber connected to a fraction collector. Practically all urinary excretion is restricted to the light phase of the light–dark cycle in this diurnal animal. (Source: Data collected by R. Refinetti at M. Menaker's laboratory at the University of Virginia in 1991.)

5.2.3 Sensation and Perception

Despite the importance of the senses as sources of information about the environment, circadian physiologists have spent little time studying daily and circadian variations in sensory processes. A few studies have shown that human subjects perceive a noxious stimulus (such as immersion of a hand in freezing water) as more painful at night than during the day.[341,342] Patients suffering from fibromyalgia (widespread muscle pain) report more pain in the early morning,[343] although the daily variation in pain in this case may 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 investigated occasionally,[344–348] but most animal studies have concentrated on pain. Figure 5.23 provides one example of a pain study. 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 figure shows data from two representative hamsters. Note that clear daily rhythmicity is present, with shorter latencies (and, therefore, greater pain sensation) during the dark phase of the light–dark cycle, which is when the animals are active. Observe also that the response latency falls before the lights go off, which suggests that the process

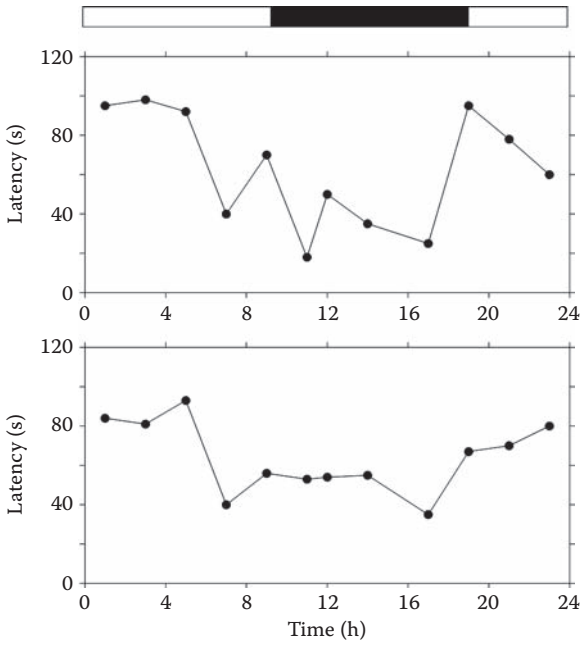


FIGURE 5.23 Daily rhythm of nociception. The graphs show the daily variation in pain sensitivity (nociception) of two golden hamsters maintained under a light–dark cycle (as indicated by the horizontal bars at the top). The data points correspond to measures of latency of the paw-licking response evoked by heating 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. (Source: Pickard, G. E. (1987). Circadian rhythm of nociception in the golden hamster. *Brain Research* 425: 395–400.)

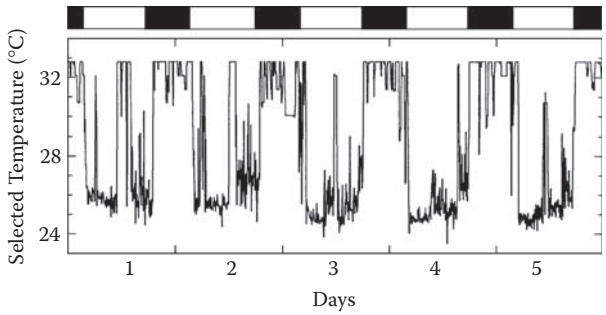


FIGURE 5.24 Daily rhythm of temperature selection. The graph shows the daily rhythm of temperature selection of a tree shrew (*Tupaia belangeri*) maintained under a light–dark cycle (as indicated by the horizontal bars at the top). The animal was housed in a long cage with a gradient of ambient temperature from 14 to 33°C. Tree shrews, which are diurnal, consistently select higher temperatures during the night than during the day. (Source: Refinetti, R. (1998). Body temperature and behavior of tree shrews and flying squirrels in a thermal gradient. *Physiology and Behavior* 63: 517–520.)

FIGURE 5.25 Basking lizard. Many animals select cooler or warmer environments at different times of the day in a consistent, rhythmic manner. (Source: © ArtToday, Tucson, AZ.)

has an endogenous component. Indeed, rhythmicity in response latency was observed in hamsters free-running in constant light.[349] The latencies were shorter during subjective day, however, rather than during subjective night, which is inconsistent with findings obtained with tests conducted under the light–dark cycle. Inconsistent results were also obtained in studies conducted under light–dark cycles in rats and mice: shorter latencies occurred at night,[350,351] during the day,[352] or with two daily troughs — one during the day and the other during the night.[353] A study in horses, which are diurnal animals, revealed shorter response latencies at the end of the day.[354] A study in mice identified daily oscillation in pain reactions in tests conducted during the spring but not in tests conducted during the winter.[355] Further studies are needed to clarify the inconsistencies.

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 crustaceans,[16,62,356,357] fishes,[15,358–361] reptiles,[80,362–374] rodents,[30,123,154,161,170,192,375–379] and other mammals.[24,380,381] Figure 5.24 shows the data for a tree shrew 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. The reason a diurnal animal selects higher temperatures during the night, instead of during the day, is not immediately obvious. Chapter 10 discusses this issue.

Reptiles, such as lizards (Figure 5.25), rely much more than mammals on the selection of adequate thermal environments to optimize bodily functions. Consequently, their temperature-selection behavior has been studied extensively.[80,362–374,382] Figure 5.26 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). Because lizards are cold-blooded animals, the circadian rhythm of ambient temperature

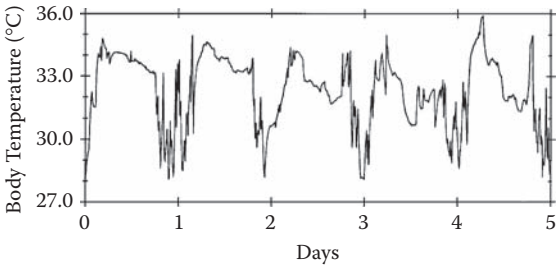


FIGURE 5.26 Circadian rhythm of temperature selection. The graph shows the body temperature rhythm of a small iguana lizard (*Iguana iguana*) maintained under constant light in a room at 22°C. The animal could change its body temperature by moving on and off a "hot rock" (i.e., an electric heater). The records show a clear oscillation in temperature with a period close to 24 hours. (Source: Data collected by R. Refinetti at M. Menaker's laboratory at the University of Virginia in 1991.)

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 5.27). These instruments allow individuals to time events without constantly thinking about them. However, humans and other animals are quite capable of estimating the passage of time in the range of seconds, minutes, or hours.[383] 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 5.28. Fourteen young men were each kept in bed from 7 A.M. to 11 P.M. Each was 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.[384] Greater overestimation in early morning also was seen in other studies in which subjects were asked to estimate the duration of 10 seconds[385] or of 1 hour.[386] However, in one study the highest estimate occurred early in the night instead,[249] and in another the peak times were not consistent across subjects, even though all subjects exhibited daily rhythmicity in their estimates.[387] 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.[388] It seems well established that time perception exhibits daily rhythmicity, but further studies are necessary to determine the precise waveform of the rhythm.

Time perception also depends on circadian rhythms in subjects kept in temporal isolation. Estimates of the duration of an hour were found to be longer when the

FIGURE 5.27 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. (Source: © ArtToday, Tucson, AZ.)

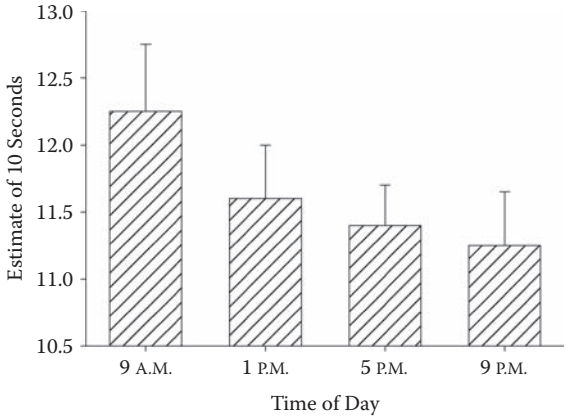


FIGURE 5.28 It's shorter than you think! When people are asked to estimate the duration of a 10-second interval, they always overestimate it. The extent of the overestimation is greater earlier in the day. The data shown in this graph are the means (± SE) for 14 young men under a constant routine protocol in the laboratory. (Source: Kuriyama, K., Uchiyama, M., Suziki, H., Tagaya, H., Ozaki, A., Aritake, S., Kamei, Y., Nishikawa, T. & Takahashi, K. (2003). Circadian fluctuation of time perception in healthy human subjects. *Neuroscience Research* 46: 23–31.)

subjects were free-running with circadian periods longer than 24 hours.[389,390] These data indicate that humans perceive an hour as 1/24 of a day, not necessarily as 60 minutes. As shown in Figure 5.29, this perceptual distortion holds true for estimates of an hour but not for estimates

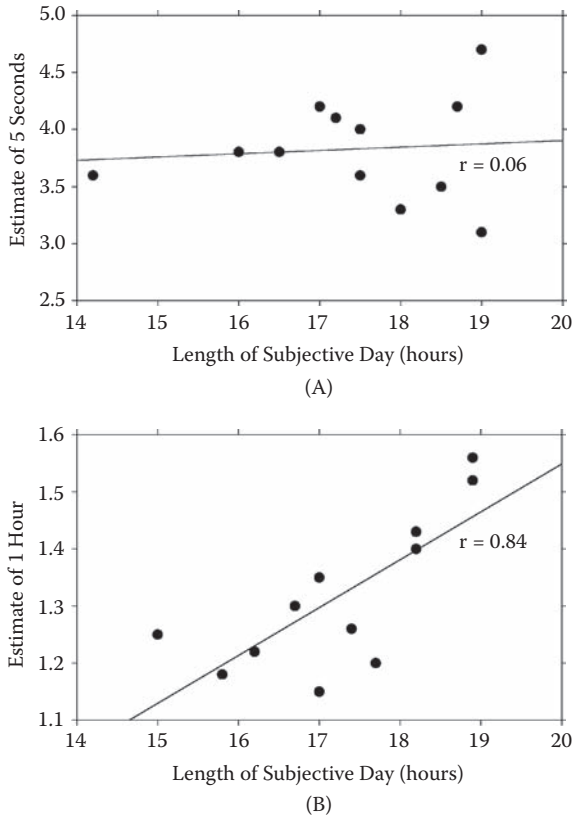


FIGURE 5.29 Time is relative. Circadian rhythms of human subjects maintained under constant conditions normally freerun with a period longer than 24.0 hours. When the circadian period is long, both subjective day and subjective night are longer. Subjects experiencing long subjective days estimate the 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. (Source: 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.)

of brief intervals of a few seconds. A large body of research on humans and other animals indicates that short intervals are timed by a mechanism distinct from the one that times circadian rhythms,[391] and a recent 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.[392] Estimates of short intervals are affected by body temperature, suggesting that the clock runs faster at higher temperatures,[384,385,393,394] while estimates of an hour or longer are temperature-compensated.[390]

Researchers who investigate short-interval timing usually are not circadian physiologists, and they have their own theories about how timing is achieved. A very popular theory, called *scalar timing*, assumes the existence of a central clock that beats with a constant frequency, an

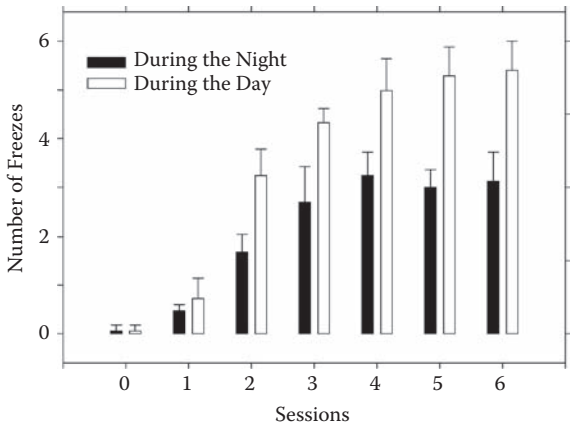


FIGURE 5.30 Daily rhythm of learning ability. Mice can be taught to fear an originally innocuous auditory tone if the tone is repeatedly associated with an electric shock to the foot (fear conditioning). A convenient fear response is "freezing" (immobilization). The graph shows that learning is more effective during the day than during the night. Each bar corresponds to the mean (± SE) of eight mice. (Source: Chaudhury, D. & Colwell, C. S. (2002). Circadian modulation of learning and memory in fear-conditioned mice. *Behavioural Brain Research* 133: 95–108.)

accumulator that counts the beats between two external events, and a memory register with which the accumulator counts are compared.[395] Some researchers, however, feel that a central clock in not necessary[396] and that timing may be "distributed," meaning that different brain circuits are involved in the timing of task- and modality-specific processes.[397] 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.[398]

5.2.4 Learning and Other Processes

Few investigators have studied daily or circadian oscillations in learning and memory.[399–405] Figure 5.30 shows the results from a well conducted study on mice subjected to fear conditioning, a procedure 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). They gradually learn to freeze in response to the tone (which initially did not induce freezing when used alone). Note that the mice in this study approached an asymptotic level of performance after about five training sessions. More important, animals taught during the day learned better than animals taught during the night. Both learning and memory-recall were better during the day (when mice are normally resting), and similar results were obtained in free-running animals.[401]

Results from another interesting study, this one conducted in the marine snail *Aplysia californica*, are shown

in Figure 5.31. This study focused on "long-term sensitization" of the siphon-withdrawal reflex. The animals naturally respond to a noxious stimulus (such as a shock) by retracting the siphon. If the stimulus is presented repeatedly, the duration of the retraction is gradually increased. In the study, 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 did this finding mean that CT 9 is the best time for learning or the time when memory recall is best? 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 an obvious loss of memory occurred 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 due to a circadian variation in learning, not in memory recall. The snails learned better at CT 9. Because these snails are diurnal, they learn better during their active phase, unlike mice, which — as described earlier — learn better during the rest phase of the circadian cycle.

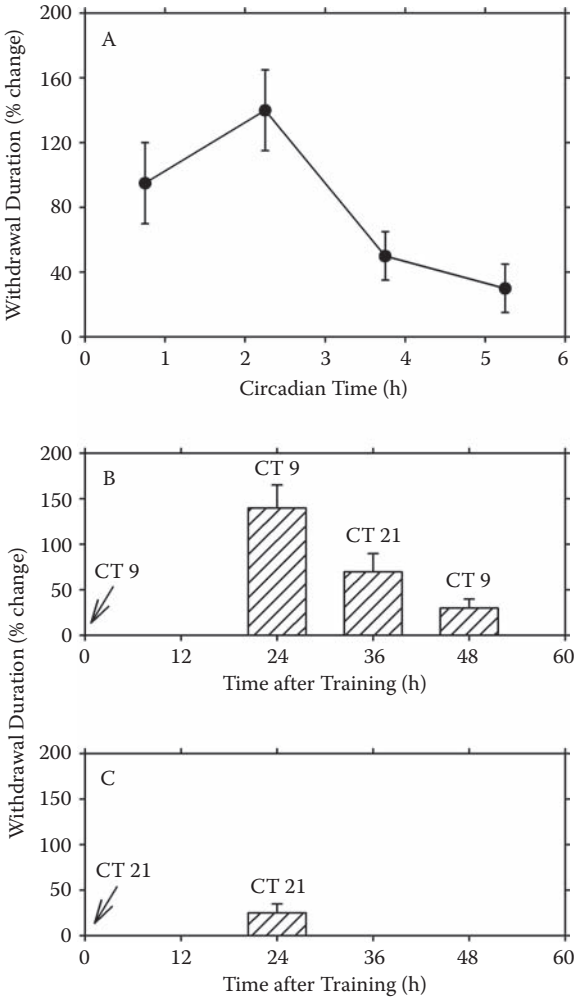


FIGURE 5.31 Circadian rhythm of learning ability. The marine snail *Aplysia californica* exhibits a simple form of learning called long-term sensitization, in which the duration of the siphon-withdrawal reflex in response to a noxious stimulus (electric shock) increases after repeated presentation of 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 CT 9 than at CT 21 when tests are conducted the same number of hours after training (B, C). (Source: Fernandez, R. I. et al. (2003). Circadian modulation of long-term sensitization. *Aplysia*. *Proceedings of the National Academy of Sciences U.S.A.* 100:14415–14420.)

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.[406,407] Tunas, seals, and penguins exhibit a daily rhythm of ocean diving.[408–412] Flies, rodents, and humans mate preferentially at particular times of the day.[43,183,413] Mother rats and mother rabbits also nurse their pups preferentially at particular times of the day.[199,414] Marmosets exhibit a daily rhythm of autogrooming.[11,227] Male hamsters temporarily placed together in the same cage engage in more sparring at night than during the day.[415] Mice kept in groups in a cold environment huddle together more often during the day than during the night.[137] 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,[416] as shown in Figure 5.32.

In humans, daily rhythmicity in alertness is particularly well documented. Alertness is lowest at wake time.[417–424] Other cognitive functions known to exhibit daily rhythmicity include mathematical-calculation performance and memory recall.[417,419,421,425] Regular changes in mood have also been described, although researchers disagree about the details. A common distinction in mood assessment is that between *positive affect* and *negative affect*.[426] Positive affect can vary from joviality/enthusiasm to drowsiness/dullness, while 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 observed in some studies[427,428] but not in others.[429,430]

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,

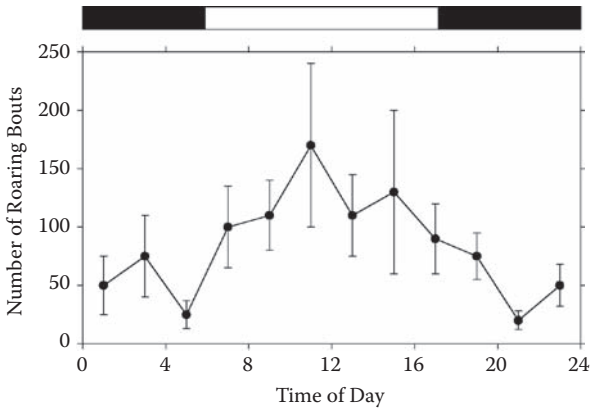


FIGURE 5.32 Roar like a lion. Red deer (*Cervus elaphus*) emit vocal sounds that resemble the roaring of lions. The graph shows the daily distribution of roaring bouts of a male red deer. Each data point is the median (± half ranges) of values collected over 2 weeks. The horizontal dark and white bars at the top indicate the duration of darkness and sunlight, respectively. Roaring bouts are clearly more common during daytime than nighttime. (Source: Pépin, D., Cargnelutti, B., Gonzalez, G., Joachim, J. & Reby, D. (2001). Diurnal and seasonal variations of roaring activity of farmed red deer stags. *Applied Animal Behaviour Science* 74: 233–239.)

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 example, 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. No single rhythm 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 important for the operation of the organism. This section discusses 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 most often as a synonym of *body core temperature*. However, because most heat-producing organs are located in the core of the body, and because ambient temperature is normally lower than core temperature, a temperature gradient exists in the body. The existence of this gradient means that different organs have different temperatures.[431–435] Regional differences in temperature are evident even at the body surface, as illustrated by the thermograph in Figure 5.33. In this black-and-white image,

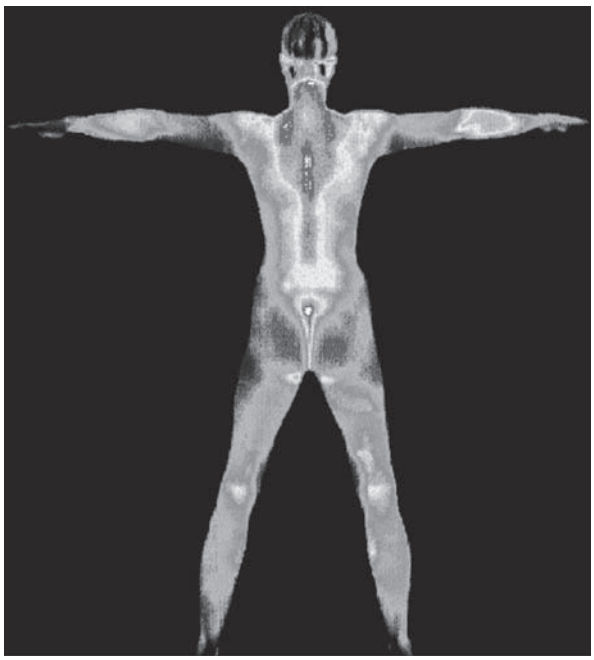


FIGURE 5.33 Show me your heat! This whole-body infrared thermography image of a human patient shows regional differences in skin temperature. Higher temperatures are indicated by lighter shades of grey. (Source: Image courtesy of Meditherm, Medical Monitoring Systems, Lake Oswego, OR.)

lighter shades of grey generally indicate higher temperatures, while darker shades indicate lower temperatures. 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 usually registers below 33°C, while core temperature measures about 37°C (98.6°F). Thus, what *thermal physiologists* usually call *body* temperature is a weighed average of *core* and *skin* temperatures. Although core temperature is an intangible concept (usually estimated by the measurement of rectal temperature), and although skin temperature varies considerably from one body site to another, a reasonable approximation of body temperature (T_b) is given by $0.8\ T_r + 0.2\ T_s$, where T_r is rectal temperature and T_s is mean skin temperature.[436] This strict definition of body temperature will *not* be used here, however. Instead, *body temperature* will be used to mean "temperature of a central part of the body." This use is justified by tradition in circadian physiology and by the fact that control of peripheral temperature is often sacrificed in favor of the maintenance of central temperature in homeothermic animals. Chapters 10 and 11 address this issue.

Daily rhythmicity of body temperature has been extensively documented in laboratory rats,[9,30,104–106,109,112,113,115,116,120,123–128,130,132–135,311,375,437–461] as well as in domestic mice,[138,139,143,144,146,147,149,462–468] golden ham-

sters,[150,152–155,157,277,469–471] and many other rodent species.[19,160,161,165,167–172,176,181,183,184,186,187,190–193,472–483] A large number of studies has also been conducted on primates,[229,230,233–235,237,240,326–328,484–488] including humans,[5,6,27,34,241,247–249,335–337,340,388,418,423,430,489–512] as well as in dogs,[203,513,514] cats,[201,202,515] goats,[516–519] sheep,[205,520–524] cattle,[10,525–528] other mammals,[209,211–213,216,218–220,223,529–541] and many species of birds.[88,91,92,94–96,100,314,435,542–549] 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 (*Iguana iguana*) — is capable of generating a small-amplitude rhythm of body temperature even when housed in a homogeneous thermal environment.[78,262]

Figure 5.34 shows an example of a daily rhythm of body temperature in birds. A hen (*Gallus domesticus*) was kept under a 24-hour light–dark cycle, and its body temperature was continuously recorded. Note 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 shown 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, making it less reproducible than the rhythm observed in roosters. The estrous cycles of other species also disrupt the daily rhythm of body temperature — but not as much as in species that ovulate every day. For this reason, most studies of daily and circadian rhythms of body temperature (or even of locomotor activity) are conducted on males.

Figure 5.35 provides examples of body temperature rhythms of male individuals of five mammalian species. To facilitate visualization of the daily patterns of oscillation, the data are plotted in 2-hour intervals (thus eliminating 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. Consistent 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. Note, however, that in these species body temperature consistently rises throughout the day and falls during the night.

A closer look at interspecies differences is possible in Figure 5.36, where the data are plotted with greater temporal resolution (6 minutes) and the Y-axis is scaled uniformly for the different species. Again, the rhythms of the nocturnal animals (laboratory rat and fat-tailed gerbil)

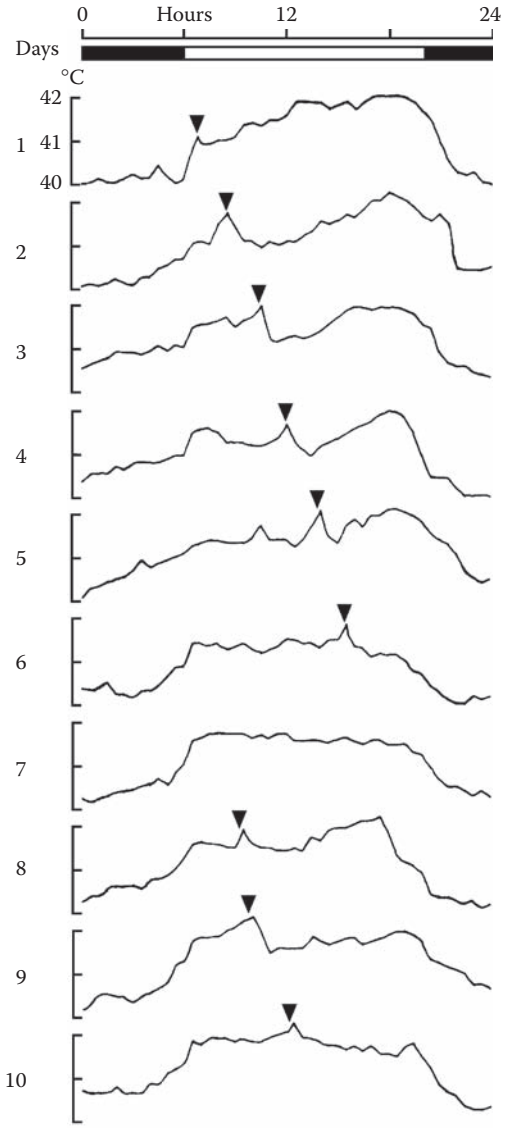


FIGURE 5.34 Daily rhythm of body temperature in a chicken. This actogram-like graph shows a 10-day segment of the body temperature records of a hen (*Gallus domesticus*) measured by telemetry. The bird was under a light–dark cycle, as indicated by the horizontal bars at the top. A daily rhythm of body 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. Note 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). (Source: Kadono, H., Besch, E. L. & Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.)

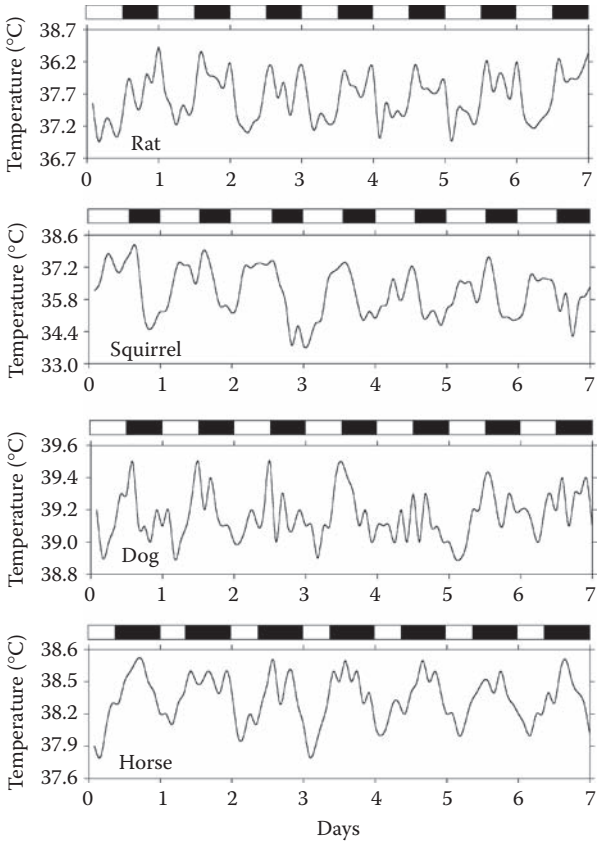


FIGURE 5.35 Daily rhythms of body temperature in mammals. The graphs show 7-day segments (with 2-hour resolution) of the body core temperature records of representative individuals of four mammalian species. The white and dark horizontal bars at the top of each graph indicate the duration of the light and dark phases of the prevailing light–dark cycles. Interspecies differences in waveform and acrophase are evident in the temperature records. (Source: Refinetti, R. & 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.)

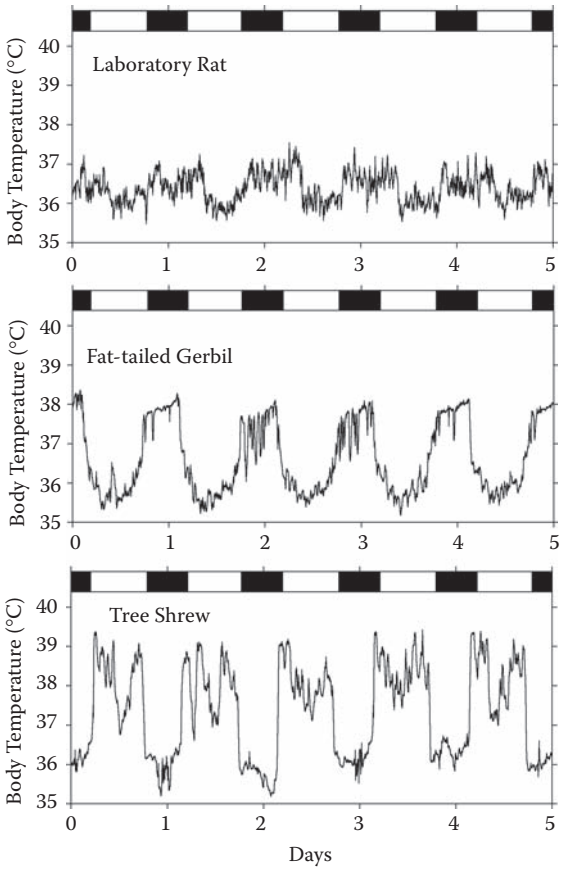


FIGURE 5.36 Interspecies differences in the amplitude of the temperature rhythm. The graphs show 5-day segments (with 6-minute resolution) of the body core temperature records of representative individuals of three mammalian species. The white and dark horizontal bars at the top indicate the duration 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. (Source: Refinetti, R. (1999). Amplitude of the daily rhythm of body temperature in eleven mammalian species. *Journal of Thermal Biology* 24: 477–481.)

are characterized by higher temperatures during the night, while 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 63 species of mammals and birds. As 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 inter-species 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, considerable inter-species variability exists. 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 5.37) exhibit the most robust rhythm

TABLE 5.3
Parameters of the Daily Rhythm of Body Temperature

Species[a]	Mean level (°C)	Range (°C)	Acrophase (hours)[b]	Source[c]
Acomys russatus	37.1	2.5	18	472
Aethomys namaquensis	36.8	3.9	18	186
Antechinus stuartii	36.5	3.1	19	212
Aotus trivirgatus	37.8	1.4	18	326
Apodemus flavicollis	37.4	1.7	17	187
Apodemus mystacinus	38.4	2.2	17	472
Arvicanthis niloticus	37.5	2.2	6	183
	37.6	1.7	5	184
Bettongia gaimardi	37.4	1.7	22	529
Bos Taurus	38.2	0.9	18	527
	38.3	1.4	14	525
	38.7	0.8	10	10
	39.2	0.9	12	526
	39.8	1.0	19	528
Canis familiaris	39.1	0.5	11	514
Capra hircus	38.5	0.7	13	519
	38.8	1.0	10	516
	38.9	0.7	14	518
	39.0	0.4	16	524
Cebus albifrons	37.2	2.7	6	484
Columba livia	40.0	2.1	6	542
	40.3	2.7	6	93
	41.5	1.5	6	91
Coturnix coturnix	41.0	1.3	15	545
Cynomys ludovicianus	37.4	2.5	7	216
Dasypus novemcinctus	35.5	2.6	18	223
Dasyurus viverrinus	36.5	3.6	18	213
Didelphis marsupialis	35.5	2.5	19	530
Didelphis virginiana	35.4	4.0	20	530
Equus caballus	38.3	1.0	14	532
Erinaceus europaeus	35.4	1.2	16	533
Felis catus	37.9	1.3	16	515
	38.3	1.0	15	201
	38.4	0.5	14	202
Gallus domesticus	40.2	1.1	12	549
	40.2	1.5	6	95
	40.7	2.2	8	314
	40.8	0.8	6	96
Glaucomys volans	37.1	2.1	17	161
Heterocephalus glaber	33.8	3.8	15	218
Homo sapiens	36.5	1.2	10	489
	36.7	1.1	10	496
	36.8	0.7	10	337
	36.8	0.8	8	501
	36.8	0.8	10	336
	36.8	1.2	10	499
	36.9	1.2	10	510
	36.9	1.0	9	493
	37.0	0.8	10	505
	37.0	1.0	9	507

(continued)

TABLE 5.3 (CONTINUED)
Parameters of the Daily Rhythm of Body Temperature

Species[a]	Mean level (°C)	Range (°C)	Acrophase (hours)[b]	Source[c]
	37.0	1.0	8	34
	37.0	1.0	10	767
	37.0	1.1	10	495
	37.0	1.2	9	768
	37.0	1.2	10	241
	37.0	1.3	10	497
	37.1	1.0	11	506
	37.6	1.6	10	500
Isoodon macrouros	36.2	2.5	16	530
Lasiorhinus latifrons	35.3	2.9	16	211
Macaca fuscata	37.0	2.4	9	229
Macaca mulatta	36.8	1.4	10	230
	37.2	1.0	10	557
	38.1	1.6	10	485
Macropus giganteus	34.6	2.8	19	534
Macropus rufus	36.3	1.7	17	534
Marmota monax	37.7	1.3	10	190
Meleagris gallopavo	40.2	1.2	12	88
Mephitis mephitis	36.4	1.3	12	469
Meriones unguiculatus	37.4	2.7	14	437
Mesocricetus auratus	36.0	2.9	14	437
	36.8	1.7	18	471
	36.9	2.5	17	154
	38.0	1.3	17	150
Microcebus murinus	36.3	2.8	18	235
	36.5	2.5	17	234
	36.6	2.5	18	237
	36.8	2.0	16	233
Mus musculus	36.3	2.2	16	139
	36.6	2.1	19	462
	36.6	2.2	18	144
	36.7	1.6	19	463
	36.8	1.7	18	138
	36.9	2.2	16	466
	37.0	2.0	17	438
Myrmecobius fasciatus	35.0	5.8	10	535
Nasua nasua	37.5	1.9	7	536
Octodon degus	36.5	2.0	5	172
	36.8	2.5	11	437
	37.0	1.7	5	168
	37.2	1.8	8	170
	37.3	2.0	6	169
Oryctolagus cuniculus	38.9	0.9	20	537
	39.8	0.8	12	469
Ovis aries	38.7	1.0	9	521
	39.3	0.3	14	524
	40.4	1.3	9	520
Pachyuromys duprasi	36.5	2.5	18	192
Procyon lotor	38.1	1.4	1	469
Rattus norvegicus	36.8	2.5	16	449
	36.9	1.8	18	115
	37.0	1.7	18	444

(continued)

TABLE 5.3 (CONTINUED)
Parameters of the Daily Rhythm of Body Temperature

Species[a]	Mean level (°C)	Range (°C)	Acrophase (hours)[b]	Source[c]
	37.0	1.8	18	124
	37.0	1.9	19	113
	37.0	2.1	18	107
	37.1	1.8	18	458
	37.2	1.5	17	440
	37.2	1.5	17	112
	37.3	1.0	18	30
	37.3	1.4	18	555
	37.3	2.1	16	437
	37.4	1.2	18	553
	37.4	1.3	18	453
	37.4	1.4	18	106
	37.4	1.4	18	127
	37.5	1.3	18	438
	37.5	1.4	18	116
	37.5	1.4	18	462
	37.5	1.5	18	461
	37.5	2.0	18	439
	37.6	1.1	18	104
	37.6	1.2	16	441
	37.6	1.7	19	23
	37.7	1.3	17	109
Saimiri sciureus	37.5	2.0	8	486
	37.5	2.7	6	487
	37.9	2.0	7	289
Sarcophilus harrisii	35.7	4.2	18	213
Sminthopsis macroura	36.2	5.5	18	769
Spalax ehrenbergi	36.4	1.5	5	176
Spermophilus beecheyi	36.4	2.4	5	481
Spermophilus lateralis	36.5	4.0	6	165
Spermophilus richardsonii	36.2	3.3	10	437
Spermophilus tridecemlineatus	36.4	5.0	7	482
	36.7	4.2	8	437
Struthio camelus	39.1	1.8	9	435
Suncus murinus	35.0	6.0	14	219
Sus scrofa	39.0	1.4	14	220
	39.6	0.5	9	538
Thallomys nigricauda	36.8	2.1	18	483
Thallomys paedulcus	36.6	2.9	18	186
Trichosurus vulpecula	37.4	2.9	16	530
Tupaia belangeri	37.4	4.2	6	161
	38.0	5.0	5	209
Vombatus ursinus	34.7	1.4	18	541

[a] For common English equivalents of scientific species names, refer to the *Organisms Used* appendix 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.
[c] Refer to *Literature Cited* section of this chapter.

of body temperature of all species tested so far,[525] 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. In contrast, one can obtain a realistic impression of the reproducibility of determinations *within* a species by inspecting Figure 5.38, which shows the body temperature records of 10 individual golden hamsters

FIGURE 5.37 A dairy cow. Domestic cattle (*Bos taurus*) exhibit the most robust daily rhythm of body temperature of all animal species studied so far. (Source: Photograph by Keith Weller, Agricultural Research Service, U.S. Department of Agriculture.)

maintained under a 24-hour light–dark cycle (LD 14:10). Although some obvious intraspecies differences are present, 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 similarity allows the creation of *educed rhythms* — that is, 1-day patterns that reasonably describe the rhythmicity of most members of a species. Figure 5.39 shows an educed rhythm of human body temperature. The data were obtained from eight healthy men living a normal sedentary life in a hospital ward and who had access to three meals a day, at 8:15 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 A.M. each morning, your own body temperature rhythm should resemble the one in the figure (see Exercise 1.3 in Chapter 1). If you wake up much earlier or much later, your rhythm should still 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,[88,91,92,94–96,100,314,544,545,547,550,551] rodents,[104,115,124–126,128,133,157,171,172,176,183,274,276,277,281,444,460,464,482,552–556] primates,[235,237,289,326–328,485,488,557] including humans,[241,247,249,291–295,499,507,558,559] and other mammals.[201,202,209,220,223,515,520,532,560–562] Figure 5.40 shows an example of a thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) kept under constant illumination (LL) in an environmental chamber at a constant temperature of 24°C. Its body temperature was monitored by telemetry. Note that body temperature oscillates regularly with a circadian period shorter than 24 hours (i.e., 23.3 hours).

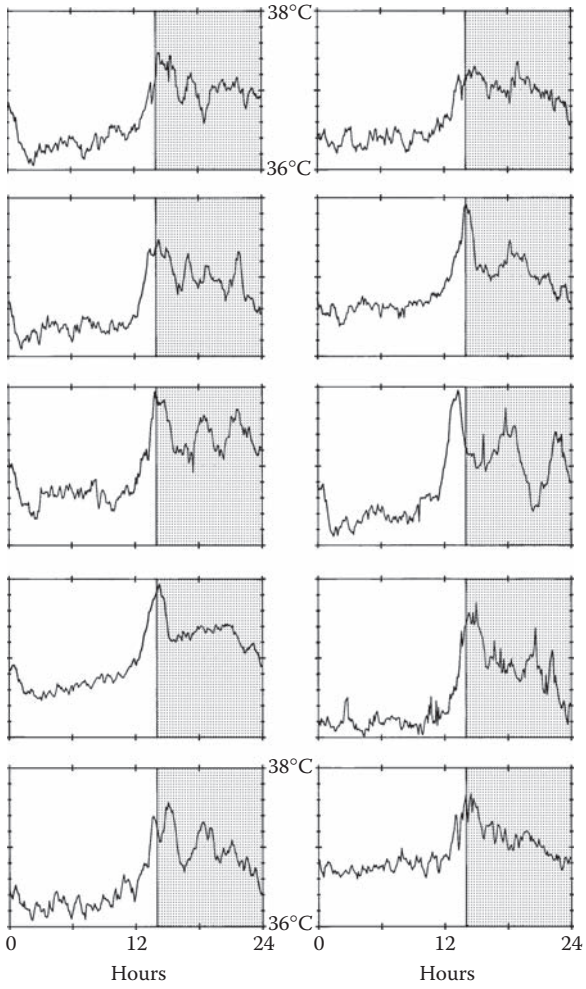


FIGURE 5.38 Intraspecies consistency of the temperature rhythm. The graphs show 24-hour segments (with 6-minute resolution) of the body core temperature records of ten individual golden hamsters (*Mesocricetus auratus*) maintained in an environment kept at 24°C under a light–dark cycle with 14 hours of light and 10 hours of darkness per day. Although individual differences can be seen, the rhythmic parameters are similar in all ten animals. (Source: Archives of the Refinetti lab.)

5.3.2 Cardiovascular Function

Daily rhythmicity in cardiovascular function (heart rate and blood pressure) has been studied most often in rodents,[30,112,118,121,122,131,133,135,139,147,181,191,452,563–571] but also in various species of birds and mammals,[96,219,484,523,572–575] including humans.[27,248,249,337,388,501,506,576–587] Although few studies have differentiated daily rhythms from circadian rhythms, free-running rhythms have occasionally been documented.[133,249,281]

Chapter 4 showed 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 5.41. Heart rate measurements

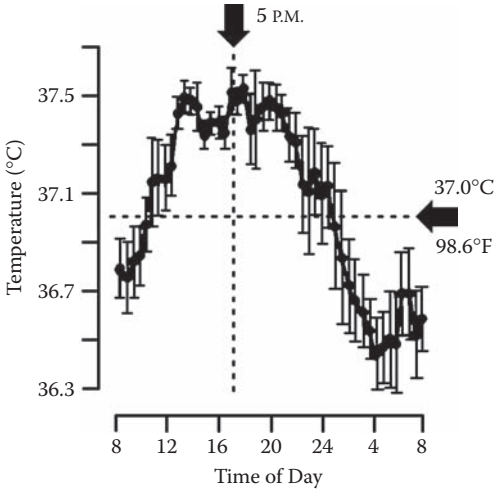


FIGURE 5.39 Human body temperature rhythm. The graph shows the typical rhythm of core body temperature of humans (*Homo sapiens*) living a normal, sedentary life with three meals per day (breakfast at 8:15 A.M.) and 8 hours of sleep at night. The data points correspond to the mean (± SE) temperature of eight 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. (Source: Scales, W. E., Vander, A. J., Brown, M. B. & Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.)

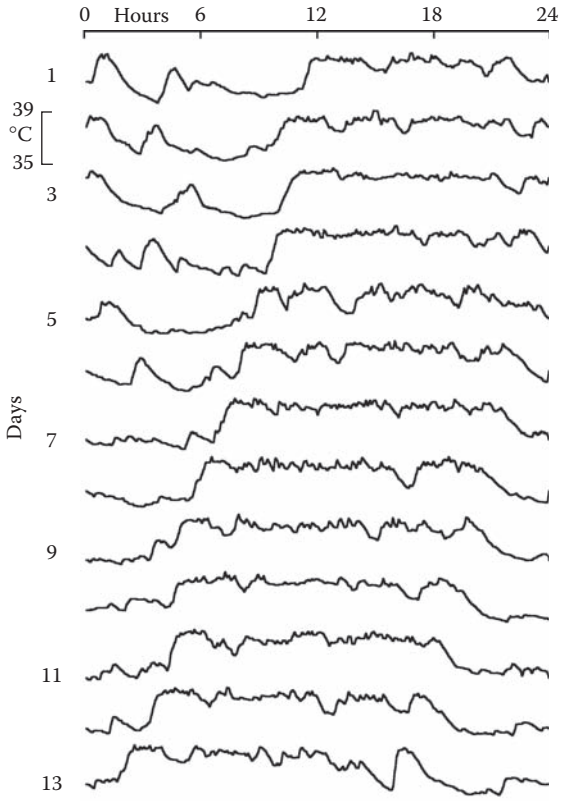


FIGURE 5.40 Circadian rhythm of body temperature. This actogram-like graph shows a 13-day segment of the body temperature records of a thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) maintained in constant light. Temperature was recorded by telemetry with 6-minute resolution. A free-running rhythm with a period of 23.3 hours can be easily identified. (Source: Refinetti, R. (1996). The body temperature rhythm of the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *Physiological Zoology* 69: 270–275.)

were taken from a mongrel dog in hourly intervals for several days. Note that heart rate increases each day from about 55 beats per minute to about 80 beats per minute.

Blood pressure is the pressure in the blood that flows through arteries and veins. In medical terms, however, blood pressure usually refers to *arterial* blood pressure, which is essentially the result of contractions of the heart. As shown in Figure 5.42, 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 5.43 shows mean values of systolic and diastolic arterial pressure of a group of 11 laboratory rats. Note that both measures are higher at night and lower during the day.

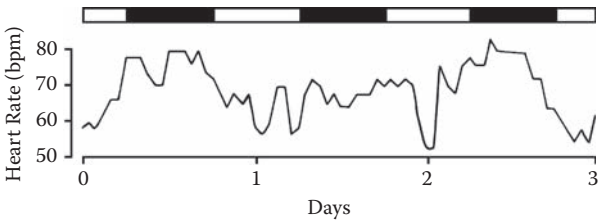


FIGURE 5.41 Daily rhythm of heart rate. The graph shows a 3-day segment of the heart rate records of a mongrel dog (*Canis familiaris*). Measurements were made by telemetry at hourly intervals. The duration of the prevailing light–dark cycle is indicated by the horizontal light and dark bars. A clear daily rhythm, with high values during the night, can be observed. (Source: Ashkar, E. (1979). Twenty-four-hour pattern of circulation by radiotelemetry in the unrestrained dog. *American Journal of Physiology* 236: R231–R236.)

5.3.3 Melatonin and Cortisol Secretion

As mentioned in Chapter 4, *melatonin* is a hormone synthesized mainly in the *pineal gland* — but also in the eyes — and 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

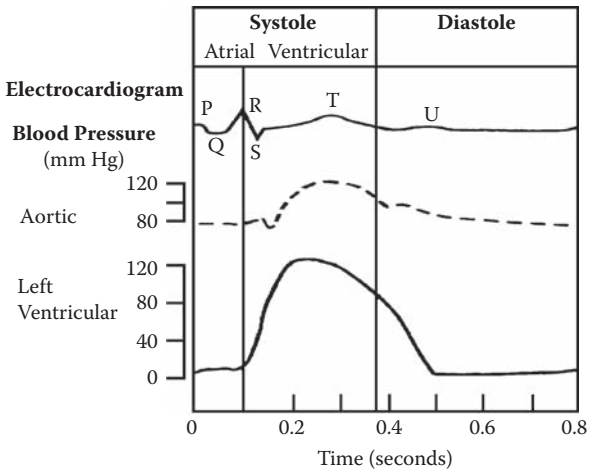


FIGURE 5.42 Systolic and diastolic blood pressure. Systolic blood pressure is measured during contraction of the left ventricle of the heart (systole). Diastolic blood pressure is measured during relaxation of the ventricle (diastole). The letters P, Q, R, S, T, and U designate the deflections of the electrocardiogram. (Source: Adapted from Ganong, W. F. (2001). *Review of Medical Physiology*, 20th Edition. New York: Lange Medical.)

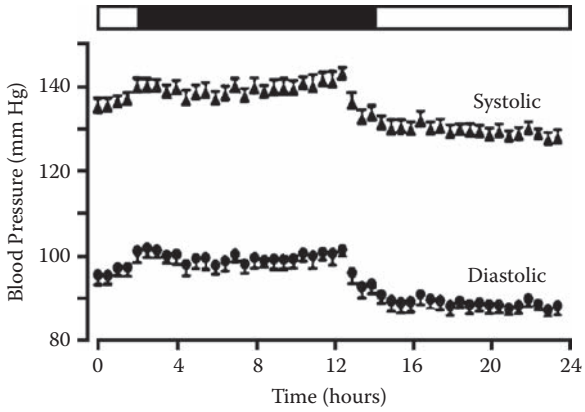


FIGURE 5.43 Daily rhythm of blood pressure. The graph shows the daily variation in systolic and diastolic blood pressure of laboratory rats (normotensive Wistar-Kyoto strain) measured at 30-minute intervals through chronically implanted catheters. Each data point corresponds to the mean (± SE) of 11 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. (Source: Henry, R. et al. (1990). Diurnal cardiovascular patterns in spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* 16: 422–428.)

systems in photoperiodic organisms. As 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 pacemaker in many nonmammalian vertebrates. Daily rhythmicity of melatonin secretion (as measured by blood melatonin concentration)

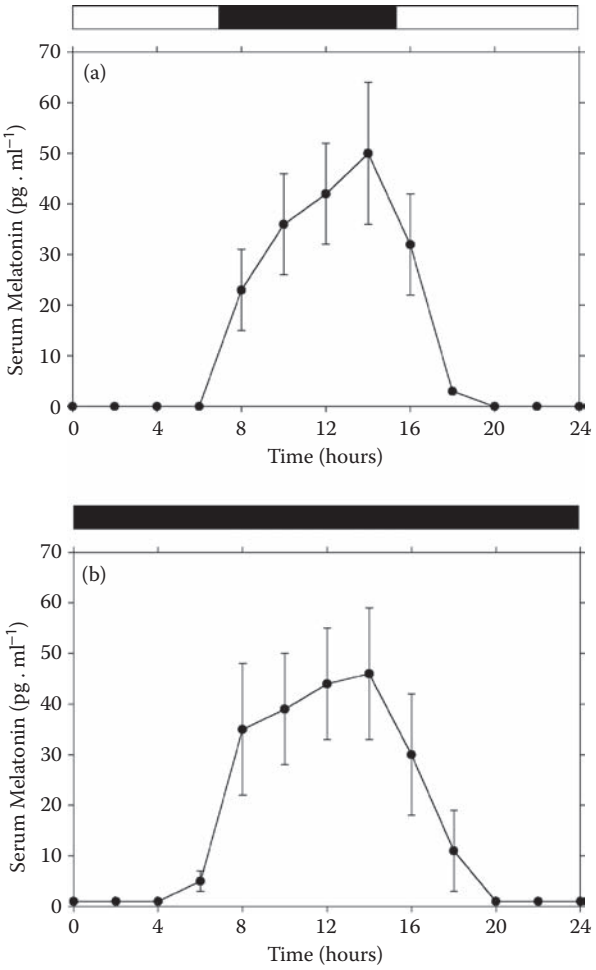


FIGURE 5.44 Daily and circadian rhythms of melatonin secretion. In goats (*Capra hircus*), as in many other animals, the hormone melatonin is secreted rhythmically under a light–dark cycle (A) as well as in constant darkness (B). Each data point corresponds to the mean (± SE) of seven goats. Melatonin secretion was measured as the concentration of melatonin in the serum at 2-hour intervals. (Source: Alila-Johansson, A. et al. (2001). Seasonal variation in endogenous serum melatonin profiles in goats: a difference between spring and fall? *Journal of Biological Rhythms* 16: 254–263.)

has been documented in fish,[18,84,588] reptiles,[262,589,590] birds,[20,101,591,592] and mammals,[206,593–597] including rodents[160,598–610] and humans.[245,427,503,504,508,509,611–619] An example is shown in the upper panel of Figure 5.44. 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. A clear daily rhythm of melatonin concentration is present, with greater concentrations 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 5.45. The data in this figure were recorded from a different species

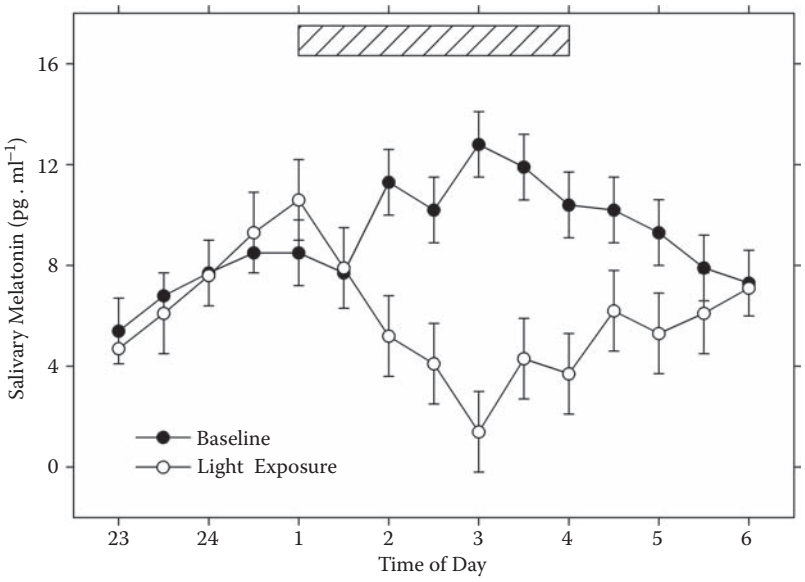


FIGURE 5.45 Melatonin suppression by light. Although melatonin secretion follows a circadian rhythm in the absence of an environmental light–dark cycle, environmental light does have a suppressive effect on melatonin secretion. The data points in the graph are the means (± SE) of seven human subjects 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. Note that both curves exhibit circadian rhythmicity, but the curve for the Light Exposure condition also exhibits light-induced suppression. (Source: Hébert, M. et al. (2002). The effects of prior light history on the suppresion of melatonin by light in humans. *Journal of Pineal Research* 33: 198–203.)

(humans), but they clearly show that light exposure at night can drastically inhibit melatonin secretion. 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. The lower panel in Figure 5.44 (with data from goats again) confirms the persistence of rhythmicity in constant darkness. Circadian rhythmicity of melatonin secretion has been documented in many studies in various species.[101,262,590–592,596,599,601,603,613,620–623]

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.[624,625] The adrenal cortex secretes many other hormones, including *corticosterone*, which is structurally similar to cortisol. Many rodents secrete corticosterone almost exclusively, while humans secrete seven times as much cortisol as corticosterone. Unlike the secretion of melatonin, the secretion of cortisol/costicosterone 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[18,20,589] and mammalian[238,354,557,626,627] vertebrates, particularly rodents[124,129,130,149,456,568,601,605,607,623,628–638] and humans.[243,336,418,427,509–511,614–620,622,639–644] Figure 5.46 shows the average daily rhythm of cortisol secretion (as measured by serum cortisol concentration) of 31 young men. Note that serum concentration starts to rise in the middle of the night, reaches the daily peak at wake time, and falls throughout the day.

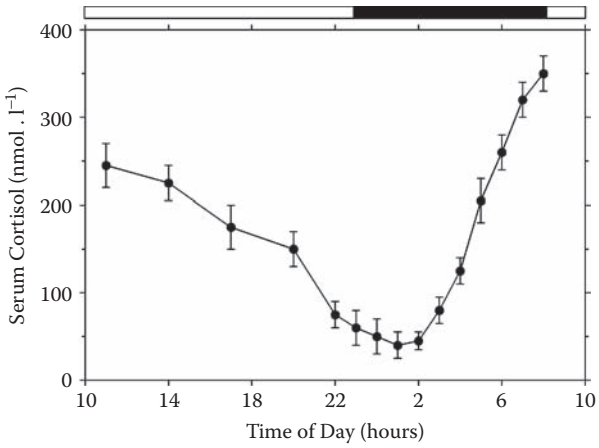


FIGURE 5.46 Daily rhythm of cortisol secretion. The graph shows the daily variation in serum cortisol concentration of human subjects. Each data point corresponds to the mean (± SE) of 31 young men. The duration of the prevailing light–dark cycle is shown by the horizontal light and dark bars. Serum cortisol concentration starts rising in the middle of the night and reaches a peak at wake time. (Source: Selmaoui, B. & 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.)

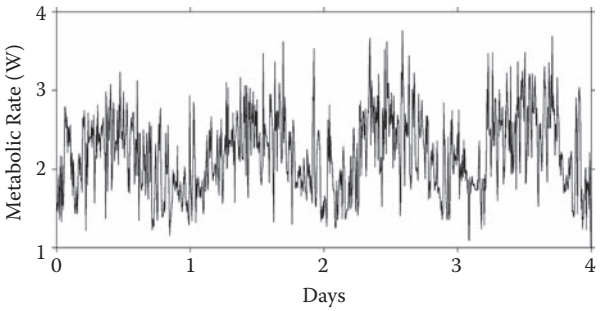


FIGURE 5.47 Circadian rhythm of metabolism. The graph shows a 4-day segment of the records of whole-organism metabolism of a laboratory rat (*Rattus norvegicus*) housed at 24°C in constant darkness. Metabolic rate was computed from measurements of oxygen consumption performed at 6-minute intervals. A clear circadian rhythm, accompanied by large ultradian oscillations, can be seen. (Source: Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423-429.)

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,[357,645–651] lower vertebrates,[368,652] birds,[93,549,653,654] and mammals,[218,223,289,534,536,540,655–658] including rats,[105,106,108,116,118,120,311,455,553,659–663] other rodents,[155,192,193,472,475,479,483,664–667] and humans.[337,511,668] Figure 5.47 shows a 4-day segment of the circadian rhythm of metabolism of a laboratory rat. Despite considerable biological noise, a regular pattern of rising and falling metabolic rate can be seen, 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,[669] sleep is a pervasive function in the animal kingdom. As illustrated in Figure 5.48, some bats sleep as much as 20 hours each day, while horses usually sleep only 3 hours. Episodes of sleep are not randomly distributed over time, however. Electroencephalographic monitoring of sleep indicates a very unequal distribution of sleep stages, as exemplified in Figure 5.49. The figure shows the percentages of slow-wave sleep (SWS) and rapid-eye-movement sleep (REM) of laboratory rats over the 24-hour cycle. In this nocturnal species, both SWS and REM are much more frequent during the light phase than during the dark phase of the light–dark cycle. Daily or circadian rhythmicity of sleep monitored with electroencephalograms has been documented in a variety of animals, including rodents,[31,110,123,126,143,168,271,273,443,459,464,554,670–676] humans,[292,499,509,512,677–679] and other vertebrates.[92,515,543,680–682]

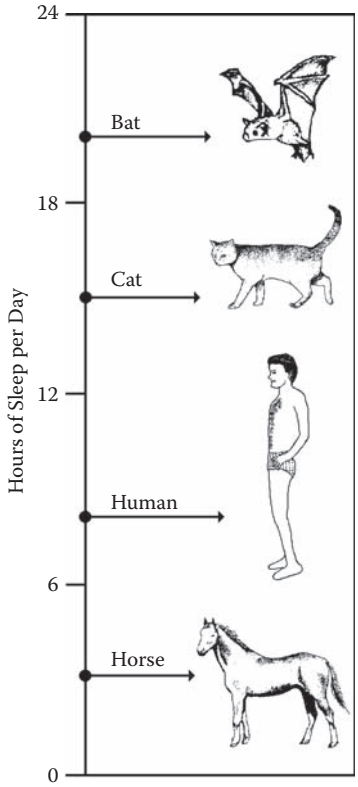


FIGURE 5.48 How much do you sleep? The usual number of hours of sleep per day varies greatly from species to species, as exemplified by these four species. (Source: Siegel, J. M. (2001). The REM sleep-memory consolidation hypothesis. *Science* 294: 1058–1063.)

Subjective sleepiness exhibits daily rhythmicity despite the amount of sleep experienced on a given day. For example, Figure 5.50 shows the mean sleepiness scores of two groups of six people kept in bed but awake for 24 consecutive hours. To partially control for 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 data curves appear very similar, showing greater sleepiness around the usual sleep time. Therefore, sleep deprivation did not significantly alter the circadian rhythmicity of sleepiness. Note, however, that sleepiness is not sharply reduced between 10:00 A.M. and 6:00 P.M. (at the end of the protocol) in the evening-start group. As discussed in greater detail in Chapter 10, sleepiness (and actual sleep, if allowed) is controlled by 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,[526,557,636,641,643,683–687] ingestive/digestive hormones,[238,632,640,641,688–690] and thyroid

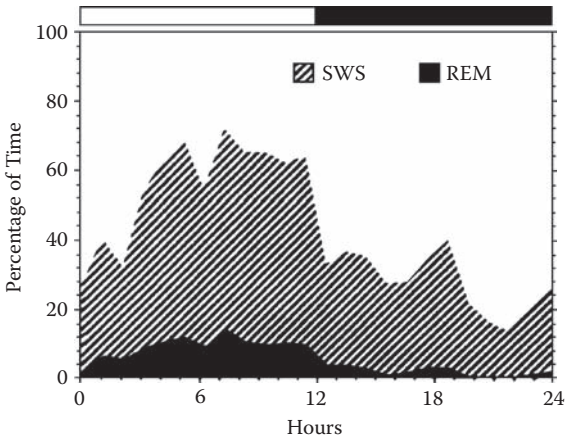


FIGURE 5.49 Daily rhythm of sleep stages. The graph shows the daily variation in the percentage of time spent each day on slow-wave sleep (SWS) and REM sleep (REM) by laboratory rats (*Rattus norvegicus*). Shown are the mean values for six adult rats (7 months old) at hourly intervals. The duration of 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. Note that, unlike humans, rats do not consolidate their sleep into a single 8-hour interval. (Source: Li, H. & Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.)

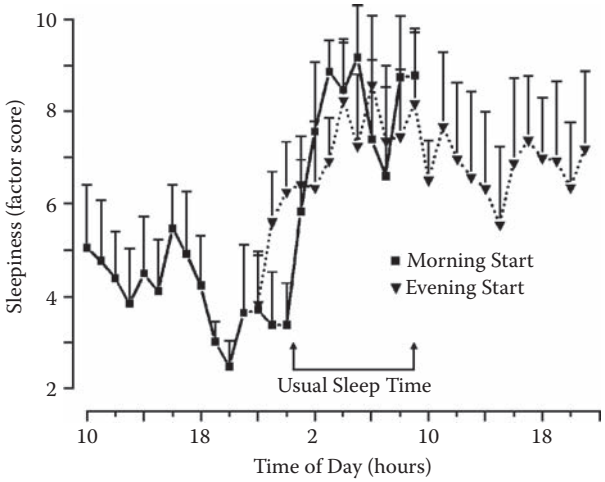


FIGURE 5.50 Circadian rhythm of sleepiness. The graph shows the variation of subjective sleepiness of human subjects in a constant routine protocol (that is, in bed rest, with no sleep allowed, and with small meals provided every hour) for over 24 consecutive hours. Each data point corresponds to the mean (± SE) of six 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. (Source: Varkevisser, M. & Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: validating a self-constructed test battery. *Chronobiology International* 20: 109–121.)

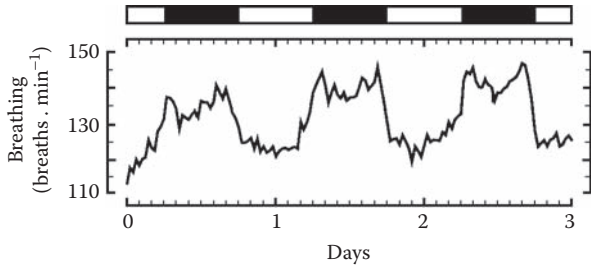


FIGURE 5.51 Daily rhythm of breathing rate. The graph displays the daily variation in the breathing rate of laboratory rats (*Rattus norvegicus*) on 3 successive days. Shown are the mean values for 23 rats with 15-minute resolution. The duration of the prevailing light–dark cycle is indicated by the horizontal light and dark bars. As nocturnal animals, rats breathe at a higher rate during the night than during the day. (Source: Mortola, J. P. & Seifert, E. L. (2002). Circadian patterns of breathing. *Respiratory Physiology and Neurobiology* 131: 91–100.)

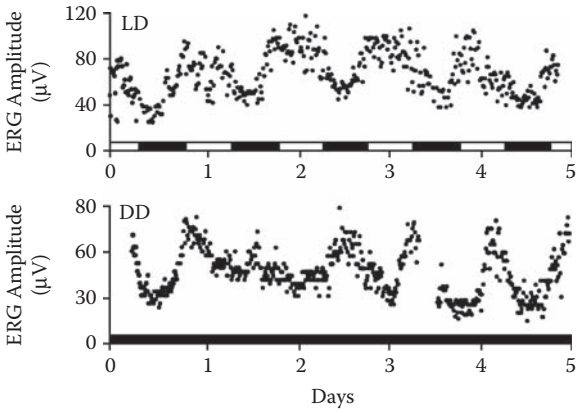


FIGURE 5.52 Daily and circadian rhythms of activity of retinal cells. The graph shows the oscillation of the amplitude of the b-wave of the electroretinogram (ERG, the compound electrical activity of neurons in the retina of the eye) of two green iguanas (*Iguana iguana*). One iguana was kept under a light–dark cycle (LD); the other iguana was kept in constant darkness (DD), 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 free-runs in constant darkness. (Source: Miranda-Anaya, M., Bartell, P. A., Yamazaki, S. & Menaker, M. (2000). Circadian rhythm of ERG in *Iguana iguana*: role of the pineal. *Journal of Biological Rhythms* 15: 163–171.)

hormones.[496,615,620,637,641,663] Rhythms in breathing have been recorded as well,[120,455,511,691] as illustrated in Figure 5.51. Also documented have been daily changes in properties of the visual system,[692–698] as exemplified by the iguana electroretinogram data in Figure 5.52. Note that the rhythm of electrical activity can be observed when the animals are kept under a light–dark cycle *and* when they are kept in constant darkness.

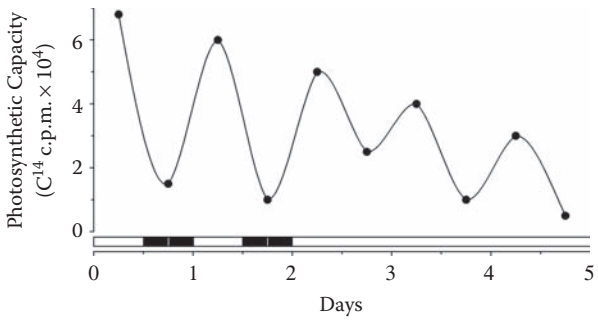


FIGURE 5.53 Daily and circadian rhythms of photosynthetic capacity. Photosynthesis cannot occur in the absence of light. Consequently, all plants exhibit a daily rhythm of photosynthetic activity. A daily rhythm of photosynthetic capacity can be demonstrated if nocturnal measurements are conducted during a brief exposure to light. The graph shows the daily variation in photosynthetic capacity of the alga *Phaedactylum 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. (Source: Palmer, J. D., Livingston, L. & Zusy, F. D. (1964). A persistent diurnal rhythm in photosynthetic capacity. *Nature* 203: 1087–1088.)

Many other rhythms have also been described. These include rhythms of growth and various cellular processes in bacteria,[699–701] resistance to herbicides in weeds,[702] photosynthetic capacity in algae and plants,[7,703,704] root pressure in sunflower plants,[705] leaf movement in mustard plants,[706] mold growth,[707,708] mitosis in mammalian cells,[470,630,709] bioluminescence (glow) in protists and insects,[710,711] eclosion (emergence) of fruit flies from the pupal stage,[712–715] heat resistance in invertebrates and vertebrates,[14,454,716] blood eosinophil count in mammals,[446,717,718] glycogen concentration,[629,719,720] cholesterol synthesis,[518,689,721–724] intraocular pressure,[725,726] susceptibility to anesthetics,[727,728] dentin increment,[729] alcohol-induced hypothermia,[730] gastric acid secretion,[731] concentration of serum fat-soluble vitamins,[732] glucose tolerance,[733] contents of stomach and intestine,[734] and concentration of plasma aldosterone.[735] Figure 5.53 provides an example of the rhythm of photosynthetic capacity in the alga *Phaedactylum tricornutum*. Photosynthesis cannot occur without light, so daily rhythmicity of photosynthesis under a light–dark cycle is ineluctable. To evaluate 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 procedure 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, I believe they sufficiently document the pervasiveness of circadian rhythmicity in behavioral and autonomic processes. Part III of this book examines 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

Exercise 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 1.3, 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 2 or more consecutive days. If you have difficulty taking the pulse at the wrist, try

taking it at your neck (or borrow a stethoscope and listen to your heart to determine your pulse rate). You may count the beats for 1 minute, or count them for 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 be calm and at rest 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 or your pulse may be disturbed). When you are finished recording data for this exercise, 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.

Exercise 5.2 Determining mean value, amplitude, and acrophase

This exercise uses the program Acro to determine the mean level, amplitude, and acrophase of circadian rhythms. As explained in Section 3.2, the 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.

1. Double-click on the Circadian icon to open the program banner, then click on Acro (the seventh icon from the left).
2. Choose "Load data from disk."
3. Open the Data subfolder by double-clicking on it.
4. Select the data file A16. This file contains the body temperature records of a fat-tailed gerbil, measured by telemetry every 6 minutes for a week under a 24-hour light–dark cycle. The file contains only values for the ordinate. You may want to inspect the data set with Plot before proceeding.
5. In Acro, click on OK (or double-click on the file name) to load A16.
6. Click on "File contains data only."
7. Because the data were collected every 6 minutes, and the file starts at midnight (or 0 hours), you can use the default values. Click on OK.
8. Because the animal was under a 24-hour light–dark cycle, you can assume that the Period of the cycle is 24.0 hours and you can use the default value (24). If the period is not 24.0 hours, there will be more (or less) than one full cycle in each 24 hours, and the program must know about it 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 (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. Choose One cycle.
10. If 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. Note the box entitled "Compute also threshold." When checked, this box indicates that the program should calculate the time at which the data series passes above an arbitrary value (or threshold) set by you. Leave this box unchecked. 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. For this exercise, 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, you 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 12:05 A.M. and 3:07 A.M.). 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. Because the file contains 7 days of data, 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 more than one cycle was used, the program was able to provide 95% confidence intervals not only for the acrophase but also for the mean and the amplitude. Note 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). These values are more accurate, 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. Because you used the same parameters (but with a different file), you do not need to enter all the information again (the low and high values, which 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. The body temperature rhythm of the diurnal tree shrew peaks during the day, while the rhythm of the nocturnal gerbil peaked at night.
18. For your last example, you will 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. 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 value is 4.3 hours after lights-on, which is consistent with the diurnal habits of this squirrel species.

Exercise 5.3 Calculating circadian period by the chi square periodogram procedure

This exercise uses the program Tau to calculate the period of circadian rhythms using the chi square periodogram procedure described in Section 3.3. The program requires equally spaced data points in a data file without time tags.

1. Double-click on the Circadian icon to open the program banner, then click on Tau (the eighth icon from the left).
2. Note 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, open the Data subfolder, then select 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. 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. The chi-square test determines whether the Q_P value is statistically significant. As shown at the top of the panel, a Q_P of 2350 is significantly different from noise at a significance level lower than 0.0061.
5. You should be warned that the test of statistical significance is rather conservative. Because 61 periods are being tested simultaneously (i.e., 20.0 to 26.0 in steps of 0.1), the level of significance is adjusted upward. Try this: in the Period panel, click on the scroll bars for "Start at" and "End at" so as to set the range at 23.1 to 23.9 hours. Then click OK again. The Q_P remains 2350, but the significance level is now lower than 0.0009. Thus, the shorter the range, the more sensitive the test. Of course, you should always set the range of the periodogram based on an honest expectation. To maliciously reduce the range based on a previous test defeats the purpose of statistical testing.
6. Set "Start at" back to 20 and "End at" back to 26. Select data file A09. This file also contains artificial data, 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 0.0061, however.
7. 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 h. This happened by chance, as indicated by the nonsignificance reported right below it ($p > 0.05$). Is

85% noise too much noise? Click on the "Bins to use" scroll bar in the Data panel. A window opens indicating 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 value corresponds to 20 days rather than 10 days. Click on OK. The period estimate is now very close (23.4 hours as compared to 23.5 hours), but it is still non-significant. Why use a range from 20 to 26 hours? Is it not reasonable to use a range from 21 to 25 instead? Try it! You will be pleased.

8. 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 note 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.
9. In the program 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.
10. 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 the expectation of significant rhythmicity with a period shorter than 24.0 hours (i.e., 23.4 hours).

Exercise 5.4 Calculating circadian period by the Lomb–Scargle periodogram procedure

This exercise is similar to Exercise 5.3, but it uses data files containing unequally spaced data points, which will require the use of a different program. LSP calculates the period of circadian rhythms using the Lomb–Scargle periodogram procedure.

1. Double-click on the Circadian icon to open the program banner, then click on LSP (the ninth icon from the left).
2. Note that you must follow 5 steps: 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 file A08, 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 to 30.0 hours, click on "See all." When you are finished viewing the values, click on Close Window to return to the main window.
5. Next, select the file A12. This file also contains artificial data, 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 program was unable to detect significant rhythmicity in the data set (note 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? Remember that some parameters were changed in the program to enhance the results of the chi square periodogram procedure, so you will need to make some changes here also. Start by using one of the files from 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). 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! Significant rhythmicity is present! 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 it is still acceptable).
10. For your last example, you will 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 body temperature records 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 want to do so now to make sure that the period calculated by LSP is consistent with your subjective evaluation of it.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.). (2001). *Circadian Clocks* (Volume 12 of *Handbook of Behavioral Neurobiology*). New York: Kluwer/Plenum. A dense but authoritative volume with contributions from experts in virtually all aspects 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: Profile. A delightful book! In contrast to the book by Takahashi and colleagues, this short text is targeted at general audiences. Topics are covered less systematically and in less detail, but the book is more readable. An excellent introduction to the field.

Binkley, S. (1997). *Biological Clocks: Your Owner's Manual.* Amsterdam: Harwood Academic. Another general-audience book. Written 7 years before Foster and Kreitzman's book and with a different perspective, this text may be of interest to some readers.

Meck, W. H. (Ed.). (2003). *Functional and Neural Mechanisms of Interval Timing.* Boca Raton, FL: CRC Press. A collection of review articles dealing with time perception (brief interval timing rather than circadian timing).

WEB SITES TO EXPLORE

Circadian Rhythm Laboratory (Refinetti):
http://www.circadian.org

HealthLink (Medical College of Wisconsin):
http://healthlink.mcw.edu/article/922567322.html

Journal of Circadian Rhythms:
http://www.jcircadianrhythms.com

Millar Research Group (University of Warwick):
http://www.amillar.org

Weather History (United States):
http://www.almanac.com/weatherhistory/index.php

LITERATURE CITED

1. Pritchard, D. R. (Ed.) (1994). *The American Heritage Dictionary*, 3rd Edition. Boston: 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. & 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*, Volume 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. & Horvath, S. M. (1951). Diurnal variations in body temperature. *Journal of Applied Physiology* 3: 665–675.
7. Palmer, J. D., Livingston, L. & Zusy, F. D. (1964). A persistent diurnal rhythm in photosynthetic capacity. *Nature* 203: 1087–1088.
8. Lobban, M. C. & 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., & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & Thompson, J. C. (1983). *Elements of Meteorology*, 4th Edition. Columbus: Charles E. Merrill.
37. Trewartha, G. T. & Horn, L. H. (1980). *An Introduction to Climate*, 5th Edition. New York: McGraw-Hill.
38. Couderc, P. (1971). Calendar. *Encyclopedia Americana* 5: 184–190.
39. Audoin, C. & Guinot, B. (2001). *The Measurement of Time* (Trans. by S. Lyle). Cambridge, UK: Cambridge University Press.
40. Zaha, M. A. (1972). Shedding some needed light on optical measurements. *Electronics* Nov 6: 91–96.
41. Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., Salinger, M. J., Razuvayev, V., Plummer, N., Jamason, P. & Folland, C. K. (1997). Maximum and minimum temperature trends for the globe. *Science* 277: 364–367.
42. Laumann, E. O., Gagnon, J. H., Michael, R. T. & Michaels, S. (1994). *The Social Organization of Sexuality: Sexual Practices in the United States*. Chicago: University of Chicago Press.

43. Palmer, J. D., Udry, J. R. & Morris, N. M. (1982). Diurnal and weekly, but no lunar rhythm in human copulation. *Human Biology* 54: 111–121.
44. Fraser, W. D., McLean, F. H. & Usher, R. H. (1989). Diurnal variation in admission to hospital of women in labour. *Canadian Journal of Surgery* 32: 33–35.
45. Ruffieux, C., Marazzi, A. & Paccaud, F. (1992). The circadian rhythm of the perinatal mortality rate in Switzerland. *American Journal of Epidemiology* 135: 936–951.
46. Anderka, M., Declercq, E. R. & Smith, W. (2000). A time to be born. *American Journal of Public Health* 90: 124–126.
47. Goldstick, O., Weissman, A. & Drugan, A. (2003). The circadian rhythm of "urgent" operative deliveries. *Israel Medical Association Journal* 5: 564–566.
48. Åkerstedt, T., Kecklund, G. & Hörte, L. G. (2001). Night driving, season, and the risk of highway accidents. *Sleep* 24: 401–406.
49. Haikonen, H. & Summala, H. (2001). Deer-vehicle crashes: extensive peak at 1 hour after sunset. *American Journal of Preventive Medicine* 21: 209–213.
50. Gallerani, M., Avato, F. M., Dal Monte, D., Caracciolo, S., Fersini, C. & Manfredini, R. (1996). The time for suicide. *Psychological Medicine* 26: 867–870.
51. Preti, A. & Miotto, P. (2001). Diurnal variations in suicide by age and gender in Italy. *Journal of Affective Disorders* 65: 253–261.
52. Fromm, R. E., Levine, R. L. & Pepe, P. E. (1992). Circadian variation in the time of request for helicopter transport of cardiac patients. *Annals of Emergency Medicine* 21: 1196–1199.
53. Spielberg, C., Falkenhahn, D., Willich, S. N., Wegscheider, K. & 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.
54. López-Messa, J. B., Garmendia-Leiza, J. R., Aguilar-García, M. D., Andrés-de-Llano, J. M., Alberola-López, C. & Ardura-Fernández, J. (2004). Factores de riesgo cardiovascular en el ritmo circadiano del infarto agudo de miocardio. *Revista Española de Cardiología* 57: 850–858.
55. Rosenberg, J., Pedersen, M. H., Ramsing, T. & Kehlet, H. (1992). Circadian variation in unexpected postoperative death. *British Journal of Surgery* 79: 1300–1302.
56. Manfredini, R., Vanni, A., Peron, L., la Cecilia, O., Smolensky, M. H. & Grassi, L. (2001). Day-night variation in aggressive behavior among psychiatric inpatients. *Chronobiology International* 18: 503–511.
57. Reinberg, O., Reinberg, A., Téhard, B. & Mechkouri, M. (2002). Accidents in children do not happen at random: predictable time-of-day incidence of childhood trauma. *Chronobiology International* 19: 615–631.
58. Koch, H. J., Vogel, M. & Raschka, C. (2003). Circadian rhythm of accidents in children: a basic activity periodicity. *Chronobiology International* 20: 157–159.
59. Parks, D. K., Yetman, R. J., McNeese, M. C., Burau, K. & Smolensky, M. H. (2000). Day-night pattern in accidental exposures to blood-borne pathogens among medical students and residents. *Chronobiology International* 17: 61–70.
60. 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.
61. 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.
62. Crawshaw, L. I. (1971). Temperature selection and activity in the crayfish, *Orconectes immunis*. *Journal of Comparative Physiology* 95: 315–322.
63. 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.
64. Refinetti, R. (2000). Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 73: 575–583.
65. Aguzzi, J., Company, J. B. & Abelló, P. (2004). Locomotor activity rhythms of continental slope *Nephrops norvegicus* (Decapoda: Nephropidae). *Journal of Crustacean Biology* 24: 282–290.
66. Bernardi, N. (1990). Temperature influence upon food ingestion and spontaneous locomotion of the freshwater prawn *Macrobrachium acanthurus*. *Journal of Thermal Biology* 15: 33–36.
67. Dreisig, H. (1980). Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia* 44: 376–389.
68. Pivnick, K. A. & McNeil, J. N. (1986). Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). *Ecology* 67: 1024–1035.
69. Shaw, P. J., Cirelli, C., Greenspan, R. J. & Tononi, G. (2000). Correlates of sleep and waking in *Drosophila melanogaster*. *Science* 287: 1834–1837.
70. Hamblen-Coyle, M. J., Wheeler, D. A., Rutila, J. E., Rosbash, M. & 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.
71. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T. & 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.
72. Meyer-Peters, H. (1993). Seasonality of circadian locomotor activity in an insect. *Journal of Comparative Physiology A* 171: 713–724.
73. Sharma, V. K., Lone, S. R., Mathew, D., Goel, A. & 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.

74. Shimizu, I., Kawai, Y., Taniguchi, M. & Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
75. North, R. D. (1993). Entrainment of the circadian rhythm of locomotor activity in wood ants by temperature. *Animal Behaviour* 45: 393–397.
76. Koilraj, A. J., Sharma, V. K., Marimuthu, G. & 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.
77. Crawshaw, L. I., Johnston, M. H. & Lemons, D. E. (1980). Acclimation, temperature selection, and heat exchange in the turtle, *Chrysemys scripta*. *American Journal of Physiology* 238: R443–R446.
78. Tosini, G. & Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *Journal of Biological Rhythms* 10: 248–255.
79. Bartell, P. A., Miranda-Anaya, M. & Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
80. Refinetti, R. & Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.
81. Bertolucci, C., Sovrano, V. A., Magnone, M. C. & Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
82. Iigo, M. & Tabata, M. (1996). Circadian rhythms of locomotor activity in the goldfish *Carassius auratus*. *Physiology and Behavior* 60: 775–781.
83. Hurd, M. W., Debruyne, J., Straume, M. & Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiology and Behavior* 65: 465–472.
84. Bayarri, M. J., Muñoz-Cueto, J. A., López-Olmeda, J. F., Vera, L. M., Rol-de-Lama, M. A., Madrid, J. A. & Sánchez-Vázquez, F. J. (2004). Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). *Physiology and Behavior* 81: 577–583.
85. Herrero, M. J., Madrid, J. A. & Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
86. Chen, W. M. & Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
87. Tieleman, B. I. & Williams, J. B. (2002). Effects of food supplementation on behavioural decisions of hoopoelarks in the Arabian Desert: balancing water, energy and thermoregulation. *Animal Behaviour* 63: 519–529.
88. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W. & 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.
89. Ebihara, S. & Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
90. King, V. M., Bentley, G. E. & 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.
91. Oshima, I. & 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.
92. Berger, R. J. & 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.
93. Rashotte, M. E., Basco, P. S. & 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.
94. Oshima, I., Yamada, H., Goto, M., Sato, K. & 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. Aschoff, J. & von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
96. Winget, C. M. & Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
97. 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.
98. Hau, M. & Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
99. Heigl, S. & Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
100. Underwood, H. & Edmonds, K. (1995). The circadian rhythm of thermoregulation in Japanese quail. II. Multioscillator control. *Journal of Biological Rhythms* 10: 234–247.
101. Kumar, V., Gwinner, E. & 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.
102. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
103. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K. & 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.

104. Mistlberger, R. E., Lukman, H. & Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: assessment and intervention. *Appetite* 30: 255–267.
105. Shido, O., Sakurada, S. & Nagasaka, T. (1991). Effect of heat acclimation on diurnal changes in body temperature and locomotor activity in rats. *Journal of Physiology* 433: 59–71.
106. Shido, O., Sakurada, S., Kohda, W. & Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
107. Satinoff, E., Kent, S. & Hurd, M. (1991). Elevated body temperature in female rats after exercise. *Medicine and Science in Sports and Exercise* 23: 1250–1253.
108. Keesey, R. E., Swiergiel, A. H. & 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.
109. Morley, R. M., Conn, C. A., Kluger, M. J. & Vander, A. J. (1990). Temperature regulation in biotelemetered spontaneously hypertensive rats. *American Journal of Physiology* 258: R1064–R1069.
110. Borbély, A. A. & 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.
111. Albers, H. E., Gerall, A. A. & Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
112. Meinrath, M. & D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
113. Kittrell, E. M. W. & Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.
114. Isobe, Y., Aoki, K., Furuyama, F. & 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.
115. Ikeda, M. & 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.
116. Nagashima, K., Nakai, S., Matsue, K., Konishi, M., Tanaka, M. & Kanosue, K. (2003). Effects of fasting on thermoregulatory processes and the daily oscillations in rats. *American Journal of Physiology* 284: R1486–R1493.
117. Francis, A. J. P. & 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.
118. Williams, T. D., Chambers, J. B., May, O. L., Henderson, R. P., Rashotte, M. E. & 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.
119. López, L., González-Pardo, H., Cimadevilla, J. M., Cavas, M., Aller, M. A., Arias, J. & Arias, J. L. (2002). Cytochrome oxidase activity of the suprachiasmatic nucleus and pineal gland in rats with portacaval shunt. *Experimental Neurology* 173: 275–282.
120. Seifert, E. L. & Mortola, J. P. (2002). The circadian pattern of breathing in conscious adult rats. *Respiration Physiology* 129: 297–305.
121. Witte, K., Grebmer, W., Scalbert, E., Delagrande, P., Guardiola-Lemaître, B. & Lemmer, B. (1998). Effects of melatoninergic agonists on light-supressed circadian rhythms in rats. *Physiology and Behavior* 65: 219–224.
122. Witte, K. & Lemmer, B. (1999). Development of inverse circadian blood pressure pattern in transgenic hypertensive TGR(mREN2)27 rats. *Chronobiology International* 16: 293–303.
123. Ray, B., Mallick, H. N. & 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.
124. Honma, K. & Hiroshige, T. (1978). Internal synchronization among several circadian rhythms in rats under constant light. *American Journal of Physiology* 235: R243–R249.
125. Honma, K. & Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
126. Ikeda, M., Sagara, M. & 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.
127. Benstaali, C., Bogdan, A. & 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.
128. Deprés-Brummer, P., Lévi, F., Metzger, G. & Touitou, Y. (1995). Light-induced suppression of the rat circadian system. *American Journal of Physiology* 268: R1111–R1116.
129. Honma, K., von Goetz, C. & Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.
130. Fischette, C. T., Edinger, H. M. & Siegel, A. (1981). Temporary desynchronization among circadian rhythms with lateral fornix ablation. *Brain Research* 229: 85–101.
131. Lemmer, B., Hauptfleisch, S. & 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.
132. Scales, W. E. & Kluger, M. J. (1987). Effect of antipyretic drugs on circadian rhythm in body temperature of rats. *American Journal of Physiology* 253: R306–R313.

133. Warren, W. S. & Cassone, V. M. (1995). The pineal gland: photoreception and coupling of behavioral, metabolic, and cardiovascular circadian outputs. *Journal of Biological Rhythms* 10: 64–79.
134. Gordon, C. J. & Rezvani, A. H. (2001). Genetic selection of rats with high and low body temperatures. *Journal of Thermal Biology* 26: 223–229.
135. Yang, Y. & 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.
136. Mount, L. E. & 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.
137. Batchelder, P., Kinney, R. O., Demlow, L. & Lynch, C. B. (1983). Effects of temperature and social interactions on huddling behavior in *Mus musculus*. *Physiology and Behavior* 31: 97–102.
138. Weinert, D. & Waterhouse, J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. *Physiology and Behavior* 63: 837–843.
139. Kramer, K., Voss, H. P., Grimbergen, J. & 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.
140. Possidente, B., McEldowney, S. & Pabon, A. (1995). Aging lengthens circadian period for wheel-running activity in C57BL mice. *Physiology and Behavior* 57: 575–579.
141. Davis, F. C. & Menaker, M. (1981). Development of the mouse circadian pacemaker: independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
142. Abe, H., Honma, S., Honma, K., Suzuki, T. & 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.
143. Sei, H., Oishi, K., Morita, Y. & Ishida, N. (2001). Mouse model for morningness/eveningness. *NeuroReport* 12: 1461–1464.
144. Irizarry, R. A., Tankersley, C., Frank, R. & Flanders, S. (2001). Assessing homeostasis through circadian patterns. *Biometrics* 57: 1228–1237.
145. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
146. Weinert, D. & Waterhouse, J. (1999). Daily activity and body temperature rhythms do not change simultaneously with age in laboratory mice. *Physiology and Behavior* 66: 605–612.
147. Tankersley, C. G., Irizarry, R., Flanders, S. E., Rabold, R. & Frank, R. (2003). Unstable heart rate and temperature regulation predict mortality in AKR/J mice. *American Journal of Physiology* 284: R742–R750.
148. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
149. Filipski, E., King, V. M., Etienne, M. C., Li, X. M., Claustrat, B., Granda, T. G., Milano, G., Hastings, M. H. & 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.
150. Conn, C. A., Borer, K. T. & 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.
151. Davis, F. C., Stice, S. & 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.
152. Golombek, D. A., Ortega, G. & Cardinali, D. P. (1993). Wheel running raises body temperature and changes the daily cycle in golden hamsters. *Physiology and Behavior* 53: 1049–1054.
153. 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.
154. Refinetti, R. (1995). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behavioral Neuroscience* 109: 523–527.
155. Brown, C. M. & Refinetti, R. (1996). Daily rhythms of metabolic heat production, body temperature, and locomotor activity in golden hamsters. *Journal of Thermal Biology* 21: 227–230.
156. Weinert, D., Fritzche, P. & Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
157. Boulos, Z., Macchi, M., Houpt, T. A. & Terman, M. (1996). Photic entrainment in hamsters: effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
158. Anchordoquy, H. C. & 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.
159. Tokura, H. & 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.
160. Steinlechner, S., Stieglitz, A. & 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.
161. Refinetti, R. (1998). Body temperature and behavior of tree shrews and flying squirrels in a thermal gradient. *Physiology and Behavior* 63: 517–520.

162. DeCoursey, P. (1960). Phase control of activity in a rodent. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 49–55.
163. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
164. Everts, L. G., Strijkstra, A. M., Hut, R. A., Hoffmann, I. E. & Millesi, E. (2004). Seasonal variation in daily activity patterns of free-ranging European ground squirrels (*Spermophilus citellus*). *Chronobiology International* 21: 57–71.
165. Lee, T. M., Holmes, W. G. & Zucker, I. (1990). Temperature dependence of circadian rhythms in golden-mantled ground squirrels. *Journal of Biological Rhythms* 5: 25–34.
166. Lee, T. M. & Zucker, I. (1995). Seasonal variations in circadian rhythms persist in gonadectomized golden-mantled ground squirrels. *Journal of Biological Rhythms* 10: 188–195.
167. 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.
168. Kas, M. J. H. & Edgar, D. M. (1998). Crepuscular rhythms of EEG sleep-wake in a hystricomorph rodent, *Octodon degus*. *Journal of Biological Rhythms* 13: 9–17.
169. Labyak, S. E. & Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
170. 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.
171. Kas, M. J. H. & Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.
172. Goel, N. & Lee, T. M. (1995). Social cues accelerate reentrainment of circadian rhythms in diurnal female *Octodon degus* (Rodentia: Octodontidae). *Chronobiology International* 12: 311–323.
173. Lovegrove, B. G., Heldmaier, G. & Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
174. Oosthuizen, M. K., Cooper, H. M. & 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.
175. Lovegrove, B. G. & 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.
176. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
177. Tavernier, R. J., Largen, A. L. & Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
178. Gerkema, M. P., Groos, G. A. & 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.
179. Jilge, B. (1985). The rhythm of food and water ingestion, faeces excretion and locomtor activity in the guinea pig. *Zeitschrift für Versuchstierkunde* 27: 215–225.
180. 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.
181. Akita, M., Ishii, K., Kuwahara, M. & Tsubone, H. (2001). The daily pattern of heart rate, body temperature, and locomotor activity in guinea pigs. *Experimental Animals* 50: 409–415.
182. Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. *Physiology and Behavior* 82: 285–294.
183. McElhinny, T. L., Smale, L. & 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.
184. Blanchong, J. A., McElhinny, T. L., Mahoney, M. M. & Smale, L. (1999). Nocturnal and diurnal rhythms in the unstriped Nile rate, *Arvicanthis niloticus*. *Journal of Biological Rhythms* 14: 364–377.
185. 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.
186. Lovegrove, B. G. & 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.
187. Gebczynski, A. K. & 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.
188. Mrosovsky, N., Melnyk, R. B., Lang, K., Hallonquist, J. D., Boshes, M. & Joy, J. E. (1980). Infradian cycles in dormice (*Glis glis*). *Journal of Comparative Physiology A* 137: 315–339.
189. Roxburgh, L. & Perrin, M. R. (1994). Temperature regulation and activity pattern of the round-eared elephant shrew *Macroscelides proboscideus*. *Journal of Thermal Biology* 19: 13–20.
190. 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.

191. Weinert, D., Nevill, A., Weinandy, R. & 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.
192. Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology A* 119: 295–300.
193. Sacher, G. A. & Duffy, P. H. (1978). Age changes in rhythms of energy metabolism, activity, and body temperature in *Mus* and *Peromyscus*. In: Samis, H. V. & Capobianco, S. (Eds.). *Aging and Biological Rhythms*. New York: Plenum, pp. 105–124.
194. Rezende, E. L., Cortés, A., Bacigalupe, L. D., Nespolo, R. F. & Bozinovic, F. (2003). Ambient temperature limits above-ground activity of the subterranean rodent *Spalacopus cyanus*. *Journal of Arid Environments* 55: 63–74.
195. Begall, S., Daan, S., Burda, H. & Overkamp, G. J. F. (2002). Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83: 153–158.
196. Kramm, K. R. & Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.
197. Richter, C. P. (1978). Evidence for existence of a yearly clock in surgically and self-blinded chipmunks. *Proceedings of the National Academy of Sciences U.S.A.* 75: 3517–3521.
198. Jilge, B. (1991). Restricted feeding: a nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
199. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
200. Kennedy, G. A., Hudson, R. & Armstrong, S. M. (1994). Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiology and Behavior* 55: 385–389.
201. Johnson, R. F. & Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
202. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., & 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.
203. Hawking, F., Lobban, M. C., Gammage, K. & 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.
204. Siwak, C. T., Tapp, P. D., Zicker, S. C., Murphey, H. L., Muggenburg, B. A., Head, E. & Cotman, C. W. (2003). Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience* 117: 813–824.
205. Mohr, E. G. & Krzywanek, H. (1995). Endogenous oscillator and regulatory mechanisms of body temperature in sheep. *Physiology and Behavior* 57: 339–347.
206. Lincoln, G., Messager, S., Andersson, H. & 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 U.S.A.* 99: 13890–13895.
207. Gill, J. (1991). A new method for continuous recording of motor activity in horses. *Comparative Biochemistry and Physiology A* 99: 333–341.
208. Scheibe, K. M., Berher, A., Langbein, J., Streich, W. J. & Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biological Rhythm Research* 30: 216–233.
209. Refinetti, R. & Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
210. Meijer, J. H., Daan, S., Overkamp, G. J. F. & 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.
211. Wells, R. T. (1978). Thermoregulation and activity rhythms in the hairy-nosed wombat, Lasiorhinus latifrons (Owen), (Vombatidae). *Australian Journal of Zoology* 26: 639–651.
212. Körtner, G. & Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiology and Behavior* 58: 31–36.
213. Jones, M. E., Grigg, G. C. & 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.
214. Kennedy, G. A., Coleman, G. J. & Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
215. Marimuthu, G., Rajan, S. & 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.
216. Bakko, E. B., Porter, W. P. & Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
217. Kowalczyk, R., Jedrzejewska, B. & 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.
218. Riccio, A. P. & Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
219. Ishii, K., Uchino, M., Kuwahara, M., Tsubone, H. & 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.

220. Ingram, D. L. & 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.
221. Lancia, R. A., Dodge, W. E. & Larson, J. S. (1982). Winter activity patterns of two radio-marked beaver colonies. *Journal of Mammalogy* 63: 598–606.
222. Francis, A. J. P., de Alwis, C., Peach, L. & Redman, J. R. (1999). Circadian activity rhythms in the Australian platypus, *Ornithorhynchus anatinus* (Monotremata). *Biological Rhythm Research* 30: 91–103.
223. Harlow, H. J., Phillips, J. A. & 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.
224. Merrill, S. B. & Mech, L. D. (2003). The usefulness of GPS telemetry to study wolf circadian and social activity. *Wildlife Society Bulletin* 31: 947–960.
225. Rauth-Widmann, B., Fuchs, E. & 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.
226. Häster, L. & 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.
227. Menezes, A. A. L., Moreira, L. F. S., Azevedo, C. V. M., Costa, S. F. & 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.
228. de Castro, C. S. S., Menezes, A. L. de & Moreira, L. F. S. (2003). Locomotor activity rhythm in free-ranging common marmosets (*Callithrix jacchus*). *Biological Rhythm Research* 34: 23–30.
229. Takasu, N., Nigi, H. & 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.
230. Tapp, W. N. & Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
231. Fuller, C. A., Hoban-Higgins, T. M., Klimovitsky, V. Y., Griffin, D. W. & Alpatov, A. M. (1996). Primate circadian rhythms during spaceflight: results from Cosmos 2044 and 2229. *Journal of Applied Physiology* 81: 188–193.
232. Tokura, H. & Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
233. Génin, F. & 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.
234. Aujard, F. & 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.
235. Perret, M. & 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.
236. Schilling, A., Richard, J. P. & 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.
237. Perret, M., Aujard, F., Séguy, M. & 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.
238. Zeitzer, J. M., Buckmaster, C. L., Parker, K. J., Hauck, C. M., Lyons, D. M. & 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.
239. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
240. Moore-Ede, M. C., Kass, D. A. & Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
241. Aschoff, J., Gerecke, U. & Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
242. Binkley, S., Tome, M. B., Crawford, D. & Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
243. Kennaway, D. J. & 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.
244. Steel, G. D., Callaway, M., Suedfeld, P. & 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.
245. Yoneyama, S., Hashimoto, S. & Honma, K. (1999). Seasonal changes of human circadian rhythms in Antarctica. *American Journal of Physiology* 277: R1091–R1097.
246. Natale, V. (2002). Circadian motor asymmetries in humans. *Neuroscience Letters* 320: 102–104.
247. Aschoff, J., Gerecke, U. & Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
248. Gander, P. H., Connell, L. J. & 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.

249. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F. & Pauly, J. E. (Eds.). *Chronobiology.* Tokyo: Igaku Shoin, pp. 451–459.
250. Endo, T., Honma, S., Hashimoto, S. & Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
251. Refinetti, R. (2004). The Nile grass rat as a laboratory animal. *Lab Animal* 33(9): 54–57.
252. Redlin, U. & Mrosovsky, N. (2004). Nocturnal activity in a diurnal rodent (*Arvicanthis niloticus*): the importance of masking. *Journal of Biological Rhythms* 19: 58–67.
253. Blanchong, J. A. & Smale, L. (2000). Temporal patterns of activity of the unstriped Nile rat, *Arvicanthis niloticus*. *Journal of Mammalogy* 81: 595–599.
254. Aschoff, J. (1966). Circadian activity pattern with two peaks. *Ecology* 47: 657–662.
255. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
256. Dainton, B. H. (1954). The activity of slugs. I. The induction of activity by changing temperatures. *Journal of Experimental Biology* 31: 165–187.
257. Page, T. L. (1985). Circadian organization in cockroaches: effects of temperature cycles on locomotor activity. *Journal of Insect Physiology* 31: 235–242.
258. Toma, D. P., Bloch, G., Moore, D. & 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 U.S.A.* 97: 6914–6919.
259. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
260. Lowe, C. H., Hinds, D. S., Lardner, P. J. & Justice, K. E. (1967). Natural free-running period in vertebrate animal populations. *Science* 156: 531–534.
261. Janik, D. S., Pickard, G. E. & 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.
262. Tosini, G. & Menaker, M. (1998). Multioscillatory circadian organization in a vertebrate, *Iguana iguana*. *Journal of Neuroscience* 18: 1105–1114.
263. Pohl, H. (1993). Does aging affect the period of the circadian pacemaker in vertebrates? *Naturwissenschaften* 80: 478–481.
264. Sridaran, R. & 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.
265. Weber, A. L. & Adler, N. T. (1978). Dissociation of persistent estrus and a free-running activity cycle in different light cycles. *Biology of Reproduction* 19: 425–430.
266. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S. & 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.
267. Bauer, M. S. (1992). Irradiance responsivity and unequivocal type-1 phase responsivity of rat circadian activity rhythms. *American Journal of Physiology* 263: R1110–R1114.
268. Stokes, M. A., Kent, S. & 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.
269. Edgar, D. M., Kilduff, T. S., Martin, C. E. & 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.
270. Hofstetter, J. R., Grahame, N. J. & Mayeda, A. R. (2003). Circadian activity rhythms in high-alcohol-preferring and low-alcohol-preferring mice. *Alcohol* 30: 81–85.
271. Edgar, D. M., Martin, C. E. & 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.
272. Yoshimura, T., Nishio, M., Goto, M. & 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.
273. Edgar, D. M. & Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
274. Refinetti, R. (1996). Ultradian rhythms of body temperature and locomotor activity in wild-type and tau-mutant hamsters. *Animal Biology* 5: 111–115.
275. Fitzgerald, K. M. & Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences U.S.A.* 73: 2923–2927.
276. Refinetti, R. & Menaker, M. (1992). The circadian rhythm of body temperature of normal and tau-mutant golden hamsters. *Journal of Thermal Biology* 17: 129–133.
277. DeCoursey, P. J., Pius, S., Sandlin, C., Wethey, D. & Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiology and Behavior* 65: 457–463.
278. Zucker, I., Fitzgerald, K. M. & Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.
279. Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology A* 162: 35–46.
280. Oklejewicz, M., Hut, R. A., Daan, S, Loudon, A. S. I. & Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.

281. Refinetti, R. & Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
282. DeCoursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science* 131: 33–35.
283. Mrosovsky, N., Boshes, M., Hallonquist, J. D. & Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.
284. Lee, T. M. & 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.
285. Honma, S. & Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
286. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P. & 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.
287. 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.
288. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
289. Robinson, E. L. & Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
290. Boulos, Z., Frim, D. M., Dewey, L. K. & Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
291. Chandrashekaran, M. K., Marimuthu, G. & 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.
292. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C. & Knauer, R. S. (1980). Human sleep: its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
293. Zulley, J., Wever, R. & Aschoff, J. (1981). The dependence of onset and duration of sleep on the circadian rhythm of rectal temperature. *Pflügers Archiv* 391: 314–318.
294. Lund, R. (1974). Personality factors and desynchronization of circadian rhythms. *Psychosomatic Medicine* 36: 224–228.
295. Campbell, S. S., Dawson, D. & Zulley, J. (1993). When the human circadian system is caught napping: evidence for endogenous rhythms close to 24 hours. *Sleep* 16: 638–640.
296. Jespersen, J. & Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency,* 2nd Edition. Mineola, NY: Dover.
297. Barnett, J. E. (1998). *Time's Pendulum: From Sundials to Atomic Clocks*. New York: Plenum.
298. Daan, S., Merrow, M. & Roenneberg, T. (2002). External time, internal time. *Journal of Biological Rhythms* 17: 107–109.
299. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th Edition. New York: Cambridge University Press.
300. Drewnowski, A., Grinker, J. A., Gruen, R. & 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.
301. Balagura, S. & Coscina, D. V. (1968). Periodicity of food intake in the rat as measured by an operant response. *Physiology and Behavior* 3: 641–643.
302. Prins, A. A., Jong-Nagelsmit, A. de, Keijser, J. & Strubbe, J. H. (1986). Daily rhythms of feeding in the genetically obese and lean Zucker rats. *Physiology and Behavior* 38: 423–426.
303. Cunningham, J. T., Beltz, T., Johnson, R. F. & 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.
304. Velasco-Plaza, A., Granda, T. G. & Cachero, M. T. G. (1993). Circadian rhythms of food and water intake and urine excretion in diabetic rats. *Physiology and Behavior* 54: 665–670.
305. Zucker, I. (1971). Light-dark rhythms in rat eating and drinking behavior. *Physiology and Behavior* 6: 115–126.
306. Strubbe, J. H., Spiteri, N. J. & Prins, A. J. A. (1986). Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats. *Physiology and Behavior* 36: 647–651.
307. Galbicka, G., Smurthwaite, S., Riggs, R. & Tang, L. W. (1997). Daily rhythms in a complex operant: targeted percentile shaping of run lengths in rats. *Physiology and Behavior* 62: 1165–1169.
308. Rosenwasser, A. M., Boulos, Z. & Terman, M. (1983). Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiology and Behavior* 30: 353–359.
309. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
310. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M. & Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
311. Shido, O., Sugano, Y., & Nagasaka, T. (1986). Circadian change of heat loss in response to change in core temperature in rats. *Journal of Thermal Biology* 11: 199–202.

312. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A. & Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
313. Shapiro, C. J. & Weathers, W. W. (1981). Metabolic and behavioral responses of American kestrels to food deprivation. *Comparative Biochemistry and Physiology A* 68: 11–114.
314. Kadono, H., Besch, E. L. & Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
315. Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.
316. Lumineau, S. & 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.
317. Guyomarc'h, C., Lumineau, S. & 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.
318. Lumineau, S. & Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
319. Reierth, E. & Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
320. Casey, T. M., Joos, B., Fitzgerald, T. D., Yurlina, M. E. & Young, P. A. (1988). Synchronized group foraging, thermoregulation, and growth of Eastern tent caterpillars in relation to microclimate. *Physiological Zoology* 61: 372–377.
321. Ryer, C. H. & 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.
322. Chen, W. M., Naruse, M. & 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.
323. Sánchez-Vázquez, F. J., Iigo, M., Madrid, J. A. & 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.
324. Bovet, J. (1994). Missed opportunities in the study of activity and body temperature of beaver (*Castor canadensis*). *Canadian Journal of Zoology* 72: 567–569.
325. Bobbert, A. C. & 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.
326. Hoban, T. M., Levine, A. H., Shane, R. B. & Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
327. Fuller, C. A. & 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.
328. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Feeding time synchronizes primate circadian rhythms. *Physiology and Behavior* 18: 775–779.
329. Green, J., Pollak, C. P. & Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
330. De Castro, J. M. (2001). Heritability of diurnal changes in food intake in free-living humans. *Nutrition* 17: 713–720.
331. De Castro, J. M. (1987). Circadian rhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology and Behavior* 40: 437–446.
332. Gauvin, D. V., Baird, T. J., Vanacek, S. A., Briscoe, R. J., Vallett, M. & 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.
333. Roelfsema, F., van der Heide, D. & Smeenk, D. (1980). Circadian rhythms of urinary electrolyte excretion in freely moving rats. *Life Sciences* 27: 2303–2309.
334. Schmidt, F., Yoshimura, Y., Ni, R. X., Kneesel, S. & 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.
335. Elliott, A. L., Mills, J. N., Minors, D. S. & 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.
336. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N. & Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.
337. Kräuchi, K. & 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.
338. Le Marchand, L., Wilkens, L. R., Harwood, P. & 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.
339. Aschoff, J. (1994). The timing of defecation within sleep-wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
340. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.

341. Hildebrandt, G., Strempel, H. & Will, H. D. (1992). Circadian variations of the subjective habituation to cold pain. *Journal of Interdisciplinary Cycle Research* 23: 222–224.
342. Koch, H. J. & Raschka, C. (2004). Diurnal variation of pain perception in young volunteers using the tourniquet pain model. *Chronobiology International* 21: 171–173.
343. Bellamy, N., Sothern, R. B. & Campbell, J. (2004). Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. *Journal of Rheumatology* 31: 379–389.
344. Bassi, C. J. & Powers, M. K. (1986). Daily fluctuations in the detectability of dim lights by humans. *Physiology and Behavior* 38: 871–877.
345. Chabot, C. C. & Taylor, D. H. (1992). Circadian modulation of the rat acoustic startle response. *Behavioral Neuroscience* 106: 846–852.
346. Page, T. L. & 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.
347. Barrozo, R. B., Minoli, S. A. & 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.
348. Nordin, S., Lotsch, J., Murphy, C., Hummel, T. & Kobal, G. (2003). Circadian rhythm and desensitization in chemosensory event-related potentials in response to odorous and painful stimuli. *Psychophysiology* 40: 612–619.
349. Pickard, G. E. (1987). Circadian rhythm of nociception in the golden hamster. *Brain Research* 425: 395–400.
350. Martínez-Gómez, M., Cruz, Y., Salas, M., Hudson, R. & Pacheco, P. (1994). Assessing pain threshold in the rat: changes with estrus and time of day. *Physiology and Behavior* 55: 651–657.
351. Christina, A. J. M., Merlin, N. J., Vijaya, C., Jayaprakash, S. & Murugesh, N. (2004). Daily rhythm of nociception in rats. *Journal of Circadian Rhythms* 2: art. 2.
352. Rosenfeld, J. P. & Rice, P. E. (1979). Diurnal rhythms in nociceptive thresholds of rats. *Physiology and Behavior* 23: 419–420.
353. Konecka, A. M. & Sroczynska, I. (1998). Circadian rhythm of pain in male mice. *General Pharmacology* 31: 809–810.
354. Hamra, J. G., Kamerling, S. G., Wolfsheimer, K. J. & Bagwell, C. A. (1993). Diurnal variation in plasma ir-β-endorphin levels and experimental pain thresholds in the horse. *Life Sciences* 53: 121–129.
355. Perissin, L., Facchin, P. & Porro, C. A. (2003). Tonic pain response in mice: effects of sex, season and time of day. *Life Sciences* 72: 897–907.
356. Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemistry and Physiology A* 77: 513–517.
357. Díaz-Iglesias, E., Díaz-Herrera, F., Re-Araujo, A. D., Báez-Hidalgo, M., López-Zenteno, M., Valdés-Sáanchez, G. & López-Murillo, A. K. (2004). Temperature preferids y consumo de oxígeno circadiano de la langosta roja, *Panulirus interruptus* (Randall, 1842). *Ciencias Marinas* 30: 169–178.
358. Neill, W, H., Magnuson, J. J. & Chipman, G. G. (1972). Behavioral thermoregulation by fishes: a new experimental approach. *Science* 176: 1443–1445.
359. Beitinger, T. L. & 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.
360. Kavaliers, M. & 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.
361. Schurmann, H. & 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.
362. Innicenti, A., Minutini, L. & Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
363. Tosini, G. & 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.
364. Sievert, L. M. & Hutchison, V. H. (1988). Light versus heat: thermoregulatory behavior in a nocturnal lizard (*Gekko gecko*). *Herpetologica* 44: 266–273.
365. Brown, R. P. (1996). Thermal biology of the gecko *Tarentola boettgeri*: comparisons among populations from different elevations within Gran Canaria. *Herpetologica* 52: 396–405.
366. Regal, P. J. (1967). Voluntary hypothermia in reptiles. *Science* 155: 1551–1553.
367. Cowgell, J. & Underwood, H. (1979). Behavioral thermoregulation in lizards: a circadian rhythm. *Journal of Experimental Zoology* 210: 189–194.
368. Rismiller, P. D. & Heldmaier, G. (1991). Seasonal changes in daily metabolic patterns of *Lacerta viridis*. *Journal of Comparative Physiology B* 161: 482–488.
369. Christian, K. A., Tracy, C. R., & 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.
370. Sievert, L. M. & 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.
371. Jarling, C., Scarperi, M., & 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.

372. Shine, R. & Lambeck, R. (1990). Seasonal shifts in the thermoregulatory behaviour of Australian blacksnakes, *Pseudechis porphyriacus* (Serpentes: Elapidae). *Journal of Thermal Biology* 15: 301–305.

373. 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.

374. Sievert, L. M. & 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.

375. Briese, E. (1985). Rats prefer ambient temperatures out of phase with their body temperature circadian rhythm. *Brain Research* 345: 389–393.

376. Gordon, C. J., Becker, P. & Ali, J. S. (1998). Behavioral thermoregulatory responses of single- and group-housed mice. *Physiology and Behavior* 65: 255–262.

377. Murakami, H. & Kinoshita, K. (1977). Spontaneous activity and heat avoidance of mice. *Journal of Applied Physiology* 43: 573–576.

378. Gordon, C. J. (1993). Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiology and Behavior* 53: 257–263.

379. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T. & Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *Journal of Physiology* 526: 417–424.

380. Beste, R., Kaiser, K., Issing, K. & Hensel, H. (1978). Perception of local thermal stimuli in the course of day. *Pflügers Archiv* 377S: R56.

381. Baldwin, B. A. & Ingram, D. L. (1968). Factor influencing behavioral thermoregulation in the pig. *Physiology and Behavior* 3: 409–415.

382. Christian, K. & Bedford, G. (1996). Thermoregulation by the spotted tree monitor, *Varanus scalaris*, in the seasonal tropics of Australia. *Journal of Thermal Biology* 21: 67–73.

383. 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.

384. Kuriyama, K., Uchiyama, M., Suziki, H., Tagaya, H., Ozaki, A., Aritake, S., Kamei, Y., Nishikawa, T. & Takahashi, K. (2003). Circadian fluctuation of time perception in healthy human subjects. *Neuroscience Research* 46: 23–31.

385. Miró, E., Cano, M. C., Espinosa-Fernández, L. & Buela-Casal, G. (2003). Time estimation during prolonged sleep deprivation and its relation to activation measures. *Human Factors* 45: 148–159.

386. Campbell, S. S., Murphy, P. J. & Boothroyd, C. E. (2001). Long-term time estimation is influenced by circadian phase. *Physiology and Behavior* 72: 589–593.

387. Pati, A. K. & Gupta, S. (1994). Time estimation circadian rhythm in shift workers and diurnally active humans. *Journal of Biosciences* 19: 325–330.

388. Moussay, S., Dosseville, F., Gauthier, A., Larue, J., Sesboüe, B. & Davenne, D. (2002). Circadian rhythms during cycling exercise and finger-tapping task. *Chronobiology International* 19: 1137–1149.

389. Aschoff, J. (1992). On the dilatability of subjective time. *Perspectives in Biology and Medicine* 35(2): 276–280.

390. 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.

391. 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.

392. Lewis, P. A., Miall, R. C., Daan, S. & Kacelnik, A. (2003). Interval timing in mice does not rely upon the circadian pacemaker. *Neuroscience Letters* 348: 131–134.

393. Hancock, P. A. (1993). Body temperature influence on time perception. *Journal of General Psychology* 120: 197–215.

394. Wearden, J. H. & Penton-Voak, I. S. (1995). Feeling the heat: body tempeature and the rate of subjective time revisited. *Quarterly Journal of Experimental Psychology* 48B: 129–141.

395. 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.

396. Dragoi, V., Staddon, J. E. R., Palmer, R. G. & Buhusi, C. V. (2003). Interval timing as an emergent learning property. *Psychological Review* 110: 126–144.

397. Mauk, M. D. & Buonomano, D. V. (2004). The neural basis of temporal processing. *Annual Review of Neuroscience* 27: 307–340.

398. 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.

399. Valentinuzzi, V. S., Kolker, D. E., Vitaterna, M. H., Ferrari, E. A. M., Takahashi, J. S. & 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.

400. Oklejewicz, M., van der Zee, E. A., Gerkema, M. P. & Daan, S. (2001). Memory retention in wild-type and tau mutant Syrian hamsters. *Behaviour* 138: 789–796.

401. Chaudhury, D. & Colwell, C. S. (2002). Circadian modulation of learning and memory in fear-conditioned mice. *Behavioural Brain Research* 133: 95–108.

402. Fernadez, R. I., Lyons, L. C., Levenson, J., Khabour, O. & Eskin, A. (2003). Circadian modulation of long-term sensitization in *Aplysia*. *Proceedings of the National Academy of Sciences U.S.A.* 100: 14415–14420.

403. Winocur, G. & 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.

404. Wagatsuma, A., Sugai, R., Chono, K., Azami, S., Hatakeyama, D., Sadamoto, H. & Itoi, 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.
405. Cajochen, C., Knoblauch, V., Wirz-Justice, A., Kräuchi, K., Graw, P. & Wallach, D. (2004). Circadian modulation of sequence learning under high and low speed pressure conditions. *Behavioural Brain Research* 151: 167–176.
406. 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.
407. Roces, F. & 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.
408. Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A., Teo, S. L. H., Seitz, A., Walli, A. & Fudge, D. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293: 1310–1314.
409. Bennett, K. A., McConnell, B. J. & 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.
410. Kunnasranta, M., Hyvärinen, H., Häkkinen, J. & 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.
411. Handrich, Y., Bevan, R. M., Charrassin, J. B., Butler, P. J., Pütz, K., Woakes, A. J., Lage, J. & Le Maho, Y. (1997). Hypothermia in foraging king penguins. *Nature* 388: 64–67.
412. Butler, P. J. (2001). Diving beyond the limits. *News in Physiological Sciences* 16: 222–227.
413. Sakai, T. & Ishida, N. (2001). Circadian rhythms of female mating activity governed by clock genes in *Drosophila*. *Proceedings of the National Academy of Sciences U.S.A.* 98: 9221–9225.
414. Leon, M., Adels, L., Coopersmith, R. & Woodside, B. (1984). Diurnal cycle of mother-young contact in Norway rats. *Physiology and Behavior* 32: 999–1003.
415. Lerwill, C. J. (1977). Diurnal variations in the agonistic behaviour of the golden hamster (*Mesocricetus auratus*). *Journal of Zoology* 182: 159–161.
416. Pépin, D., Cargnelutti, B., Gonzalez, G., Joachim, J. & Reby, D. (2001). Diurnal and seasonal variations of roaring activity of farmed red deer stags. *Applied Animal Behaviour Science* 74: 233–239.
417. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M. & 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.
418. Van Dongen, H. P. A., Kerkhof, G. A. & Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
419. Kraemer, S., Danker-Hopfe, H., Dorn, H., Schmidt, A., Ehlert, I. & 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.
420. De Gennaro, L., Ferrara, M., Curcio, G. & 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.
421. Wright, K. P., Hull, J. T. & Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *American Journal of Physiology* 283: R1370–R1377.
422. Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M. & Tisak, J. (2002). Investigation of morning-evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.
423. Varkevisser, M. & Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: validating a self-constructed test battery. *Chronobiology International* 20: 109–121.
424. Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A. & Cajochen, C. (2004). Circadian and wake-dependent modulation of fastest and slowest reaction times during psychomotor vigilance task. *Physiology and Behavior* 80: 695–701.
425. Youngstedt, S. D., Perlis, M. L., O'Brien, P. M., Palmer, C. R., Smith, M. T., Orff, H. J. & Kripke, D. F. (2003). No association of sleep with total daily physical activity in normal sleepers. *Physiology and Behavior* 78: 395–401.
426. Watson, D. & Clark, L. A. (1997). Measurement and mismeasurement of mood: recurrent and emergent issues. *Journal of Personality Assessment* 68: 267–297.
427. Mitsutake, G., Otsuka, K., Cornélissen, G., Herold, M., Günther, R., Dawes, C., Burch, J. B., Watson, D. & Halberg, F. (2001). Circadian and infradian rhythms in mood. *Biomedicine and Pharmacotherapy* 55: 94–100.
428. Mitsutake, G., Cornélissen, G., Otsuka, K., Dawes, C., Burch, J., Rawson, M. J., Siegelová, J., Jancik, J., Masek, M., Pazdirek, J. & Halberg, F. (2002). Relationship between positive and negative moods and blood pressure in a clinically healthy man. *Scripta Medica* 75: 315–320.
429. Watson, D., Wiese, D., Vaidya, J. & 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.
430. Murray, G., Allen, N. B. & Trinder, J. (2002). Mood and the circadian system: investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.

431. Kiley, J. P., Eldridge, F. L. & Millhorn, D. E. (1984). Brain, blood and rectal temperature during whole body cooling. *Comparative Biochemistry and Physiology A* 79: 631–634.
432. Brinnel, H. & Cabanac, M. (1989). Tympanic temperature is a core temperature in humans. *Journal of Thermal Biology* 14: 47–53.
433. Kolka, M. A., Quigley, M. D., Blanchard, L. A., Toyota, D. A. & Stephenson, L. A. (1993). Validation of a temperature telemetry system during moderate and strenuous exercise. *Journal of Thermal Biology* 18: 203–210.
434. Audet, D. & Thomas, D. W. (1996). Evaluation of the accuracy of body temperature measurement using external radio transmitters. *Canadian Journal of Zoology* 74: 1778–1781.
435. Fuller, A., Kamerman, P. R., Maloney, S. K., Mitchell, G. & 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.
436. Minard, D. (1970). Body heat content. In: Hardy, J. D., Gagge, A. P. & Stolwijk, J. A. J. (Eds.). *Physiological and Behavioral Temperature Regulation*. Springfield, IL: Charles C Thomas, pp. 345–357.
437. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
438. Kluger, M. J., Conn, C. A., Franklin, B., Freter, R. & Abrams, G. D. (1990). Effect of gastrointestinal flora on body temperature of rats and mice. *American Journal of Physiology* 258: R552–R557.
439. Georgiev, J. (1978). Influence of environmental conditions and handling on the temperature rhythm of the rat. *Biotelemetry and Patient Monitoring* 5: 229–234.
440. Berkey, D. L., Meeuwsen, K. W. & Barney, C. C. (1990). Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. *American Journal of Physiology* 258: R743–R749.
441. Peloso, E., Wachulec, M. & Satinoff, E. (2002). Stress-induced hyperthermia depends on both time of day and light condition. *Journal of Biological Rhythms* 17: 164–170.
442. Bruguerolle, B. & Roucoules, X. (1994). Time-dependent changes in body temperature rhythm induced in rats by brewer's yeast injection. *Chronobiology International* 11: 180–186.
443. Yoshida, Y., Fujiki, N., Nakajima, T., Ripley, B., Matsumura, H., Yoneda, H., Mignot, E. & Nishino, S. (2001). Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activitties. *European Journal of Neuroscience* 14: 1075–1081.
444. Refinetti, R. (1990). Experimentally induced disruption of the diurnal rhythm of body temperature of the rat. *Biotemas* 3(2): 47–58.
445. Fioretti, M. C., Riccardi, C., Menconi, E. & Martini, L. (1974). Control of the circadian rhythm of body temperature in the rat. *Life Sciences* 14: 2111–2119.
446. Halberg, F., Zander, H. A., Houglum, M. W. & Mühlemann, H. R. (1954). Daily variations in tissue mitoses, blood eosinophils and rectal temperatures of rats. *American Journal of Physiology* 177: 361–366.
447. Thornhill, J. A., Hirst, M. & 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.
448. Abrams, R. & Hammel, H. T. (1965). Cyclic variations in hypothalamic temperature in unanesthetized rats. *American Journal of Physiology* 208: 698–702.
449. Miles, G. H. (1962). Telemetering techniques for periodicity studies. *Annals of the New York Academy of Sciences* 98: 858–865.
450. Tanaka, H., Yanase, M., Kanosue, K. & Nakayama, T. (1990). Circadian variation of thermoregulatory responses during exercise in rats. *American Journal of Physiology* 258: R836–R841.
451. Roussel, B., Chouvet, G. & Debilly, G. (1976). Rythmes circadiens des températures internes et ambiance thermique chez le rat. *Pflügers Archiv* 365: 183–189.
452. Tornatzky, W. & Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior* 53: 983–993.
453. Yoda, T., Crawshaw, L. I., Yoshida, K., Su, L., Hosono, T., Shido, O., Sakurada, S., Fukuda, Y. & 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.
454. Isobe, Y., Takaba, S. & Ohara, K. (1980). Diurnal variation of thermal resistance in rats. *Canadian Journal of Physiology and Pharmacology* 58: 1174–1179.
455. Stephenson, R., Liao, K. S., Hamrahi, H. & Horner, R. L. (2001). Circadian rhythms and sleep have additive effects on respiration in the rat. *Journal of Physiology* 536: 225–235.
456. Krieger, D. T. (1974). Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* 95: 1195–1201.
457. Murphy, H. M., Wideman, C. H. & Nadzam, G. R. (2003). A laboratory animal model of human shift work. *Integrative Physiological and Behavioral Science* 38: 316–328.
458. Refinetti, R., Ma, H. & Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
459. Li, H. & Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
460. Spencer, F., Shirer, H. W. & Yochim, J. M. (1976). Core temperature in the female rat: effect of pinealectomy or altered lighting. *American Journal of Physiology* 231: 355–360.
461. Severinsen, T. & Øritsland, N. A. (1991). Endotoxin induced prolonged fever in rats. *Journal of Thermal Biology* 16: 167–171.

462. Leon, L. R., Walker, L. D., DuBose, D. A. & Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
463. Keeney, A. J., Hogg, S. & 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.
464. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D. & Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
465. Berezkin, M. V., Kudinova, V. F., Batygov, A. N., Ponomareva, L. E. & 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.
466. Conn, C. A., Franklin, B., Freter, R. & 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.
467. Connolly, M. S. & Lynch, C. B. (1983). Classical genetic analysis of circadian body temperature rhythms in mice. *Behavior Genetics* 13: 491–500.
468. Nomoto, S., Ohta, M., Kanai, S., Yoshida, Y., Takiguchi, S., Funakoshi, A. & 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.
469. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
470. Chaudhry, A. P., Halberg, F., Keenan, C. E., Harner, R. N. & 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.
471. Watts Jr., R. H. & Refinetti, R. (1996). Circadian modulation of cold-induced thermogenesis in the golden hamster. *Biological Rhythm Research* 27: 87–94.
472. Rubal, A., Choshniak, I. & Haim, A. (1992). Daily rhythms of metabolic rate and body temperature of two murids from extremely different habitats. *Chronobiology International* 9: 341–349.
473. Elvert, R., Kronfeld, N., Dayan, T., Haim, A., Zisapel, N. & 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.
474. Haim, A., Yedidia, I., Haim, D. & 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.
475. Haim, A. & 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.
476. Haim, A., McDevitt, R. M. & 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.
477. Saarela, S. & 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.
478. Halberg, E., Halberg, F., Timm, R. M., Regal, P. J., Cugigni, P. & de Remigis, P. (1982). Socially-related and spontaneous circadian thermo-acrophase shifts in *Rhabdomys pumilio*: complications for chronopharmacologists. In: Takahashi, R., Halberg, F. & Walker, C. A. (Eds.). *Toward Chronopharmacology*. Oxford, UK: Pergamon, pp. 357–368.
479. Haim, A., Ellison, G. T. H. & Skinner, J. D. (1988). Thermoregulatory circadian rhythms in the pouched mouse (*Saccostomus campestris*). *Comparative Biochemistry and Physiology A* 91: 123–127.
480. 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.
481. Muchlinski, A. E., Baldwin, B. C., Padick, D. A., Lee, B. Y., Salguero, H. S. & 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.
482. Refinetti, R. (1996). The body temperature rhythm of the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *Physiological Zoology* 69: 270–275.
483. Haim, A., Downs, C. T. & 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.
484. Winget, C. M., Card, D. H. & Hetherington, N. W. (1968). Circadian oscillations of deep-body temperature and heart rate in a primate (*Cebus albafrons*). *Aerospace Medicine* 39: 350–353.
485. Simpson, S. & 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.
486. Fuller, C. A. (1984). Circadian brain and body temperature rhythms in the squirrel monkey. *American Journal of Physiology* 246: R242–R246.
487. Fuller, C. A., Sulzman, F. M. & 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.
488. Fuller, C. A., Sulzman, F. M. & Moore-Ede, M. C. (1978). Thermoregulation is impaired in an environment without circadian time cues. *Science* 199: 794–796.

489. Wever, R. & Zink, R. A. (1971). Fortlaufende Registrierung der Rectaltemperatur des Menschen unter extremen Bedingungen. *Pflügers Archiv* 327: 186–190.
490. Stephenson, L. A., Wenger, C. B., O'Donovan, B. H. & Nadel, E. R. (1984). Circadian rhythm in sweating and cutaneous blood flow. *American Journal of Physiology* 246: R321–R324.
491. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.
492. 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.
493. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
494. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
495. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K. & Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
496. Souetre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B. & 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.
497. Scales, W. E., Vander, A. J., Brown, M. B. & Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
498. Marotte, H. & Timbal, J. (1981). Circadian rhythm of temperature in man: comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
499. Weitzman, E. D., Moline, M. L., Czeisler, C. A. & Zimmerman, J. C. (1982). Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
500. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T. & Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
501. Kleitman, N. & Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
502. Cisse, F., Martineaud, R. & 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.
503. Shanahan, T. L. & 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.
504. Honma, K., Honma, S., Kohsaka, M. & Fukuda, N. (1992). Seasonal variation in the human circadian rhythm: dissociation between sleep and temperature rhythm. *American Journal of Physiology* 262: R885–R891.
505. Barrett, J., Lack, L. & Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
506. Lee, Y. H. & 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.
507. Pollak, C. P. & Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
508. Honma, K., Honma, S., Nakamura, K., Sasaki, M., Endo, T. & Takahashi, T. (1995). Differential effects of bright light and social cues on reentrainment of human circadian rhythms. *American Journal of Physiology* 268: R528–R535.
509. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J. & 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.
510. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J., Elliott, A. R., Prisk, G. K., West, J. B. & Czeisler, C. A. (2001). Sleep, performance, circadian rhythms, and light-dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
511. Spengler, C. M., Czeisler, C. A. & Shea, S. A. (2000). An endogenous circadian rhythm of respiratory control in humans. *Journal of Physiology* 526: 683–694.
512. Gradisar, M. & 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.
513. Rawson, R. O., Stolwijk, J. A. J., Graichen, H. & Abrams, R. (1965). Continuous radio telemetry of hypothalamic temperatures from unrestrained animals. *Journal of Applied Physiology* 20: 321–325.
514. Refinetti, R. & Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.
515. Kuwabara, N., Seki, K. & 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.
516. Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D. & Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflügers Archiv* 436: 659–666.
517. Ayo, J. O., Oladele, S. B., Ngam, S., Fayomi, A. & 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.

518. Piccione, G., Caola, G. & 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.
519. Jessen, C. & Kuhnen, G. (1996). Seasonal variations of body temperature in goats living in an outdoor environment. *Journal of Thermal Biology* 21: 197–204.
520. Recabarren, S. E., Vergara, M., Llanos, A. J. & Serón-Ferré, M. (1987). Circadian variation of rectal temperature in newborn sheep. *Journal of Developmental Physiology* 9: 399–408.
521. Bligh, J., Ingram, D. L., Keynes, R. D. & 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.
522. Mohr, E. & Krzywanek, H. (1990). Variations of core-temperature rhythms in unrestrained sheep. *Physiology and Behavior* 48: 467–473.
523. Lowe, T. E., Cook, C. J., Ingram. J. R. & Harris, P. J. (2001). Impact of climate on thermal rhythm in pastoral sheep. *Physiology and Behavior* 74: 659–664.
524. Piccione, G., Caola, G. & Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
525. Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
526. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L. & 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.
527. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A. & 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.
528. Hahn, G. L., Chen, Y. R., Nienaber, J. A., Eigenberg, R. A. & Parkhurst, A. M. (1992). Characterizing animal stress through fractal analysis of thermoregulatory responses. *Journal of Thermal Biology* 17: 115–120.
529. Rose, R. W., Swain, R. & 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.
530. Gemmell, R. T., Turner, S. J. & Krause, W. J. (1997). The circadian rhythm of body temperature of four marsupials. *Journal of Thermal Biology* 22: 301–307.
531. Piccione, G., Caola, G. & Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
532. Piccione, G., Caola, G. & Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
533. Fowler, P. A. & 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.
534. McCarron, H. C. K., Buffenstein, R., Fanning, F. D. & 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.
535. Cooper, C. E. & 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.
536. Chevillard-Hugot, M. C., Müller, E. F. & Kulzer, E. (1980). Oxygen consumption, body temperature and heart rate in the coati (*Nasua nasua*). *Comparative Biochemistry and Physiology A* 65: 305–309.
537. Varosi, S. M., Brigmon, R. L. & Besch, E. L. (1990). A simplified telemetry system for monitoring body temperature in small animals. *Laboratory Animal Science* 40: 299–302.
538. Macari, M. (1983). Efeito do cruzamento de suínos sobre o comportamento termoregulador. *Ciência e Cultura* 35: 1145–1150.
539. Bligh, J. & 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.
540. Hildwein, G. & 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.
541. Peters, D. G. & 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.
542. Rashotte, M. E. & 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.
543. Rashotte, M. E., Pastukhov, I. F., Poliakov, E. L. & 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.
544. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. I. Effector mechanisms. *Pflügers Archiv* 386: 173–179.
545. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
546. Bobr, L. W. & 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.

547. Kadono, H. & 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: Springer, pp. 91–99.
548. Wilson, H. R., Mather, F. B., Brigmon, R. L., Besch, E. L., Dugan, V. P. & Boulos, N. Z. (1989). Feeding time and body temperature interactions in broiler breeders. *Poultry Science* 68: 608–616.
549. Michels, H., Herremans, M. & 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.
550. Winget, C. M., Averkin, E. G. & Fryer, T. B. (1965). Quantitative measurement by telemetry of ovulation and oviposition in the fowl. *American Journal of Physiology* 209: 853–858.
551. MacMillen, R. E. & Trost, C. H. (1967). Nocturnal hypothermia in the Inca dove, *Scardafella inca*. *Comparative Biochemistry and Physiology* 23: 243–253.
552. Pickard, G. E., Kahn, R. & Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiology and Behavior* 32: 763–766.
553. Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429.
554. Eastman, C. & Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
555. Deprés-Brummer, P., Metzger, G. & Lévi, F. (1996). Analyses des rythmes de la température corporelle du rat en libre cours. *Pathologie Biologie* 44: 150–156.
556. Kas, M. J. H. & 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.
557. Honnebier, M. B. O. M., Jenkins, S. L. & 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.
558. Reinberg, A., Halberg, F., Ghata, J. & 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.
559. Colin, J., Timbal, J., Boutelier, C., Houdas, Y. & Siffre, M. (1968). Rhythm of the rectal temperature during a 6-month free-running experiment. *Journal of Applied Physiology* 25: 170–176.
560. Menaker, M. (1959). Endogenous rhythms of body temperature in hibernating bats. *Nature* 184: 1251–1252.
561. 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.
562. Jilge, B., Kuhnt, B., Landerer, W. & Rest, S. (2001). Circadian temperature rhythms in rabbit pups and in their does. *Laboratory Animals* 35: 364–373.
563. Kramer, K., Grimbergen, J., Brockway, B., Brockway, B. & Voss, H. P. (1999). Circadian respiratory rate rhythms in freely moving small laboratory animals using radio telemetry. *Lab Animal* 28(4): 38–41.
564. Witte, K., Engelhardt, S., Janssen, B. J. A., Lohse, M. & 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.
565. Henry, R., Casto, R. & Printz, M. P. (1990). Diurnal cardiovascular patterns in spontaneously hypertensive and Wistar-Kyoto rats. *Hypertension* 16: 422–428.
566. Smith, T. L., Coleman, T. G., Stanek, K. A. & Murphy, W. R. (1987). Hemodynamic monitoring for 24 h in unanesthetized rats. *American Journal of Physiology* 253: H1335–H1341.
567. Su, D. F., Julien, C., Kandza, P. & Sassard, J. (1987). Variation circadienne de la sensibilité du baroréflexe chez le rat conscient. *Comptes Rendus de l'Académie des Sciences* 305: 683–686.
568. Brown, G. M. & Seggie, J. (1988). Effects of antidepressants on entrainment of circadian rhythms. *Progress in Neuro-psychopharmacology and Biological Psychiatry* 12: 299–306.
569. Sato, K., Chatani, F. & 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.
570. Halberg, J., Halberg, E., Hayes, D. K., Smith, R. D., Halberg, F., Delea, C. S., Danielson, R. S. & 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.
571. Scheer, F. A. J. L., Ter Horst, G. J., van der Vliet, J. & 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.
572. Matsunaga, T., Harada, T., Mitsui, T., Inokuma, M., Hashimoto, M., Miyauchi, M., Murano, H. & Shibutani, Y. (2001). Spectral analysis of circadian rhythms in heart rate variability of dogs. *American Journal of Veterinary Research* 62: 37–42.
573. Ashkar, E. (1979). Twenty-four-hour pattern of circulation by radiotelemetry in the unrestrained dog. *American Journal of Physiology* 236: R231–R236.
574. Mishina, M., Watanabe, T., Matsuoka, S., Shibata, K., Fujii, K., Maeda, H. & Wakao, Y. (1999). Diurnal variations of blood pressure in dogs. *Journal of Veterinary Medical Sciences* 61: 643–647.
575. Eloranta, E., Norberg, H., Nilsson, A. & Säkkinen, H. (2002). Individually coded telemetry: a tool for studying heart rate and behaviour in reindeer calves. *Acta Veterinaria Scandinavica* 43: 135–144.
576. Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K. & Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s–44s.

577. Scheer, F. A. J. L., van Doornen, L. J. P. & 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.
578. Hermida, R. C., Ayala, D. E., Fernández, J. R., Mojón, A., Alonso, I. & Calvo, C. (2002). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiology International* 19: 461–481.
579. Kumagai, Y., Shiga, T., Sunaga, K., Cornélissen, G., Ebihara, A. & Halberg, F. (1992). Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 19: 43–58.
580. Leary, A. C., Struthers, A. D., Donnan, P. T., MacDonald, T. M. & 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.
581. Viola, A. U., Simon, C., Ehrhart, J., Geny, B., Piquard, F., Muzet, A. & 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.
582. O'Sullivan, C., Duggan, J., Atkins, N. & 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.
583. Van Eekelen, A. P. J., Houtveen, J. H. & Kerkhof, G. A. (2004). Circadian variation in cardiac autonomic activity: reactivity measurements to different types of stressors. *Chronobiology International* 21: 107–129.
584. Hermida, R. C., Fernández, J. R., Ayala, D. E., Mojón, A., Alonso, I. & Calvo, C. (2004). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiology International* 21: 147–160.
585. Hadtstein, C., Wühl, E., Soergel, M., Witte, K. & Schaefer, F. (2004). Normative values for circadian and ultradian cardiovascular rhythms in childhood. *Hypertension* 43: 547–554.
586. Taillard, J., Sanchez, P., Lemoine, P. & 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.
587. Hermida, R. C., Calvo, C., Ayala, D. E., Domínguez, M. J., Covelo, M., Fernández, J. R., Fontao, M. J. & López, J. E. (2004). Administration-time-dependent effects of doxazosin GITS on ambulatory blood pressure of hypertensive subjects. *Chronobiology International* 21: 277–296.
588. Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K. & 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.
589. Jessop, T. S., Limpus, C. J. & Whittier, J. M. (2002). Nocturnal activity in the green sea turtle alters daily profiles of melatonin and corticosterone. *Hormones and Behavior* 41: 357–365.
590. Bertolucci, C., Foà, A. & Van't Hof, T. J. (2002). Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. *Hormones and Behavior* 41: 414–419.
591. Hasegawa, M. & Ebihara, S. (1992). Circadian rhythms of pineal melatonin release in the pigeon measured by *in vivo* microdialysis. *Neuroscience Letters* 148: 89–92.
592. Brandstätter, R., Kumar, V., Abraham, U. & 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 U.S.A.* 97: 12324–12328.
593. Thrun, L. A., Moenter, S. M., O'Callaghan, D., Woodfill, C. J. I. & Karsch, F. J. (1995). Circannual alterations in the circadian rhythm of melatonin secretion. *Journal of Biological Rhythms* 10: 42–54.
594. Guerin, M. V., Deed, J. R., Kennaway, D. J. & 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.
595. Aarseth, J. J., Van't Hof, T. J. & Stokkan, K. A. (2003). Melatonin is rhythmic in newborn seals exposed to continuous light. *Journal of Comparative Physiology B* 173: 37–42.
596. Alila-Johansson, A., Eriksson, L., Soveri, T. & 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.
597. García, A., Landete-Castillejos, T., Zaragata, L., Garde, J. & Gallego, L. (2003). Seasonal changes in melatonin concentrations in female Iberian red deer (*Cervus elaphus hispanicus*). *Journal of Pineal Research* 34: 161–166.
598. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J. & 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.
599. Maywood, E. S., Hastings, M. H., Max, M., Ampleford, E., Menaker, M. & 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.
600. Pitrosky, B., Kirsch, R., Vivien-Roels, B., Georg-Bentz, I., Canguilhem, B. & 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.
601. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N. & Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
602. Vivien-Roels, B., Malan, A., Rettori, M. C., Delagrange, P., Jeanniot, J. P. & Pévet, P. (1998). Daily variations in pineal melatonin concentrations in inbred and outbred mice. *Journal of Biological Rhythms* 13: 403–409.

603. Nakahara, D., Nakamura, M., Iigo, M. & Okamura, H. (2003). Bimodal circadian secretion of melatonin from the pineal gland in a living CBA mouse. *Proceedings of the National Academy of Sciences U.S.A.* 100: 9584–9589.
604. Stepien, J. M. & Kennaway, D. J. (2001). Phase response relationships between light pulses and melatonin rhythms in rats. *Journal of Biological Rhythms* 16: 234–242.
605. Challet, E., Pévet, P., Vivien-Roels, B. & 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.
606. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M. & 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.
607. Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., van der Vliet, J., van Heijningen, C., Simonneaux, V., Pévet, P. & Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. *European Journal of Neuroscience* 17: 221–228.
608. McEachron, D. L., Kripke, D. F., Sharp, F. R., Lewy, A. J. & McClellan, D. E. (1985). Lithium effects on selected circadian rhythms in rats. *Brain Research Bulletin* 15: 347–350.
609. Barassin, S., Saboureau, M., Kalsbeek, A., Bothorel, B., Vivien-Roels, B., Malan, A. Buijs, R. M., Guardiola-Lemaitre, B. & Pévet, P. (1999). Interindividual differences in the pattern of melatonin secretion of the Wistar rat. *Journal of Pineal Research* 27: 193–201.
610. Sun, X., Liu, T., Deng, J. & Borjigin, J. (2003). Long-term *in vivo* pineal microdialisis. *Journal of Pineal Research* 35: 118–124.
611. Martin, S. K. & 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.
612. Voultsios, A., Kennaway, D. J. & Dawson, D. (1997). Salivary melatonin as a circadian phase marker: validation and comparison to plasma melatonin. *Journal of Biological Rhythms* 12: 457–466.
613. Wright, K. P., Hughes, R. J., Kronauer, R. E., Dijk, D. J. & 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 U.S.A.* 98: 14027–14032.
614. Jelínková-Vondrasová, D., Hájek, I. & 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.
615. Lindblom, N., Heiskala, H., Hätönen, T., Mustanoja, S., Alfthan, H., Alila-Johansson, A. & Laakso, M. L. (2000). No evidence for extraocular light induced phase shifting of human melatonin, cortisol and thyrotropin rhythms. *NeuroReport* 11: 713–717.
616. Roden, M., Koller, M., Pirich, K., Vierhapper, H. & Waldhauser, F. (1993). The circadian melatonin and cortisol secretion pattern in permanent night shift workers. *American Journal of Physiology* 265: R261–R267.
617. Whitson, P. A., Putcha, L., Chen, Y. M. & Baker, E. (1995). Melatonin and cortisol assessment of circadian shifts in astronauts before flight. *Journal of Pineal Research* 18: 141–147.
618. Rajaratnam, S. M. W., Dijk, D. J., Middleton, B., Stone, B. M. & 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.
619. Selmaoui, B. & 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.
620. Goichot, B., Weibel, L., Chapotot, F., Gronfier, C., Piquard, F. & 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.
621. Kelly, T. L., Neri, D. F., Grill, J. T., Ryman, D., Hunt, P. D., Dijk, D. J., Shanahan, T. L. & Czeisler, C. A. (1999). Nonentrained circadian rhythms of melatonin in submariners scheduled to an 18-hour day. *Journal of Biological Rhythms* 14: 190–196.
622. Hack, L. M., Lockley, S. W., Arendt, J. & 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.
623. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M. & 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.
624. Ganong, W. F. (2001). *Review of Medical Physiology*, 20th Edition. New York: McGraw-Hill.
625. Hadley, M. E. (2000). *Endocrinology*, 5th Edition. Upper Saddle River, NJ: Prentice Hall.
626. Irvine, C. H. G. & Alexander, S. L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology* 11: 227–238.
627. Suzuki, M., Uchida, S., Ueda, K., Tobayama, T., Katsumata, E., Yoshioda, M. & 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.
628. Verhagen, L. A. W., Pévet, P., Saboureau, M., Sicard, B., Nesme, B., Claustrat, B., Buijs, R. M. & 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.

629. Halberg, F., Barnum, C. P., Silber, R. H. & 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.

630. Scheving, L. E., Tsai, T. H., Powell, E. W., Pasley, J. N., Halberg, F. & 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.

631. Marcilhac, A., Maurel, D., Anglade, G., Ixart, G., Mekaouche, M., Héry, F. & 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.

632. Kalsbeek, A., Fliers, E., Romijn, J. A., la Fleur, S. E., Wortel, J., Bakker, O., Endert, E. & Buijs, R. M. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685.

633. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O. & Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.

634. Ruiter, M., La Fleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A. & 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.

635. Mitome, M., Honma, S., Yoshihara, T. & 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.

636. Greco, A. M., Gambardella, P., Sticchi, R., D'Aponte, D., di Renzo, G. & de Franciscis, P. (1989). Effects of individual housing on circadian rhythms of adult rats. *Physiology and Behavior* 45: 363–366.

637. Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J. & 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.

638. Mohawk, J. A., Cashen, K. & Lee, T. M. (2005). Inhibiting cortisol response accelerates recovery from a photic phase shift. *American Journal of Physiology* 288: R221–R228.

639. Van Eekelen, A. P. J., Kerkhof, G. A. & van Amsterdam, J. G. C. (2003). Circadian variation in cortisol reactivity to acute stressor. *Chronobiology International* 20: 863–878.

640. Dzaja, A., Dalal, M. A., Himmerich, H., Uhr, M., Pollmächer, T. & Schuld, A. (2004). Sleep enhances nocturnal plasma ghrelin levels in healthy subjects. *American Journal of Physiology* 286: E963–E967.

641. Holmbäck, U., Forslund, A., Lowden, A., Forslund, J., Åkerstedt, T., Lennernäs, M., Hambreus, L. & Stridsberg, M. (2003). Endocrine responses to nocturnal eating: possible implications for night work. *European Journal of Nutrition* 42: 75–83.

642. Weibel, L., Spiegel, K., Follenius, M., Ehrhart, J. & Branderberger, G. (1996). Internal dissociation of the circadian markers of the cortisol rhythm in night workers. *American Journal of Physiology* 270: E608–E613.

643. Bao, A. M., Ji, Y. F., van Someren, E. J. W., Hofman, M. A., Liu, R. Y. & 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.

644. Koenigsberg, H. W., Teicher, M. H., Mitropoulou, V., Navalta, C., New, A. S., Trestman, R. & 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.

645. Hillman, W. S. (1970). Carbon dioxide output as an index of circadian timing in *Lemna* photoperiodism. *Plant Physiology* 45: 273–279.

646. Gromysz-Kalkowska, K. & Szubartowska, E. (1984). Oxygen consumption in two terrestrial species of crustaceans (Isopoda). *Bulletin of the Polish Academy of Sciences, Biological Sciences* 32: 47–56.

647. Gromysz-Kalkowska, K., Oder, M. & Szubartowska, E. (1984). Oxygen consumption in *Armadillidium nasutum* Buddle-Lund (Isopoda). *Folia Biologica Kraków* 32: 89–108.

648. Aguzzi, J., Company, J. B., Sardà, F. & 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.

649. Kronenberg, F. & Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *Journal of Comparative Physiology* 148: 65–76.

650. Takahashi-del-Bianco, M., Benedito-Silva, A. A., Hebling, J. A., Marques, N. & Marques, M. D. (1992). Circadian oscillatory patterns of oxygen uptake in individual workers of the ant *Camponotus rufipes*. *Physiological Entomology* 17: 377–383.

651. Moritz, R. F. A. & Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.

652. Chakraborty, S. C., Ross, L. G. & Ross, B. (1992). The effect of photoperiod on the resting metabolism of carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology A* 101: 77–82.

653. Aschoff, J. & Pohl, H. (1970). Rhythmic variations in energy metabolism. *Federation Proceedings* 29: 1541–1552.

654. Prinzinger, R., Schäfer, T. & 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.

655. Randolph, J. C. (1980). Daily metabolic patterns of short-tailed shrews (*Blarina*) in three natural seasonal temperature regimes. *Journal of Mammalogy* 61: 628–638.
656. Henken, A. M., Brandsma, H. A., van der Hel, W. & 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.
657. Verstegen, M. W. A., van der Hel, W., Duijghuisen, R., & Geers, R. (1986). Diurnal variation in thermal demand of growing pigs. *Journal of Thermal Biology* 11: 131–135.
658. Kemp, B., de Greef-Lammers, F. J., Verstegen, M. W. A. & van der Hel, W. (1990). Some aspects of daily pattern in thermal demand of breeding boars. *Journal of Thermal Biology* 15: 103–108.
659. Armitage, G., Harris, R. B. S., Hervey, G. R. & 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.
660. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G. & Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
661. Carlisle, H. J., Wilkinson, C. W., Laudenslager, M. L. & Keith, L. D. (1979). Diurnal variation of heat intake in ovariectomized, steroid-treated rats. *Hormones and Behavior* 12: 232–242.
662. 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.
663. Hervey, G. R., Hussain, S. H., Rayfield, K. M., Tobin, G. & Whitaker, E. M. (1987). The role of thyroid hormones in the metabolic response to cold in the rat. *Journal of Physiology* 386: 73P.
664. Tschöp, M., Smiley, D. L. & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407: 908–913.
665. Stupfel, M., Gourlet, V., Court, L., Mestries, J., Parramon, A. & 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.
666. Vogt, F. D. & Lynch, G. R. (1982). Influence of ambient temperature, nest availability, huddling, and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus*. *Physilogical Zoology* 55: 56–63.
667. Haim, A., Saarela, S., Hohtola, E. & 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.
668. 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.
669. Kavanau, J. L. (1998). Vertebrates that never sleep: implications for sleep's basic function. *Brain Research Bulletin* 46: 269–279.
670. Naylor, E., Bergmann, B. M., Krauski, K., Zee, P. C., Takahashi, J. S., Vitaterna, M. H. & Turek, F. W. (2000). The circadian clock mutation alters sleep homeostasis in the mouse. *Journal of Neuroscience* 20: 8138–8143.
671. Wisor, J. P., O'Hara, B. F., Terao, A., Selby, C. P., Kilduff, T. S., Sancar, A., Edgar, D. M. & Franken, P. (2002). A role for *cryptochromes* in sleep regulation. *BMC Neuroscience* 3: art. 20.
672. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P. & Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
673. Franken, P., Dijk, D. J., Tobler, I. & 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.
674. Adrien, J., Dugovic, C. & Martin, P. (1991). Sleep-wakefulness patterns in the helpless rat. *Physiology and Behavior* 49: 257–262.
675. Stephan, F. K. & 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.
676. Deboer, T., Vansteensel, M. J., Détári, L. & Meijer, J. H. (2003). Sleep states alter activity of suprachiasmatic nucleus neurons. *Nature Neuroscience* 6: 1086–1090.
677. Tzischinsky, O. & Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.
678. Hayashi, M., Morikawa, T. & Hori, T. (2002). Circasemidian 12 h cycle of slow wave sleep under constant darkness. *Clinical Neurophysiology* 113: 1505–1516.
679. Tan, X., Uchiyama, M., Shibui, K., Tagaya, H., Suzuki, H., Kamei, Y., Kim, K., Aritaka, S., Ozaki, A. & Takahashi, K. (2003). Circadian rhythms in human's delta sleep electroencephalogram. *Neuroscience Letters* 344: 205–208.
680. 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.
681. Lancel, M., van Riezen, H. & 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.
682. Marks, G. A. & 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.
683. Baranczuk, R. & Greenwald, G. S. (1973). Peripheral levels of estrogen in the cyclic hamster. *Endocrinology* 92: 805–812.
684. Seegal, R. F. & Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. *Biology of Reproduction* 12: 223–231.

685. Stetson, M. H. & Gibson, J. T. (1977). The estrous cycle in golden hamsters: a circadian pacemaker times preovulatory gonadotropin release. *Journal of Experimental Zoology* 201: 289–294.
686. Duvilanski, B. H., Alvarez, M. P., Castrillón, P. O., Cano, P. & 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.
687. Stern, J. M. & Reichlin, S. (1990). Prolactin circadian rhythm persists throughout lactation in women. *Neuroendocrinology* 51: 31–37.
688. Inoue, S., Satoh, S., Saito, M., Naitoh, M., Suzuki, H. & 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.
689. Wasan, K. M., Brunner, L. J., Berens, K. L., Meltzer, A. A. & Luke, D. R. (1989). Circadian assessment of lipids in the hyperphagic obese rat compared with lean litter-mates. *Chronobiology International* 6: 223–228.
690. Randeva, H. S., Karteris, E., Lewandowski, K. C., Sailesh, S., O'Hare, P. & Hillhouse, E. W. (2003). Circadian rhythmicity of salivary leptin in healthy subjects. *Molecular Genetics and Metabolism* 78: 229–235.
691. Stephenson, R., Mohan, R. M., Duffin, J. & Jarsky, T. M. (2000). Circadian rhythms in the chemoreflex control of breathing. *American Journal of Physiology* 278: R282–R286.
692. Terman, M. & Terman, J. (1985). A circadian pacemaker for visual sensitivity? *Annals of the New York Academy of Sciences* 453: 147–161.
693. Cellier-Michel, S. & Berthon, J. L. (2003). Rhythmicity of the pigments in the compound eye of *Daphnia longispina* (Cladocera). *Journal of Freshwater Ecology* 18: 445–450.
694. Hankins, M. W., Jones, R. J. M. & Ruddock, K. H. (1998). Diurnal variation in the *b*-wave implicit time of the human electroretinogram. *Visual Neuroscience* 15: 55–67.
695. Hankins, M. W., Jones, S. R., Jenkins, A. & 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.
696. Pardo, B. F. & 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.
697. Miranda-Anaya, M., Bartell, P. A., Yamazaki, S. & Menaker, M. (2000). Circadian rhythm of ERG in *Iguana iguana*: role of the pineal. *Journal of Biological Rhythms* 15: 163–171.
698. Doyle, S. E., Grace, M. S., McIvor, W. & Menaker, M. (2002). Circadian rhythms of dopamine in mouse retina: the role of melatonin. *Visual Neuroscience* 19: 593–601.
699. Halberg, F. & 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.
700. Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S. & 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 U.S.A.* 90: 5672–5676.
701. Mihalcescu, I., Hsing, W. & Leibler, S. (2004). Resilient circadian oscillator revealed in individual cyanobacteria. *Nature* 430: 81–85.
702. Miller, R. P., Martinson, K. B., Sothern, R. B., Durgan, B. R. & 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.
703. Yen, U. C., Huang, T. C. & Yen, T. C. (2004). Observation of the circadian photosynthetic rhythm in cyanobacteria with a dissolved-oxygen meter. *Plant Science* 166: 949–952.
704. Hennessey, T. L. & Field, C. B. (1992). Evidence of multiple circadian oscillators in bean plants. *Journal of Biological Rhythms* 7: 105–113.
705. Vaadia, Y. (1960). Autonomic diurnal fluctuations in rate of exudation and root pressure of decapitated sunflower plants. *Physiologia Plantarum* 13: 701–717.
706. Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-ike, N. & 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.
707. Feldman, J. F. & Hoyle, M. N. (1973). Isolation of circadian clock mutants of *Neurospora crassa*. *Genetics* 75: 605–613.
708. Vitalini, M. W., Morgan, L. W., March, I. J. & Bell-Pedersen, D. (2004). A genetic selection for circadian output pathway mutations in *Neurospora crassa*. *Genetics* 167: 119–129.
709. Matsuo, T., Yamaguchi, S., Mitsui, S., Emi, A., Shimoda, F. & Okamura, H. (2003). Control mechanism of the circadian clock for timing of cell division *in vivo*. *Science* 302: 255–259.
710. Von der Heide, F., Wilkens, A. & 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.
711. Perkins, M. (1931). Light of glow-worms. *Nature* 128: 905.
712. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences U.S.A.* 40: 1018–1029.
713. 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.
714. Myers, E. M., Yu, J. & Sehgal, A. (2003). Circadian control of eclosion: interaction between a central and peripheral clock in *Drosophila melanogaster*. *Current Biology* 13: 526–533.

715. Paranjpe, D. A., Anitha, D., Joshi, A. & 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.
716. Johnson, C. R. (1971). Daily variation in the thermal tolerance of *Litoria caerulea* (Anura: Hylidae). *Comparative Biochemistry and Physiology A* 40: 1109–1111.
717. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K. & Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *Journal-Lancet* 71: 312–319.
718. Halberg, F., Visscher, M. B. & Bittner, J. J. (1953). Eosinophil rhythm in mice: range of occurrence, effects of illumination, feeding and adrenalectomy. *American Journal of Physiology* 174: 109–122.
719. Nelson, W., Scheving, L. & Halberg, F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *Journal of Nutrition* 105: 171–184.
720. Héroux, O., Bhasin, J., Jobin, M. & 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.
721. Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C. & 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.
722. Dugan, R. E., Slakey, L. L., Briedis, A. V. & 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.
723. Lanza-Jacoby, S., Stevenson, N. R. & 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.
724. Jurevics, H., Hostettler, J., Barrett, C., Morell, P. & 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.
725. Liu, J. H. K. & Dacus, A. C. (1991). Endogenous hormonal changes and circadian elevation of intraocular pressure. *Investigative Ophthalmology and Visual Science* 32: 496–500.
726. Aihara, M., Lindsey, J. D. & Weinreb, R. N. (2003). Twenty-four-hour pattern of mouse intraocular pressure. *Experimental Eye Research* 77: 681–686.
727. Scheving, L. E., Vedral, D. F. & Pauly, J. E. (1968). A circadian susceptibility rhythm in rats to pentobarbital sodium. *Anatomical Record* 160: 741–750.
728. Rebuelto, M., Ambros, L., Waxman, S. & Montoya, L. (2004). Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. *Chronobiology International* 21: 591–600.
729. Ohtsuka-Isoya, M., Hayashi, H. & Shinoda, H. (2001). Effect of suprachiasmatic nucleus lesion on circadian dentin increment in rats. *American Journal of Physiology* 280: R1364–R1370.
730. Williams, R. L., Soliman, K. F. A. & Mizinga, K. M. (1993). Circadian variation in tolerance to the hypothermic action of CNS drugs. *Pharmacology, Biochemistry and Behavior* 46: 283–288.
731. Saitoh, T., Watanabe, Y., Kubo, Y., Shinagawa, M., Otsuka, K., Ohkawa, S. I. & Watanabe, T. (2002). Effect of H2 blockers on the circadian rhythm of intragastric acidity. *Biomedicine and Pharmacotherapy* 56: 349s–352s.
732. Piccione, G., Assenza, A., Grasso, F. & 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.
733. La Fleur, S. E., Kalsbeek, A., Wortel, J., Fekkes, M. L. & Buijs, R. M. (2001). A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. *Diabetes* 50: 1237–1243.
734. Armstrong, S., Clarke, J. & Coleman, G. (1978). Light-dark variation in laboratory rat stomach and small intestine content. *Physiology and Behavior* 21: 785–788.
735. Khaldoun, M., Khaldoun, T., Mellado, M., Cambar, J. & 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.
736. Mahoney, M., Bult, A. & 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.
737. Glass, J. D., Tardif, S. D., Clements, R. & Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
738. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S. & 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.
739. Sheeba, V., Chandrashekaran, M. K., Joshi, A. & Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *Journal of Insect Physiology* 48: 25–32.
740. Rajaratnam, S. M. W. & Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
741. Aschoff, J. (1994). Naps as integral parts of the wake time within the human sleep-wake cycle. *Journal of Biological Rhythms* 9: 145–155.
742. Czeisler, C. A., Duffy, J. F., Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M., Silva, E. J., Allan, J. S., Emens, J. S., Dijk, D. J. & Kronauer, R. E. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177–2181.

743. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A. & 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.
744. Wever, R. A. (1989). Light effects on human circadian rhythms: a review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
745. Honma, K., Honma, S. & Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
746. Lockley, S. W., Skene, D. J., Butler, L. J. & Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 22: 616–623.
747. Sack, R. L., Brandes, R. W., Kendall, A. R. & Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
748. Page, T. L., Mans, C. & Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.
749. Shimomura, K., Nelson, D. E., Ihara, N. L. & Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
750. Ralph, M. R. & Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
751. Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
752. Daan, S. & 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.
753. Rosenberg, R. S., Zee, P. C. & Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.
754. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
755. Refinetti, R., Nelson, D. E. & 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.
756. Von Gall, C., Duffield, G. E., Hastings, M. H., Kopp, M. D. A., Dehghani, F., Korf, H. W. & 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.
757. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S. & 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.
758. Benloucif, S. & 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.
759. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences U.S.A.* 59: 414–421.
760. Puchalski, W. & Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.
761. Hamasaka, Y., Watari, Y., Arai, T., Numata, H. & 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.
762. Summer, T. L., Ferraro, J. S. & McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
763. Canal-Corretger, M. M., Vilaplana, J., Cambras, T. & 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.
764. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M. & Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.
765. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M. & 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.
766. Underwood, H. (1983). Circadian pacemakers in lizards: phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
767. Terai, Y., Asayama, M., Ogawa, T., Sugenoya, J. & Miyagawa, T. (1985). Circadian variation of preferred environmental temperature and body temperature. *Journal of Thermal Biology* 10: 151–156.
768. Kattapong, K. R., Fogg, L. F. & Eastman, C. I. (1995). Effect of sex, menstrual phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International* 12: 257–266.
769. Song, X., Körtner, G. & Geiser, F. (1998). Temperature selection and use of torpor by the marsupial *Sminthopsis macroura*. *Physiology and Behavior* 64: 675–682.

Part III

Mechanisms

A photographic collage of a golden hamster and a clock symbolizing the mechanism of circadian timing. (Photographs and collage by R. Refinetti.)

6 Endogenous Mechanisms

CHAPTER OUTLINE

6.1 Endogenous Rhythmicity
6.2 Inheritance Mechanisms
6.3 Single or Multiple Oscillators

6.1 ENDOGENOUS RHYTHMICITY

Part II of this book looked at the phenomenology of biological rhythms. Ultradian and infradian rhythms were examined in Chapter 4, and daily and circadian rhythms were discussed in Chapter 5. Part II showed that nearly every biological function ever measured exhibits daily rhythmicity. While all daily rhythms are modulated by the alternation of day and night, many rhythms are generated endogenously. Under constant environmental conditions, the rhythms freerun with periods slightly different from 24.0 hours. For example, the rhythm of locomotor activity can freerun in numerous species of invertebrates,[1–13] reptiles,[14–21] fishes,[22–25] birds,[26–39] and mammals,[40–91] including humans.[92–102]

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. In addition, circadian rhythms have been recorded in humans kept in underground bunkers and caves,[100,103] as well as in space.[104–106] Also, free-running rhythms were recorded in people living in Arctic and Antarctic field camps, where Earth's influence is just as strong but continuous sunlight exists throughout the summer and continuous darkness occurs throughout the winter.[95,96]

6.1.1 The Concept of a Pacemaker

If circadian rhythms are endogenously generated, a clock that generates them must exist. Everyone knows what a clock is, but no one expects to find a clock such as that shown in Figure 6.1 inside an organism. Let me clarify what circadian physiologists mean by the word *clock*. Three basic elements of time measurement include 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. The three circles in Figure 6.2 represent these elements. The lower circle represents the ability to undergo a constant change of state

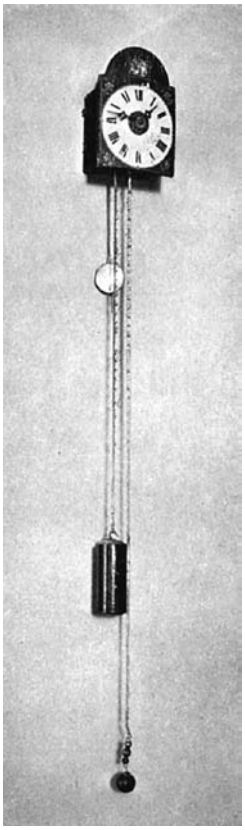


FIGURE 6.1 An old clock. This pendulum clock is a good example of a device that measures the passage of time. (Source: © ArtToday, Tucson, AZ.)

over time. Many entities in the world do not demonstrate this capability. For example, 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*. Two examples of timers include an hourglass and a mechanical count-down timer.

The circle on the right in Figure 6.2 represents the ability to display absolute time. For our purposes absolute time can be defined as "time in relation to the Earth's position relative to the Sun." The ability to display absolute time, then, 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 a basic clock.

The circle on the left in Figure 6.2 represents the ability to generate a self-sustaining oscillation (that is, the

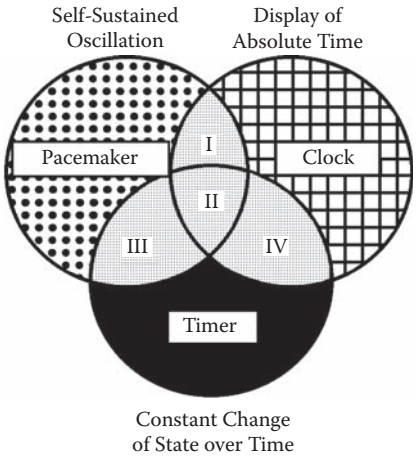


FIGURE 6.2 Timing instruments. A pacemaker is an instrument capable of generating a self-sustained oscillation. A clock is an instrument that can display absolute time. A timer is an instrument that undergoes a constant change of state over time. The functions of these instruments may overlap, but no special names exist for the overlaps.

ability to repeat a process over and over without external input). This property is sufficient to define a *pacemaker*. A drummer in a rock-and-roll band is an example of a basic pacemaker. Note 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.

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.[107,108] A clepsydra was a ceramic pot with a small hole at the bottom. Etchings on the inside of the pot allowed the 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. With appropriate calibration, it could also display absolute time and, therefore, qualified as a clock (grey area 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 (grey area III). Most wristwatches available today are sophisticated instruments that display absolute time and possess a mechanism that undergoes a constant change of state over time as part of a self-sustained oscillatory process (grey area 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 — mechanisms that do *not* possess the properties of a pacemaker or a clock. The term *pacemaker* (or *oscillator*) is used to describe pure pacemakers as well as pacemaker–timer combinations. The term *clock* is often used in a nontechnical sense that includes timer, pacemaker, clock, and any of their combinations.[109] 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*, because the circadian pacemaker, when synchronized to the alternation of day and night, can estimate the 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.

Part IV of this book examines the anatomical identity of the circadian pacemaker and dissects its functional properties. This section, however, examines the concept of a pacemaker in general functional terms. The central question is: How does the circadian pacemaker "generate" time? The answer to this question can be simple *and* complex. Because all biochemical reactions take place over time, time 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 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.[110]

Although the full ensemble of biochemical reactions responsible for the circadian pacemaker is still not known, great progress has been achieved recently, as discussed in Chapter 12. The fundamental requirement for the creation of a biological pacemaker is a negative feedback loop with delay.[111] If, for example, 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 is rather simple and requires no magical elements. Analogies based on water flow and electricity help illustrate the concept. In the watermill in Figure 6.3, the constant flow of water falling on the wooden wheel causes the wheel to spin, creating a circular movement that runs the mill. The repetitive movement of the wheel allows the watermill to be used as a timer or even as a clock (see Exercise 6.1). It is not a pacemaker, however, because it requires an external source (the flow of water).

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 (which deactivates the relay's coil). Of

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. (Source: © ArtToday, Tucson, AZ.)

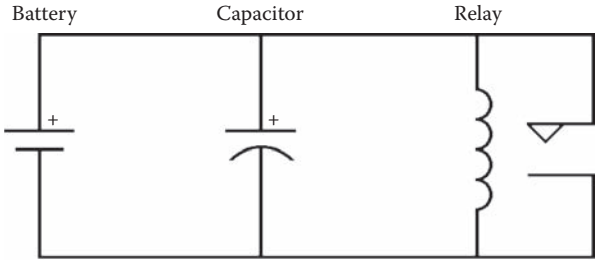


FIGURE 6.4 Let the current flow. This example of a simple pacemaker shows an unpretentious electronic circuit composed of a battery, a capacitor, and a relay.

course, nothing happens if the actions are instantaneous (that is, the relay closes and opens at the same time, which is impossible). The capacitor adds the necessary delay in the feedback loop, creating a cycle of opening and closing of the relay that repeats itself over and over until the battery dies (see Exercise 6.2).

In the early 1900s, a Dutch electrical engineer, 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.*[112,113] He used his mathematical model to study the cardiac pacemaker, and many biologists since then have used the model to study the circadian pacemaker.[114–119] This topic is discussed further in Chapter 7.

6.1.2 Free-Running Rhythms

Although the existence of free-running circadian rhythms was extensively documented in Chapter 5, the discussion

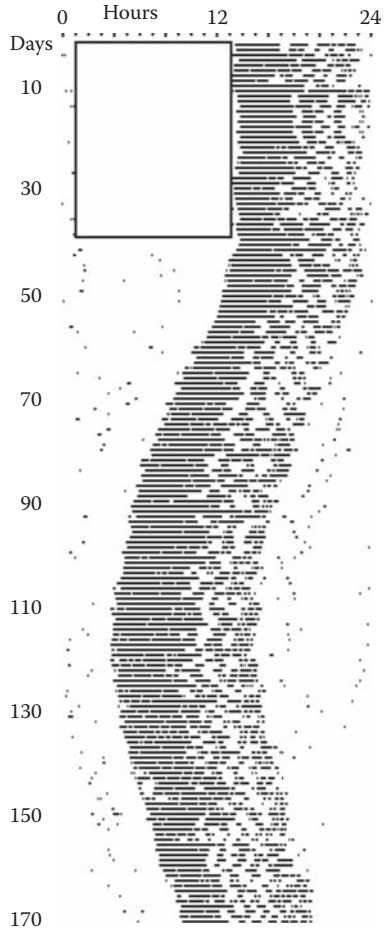


FIGURE 6.5 Run-away mouse. This actogram shows the running wheel activity records of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle for 6 weeks and in constant darkness for over 4 months. The rectangle indicates the light phase of the light–dark cycle. Note that the period of the activity rhythm differs from 24 hours in the absence of the light–dark cycle. (If you are not familiar with actograms, refer to Figure 3.19 in Chapter 3.) (Source: Archives of the Refinetti lab.)

did not address the issue of how long the rhythms can freerun. It was simply assumed that the rhythms would freerun for the life of the organism. Long free-running rhythms under constant environmental conditions have been documented in various species.[28,31,74,77,120–124] Figure 6.5 shows records of running-wheel activity of a domestic mouse (*Mus musculus*) over many months. 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. The mouse ran an average of 6 km each day, "traveling" more than 1000 km (600 miles) during the 170 days of the study. If the mouse had been running outdoors, in one direction, it could have traveled from Charleston, South Carolina, to Miami, Florida (or, for our European readers, from Paris

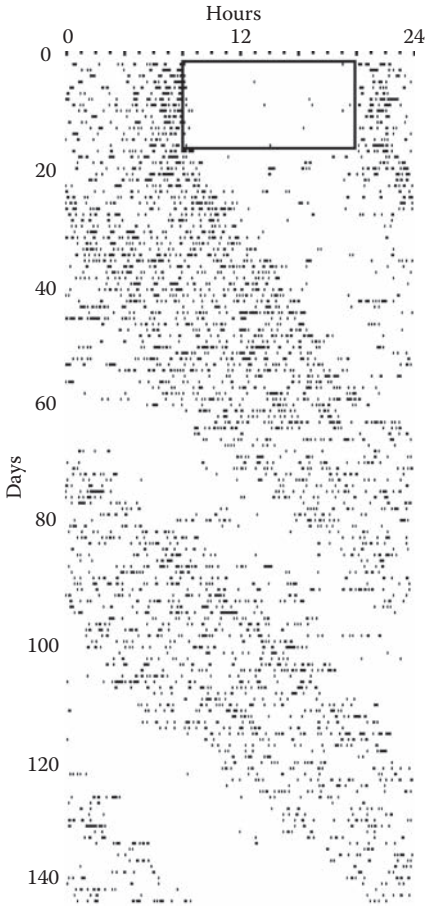


FIGURE 6.6 Run-away rat. This actogram shows the running-wheel activity records of a laboratory rat (*Rattus norvegicus*) maintained under a light–dark cycle for 2 weeks and in constant darkness for 4 months. The rectangle indicates the light phase of the light–dark cycle. Note that the period of the activity rhythm is consistently longer than 24 hours in the absence of the light–dark cycle. (Source: Archives of the Refinetti lab.)

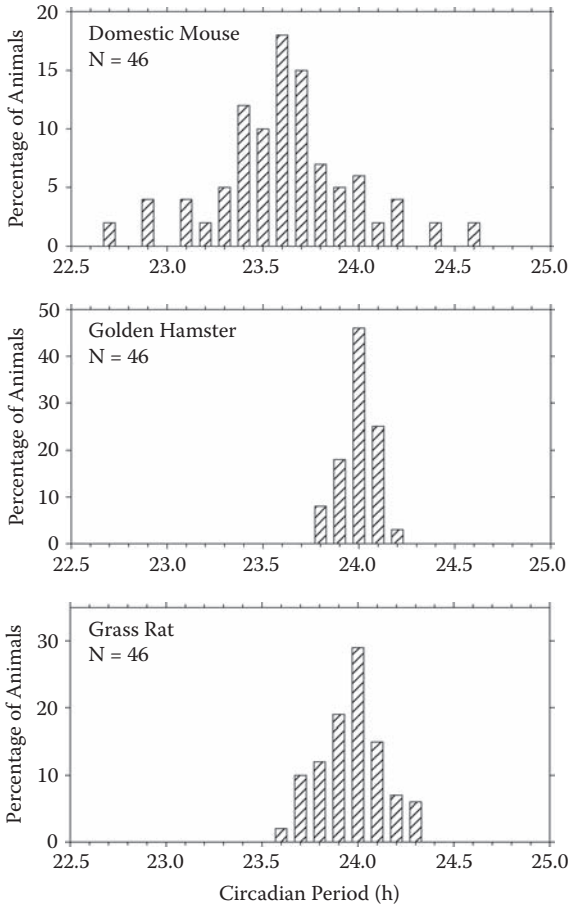


FIGURE 6.7 How long is your period? The graphs show the distributions of free-running periods of 46 individuals of three species of small rodents. Note that the species differ in their average periods and in the spread of the period distributions. (Sources: Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107; and 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.)

to Vienna)! In Figure 6.5, the change in length (shortening followed by lengthening) of the free-running period is not unusual, but it is not typical either. For example, the activity records of a laboratory rat (*Rattus norvegicus*) (Figure 6.6) exhibit a very stable circadian period of 24.4 hours throughout the 4-month freerun. I must acknowledge, however, that immediately after the rat was released from the 24.0-hour light–dark cycle, its 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 is 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 species of rodents: the domestic mouse (*Mus musculus*), the golden hamster (*Mesocricetus auratus*), and the Nile grass rat (*Arvicanthis niloticus*). Golden hamsters have a longer free-running period than mice (24.04 hours *versus* 23.62 hours), and the 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. Table 6.1 shows the mean free-running periods of 50 animal species, including invertebrates, fishes, reptiles, birds, and mammals. The species are listed in alphabetical order by Latin name. Consult the *Organisms Used* appendix if you need help identifying the common names. The mean free-running period for all species combined is 23.86 hours with a standard deviation of 0.65 hours. Thus, the mean free-running period is only 8 minutes shorter than the duration of a day, and the mean deviation from

TABLE 6.1
Circadian Period in Constant Darkness[a]

Species[b]	Period (h)	Sources[d]
Aotus trivirgatus	23.6	135
Apis cerana	23.1	11
Apis mellifera	22.8	347
Arvicanthis ansorgei	24.1	348
Arvicanthis niloticus	23.8	349
Callithrix jacchus	23.3	350
Carassius auratus	25.0	23, 351
Cavia porcellus	23.6	141, 352
Clethrionomys rutilus	23.9	136
Columba livia	23.1	29
Coturnix coturnix	23.1	132, 141, 272, 353
Danio rerio	25.0	22
Dasyuroides byrnei	23.7	91
Dimorphostylis asiatica	24.9	2
Dipodomys merriami	24.0	17
Drosophila melanogaster	24.3	12, 354
Eutamias sibiricus	23.9	294
Felis catus	23.9	79
Funambulus pennanti	23.4	355
Gekko gecko	23.1	19
Georychus capensis	24.3	296, 356
Glaucomys volans	23.8	71
Glyphiulus cavernicolus	25.7	6
Homo sapiens[c]	24.5	92–96, 101, 102, 170, 319, 320, 322, 330, 332, 333, 357–362
Iguana iguana	24.0	14, 15, 129
Leucophaea maderae	23.3	363
Lycosa tarentula	24.1	1
Macaca mulatta	23.8	364
Macaca nemestrina	23.0	87
Mesocricetus auratus	24.0	67, 70, 122, 243, 247, 252, 365–367
Microcebus murinus	23.0	83, 153, 368
Microtus arvalis	23.5	139
Mus booduga	23.4	369, 370
Mus musculus	23.6	52, 54, 55, 57, 59, 60, 365, 367, 371–373
Octodon degus	23.5	167, 325, 374
Oryctolagus cuniculus	23.9	76
Passer domesticus	24.8	31, 33, 375
Perognathus longimembris	23.6	376
Peromyscus leucopus	24.0	365
Peromyscus maniculatus	22.9	365
Phodopus sungorus	24.0	271
Podarcis sicula	23.3	377
Protophormia terraenovae	25.0	378
Rattus norvegicus	24.4	41, 44, 48, 49, 123, 144, 154, 379–384
Saimiri sciureus	24.8	169
Sceloporus occidentalis	23.6	165
Spalacopus cyanus	23.7	385
Sturnus vulgaris	23.7	27, 28
Tamias striatus	24.9	386
Tinca tinca	22.9	24
Tupaia belangeri	23.7	164

[a] In a few cases, values measured in constant darkness were not available, and the values shown were obtained in constant dim light (< 1 lux). The values shown are the means for each species; some species exhibit greater interindividual variability than others.
[b] For common English equivalents of scientific species names, see the *Organisms Used* appendix at the end of the 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 (range: 24.2 to 26.0 hours).
[d] Refer to *Literature Cited* section of this chapter.

this mean is 39 minutes. The accuracy and precision of the circadian system are remarkable, particularly since the duration of a day was several hours shorter than 24 hours when the first animals evolved, as discussed in Chapter 9.

As mentioned earlier, long freeruns like those shown in Figures 6.5 and 6.6 are not unusual, but they are not typical either. A gradual, or even abrupt, loss of rhythmicity has been reported for various species maintained in conditions of constant darkness or constant light. Figure 6.8 provides an example of gradual loss of rhythmicity. Generally, the rhythm of running-wheel activity of golden hamsters maintained in constant darkness loses robustness after 1 to 2 months. It is of historical significance that, in 1875, an Acacia-like plant, the crested wattle (*Paraserianthes lophantha*), was shown to lose rhythmicity a few days after being placed in constant darkness (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 under a light–dark cycle and in constant darkness. However, rhythm robustness was drastically reduced in the animals whose records appear in Panels C and D. Note that Nile grass rats do *not* lose rhythm robustness when maintained in constant *light*, as exemplified in Figure 6.11. Golden hamsters (which are nocturnal), however, often exhibit weak activity rhythms

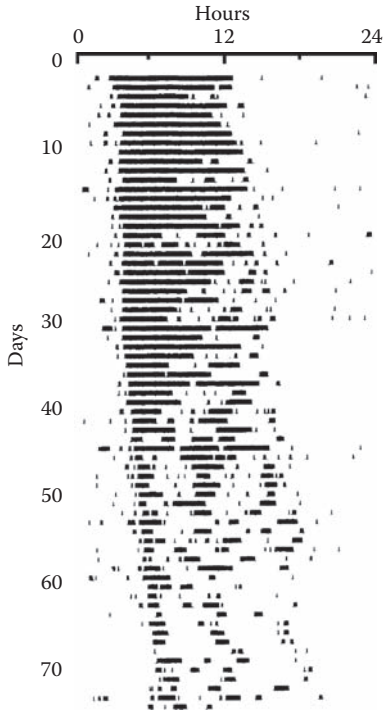


FIGURE 6.8 Nocturnal animal bothered by prolonged darkness. Although golden hamsters (*Mesocricetus auratus*) are fond of darkness and are more active during the dark phase than the light phase of a light–dark cycle, their activity rhythms usually deteriorate if they are maintained in constant darkness for many weeks. This actogram shows the rhythm of running-wheel activity of a typical male golden hamster maintained in constant darkness. Activity becomes infrequent approximately 50 days after the light–dark cycle is discontinued. (Source: Adapted from Refinetti, R., Nelson, D. E. & 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.)

in constant light (Figure 6.12). When abrupt loss of rhythmicity occurs, it generally is observed only in constant light in nocturnal organisms and in constant darkness in diurnal organisms, although gradual loss of rhythmicity may occur in constant darkness *and* in constant light. Abrupt or gradual loss of rhythmicity has been observed in bacteria,[125] plants,[126,127] invertebrate animals,[1,3–5,12] lower vertebrates,[128,129] birds,[32,120,130–133] and mammals.[41,51,68,123,124,134–149]

In principle, loss of rhythmicity may result from deterioration of the circadian pacemaker or from impairment of the overt rhythm (despite the presence of a fully functional pacemaker). The data shown in Figure 6.13 illustrate the latter situation. 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. After that point, 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 interpreted easily, 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.

In Figure 6.13 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 (discussed in Chapter 1). While reviewing numerous studies in various species, Aschoff noticed that different intensities of constant light had different effects on the free-running period.[150,151] 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 called this dependence of the free-running period on the intensity of constant illumination *Aschoff's rule*.[152]

The meaning of Aschoff's rule is discussed in Chapter 7, but one aspect of it must be discussed here. The data shown in the top panels in Figure 6.14 clearly demonstrate that domestic mice are nocturnal, while Nile grass rats are diurnal. When kept in constant darkness, both species exhibit free-running rhythmicity with periods shorter than 24 hours (middle panels). 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 (bottom panels). Evidently, domestic mice follow Aschoff's rule, while Nile grass rats do not. Constant light clearly affects the free-running period of both species, even if not in the way predicted by Aschoff. Aschoff admitted that his rule had as many exceptions as positive cases in the subgroup of diurnal mammals.[151] The results of 29 studies published after Aschoff's last review showed no exceptions to the rule among nocturnal mammals[83,124,141,144,153–161] but many exceptions among diurnal animals of various phyla and classes. Although free-running periods are shorter in constant light than in constant darkness in some diurnal vertebrates,[22,36,141,162–164] they are *longer* in constant light than in constant darkness in diurnal honey bees,[11] goldfish,[23] lizards,[165,166] quail,[132] rodents,[167,168] and primates.[87,169,170] It would seem, therefore, that Aschoff's rule does not apply to diurnal organisms. It might be better to state the 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.

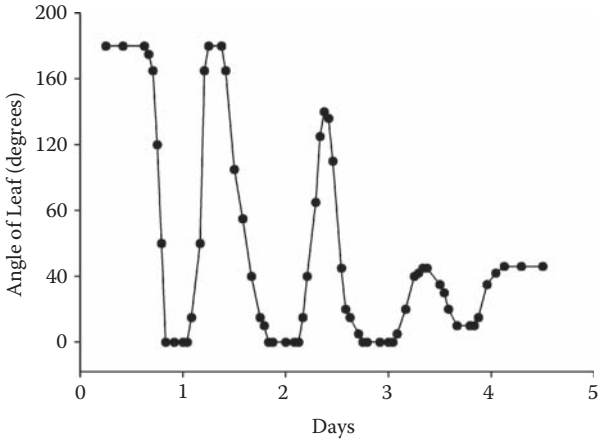


FIGURE 6.9 Plant bothered by darkness. The "sleep" movement of many plants persists in constant darkness, but only for a few days. The graph shows the daily variation in the position of a leaf of *Paraserianthes (Acacia) lophantha* in continuous darkness. The amplitude of the rhythm is greatly reduced after 2 or 3 days. (Source: Pfeffer, W. (1875). *Die periodischen Bewegungen der Blattorgane*. Leipzig: Wilhelm Engelmann.)

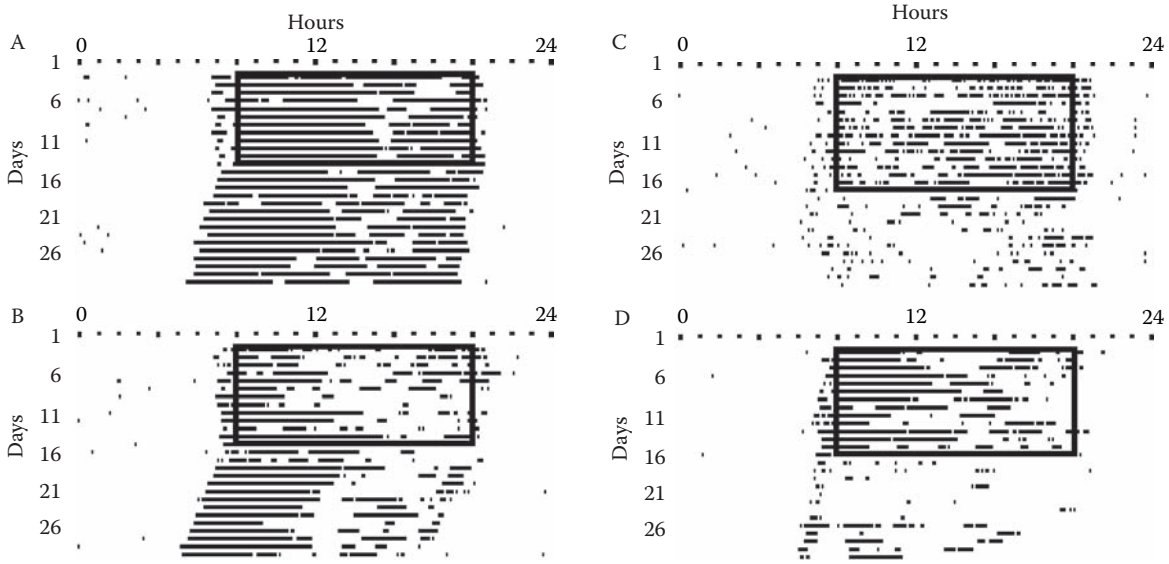


FIGURE 6.10 Diurnal animal bothered by darkness. Nile grass rats (*Arvicanthis niloticus*) are diurnal and, consequently, are more active during the light phase of a light–dark cycle. When placed in constant darkness, some individuals remain rhythmic, but others show great deterioration in the activity pattern. These actograms show the rhythms of running-wheel activity of four grass rats transferred from a light–dark cycle to constant darkness. (Source: Archives of the Refinetti lab.)

Figure 6.15 shows additional data from Nile grass rats. Note that for the 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). Figure 6.16 shows the mean values of circadian period for 16 Nile grass rats. 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.

In Figure 6.15 you may have noted that the duration of the active phase of the circadian cycle (α) seemed to shrink when the period was shorter than 24 hours. As discussed in Chapter 7, this finding may be a side effect of the action of light on the circadian pacemaker. However, actograms normally give the impression of a compression of α even when no actual compression is present. Figure 6.17 shows the same data set (running-wheel activity data from a golden hamster) plotted in three different

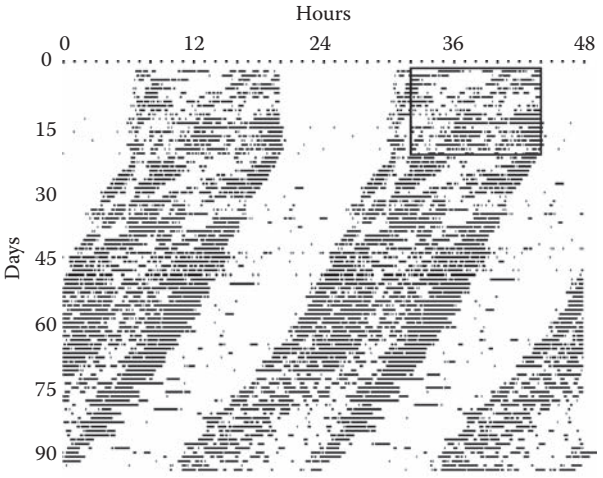


FIGURE 6.11 Diurnal animal not bothered by light. This double-plotted actogram shows the running-wheel activity pattern of a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle for 3 weeks (as indicated by the single-plotted rectangle) and in constant light (300 lux) for 10 weeks. Robust rhythmicity with a period of 23.8 hours was maintained throughout the exposure to constant light. (Source: Archives of the Refinetti lab.)

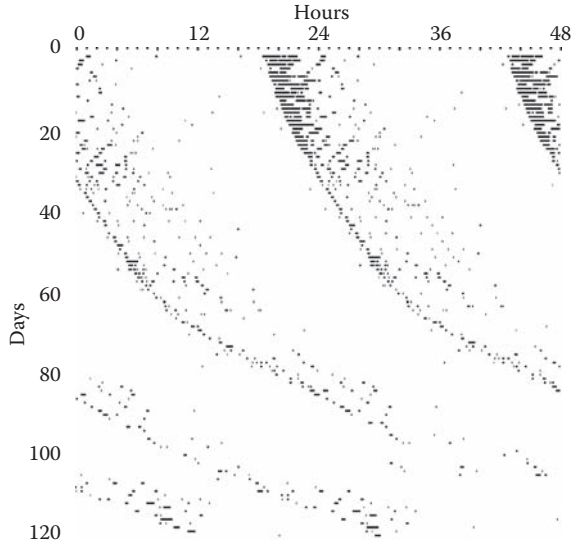


FIGURE 6.12 Hamster bothered by light. The activity rhythms of golden hamsters (*Mesocricetus auratus*) deteriorate not only under constant darkness (as seen in Figure 6.8) but also under constant light, as seen in this double-plotted actogram of the running-wheel activity of a male golden hamster maintained in constant light (300 lux) for 4 months. (Source: Archives of the Refinetti lab.)

formats: modulo 24 (the usual format), modulo 23 (to simulate a 1-hour lengthening of period), and modulo 25 (to simulate a 1-hour shortening of period). Note 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. You can easily verify this fact using the program Plot from the circadian physiology software package and the sample data set A03, both of which were used to build Figure 6.17. As you can see, actograms are not a reliable tool for the study of α. Based on comments made in several published articles, I believe that many circadian physiologists are unaware of this limitation of actograms.

The level of illumination is not the only factor that affects circadian period. Various drugs (Figure 6.18) also 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,[171] morphine,[172] and heroin.[173] Two drugs cause clear and reproducible effects on circadian period and have received special attention: methamphetamine and deuterium oxide.

Methamphetamine mixed in the drinking water, or infused intravenously over a long period of time, lengthens the free-running period of circadian rhythms in rats and mice. It adds up to 5 hours to the duration of the circadian cycle.[174,175] Methamphetamine also *induces* circadian rhythmicity in animals previously rendered arrhythmic by surgical destruction of the master circadian clock in the brain[121,176] or by mutagenic ablation of a gene essential for rhythmicity.[175,177] Figure 6.19 shows representative records from two mice. The records in Panel A correspond to a normal mouse that was maintained in constant darkness and that received methamphetamine in the drinking water during the days between "Start" and "Stop." Note that a free-running rhythm with period slightly shorter than 24 hours was present initially and persisted throughout the study. Note 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 its drinking water. In this mouse (and in 6 of 10 mice tested), 24-hour rhythmicity persisted for about a month after the administration of methamphetamine was discontinued. The reason for this persistent rhythmicity 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,[178] but period lengthening of "only" 1 or 2 hours has been observed in the fruit fly[179] and in rodents.[152,180–182]

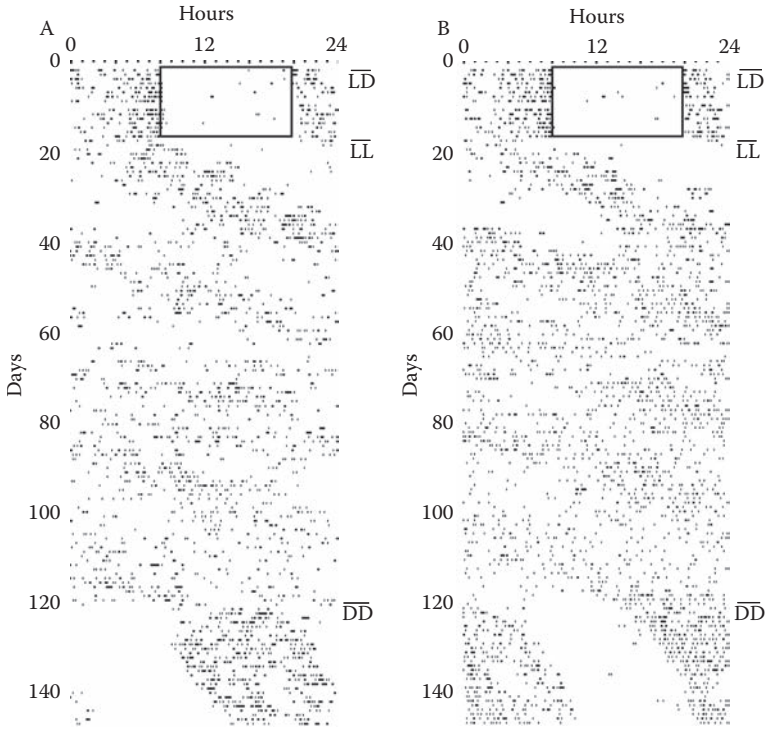


FIGURE 6.13 Rats greatly bothered by light. The activity rhythms of laboratory rats (*Rattus norvegicus*) are not disturbed by exposure to constant darkness, but they exhibit great deterioration in constant light. This actogram shows the running-wheel activity rhythms for rats exposed to a light–dark 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 exhibited free-running rhythms (with periods approaching 25 hours) in the first weeks under constant light, the rhythms later deteriorated. Exposure to constant darkness rapidly restored rhythmicity. (Source: Archives of the Refinetti lab.)

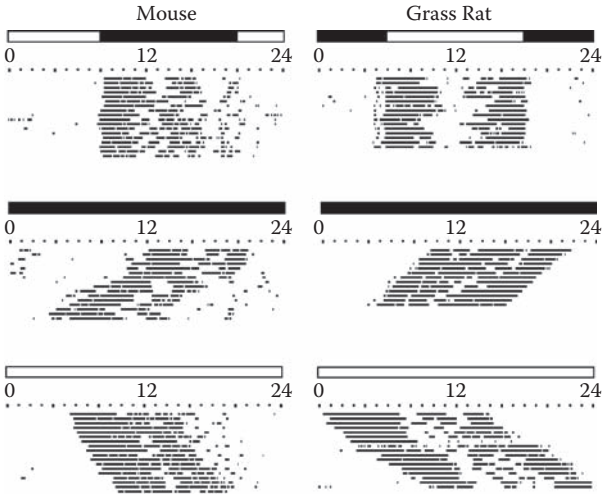


FIGURE 6.14 Effects of constant illumination on circadian period. The actograms show the rhythms of running-wheel activity of a domestic mouse (*Mus musculus*) and a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle, in constant darkness, and in constant light (as indicated by the horizontal bars above each actogram). 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). (Source: Archives of the Refinetti lab.)

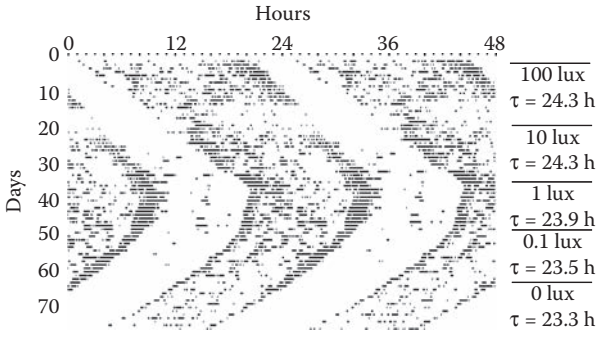


FIGURE 6.15 Constant light and circadian period. This double-plotted actogram shows the rhythm of running-wheel activity of a Nile grass rat (*Arvicanthis niloticus*) maintained under constant light of varying intensities (as indicated in the right margin). Although the circadian period is longer than 24.0 hours under bright light, it is shorter than 24.0 hours under dim light or in darkness. (Source: 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.)

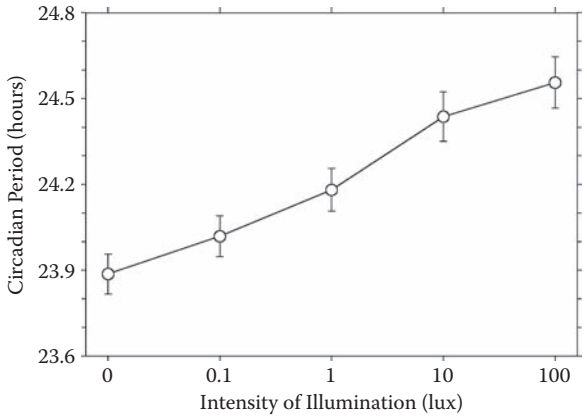


FIGURE 6.16 Intensity of illumination and circadian period. The graph shows the mean (± SE) circadian period of 16 Nile grass rats (*Arvicanthis niloticus*) maintained under constant illumination of varying intensity. Note that the abscissa is logarithmic and that, for convenience, 0 lux is shown at the position of 0.01 lux. The graph clearly shows that circadian period lengthens as the intensity of illumination increases. (Source: 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.)

Figure 6.20 shows data for activity rhythms of fruit flies. Flies were kept in constant darkness and were provided drinking water containing varying percentages of deuterium oxide. Note 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[181,182] and does not seem to induce rhythmicity in rodents rendered arrhythmic by surgical destruction of the circadian clock.[180]

Research has also been conducted on the effects of many other drugs on properties of the circadian system. Chapters 12 and 13 discuss drugs that naturally serve as neurotransmitters in the nervous system. 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 little evidence exists showing that alcohol significantly affects fundamental properties of the circadian clock.[53,183–186] *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[187–191] and, if administered chronically, have a small effect on circadian period.[192] The effects of benzodiazepines on the circadian system are believed to be mediated by their primary effect on motivation and physical activity.[193] Finally, a few studies have suggested that vitamin B_{12} may have a small effect on the circadian system.[194–196]

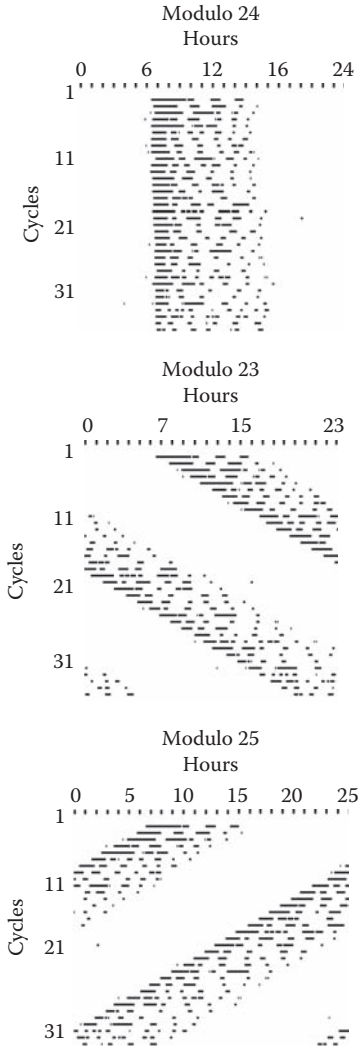


FIGURE 6.17 Optical illusion. The same data (running wheel activity of a golden hamster with a free-running period of 24.0 hours) are plotted in 24-, 23-, and 25-hour actograms (Modulo 24, Modulo 23, and Modulo 25, respectively). The duration of the activity phase (α) seems to be shorter when the data are plotted in Modulo 23 and Modulo 25 than in Modulo 24.

6.1.3 Temperature Compensation

An important aspect of endogenous rhythmicity not yet discussed is *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.[197,198] This temperature dependence is numerically expressed as a Q_{10} (the quotient of reaction rates 10°C apart), with Q_{10} about equal to 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, the Q_{10} effect can be seen in the dependence of metabolic rate of

FIGURE 6.18 Snorting cocaine. Many drugs are known to affect the operation of the circadian pacemaker. (Source: © Art-Today, Tucson, AZ.)

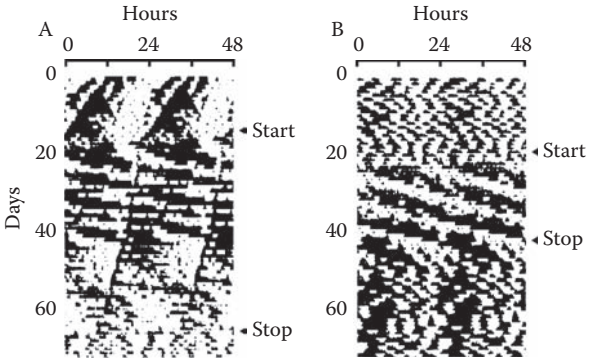


FIGURE 6.19 Methamphetamine induces circadian rhythmicity. These double-plotted actograms show the running-wheel activity rhythms of two mice (*Mus musculus*) maintained in constant darkness. Methamphetamine hydrochloride was dissolved in the drinking water of both mice, starting and ending on the days indicated in 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. (Source: Adapted from Masubuchi, S., Honma, S., Abe, H., Nakamura, W. & Honma, K. (2001). Circadian activity rhythm in methamphetamine-treated *Clock* mutant mice. *European Journal of Neuroscience* 14: 1177–1180.)

the whole organism on the temperature of the environment, as has been extensively documented in plants,[199–202] arachnids,[203,204] crustaceans,[205–212] insects,[213–216] and fishes.[217–223]

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. Even warm-blooded animals may experience fluctuations of body temperature of up to

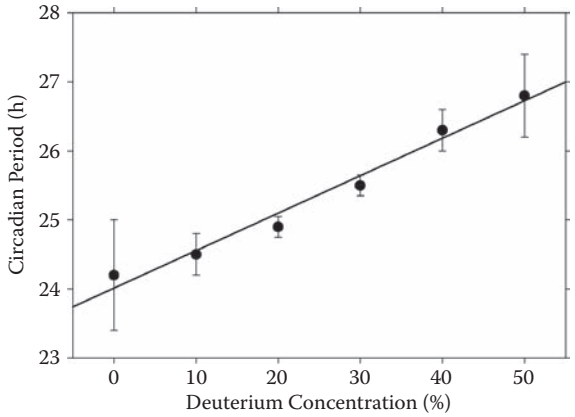


FIGURE 6.20 "Heavy" water lengthens circadian period. The graph shows the mean circadian periods of the activity rhythm of fruit flies (*Drosophila melanogaster*) maintained in constant darkness and provided with drinking water containing different amounts of deuterium oxide (D_2O). Each point corresponds to the mean (± SE) of approximately 20 flies. When the concentration of deuterium is higher, the circadian period is longer. (Source: White, L., Ringo, J. & Dowse, H. (1992). A circadian clock of *Drosophila*: effects of deuterium oxide and mutations at the *period* locus. *Chronobiology International* 9: 250–259.)

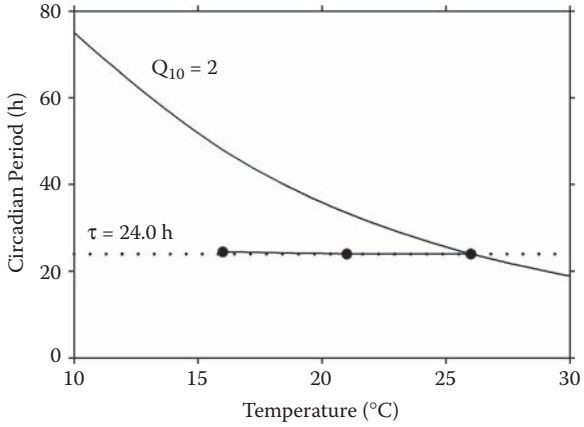


FIGURE 6.21 Temperature compensation in the circadian system of an ectotherm. The graph shows that the period of the rhythm of eclosion of the fruit fly (*Drosophila pseudoobscura*) is independent from environmental temperature, at least within the tested range. The sloped line indicates the normal temperature dependence of cellular processes (Q_{10} = 2). The dotted line indicates full temperature independence (τ = 24.0 hours). Clearly, the circadian system of the fruit fly is temperature compensated (Q_{10} = 1). (Source: Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in *Drosophila*. *Proceedings of the National Academy of Sciences U.S.A.* 40: 1018–1029.)

5°C or more as part of their normal circadian rhythmicity, which could render the circadian clock useless. If the circadian clock were not temperature compensated, high

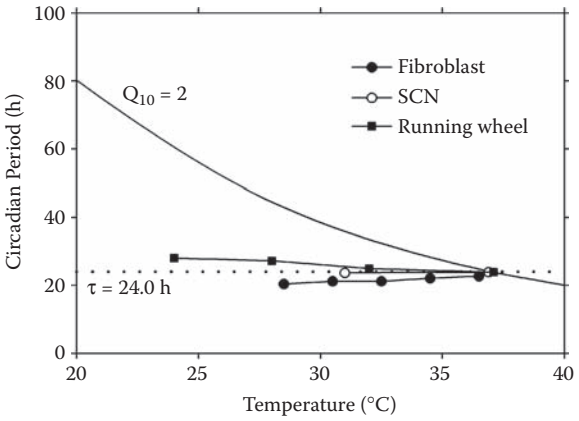


FIGURE 6.22 Temperature compensation in the circadian system of an endotherm. The graph shows that the period of circadian rhythms in the laboratory rat (*Rattus norvegicus*) is independent from tissue temperature. Data are shown for cultured fibroblasts exhibiting circadian rhythmicity *in vitro*, cultured 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 (τ = 24.0 hours). Clearly, the circadian system of the rat is temperature compensated. (Sources: Izumo, M., Johnson, C. H. & 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 U.S.A.* 100: 16089–16094; Ruby, N. F., Burns, D. E. & 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; Gibbs, F. P. (1981). Temperature dependence of rat circadian pacemaker. *American Journal of Physiology* 241: R17–R20.)

temperatures would speed it up and low temperatures would slow it down. 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 than 70 hours at a body temperature of 10°C. In contrast, observe the experimentally determined circadian period of eclosion (emergence) of fruit flies, as indicated by the line connecting the closed circles. The line 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,[151,224] including bacteria,[225] mold,[226] bean plant,[227] fruit fly,[228] zebrafish,[22] ruin lizard,[229] bat,[230] and various species of rodents.[75,84,231–236] Figure 6.22 provides examples of data collected from laboratory rats. Measurements of circadian period were made from fibroblast cultures, brain slices containing the master circadian pacemaker (SCN), and running-wheel activity. Note that, although none of the variables was absolutely temperature independent, all three exhibited a Q_{10} very close to 1. The process responsible for temperature compensation of period is clearly as active in tissues of warm-blooded animals as in tissues of cold-blooded organisms.

FIGURE 6.23 Fruit fly up close. The fruit fly (*Drosophila melanogaster*) is widely used in biological research, including research on circadian rhythms. (Source: Image courtesy of Jim Ehrman, Digital Microscopy Facility, Mount Allison University, Canada.)

6.2 INHERITANCE MECHANISMS

The endogenous nature of the circadian clock necessitates some form of genetic inheritance. Because different species have clearly distinct circadian periods, even though they live in similar environments, genetic influences must be stronger than environmental influences. Additional information needed to ascertain the strength of the genetic influence 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*,[237] and in bread mold, *Neurospora crassa*.[238] 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 to be identified shortened (per^S, τ = 19 hours), lengthened (per^L, τ = 28 hours), or eliminated (per^0) circadian rhythmicity of activity.[237] 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 in length.

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.

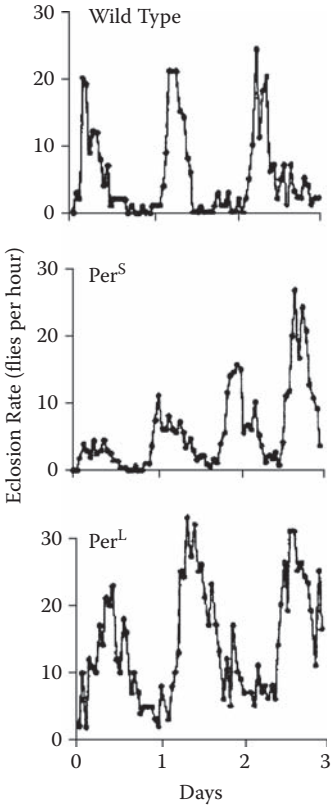


FIGURE 6.25 The mutant flies. The graphs show the variation in eclosion time of fruit flies (*Drosophila melanogaster*) kept in constant darkness. Wild type flies exhibit one cycle per day (period ≈ 24 hours), per^S mutants exhibit four cycles in 3 days (period ≈ 19 hours), and per^L mutants exhibit fewer than three cycles in 3 days (period ≈ 28 hours). (Source: Adapted from Konopka, R. J. & Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences U.S.A.* 68: 2112–2116.)

Figure 6.25 shows examples of eclosion rhythms of fruit flies maintained in constant darkness. 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. Note that one eclosion cycle occurs per day for wild types (τ = 24 hours), but four cycles are present in 3 days for per^S mutants (τ = 19 hours) and not quite three cycles occur in 3 days for per^L mutants (τ = 28 hours). The fact that a single-gene mutation can drastically affect free-running rhythms indicates that the period of the circadian pacemaker has a very strong genetic component. Several other *per* alleles were identified later,[239] and in the early 2000s three other mutant genes were created by researchers: *tim* (for timeless),[240] *tik* (for timekeeper),[240] and *andante*.[241] 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[240] — a duration so long it would not normally be considered *circadian*!

Three mutations of the gene *frq* (for frequency) were originally identified in bread mold.[238] (See Figure 6.26.) While 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. The *frq-3* mutants clearly are more "normal" than the wild types. Additional clock mutations in bread mold were identified later.[242] Of course, this research relies only on flies and fungi to show that circadian period is genetically determined. In 1989, a natural clock mutation was identified in the golden hamster,[243] and in 1994 a mutation was produced in the laboratory mouse by mutagenesis and subsequent screening.[244]

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 kept 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. Ralph was aware of the studies in fruit flies, so he resisted the impulse to discard the "defective" hamster and replace it with a "good" one. He started inbreeding successive generations of the unusual hamster. He soon found that the abnormal behavior resulted from 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

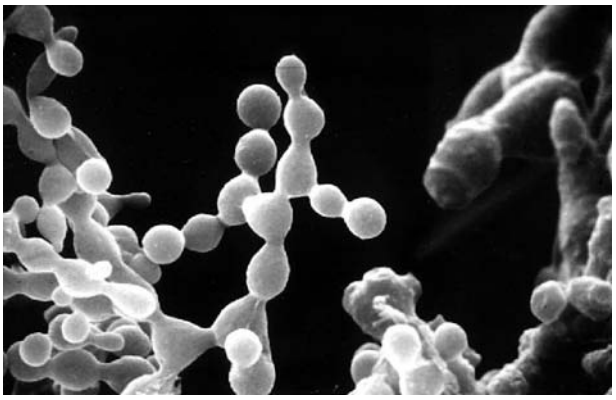


FIGURE 6.26 Mold up close. Bread mold (*Neurospora crassa*) has been used widely in the study of the genetics of circadian rhythmicity. (Source: Springer, M. & Yanofsky, C. (1992). Expression of *con* genes along the three sporulation pathways of *Neurospora crassa*. *Genes and Development* 6: 1052–1057; © 1992 Cold Spring Harbor Laboratory Press. Reproduced with permission from the publisher and the authors.)

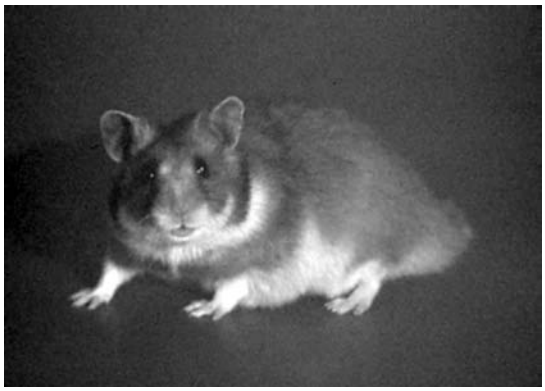


FIGURE 6.27 Mutant hamster up close. The golden hamster (*Mesocricetus auratus*) was the first mammal in which a genetic mutation that specifically affects the circadian system was found (the *tau* mutation). (Source: Photograph by R. Refinetti.)

period of 22 hours, and homozygotes (*tau*/*tau*) have a period of 20 hours.[243,245,246]

Figure 6.28 shows the dramatic effects of the mutation. 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. An 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, no obvious difference is seen 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,[247,248] but also for the body temperature rhythm,[64] the feeding rhythm,[249] rhythms of hormone secretion,[250,251] and the circadian modulation of the reproductive system's photoperiodic response.[252,253] This demonstrates that the effect of the *tau* gene is general to the circadian pacemaker rather than restricted to a particular effector mechanism. Evidence also indicates that the mutation is specific to the

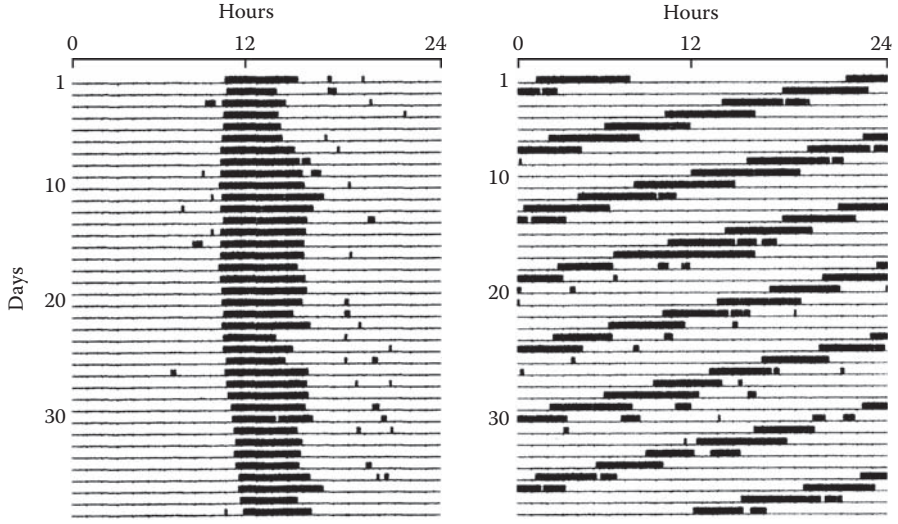


FIGURE 6.28 Hamster in a hurry. The actograms show the rhythms of running-wheel activity of a wild type golden hamster (left) and of a homozygous *tau*-mutant hamster (right) both maintained in constant darkness. The wild-type hamster completes a circadian cycle in almost exactly 24 hours, while the mutant hamster completes each circadian cycle in approximately 20 hours. (Source: Menaker, M. & 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. Reprinted by courtesy of Marcel Dekker, Inc.)

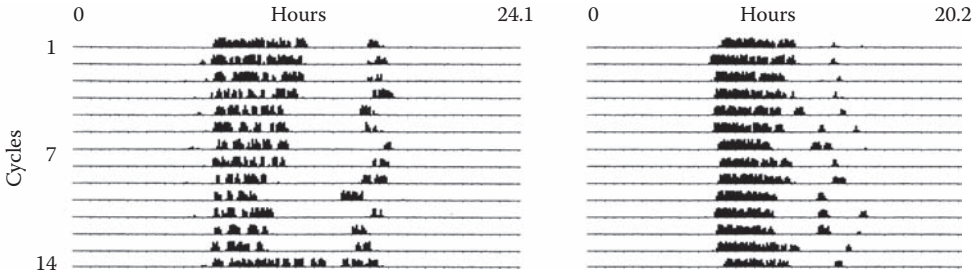


FIGURE 6.29 Proportionally short. This figure shows the running-wheel activity rhythms of a wild-type golden hamster (left) and of a homozygous *tau*-mutant hamster (right) plotted in actograms with hour-axes proportional to the circadian periods of the animals. The activity patterns of the two phenotypes are clearly similar. (Source: Adapted from Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.)

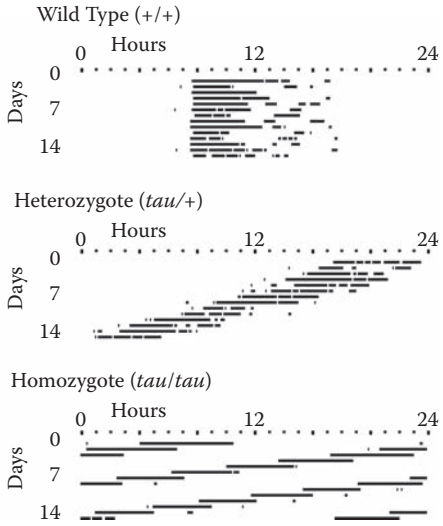


FIGURE 6.30 Three genotypes, three phenotypes. These actograms show the running-wheel activity rhythms of representative members of the three genotypes of the *tau* gene, maintained in constant darkness. Wild type hamsters have circadian periods very close to 24 hours, heterozygous mutants have periods of approximately 22 hours, and homozygous mutants have circadian periods of approximately 20 hours. (Source: Archives of the Refinetti lab.)

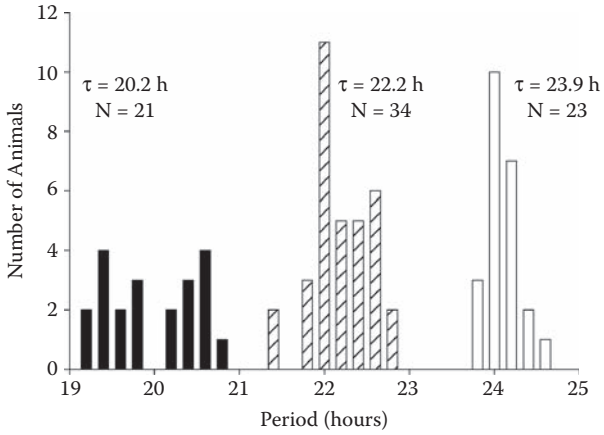


FIGURE 6.31 Hamsters and peas. The frequency distribution of free-running periods of the progenies of pairs of heterozygous *tau*-mutant hamsters delineates three groups with frequencies similar to the 1:2:1 ratio expected from the crossing of heterozygotes, as originally described for peas by Mendel. (Source: Ralph, M. R. & Menaker, M. (1988). A mutation in the circadian system of the golden hamster. *Science* 241: 1225–1227.)

circadian system and does not alter other processes of biological timing. The mutation does not affect the frequency of the pacemaker of the heart[69] or the duration of the estrous cycle.[254] Although ultradian oscillations in body temperature were shorter in the mutants than in wild types,[61] the difference was probably an artifact of Fourier analysis, as previously discussed in Chapter 3. A lengthening of the ultradian period of hormone pulsatile secretion in mutants[255] seems to be legitimate, however.

Additional studies on the effects of the *tau* gene 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.[256–258] The mutation may also affect the property of temperature compensation[236] and increase the longevity of the animals.[259] Efforts to understand these secondary effects of the mutation were deterred until recently by the lack of knowledge of the molecular aspects of the mutation. These efforts are likely to increase again soon, because the molecular identity of the *tau* gene was discovered in 2000. The *tau* allele is an allele of the casein kinase I epsilon (*CKIε*) gene,[260] which codes for an enzyme (kinase) used for the phosphorylation of proteins in animals.

Before I discuss other single-gene mutations that affect circadian period in mammals, I first want to comment briefly on 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 exhibit the phenotypic distribution expected of a semidominant allele. Figure 6.31 shows that, for a group

of 78 hamsters with heterozygous parents, the distribution of phenotypes follows the 1:2:1 proportion.

In 1994 a team of researchers led by Joseph Takahashi at Northwestern University, in Illinois, isolated the *clock* gene in the mouse. Takahashi, Menaker, and other researchers had earlier received a multimillion-dollar grant from the National Science Foundation to create a Center for Biological Timing, with headquarters at the University of Virginia. The success of the grant encouraged Takahashi to embark on a very ambitious project aimed at the production of a clock gene mutation in the mouse (whose genome was much better known than that of the hamster). After an extensive program of mutagenesis and screening of mutants, Takahashi and colleagues identified the *clock* gene, a single-locus, semidominant gene located in chromosome 5 that 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.[244,261,262] Mice having the *clock* mutant gene are excellent animals for research at the molecular level, but they have not been studied at the organismic level as extensively as the *tau* mutant, in part because the homozygotes (*clock*/*clock*) lose circadian rhythmicity a few weeks after being placed in constant darkness. Figure 6.32 shows representative actograms of the running-wheel activity rhythms of the three phenotypes. Note 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 few weeks. Curiously, *clock*/*clock* mice do not lose rhythmicity in constant light, a condition under which some wild-type mice lose rhythmicity.[124] Other nonperiod-specific effects of the *clock* mutation include a disruption of reproductive cyclicity[263] and a reduction in the responsiveness of the circadian pacemaker to photic stimulation.[264] Takahashi's work with the *clock* mutant gene gained him induction into the U.S. National Academy of Sciences in 2003.

Another team of researchers, led by Gary Pickard at the University of Pennsylvania, conducted a mutagenesis program similar to Takahashi's program. They identified the *wheels* mutant gene, located in chromosome 4. The *wheels* mutant gene 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.[265] A third team, 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 have yet to be fully characterized.[266]

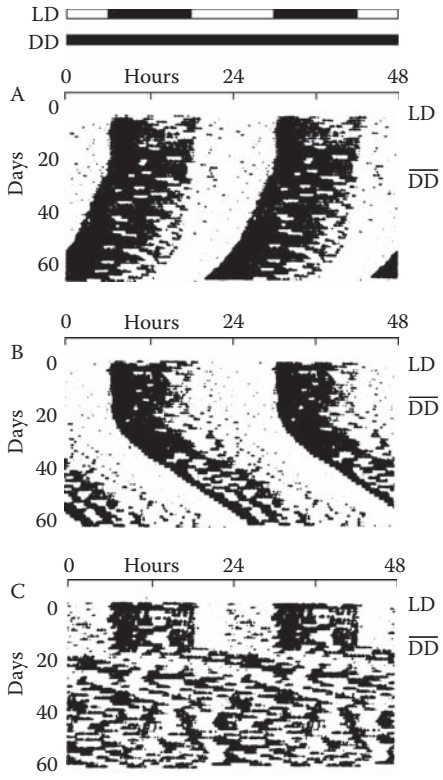


FIGURE 6.32 Mutant mice. These double-plotted actograms show the running-wheel activity rhythms of three representative mice bearing different genotypes of the *Clock* gene. The animals were maintained under a light–dark cycle for 2 weeks (as indicated by the top horizontal bar above the actograms) and 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 later became arrhythmic. (Source: Adapted from 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. & Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264: 719–725.)

The discovery of several genes that are at the core of the circadian pacemaker (see Chapter 12) allowed various research groups to generate knock-out mice (i.e., mice with targeted deletions of particular genes) that are arrhythmic or have abnormal free-running periods.[267–269] Several selective-breeding studies have also been conducted,[270–272] although this approach is too crude to allow single-gene selection. A mutagenesis program in the mustard plant (*Arabidopsis thaliana*) yielded 6 lines of arrhythmic mutants and 29 lines of period mutants with periods ranging from 16 to 27 hours.[273]A study of human twins provided evidence in support of the heritability of the daily eating pattern of men and women.[274]

Circadian period clearly has a 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, 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 example, 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 it disappears. Would these "aftereffects" become permanent if the animals were raised from birth (or from conception) under different light–dark cycles? In vertebrates, this question has not been addressed experimentally often enough to allow a definitive answer, but two studies — one in mice[56] and one in hamsters[275] — have revealed that the early environment has no effect beyond that expected as an aftereffect of the light–dark cycle.

In Figure 6.33 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, while 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. It appears 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.[275] That is, the shortening of the free-running period of heterozygous hamsters seen in Figure 6.33 (bottom) is not a permanent effect of the early environment; it is, rather, a temporary aftereffect of the light–dark cycle to which the hamsters were previously exposed. It seems, therefore, that the old nature versus 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.[276]

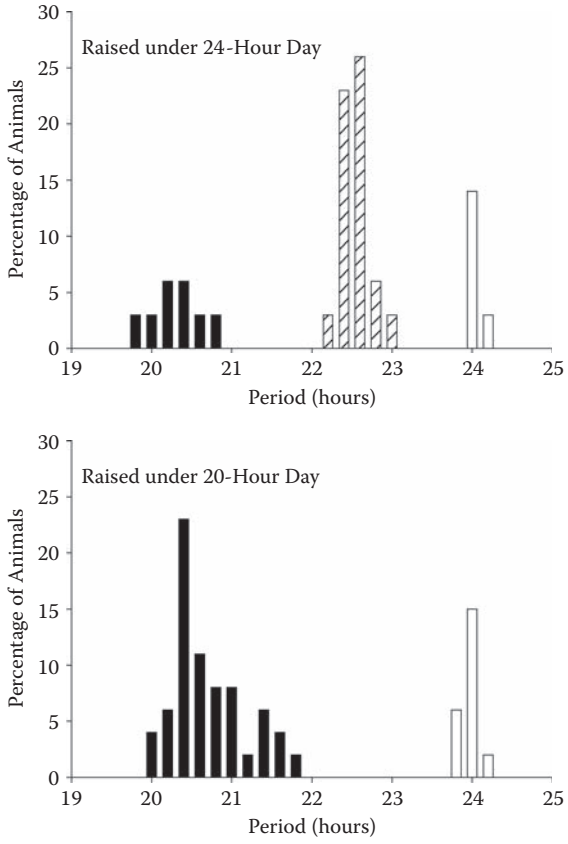


FIGURE 6.33 Nature or nurture? The graphs show the frequency distributions of free-running periods of the progenies of pairs of heterozygous *tau*-mutant hamsters conceived and raised under a 24-hour or a 20-hour light–dark cycle. The data suggest that hamsters raised under 20-hour days have shorter 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. (Source: Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.)

6.3 SINGLE OR MULTIPLE OSCILLATORS

Many biological variables exhibit circadian rhythmicity, so it is reasonable to wonder whether each variable is controlled by its own circadian clock. Multiple clocks would need to be synchronized in the organism, however, so it is also reasonable to expect the existence of a single clock. What is reasonable is not always true. At the cellular level, more than one circadian clock is present. As discussed in Chapter 12, individual cells are autonomous pacemakers, so that the human brain contains several thousand circadian clocks. Do these cells make up one unit that functions as a single clock? A large body of evidence indicates the existence of multiple clocks in protists[277,278] and lower vertebrates,[21,37] but, in my opinion, mammals have a single master circadian clock.

Over the years, many authors have proposed the existence of two clocks. Two distinct versions of the two-clock model have been suggested: one that explains the phenomenon of *splitting* in rodents, and one that explains the phenomenon of *spontaneous internal desynchronization* in humans. I discuss each version in the following sections.

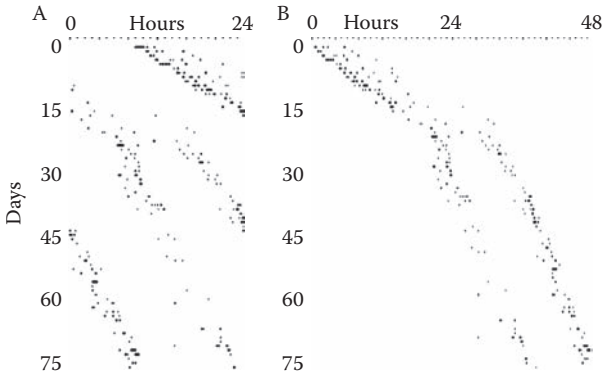


FIGURE 6.34 Splitting in constant light. When golden hamsters (*Mesocricetus auratus*) are maintained in constant light, their running-wheel activity rhythms are much less robust than when the animals are maintained under a light–dark cycle or in constant darkness. In 50% of the animals, the activity pattern splits into two separate components. This figure shows the activity records of a typical hamster, in standard actogram format (A) and in double-plot format with omission of repeated segments (B). The animal had been kept 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). (Source: Archives of the Refinetti lab.)

6.3.1 Splitting

Splitting refers to the separation of a free-running rhythm into two separate components 180° apart, as exemplified in Figure 6.34. This bizarre phenomenon has been consistently observed in many laboratories when golden hamsters are maintained in constant light for extended periods of time.[138,155,279–287] Because the two split components exhibit different circadian periods during the initial stage of splitting, some researchers have suggested that two separate clocks might be involved. According to this view, the circadian pacemaker is not a single oscillator but, rather, the result of the coupling of two distinct oscillators, often called the *evening oscillator* and the *morning oscillator*.[152,288–290] An alternative to this theory suggests that splitting is a minor anomaly yet to be explained, rather than a fundamental piece of evidence requiring a revision of the concept of the circadian pacemaker.

For a variety of reasons, I favor the "anomaly" alternative to explain 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*, only half of the hamsters kept in constant light typically develop split rhythms.[138,155,279–287] 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 observed consistently in golden hamsters, it has been observed only occasionally in other species, and usually in only a few individuals under special conditions.[12,291–294] 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.[37,57,163,295,296] *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.[155] *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 fails to demonstrate differential responsivity.[163]

Seventh, as discussed in Chapter 12, research on the neural substrates of the circadian pacemaker has not supported the concept of two distinct oscillators. 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.[280] This finding 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,[297] 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.[298] 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 were in antiphase during splitting.[299]

Additional support for the two-oscillator hypothesis might be found in electrophysiological recordings from brain slices of hamsters that were not split.[300] 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).[301] Furthermore, a bimodal pattern of activity in the brain is no more significant than a bimodal pattern of locomotor activity (which, as seen in Chapter 5, is typical of most animals). Both patterns may simply reflect the bimodal waveform of rhythms generated by a single pacemaker. Very recently, 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.[302] Although this finding may indicate that the LN_d is the evening oscillator and the adjacent LN_v is the morning oscillator, there is no indication that the LN_d is a functional oscillator capable of driving the activity rhythm in constant darkness.

For the seven reasons listed in this section, I do not believe that the phenomenon of splitting justifies the assumption of two independent oscillators. A series of recent observations, however, 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." Researchers attained "splitting" in golden hamsters by inducing daily bouts of activity in the middle of the light phase of the light–dark cycle.[303,304] Because hamsters do not normally exercise in illuminated environments, the lights were turned off during activity induction (which was encouraged by providing a novel running wheel). The animals then were exposed to two light–dark cycles each day and, not surprisingly, they 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 young researcher now at the University of California at San Diego, adapted the induced-splitting protocol[305,306] and observed that splitting could be obtained simply by the use of two daily light–dark cycles, eliminating the need for the novel running wheel.[307] Figure 6.35 shows a sample of his data. 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), activity concentrated in the single dark phase, but under LDLD 9:5:5:5 (Section B), activity split into two daily bouts. As 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.

I tried to replicate Gorman's study in hamsters, mice, and Nile grass rats, but found no evidence of splitting (Figure 6.36). Evidently, the splitting phenomenon was not robust and required specific experimental conditions that I failed to replicate. Soon after my attempt, 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.[308,309] Splitting under this protocol is quite robust, and I had no problem replicating it in mice in my laboratory, as shown in Figure 6.37. 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.

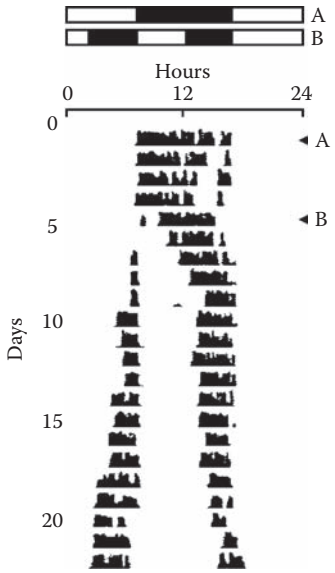


FIGURE 6.35 Splitting induced by fragmentation of the light–dark cycle. The unimodal daily pattern of activity exhibited by golden hamsters under a regular 24-hour light–dark cycle can be converted into a bimodal pattern if the light–dark cycle is split into two daily cycles. This actogram shows the 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). The actogram clearly shows splitting of the activity rhythm into two daily components. (Source: Adapted from Gorman, M. R. (2001). Exotic photoperiods induce and entrain split circadian activity rhythms in hamsters. *Journal of Comparative Physiology A* 187: 793–800.)

I see it as a curious modulation of the waveform of the oscillation generated by *the* circadian pacemaker — not as evidence that supports the two-oscillator hypothesis.

6.3.2 Spontaneous Internal Desynchronization

Most studies in circadian physiology examine only one variable at a time. If two or more variables are studied simultaneously, researchers can investigate whether the variables are mutually synchronized. The different variables usually are synchronized, but transient desynchronization can be induced by various environmental manipulations.[310–322] In studies of free-running rhythms in which two or more variables are recorded simultaneously, the rhythms generally are found to freerun together with the same period. Some amount of "biological noise" is always present and may lead a hasty observer to see a transient episode of "spontaneous" internal desynchronization.[28,323] In the long run, however, no evidence of *spontaneous internal desynchronization* has ever been found in studies of nonhuman vertebrates maintained in constant darkness or constant light for extended periods of time.[29,37,50,65,86,135,143,164,169,324,325] Neither has it been found in many studies of humans.[95,99,101–103,326,327] However, various investigators,

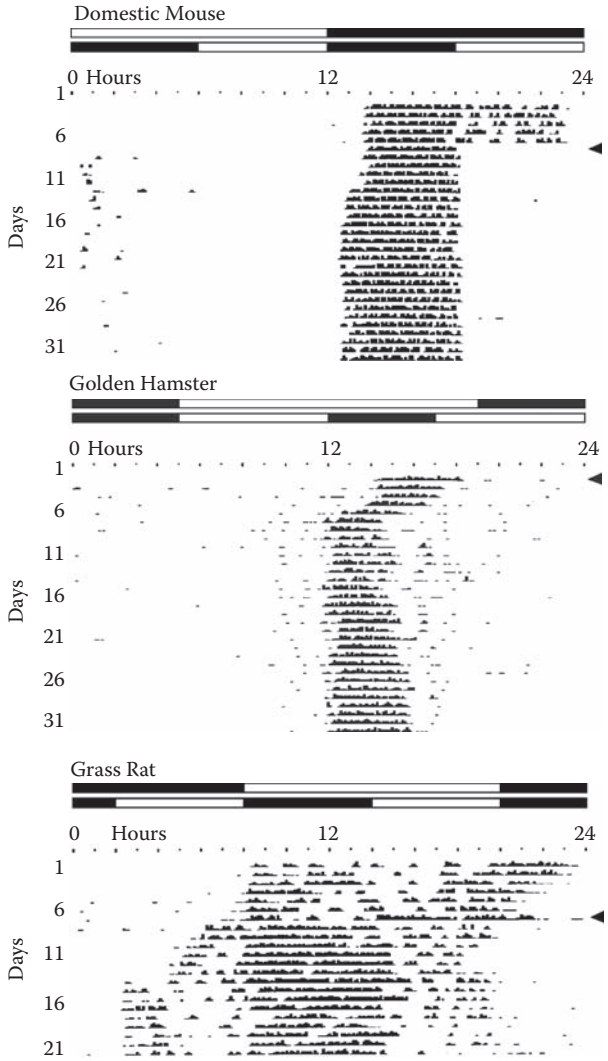


FIGURE 6.36 Refusing to split. Domestic mice (*Mus musculus*), golden hamsters (*Mesocricetus auratus*), and Nile grass rats (*Arvicanthis niloticus*) did not develop split rhythms when subjected to exotic light–dark cycles in the author's laboratory, as exemplified in these actograms of running-wheel activity. The mouse and the grass rat were transferred from LD 12:12 to LD 6:6, while 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. (Source: Archives of the Refinetti lab.)

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,[328,329] but their results were never replicated. More commonly, spontaneous internal desynchronization has been found between the sleep–wake rhythm, on one hand, and all other rhythms (although the body temperature rhythm is usually the only other rhythm measured), on the other hand.[92,94,97,100,170,330–334] Typically, the period of the sleep–wake rhythm becomes very long (up to 30 or even 50 hours) after a week or so, while the period of the body temperature rhythm (and other rhythms) remains between 24 and 25 hours. Depending on the study, 15 to 60% of the subjects exhibit this phenomenon.

Figure 6.38 shows an example of spontaneous internal desynchronization. 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. To explain the existence of rhythms with two different periods in the same individual, researchers 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.[115,335]

Synchronization is a relatively common word used every day. We often do not recognize, however, the subtle difference between the two meanings of synchronization. In the left panel (A) of Figure 6.39, the faces of two clocks are shown in four successive time points. The two clocks clearly are *not* synchronized, as the clock on the right advances 6 hours per frame while the clock on the left advances only 3 hours. The middle panel (B) contains a pair of synchronized clocks. These two clocks show exactly the same time every time. The panel on the right (C) also shows synchronized clocks, but this synchronization may not be evident at first. 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. Circadian physiology is concerned with both types of synchronization. In fact, the pattern in Panel C occurs more commonly than that in Panel B. Figure 6.40 shows the synchronized rhythms of body temperature and locomotor activity of a tree shrew. 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 synchronization has been defined, I want to make sure you understand internal desynchronization. Suppose that you go into a cave where you have no way to tell the time of day. 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 the correct time of day. In this scenario, some male subjects believe that their beard is growing faster in the cave, as the interval between daily shaves increases without their awareness. If you do not develop internal desynchronization, you will start going

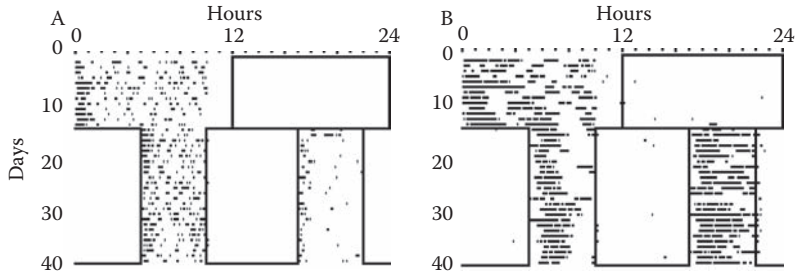


FIGURE 6.37 Splitting under 12-hour light–dark cycle with background of dim constant light. These actograms show the running-wheel activity rhythms of two mice (*Mus musculus*) maintained first under a 24-hour light–dark cycle (LD 12:12) and then under a 12-hour light–dark cycle (LD 7:5). The rectangles 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, while the records in B show a robust partition of activity into two daily blocks. (Source: Archives of the Refinetti lab.)

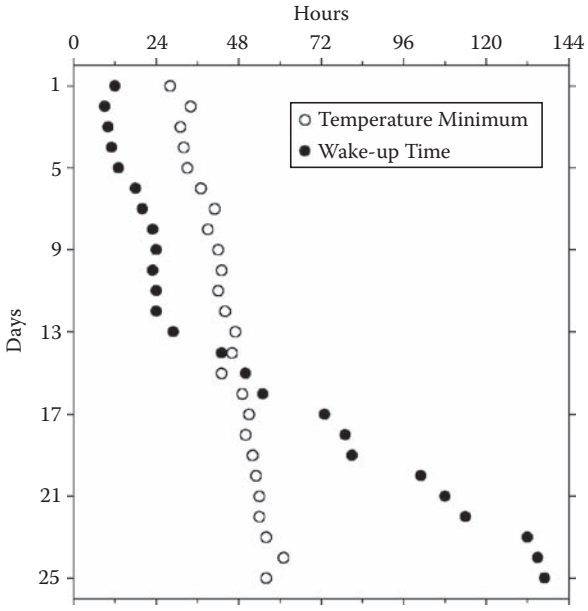


FIGURE 6.38 Spontaneous internal desynchronization. This graph shows the daily time of the body temperature rhythm trough and the daily awakening time of a human subject kept under constant environmental conditions for 1 month. The hour-axis is sextuple-plotted, and repeated segments are omitted. In such a plot, clear dissociation of the rhythms of body temperature and wake-sleep can be observed starting on Day 14. (Source: Aschoff, J. & Wever, R. (1981). The circadian system of man. In: Aschoff, J. *Biological Rhythms*. New York: Plenum, pp. 311–331.)

to bed at noon after 2 weeks in the cave. This time of day will also be when you feel sleepy. However, if you 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.

Several researchers have suggested that internal desynchronization is unlikely to reflect a true dissociation of rhythms that results from the dissociation of two biological clocks. Instead, they suggest that the phenomenon may be due simply to a lot of "noise" in the expression of the sleep–wake rhythm.[314,327,336] Look again at Figure 6.38. Note that the hours-axis differs 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 Figure 6.38 is replotted using a standard actogram format, a more neutral view is obtained of the actual relationship between temperature and the sleep–wake rhythm. In the standard format (Figure 6.41), 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 onwards, but internal desynchronization is no longer obvious.

Figure 6.42 is a fictitious actogram built using the periods that the authors claimed to have identified in the data. Although some similarities exist between Figure 6.42 and Figure 6.41, particularly for the open circles (which correspond to the noncontroversial temperature data), many differences are also present, particularly for the closed circles (which correspond to the sleep–wake data). My interpretation is that the sleep–wake rhythm in Figure 6.41 is certainly "noisy," but it 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).

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.[94,98,327,337,338] 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 this way, then they obviously are not independent of each other. In other words, they do not freerun separately — and, if they do not freerun separately,

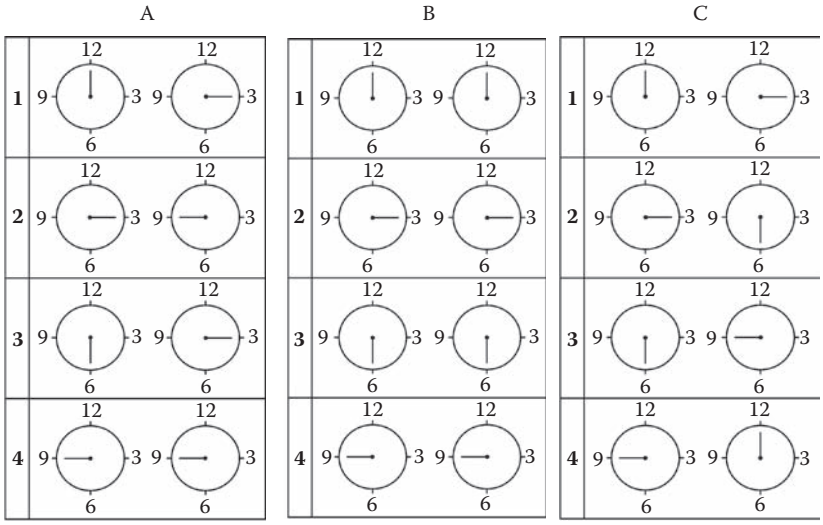


FIGURE 6.39 Too many clocks? Do not allow the apparent complexity of these diagrams to scare you off. Look closely and note that the two clocks in A are not synchronized, but the clocks in B and C are synchronized. Refer to the text for details.

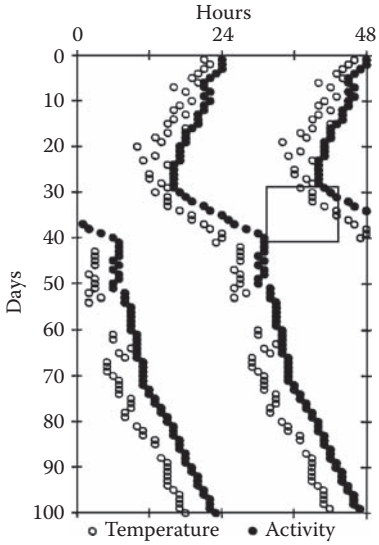


FIGURE 6.40 No internal desynchronization in tree shrews. This graph shows the daily time of the body temperature rhythm trough and the daily time of activity initiation of a tree shrew (*Tupaia belangeri*) as recorded by telemetry for over 3 consecutive months. The figure is double-plotted to facilitate 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. Note the persistent synchronization of the rhythms of body temperature and activity. (Source: Refinetti, R. & Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.)

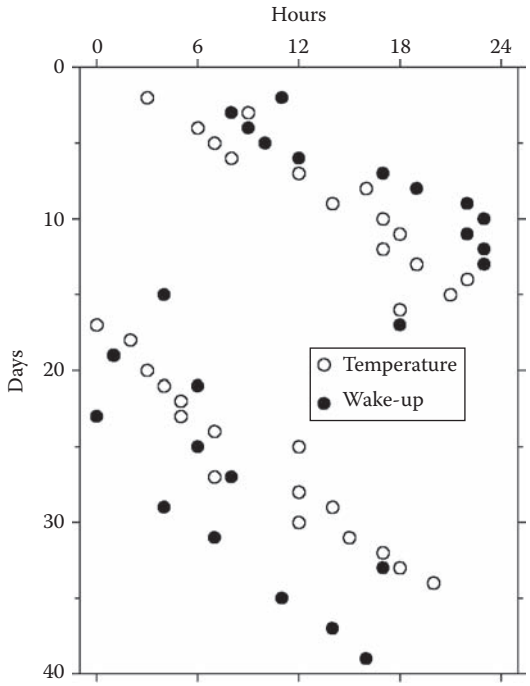


FIGURE 6.41 It depends on how you slice it. The data shown in Figure 6.38 are replotted here in actogram format. Inspection of this plot suggests that the rhythms of body temperature and wake-sleep are "noisy" but the data provide no clear indication of internal desynchronization.

they need not be generated by separate circadian clocks. Naturally, the absence of the requirement for separate pacemakers does not rule out the possibility of their existence. 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.[115,335] However, models also have been developed to show that the data can be explained by the action of a single pacemaker.[339,340] In the absence of additional evidence, parsimony leads one to conclude that only one circadian pacemaker exists.

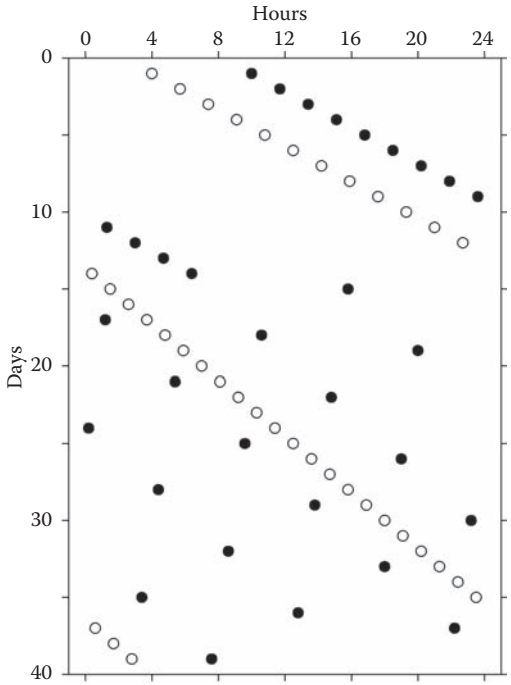


FIGURE 6.42 What would it look like? This graph, which should be compared to Figure 6.41, shows the expected outcome of actual desynchronization of the rhythms of body temperature and wake-sleep starting on Day 14. Refer to the text for a detailed discussion.

Additional animal studies suggested that destruction of the brain area believed to control circadian rhythmicity of activity does not eliminate circadian rhythmicity of body temperature.[341–345] However, the findings in these studies were later shown to result from an artifact in the analysis of rhythmicity.[346] Chapter 12 addresses the issue of the physical location of the circadian pacemaker. The next four chapters, however, continue the analysis of the functional properties of the pacemaker as revealed by the study of circadian rhythms.

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 led 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

Exercise 6.1 A very simple timing device

Section 6.1 described various models of pacemakers. To understand the concept of a pacemaker, it is helpful to first understand how a simple timing device can be built. In this exercise, you will look at a very simple device that uses a steady flow of water to generate a cyclic process. In essence, you will set up a miniature watermill. You will need a sink with a faucet, and a rodent running wheel. Place the wheel under the faucet and, before turning on the water, note the obvious: 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 be able to make it spin. Note not only that the wheel is spinning now, but also that it is spinning at a regular rate. The wheel completes a full revolution within the same interval, at the same speed, over and over again. If you calibrated the wheel, you could use it as a clock. Next, increase or decrease the flow of water. Note that you can make the clock go faster or slower. That's it! You built your own clock!

Exercise 6.2 A slightly more sophisticated timing device

Section 6.1 mentioned the use of a simple electronic circuit as a model of a pacemaker. In this exercise, you will assemble a circuit. The required parts are shown in Figure 6.43. 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 solid-state because you must be able to hear the clicking of the solenoid. Once 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 calibrated it and synchronized it to solar time. For more sophisticated electronic circuits, see the *Web Sites to Explore* section of this chapter.

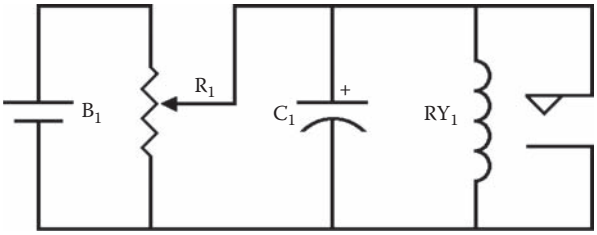


FIGURE 6.43 Building a simple clock. This schematic drawing of an electronic circuit will help you build a simple pacemaker. B_1: 9 V battery or DC power supply. R_1: 1 kΩ adjustable resistor. C_1: 2000 μF electrolytic capacitor. RY_1: 9 VDC mechanical relay.

Exercise 6.3 Using a computer model of the circadian pacemaker

This exercise uses the program Model to simulate a circadian pacemaker. Section 7.3 of Chapter 7 provides details about using this program; the Exercises section of Chapter 7 contains additional exercises that use Model.

1. Double-click on the Circadian icon to open the program banner, then click on Model (the fifth icon from the right).
2. At the top of the window, note that you can stipulate the "endogenous" period of the "organism" and can choose one to four blocks of simulations. In this exercise, you will need only one block.
3. Set the period to 24.1 hours (which is the approximate free-running period of a golden hamster), and move to Block 1. Set the Days to 60 (2 months) and the Mode to DD (constant darkness). Click on Run.
4. The program displays 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. The program now displays an actogram with a smooth drift to the left, which is consistent with a circadian period shorter than 24.0 hours.
5. Return to the main window, set the period to 23.6 hours (the typical free-running period of a mouse) and run another simulation. Then enter a period of 20.2 hours (the typical free-running period of a homozygous tau-mutant hamster). Note 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 display a perfectly vertical actogram. Then switch the Mode to LL (constant light) and run another simulation. Note 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 freerun in the sense that they do not receive temporal cues from the environment, but their expressed circadian period is not the same as their endogenous period. Section 7.2 discusses this issue in further detail.

Exercise 6.4 Recording a free-running rhythm

You can conduct this exercise if you assembled the data acquisition system in Exercise 2.4, or if you have access to a data acquisition system for rodent activity in a researcher's laboratory or to any system that measures circadian rhythms. The exercise is quite simple: you will keep your experimental subjects under a light–dark cycle for 1 to 2 weeks, transfer them to constant darkness for 2 or more weeks, and then inspect the records. If you use the data acquisition system from Exercise 2.4 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 the period is either shorter or longer in constant darkness. (In rare cases, your test animal might have an endogenous period of exactly 24.0 hours.) Observing free-running rhythms in your own experimental subjects helps 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

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the

Literature Cited section. For more general reading, the following sources may be useful.

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 Edition). 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.

WEB SITES TO EXPLORE

Center for Neurodynamics at the University of Missouri:
http://neurodyn.umsl.edu

Electronic Oscillators:
http://www.electronics-tutorials.com/oscillators/oscillators.htm

Steve Kay's Laboratory (Scripps Research Institute):
http://www.scripps.edu/cb/kay

Van der Pol Oscillator:
http://www.iro.umontreal.ca/~eckdoug/vibe/Relaxation/VanDerPol.html

LITERATURE CITED

1. 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.
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. & 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. & 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. & 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 U.S.A.* 97: 6914–6919.
11. Shimizu, I., Kawai, Y., Taniguchi, M. & 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. & 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. Tosini, G. & Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *Journal of Biological Rhythms* 10: 248–255.
15. Bartell, P. A., Miranda-Anaya, M. & Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
16. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
17. Lowe, C. H., Hinds, D. S., Lardner, P. J. & Justice, K. E. (1967). Natural free-running period in vertebrate animal populations. *Science* 156: 531–534.
18. Bertolucci, C., Sovrano, V. A., Magnone, M. C. & Foà, A. (2000). Role of suprachiasmatic nuclei in circadian and light-entrained behavioral rhythms of lizards. *American Journal of Physiology* 279: R2121–R2131.
19. Refinetti, R. & Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.
20. Janik, D. S., Pickard, G. E. & 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.
21. Tosini, G. & Menaker, M. (1998). Multioscillatory circadian organization in a vertebrate, *Iguana iguana*. *Journal of Neuroscience* 18: 1105–1114.
22. Hurd, M. W., Debruyne, J., Straume, M. & Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiology and Behavior* 65: 465–472.

23. Iigo, M. & Tabata, M. (1996). Circadian rhythms of locomotor activity in the goldfish *Carassius auratus*. *Physiology and Behavior* 60: 775–781.
24. Herrero, M. J., Madrid, J. A. & Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
25. Chen, W. M. & Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
26. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W. & 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.
27. King, V. M., Bentley, G. E. & 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.
28. Ebihara, S. & Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
29. Oshima, I. & 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.
30. Oshima, I., Yamada, H., Goto, M., Sato, K. & 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.
31. 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.
32. Berger, R. J. & 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.
33. Hau, M. & Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
34. Heigl, S. & Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
35. Aschoff, J. & von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
36. Pohl, H. (1993). Does aging affect the period of the circadian pacemaker in vertebrates? *Naturwissenschaften* 80: 478–481.
37. Underwood, H. & Edmonds, K. (1995). The circadian rhythm of thermoregulation in Japanese quail. II. Multioscillator control. *Journal of Biological Rhythms* 10: 234–247.
38. Kumar, V., Gwinner, E. & 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.
39. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
40. Mistlberger, R. E., Lukman, H. & Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: assessment and intervention. *Appetite* 30: 255–267.
41. Borbély, A. A. & 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.
42. Zucker, I., Fitzgerald, K. M. & Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.
43. Albers, H. E., Gerall, A. A. & Axelson, J. F. (1981). Effect of reproductive state on circadian periodicity in the rat. *Physiology and Behavior* 26: 21–25.
44. Sridaran, R. & 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.
45. Weber, A. L. & Adler, N. T. (1978). Dissociation of persistent estrus and a free-running activity cycle in different light cycles. *Biology of Reproduction* 19: 425–430.
46. 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.
47. Isobe, Y., Aoki, K., Furuyama, F. & 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.
48. Ikeda, M. & 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.
49. Francis, A. J. P. & 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.
50. Honma, K. & Hiroshige, T. (1978). Internal synchronization among several circadian rhythms in rats under constant light. *American Journal of Physiology* 235: R243–R249.
51. Honma, K. & Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
52. Edgar, D. M., Kilduff, T. S., Martin, C. E. & 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.

53. Hofstetter, J. R., Grahame, N. J. & Mayeda, A. R. (2003). Circadian activity rhythms in high-alcohol-preferring and low-alcohol-preferring mice. *Alcohol* 30: 81–85.
54. Edgar, D. M., Martin, C. E. & 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.
55. Possidente, B., McEldowney, S. & Pabon, A. (1995). Aging lengthens circadian period for wheel-running activity in C57BL mice. *Physiology and Behavior* 57: 575–579.
56. Davis, F. C. & Menaker, M. (1981). Development of the mouse circadian pacemaker: independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
57. Abe, H., Honma, S., Honma, K., Suzuki, T. & 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.
58. Yoshimura, T., Nishio, M., Goto, M. & 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.
59. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
60. Edgar, D. M. & Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
61. Refinetti, R. (1996). Ultradian rhythms of body temperature and locomotor activity in wild-type and tau-mutant hamsters. *Animal Biology* 5: 111–115.
62. Fitzgerald, K. M. & Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences U.S.A.* 73: 2923–2927.
63. Davis, F. C., Stice, S. & 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.
64. Refinetti, R. & Menaker, M. (1992). The circadian rhythm of body temperature of normal and tau-mutant golden hamsters. *Journal of Thermal Biology* 17: 129–133.
65. DeCoursey, P. J., Pius, S., Sandlin, C., Wethey, D. & Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiology and Behavior* 65: 457–463.
66. Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology A* 162: 35–46.
67. Weinert, D., Fritzche, P. & Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
68. Oklejewicz, M., Hut, R. A., Daan, S, Loudon, A. S. I. & Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.
69. Refinetti, R. & Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
70. Boulos, Z., Macchi, M., Houpt, T. A. & Terman, M. (1996). Photic entrainment in hamsters: effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
71. DeCoursey, P. (1960). Phase control of activity in a rodent. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 49–55.
72. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
73. DeCoursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science* 131: 33–35.
74. Mrosovsky, N., Boshes, M., Hallonquist, J. D. & Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.
75. Lee, T. M., Holmes, W. G. & Zucker, I. (1990). Temperature dependence of circadian rhythms in golden-mantled ground squirrels. *Journal of Biological Rhythms* 5: 25–34.
76. Kennedy, G. A., Hudson, R. & Armstrong, S. M. (1994). Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiology and Behavior* 55: 385–389.
77. Jilge, B. (1991). Restricted feeding: a nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
78. Johnson, R. F. & Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
79. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., & 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.
80. Hawking, F., Lobban, M. C., Gammage, K. & 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.
81. Siwak, C. T., Tapp, P. D., Zicker, S. C., Murphey, H. L., Muggenburg, B. A., Head, E. & Cotman, C. W. (2003). Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience* 117: 813–824.
82. Rauth-Widmann, B., Fuchs, E. & 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.

83. Perret, M. & 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.
84. Robinson, E. L. & Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
85. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
86. Boulos, Z., Frim, D. M., Dewey, L. K. & Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
87. Tokura, H. & Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
88. Ingram, D. L. & 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.
89. Marimuthu, G., Rajan, S. & 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.
90. Harlow, H. J., Phillips, J. A. & 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.
91. Kennedy, G. A., Coleman, G. J. & Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
92. Chandrashekaran, M. K., Marimuthu, G. & 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.
93. Aschoff, J., Gerecke, U. & Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
94. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C. & Knauer, R. S. (1980). Human sleep: its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
95. Kennaway, D. J. & 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.
96. Steel, G. D., Callaway, M., Suedfeld, P. & 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.
97. Aschoff, J., Gerecke, U. & Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
98. Zulley, J., Wever, R. & Aschoff, J. (1981). The dependence of onset and duration of sleep on the circadian rhythm of rectal temperature. *Pflügers Archiv* 391: 314–318.
99. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F. & Pauly, J. E. (Eds.). *Chronobiology.* Tokyo: Igaku Shoin, pp. 451–459.
100. Lund, R. (1974). Personality factors and desynchronization of circadian rhythms. *Psychosomatic Medicine* 36: 224–228.
101. Campbell, S. S., Dawson, D. & Zulley, J. (1993). When the human circadian system is caught napping: evidence for endogenous rhythms close to 24 hours. *Sleep* 16: 638–640.
102. Endo, T., Honma, S., Hashimoto, S. & Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
103. Colin, J., Timbal, J., Boutelier, C., Houdas, Y. & Siffre, M. (1968). Rhythm of the rectal temperature during a 6-month free-running experiment. *Journal of Applied Physiology* 25: 170–176.
104. Gundel, A., Polyakov, V. V. & Zulley, J. (1997). The alteration of human sleep and circadian rhythms during spaceflight. *Journal of Sleep Research* 6: 1–8.
105. Monk, T. H., Buysse, D. J., Billy, B. D., Kennedy, K. S. & Willrich, L. M. (1998). Sleep and circadian rhythms in four orbiting astronauts. *Journal of Biological Rhythms* 13: 188–201.
106. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J., Elliott, A. R., Prisk, G. K., West, J. B. & Czeisler, C. A. (2001). Sleep, performance, circadian rhythms, and light-dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
107. Jespersen, J. & Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency,* 2nd Edition. Mineola, NY: Dover.
108. Barnett, J. E. (1998). *Time's Pendulum: From Sundials to Atomic Clocks*. New York: Plenum.
109. Rensing, L., Meyer-Grahle, U. & Ruoff, P. (2001). Biological timing and the clock metaphor: oscillatory and hourglass mechanisms. *Chronobiology International* 18: 329–369.
110. Kondo, T., Mori, T., Lebedeva, N. V., Aoki, S., Ishiura, M. & Golden, S. S. (1997). Circadian rhythms in rapidly dividing cyanobacteria. *Science* 275: 224–227.
111. Friesen, W. O., Block, G. D. & Hocker, C. G. (1993). Formal approaches to understanding biological oscillators. *Annual Review of Physiology* 55: 661–681.
112. van der Pol, B. (1960). *Selected Scientific Papers*. Amsterdam: North Holland.
113. Hilborn, R. C. (2000). *Chaos and Nonlinear Dynamics,* 2nd Edition. New York: Oxford University Press.

114. Pavlidis, T. (1981). Mathematical models. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology, Volume 4*). New York: Plenum, pp. 41–54.
115. Kronauer, R. E., Czeisler, C. A., Pilato, S. F., Moore-Ede, M. C. & Weitzman, E. D. (1982). Mathematical model of the human circadian system with two interacting oscillators. *American Journal of Physiology* 242: R3–R17.
116. De Goede, J., Olofson, E. & Rietveld, W. J. (1992). A mathematical model of the circadian system with a delayed feedback loop. *Journal of Interdisciplinary Cycle Research* 23: 142–143.
117. Pedersen, M. & Johnsson, A. (1994). A study of the singularities in a mathematical model for circadian rhythms. *BioSystems* 33: 193–201.
118. Barrio, R. A., Zhang, L. & Maini, P. K. (1997). Hierarchically coupled ultradian oscillators generating robust circadian rhythms. *Bulletin of Mathematical Biology* 59: 517–532.
119. Kunz, H. & 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.
120. Binkley, S., Kluth, E. & Menaker, M. (1971). Pineal function in sparrows: circadian rhythms and body temperature. *Science* 174: 311–314.
121. Honma, S., Honma, K., Shirakawa, T. & Hiroshige, T. (1988). Rhythms in behavior, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. *Physiology and Behavior* 44: 247–255.
122. Refinetti, R., Nelson, D. E. & 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.
123. Canal-Corretger, M. M., Vilaplana, J., Cambras, T. & 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.
124. Spoelstra, K., Oklejewicz, M. & 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.
125. Yen, U. C., Huang, T. C. & Yen, T. C. (2004). Observation of the circadian photosynthetic rhythm in cyanobacteria with a dissolved-oxygen meter. *Plant Science* 166: 949–952.
126. Pfeffer, W. (1875). *Die periodischen Bewegungen der Blattorgane*. Leipzig: Wilhelm Engelmann.
127. Hillman, W. S. (1970). Carbon dioxide output as an index of circadian timing in *Lemna* photoperiodism. *Plant Physiology* 45: 273–279.
128. Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science* 284: 2124–2128.
129. Tosini, G. & 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.
130. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
131. Winget, C. M. & Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
132. Lumineau, S. & Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
133. Reierth, E. & Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
134. Kuwabara, N., Seki, K. & 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.
135. Hoban, T. M., Levine, A. H., Shane, R. B. & Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
136. Tavernier, R. J., Largen, A. L. & Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
137. Mrosovsky, N., Melnyk, R. B., Lang, K., Hallonquist, J. D., Boshes, M. & Joy, J. E. (1980). Infradian cycles in dormice (*Glis glis*). *Journal of Comparative Physiology A* 137: 315–339.
138. Boulos, Z. & Rusak, B. (1982). Circadian phase response curves for dark pulses in the hamster. *Journal of Comparative Physiology A* 146: 411–417.
139. Gerkema, M. P., Groos, G. A. & 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.
140. Halberg, F., Visscher, M. B. & Bittner, J. J. (1953). Eosinophil rhythm in mice: range of occurrence, effects of illumination, feeding and adrenalectomy. *American Journal of Physiology* 174: 109–122.
141. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G. & Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.
142. Witte, K., Grebmer, W., Scalbert, E., Delagrande, P., Guardiola-Lemaître, B. & Lemmer, B. (1998). Effects of melatoninergic agonists on light-supressed circadian rhythms in rats. *Physiology and Behavior* 65: 219–224.
143. Eastman, C. & Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
144. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.

145. Edelstein, K., Pfaus, J. G., Rusak, B. & Amir, S. (1995). Neonatal monosodium glutamate treatment prevents effects of constant light on circadian temperature rhythms of adults rats. *Brain Research* 675: 135–142.
146. Deprés-Brummer, P., Metzger, G. & Lévi, F. (1996). Analyses des rythmes de la température corporelle du rat en libre cours. *Pathologie Biologie* 44: 150–156.
147. Deprés-Brummer, P., Lévi, F., Metzger, G. & Touitou, Y. (1995). Light-induced suppression of the rat circadian system. *American Journal of Physiology* 268: R1111–R1116.
148. Usui, S., Okazaki, T. & Honda, Y. (2003). Interruption of the rat circadian clock by short light-dark cycles. *American Journal of Physiology* 284: R1255–R1259.
149. Granados-Fuentes, D., Prolo, L. M., Abraham, U. & Herzog, E. D. (2004). The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *Journal of Neuroscience* 24: 615–619.
150. Aschoff, J. (1958). Tierische Periodik unter dem Einfluß von Zeitgebern. *Zeitschrift für Tierpsychologie* 15: 1–30.
151. Aschoff, J. (1979). Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
152. Daan, S. & 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.
153. Schilling, A., Richard, J. P. & 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.
154. Summer, T. L., Ferraro, J. S. & McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
155. Earnest, D. J. & 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.
156. Pohl, H. (1985). The circadian system of the Turkish hamster, *Mesocricetus brandti*: responses to light. *Comparative Biochemistry and Physiology A* 81: 613–618.
157. Witting, W., Boerma, D., Koster-Van- Hoffen, G. C., Swaab, D. F. & Mirmiran, M. (1995). Light suppresses frequency and endogenous amplitude of the circadian system in nocturnal animals. *Biological Rhythm Research* 26: 477–485.
158. Morin, L. P. & 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.
159. Mrosovsky, N. (2003). Aschoff's rule in retinally degenerate mice. *Journal of Comparative Physiology A* 189: 75–78.
160. Koteja, P., Swallow, J. G., Carter, P. A. & Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.
161. Canal-Corretger, M. M., Cambras, T. & Díez-Noguera, A. (2003). Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiology International* 20: 21–35.
162. DeCoursey, P. J., Krulas, J. R., Mele, G. & Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior* 62: 1099–1108.
163. Meijer, J. H., Daan, S., Overkamp, G. J. F. & 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.
164. Refinetti, R. & Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
165. Underwood, H. (1983). Circadian pacemakers in lizards: phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
166. Miranda-Anaya, M., Bartell, P. A., Yamazaki, S. & Menaker, M. (2000). Circadian rhythm of ERG in *Iguana iguana*: role of the pineal. *Journal of Biological Rhythms* 15: 163–171.
167. Lee, T. M. & 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. 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.
169. Fuller, C. A. & 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.
170. Wever, R. A. (1989). Light effects on human circadian rhythms: a review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
171. Giorgetti, M. & Zhdanova, I. V. (2000). Chronic cocaine treatment induces dysregulation in the circadian pattern of rats' feeding behavior. *Brain Research* 877: 170–175.
172. Marchant, E. G. & Mistlberger, R. E. (1995). Morphine phase-shifts circadian rhythms in mice: role of behavioural activation. *NeuroReport* 7: 209–212.
173. Park, R. N., Hirst, M. & 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.
174. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M. & 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.
175. Masubuchi, S., Honma, S., Abe, H., Nakamura, W. & Honma, K. (2001). Circadian activity rhythm in methamphetamine-treated *Clock* mutant mice. *European Journal of Neuroscience* 14: 1177–1180.

176. Honma, S. & Honma, K. (1995). Phase-dependent phase shift of methamphetamine-induced circadian rhythm by haloperidol in SCN-lesioned rats. *Brain Research* 674: 283–290.
177. Coward, D. J., Cain, S. W. & Ralph, M. R. (2001). A circadian rhythm in mice that is unaffected by the period mutation at *Clock*. *Biological Rhythm Research* 32: 233–242.
178. Pardo, B. F. & 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.
179. White, L., Ringo, J. & Dowse, H. (1992). A circadian clock of *Drosophila*: effects of deuterium oxide and mutations at the *period* locus. *Chronobiology International* 9: 250–259.
180. Pickard, G. E. & Zucker, I. (1986). Influence of deuterium oxide on circadian activity rhythms of hamsters: role of the suprachiasmatic nuclei. *Brain Research* 376: 149–154.
181. Oklejewicz, M., Hut, R. A. & 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.
182. Mistlberger, R. E., Marchant, E. G. & Kippin, T. E. (2001). Food-entrained circadian rhythms in rats are insensitive to deuterium oxide. *Brain Research* 919: 283–291.
183. Mistlberger, R. E. & 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.
184. Eastman, C. I., Stewart, K. T. & Weed, M. R. (1994). Evening alcohol consumption alters the circadian rhythm of body temperature. *Chronobiology International* 11: 141–142.
185. Danel, T., Libersa, C. & 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.
186. Sei, H., Sakata-Haga, H., Ohta, K., Sawada, K., Morita, Y. & Fukui, Y. (2003). Prenatal exposure to alcohol alters the light response in postnatal circadian rhythm. *Brain Research* 987: 131–134.
187. Van Reeth, O. & 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. & 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. & 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. & 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. & 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. Van Reeth, O. & Turek, F. W. (1990). Daily injections of triazolam induce long-term changes in hamster circadian period. *American Journal of Physiology* 259: R514–R520.
193. Penev, P. D., Zee, P. C. & Turek, F. W. (1994). Monoamine depletion blocks triazolam-induced phase advances of the circadian clock in hamsters. *Brain Research* 637: 255–261.
194. Takahashi, Y., Usui, S. & 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.
195. Tsujimaru, S., Egami, H., Honma, G., Ida, Y., Mukasa, H. & 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.
196. Ikeda, M., Honda, K. & Inoué, S. (1996). Vitamin B_{12} amplifies circadian phase shifts induced by a light pulse in rats. *Experientia* 52: 691–694.
197. Cossins, A. R. & Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman and Hall.
198. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th Edition. New York: Cambridge University Press.
199. Priddle, J. (1980). The production ecology of benthic plants in some Arctic lakes. II. Laboratory physiology studies. *Journal of Ecology* 68: 155–166.
200. Jones, M. B., Leafe, E. L., Stiles, W. & Collett, B. (1978). Pattern of respiration of a perennial ryegrass crop in the field. *Annals of Botany* 42: 693–703.
201. Scholander, P. F., Flagg, W., Walters, V. & Irving, L. (1952). Respiration in some Arctic and tropical lichens in relation to temperature. *American Journal of Botany* 39: 707–713.
202. Wager, H. G. (1941). On the respiration and carbon assimilation rates of some arctic plants as related to temperature. *New Phytologist* 40: 1–19.
203. Humphreys, W. F. (1975). The respiration of *Geolycosa godeffroyi* (Araneae: Lycosidae) under conditions of constant and cyclic temperature. *Physiological Zoology* 48: 269–281.
204. Withers, P. C. & 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.
205. Edwards, G. A. & 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.
206. Husain, M. Z. & 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.

207. Gromysz-Kalkowska, K. & Szubartowska, E. (1984). Oxygen consumption in two terrestrial species of crustaceans (Isopoda). *Bulletin of the Polish Academy of Sciences, Biological Sciences* 32: 47–56.
208. Newell, R. C., Wieser, W. & Pye, V. I. (1974). Factors affecting oxygen consumption in the woodlouse *Porcellio scaber*. *Oecologia* 16: 31–51.
209. Gromysz-Kalkowska, K., Oder, M. & Szubartowska, E. (1984). Oxygen consumption in *Armadillidium nasutum* Buddle-Lund (Isopoda). *Folia Biologica Kraków* 32: 89–108.
210. Halcrow, K. & 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.
211. Moreira, G. S. & 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.
212. Reiber, C. L. & Birchard, G. F. (1993). Effect of temperature on metabolism and hemolymph pH in the crab *Stoliczia abbotti*. *Journal of Thermal Biology* 18: 49–52.
213. Harrison, J. F., Fewell, J. H., Roberts, S. P. & Hall, H. G. (1996). Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science* 274: 88–90.
214. Schmolz, E., Lamprecht, I. & Schricker, B, (1993). Calorimetric investigations on social thermogenesis in the hornet *Vespa crabo* L. (Hymenoptera: Vespinae). *Thermochimica Acta* 229: 173–180.
215. Singh, S. P. & Das, A. B. (1977). Seasonal variation and acclimatization of respiratory metabolism of *Periplaneta americana* (Linn.). *Comparative Physiology and Ecology* 2: 133–138.
216. Frears, S. L., Chown, S. L. & Webb, P. I. (1997). Behavioural thermoregulation in the mopane worm (Lepidoptera). *Journal of Thermal Biology* 22: 325–330.
217. Haugaard, N. & 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.
218. Fry, F. E. J. & Hart, J. S. (1948). The relation of temperature to oxygen consumption in the goldfish. *Biological Bulletin* 94: 66–77.
219. Henry, J. A. C. & Houston, A. H. (1984). Absence of respiratory acclimation to diurnally-cycling temperature conditions in rainbow trout. *Comparative Biochemistry and Physiology A* 77: 727–734.
220. Vondracek, B., Cech, J. J. & 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.
221. Duthie, G. G. & 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.
222. Refinetti, R. & Refinetti, K. Z. (1988). Absence of metabolic acclimation to cycling temperature conditions in the goldfish. *Physiology and Behavior* 43: 121–122.
223. Crawshaw, L. I., Ackerman, R. A., White, F. N. & 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.
224. Sweeney, B. M. & Hastings, J. W. (1960). Effects of temperature upon diurnal rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 87–103.
225. Kondo, T., Strayer, C. A., Kulkarni, R. D., Taylor, W., Ishiura, M., Golden, S. S. & 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 U.S.A.* 90: 5672–5676.
226. Liu, Y., Merrow, M., Loros, J. J. & Dunlap, J. C. (1998). How temperature changes reset a circadian oscillator. *Science* 281: 825–829.
227. Hennessey, T. L. & Field, C. B. (1992). Evidence of multiple circadian oscillators in bean plants. *Journal of Biological Rhythms* 7: 105–113.
228. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences U.S.A.* 40: 1018–1029.
229. Hoffmann, K. (1957). Über den Einfluß der Temperatur auf die Tagesperiodik bei einem Poikilothermen. *Naturwissenschaften* 44: 358.
230. Menaker, M. (1959). Endogenous rhythms of body temperature in hibernating bats. *Nature* 184: 1251–1252.
231. Gibbs, F. P. (1981). Temperature dependence of rat circadian pacemaker. *American Journal of Physiology* 241: R17–R20.
232. Ruby, N. F., Burns, D. E. & 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.
233. Herzog, E. D. & Huckfeldt, R. M. (2003). Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 763–770.
234. Izumo, M., Johnson, C. H. & 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 U.S.A.* 100: 16089–16094.
235. Gibbs, F. P. (1983). Temperature dependence of the hamster circadian pacemaker. *American Journal of Physiology* 244: R607–R610.
236. Tosini, G. & Menaker, M. (1998). The *tau* mutation affects temperature compensation of hamster retinal circadian oscillators. *NeuroReport* 9: 1001–1005.
237. Konopka, R. J. & Benzer, S. (1971). Clock mutants of Drosophila melanogaster. *Proceedings of the National Academy of Sciences U.S.A.* 68: 2112–2116.
238. Feldman, J. F. & Hoyle, M. N. (1973). Isolation of circadian clock mutants of *Neurospora crassa*. *Genetics* 75: 605–613.

239. Hall, J. C. (1990). Genetics of circadian rhythms. *Annual Review of Genetics* 24: 659–697.
240. Rothenfluh, A., Abodeely, M., Price, J. L. & 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.
241. Akten, B., Jauch, E., Genova, G. K., Kim, E. Y., Edery, I., Raabe, T. & Jackson, F. R. (2003). A role for CK2 in the *Drosophila* circadian oscillator. *Nature Neuroscience* 6: 251–257.
242. Dunlap, J. C. (1996). Genetic and molecular analysis of circadian rhythms. *Annual Review of Genetics* 30: 579–601.
243. Ralph, M. R. & Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
244. 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. & Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264: 719–725.
245. Mrosovsky, N. (1989). Mutant hamster in a hurry. *Nature* 337: 213.
246. Menaker, M. & 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.
247. Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
248. Osiel, S., Golombek, D. A. & 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.
249. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A. & Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
250. Tosini, G. & Menaker, M. (1996). Circadian rhythms in cultured mammalian retina. *Science* 272: 419–421.
251. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M. & 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.
252. Shimomura, K., Nelson, D. E., Ihara, N. L. & Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
253. Loudon, A. S. I., Ihara, N. & 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.
254. Refinetti, R. & Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.
255. Loudon, A. S. I., Wayne, N. L., Krieg, R., Iranmanesh, A., Veldhuis, J. D. & Menaker, M. (1994). Ultradian endocrine rhythms are altered by a circadian mutation in the Syrian hamster. *Endocrinology* 135: 712–718.
256. Shimomura, K. & Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
257. Grosse, J., Loudon, A. S. I. & 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.
258. Shimomura, K., Kornhauser, J. M., Wisor, J. P., Umezu, T., Yamazaki, S., Ihara, N. L., Takahashi, J. S. & 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.
259. Oklejewicz, M. & Daan, S. (2002). Enhanced longevity in *tau* mutant Syrian hamsters, *Mesocricetus auratus*. *Journal of Biological Rhythms* 17: 210–216.
260. Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamazaki, S., Zemenides, P. D., Ralph, M. R., Menaker, M. & Takahashi, J. S. (2000). Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* 288: 483–491.
261. King, D. P., Zhao, Y., Sangoram, A. M., Wilsbacher, L. D., Tanaka, M., Antoch, M. P., Steeves, T. D. L., Vitaterna, M. H., Kornhauser, J. M., Lowrey, P. L., Turek, F. W. & Takahashi, J. S. (1997). Positional cloning of the mouse circadian *Clock* gene. *Cell* 89: 641–653.
262. 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. & Takahashi, J. S. (1997). Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. *Cell* 89: 655–667.
263. Miller, B. H., Olson, S. L., Turek, F. W., Levine, J. E., Horton, T. H. & Takahashi, J. S. (2004). Circadian *Clock* mutation disrupts estrous ciclicity and maintenance of pregnancy. *Current Biology* 14: 1367–1373.
264. Shearman, L. P. & Weaver, D. R. (1999). Photic induction of *Period* gene expression is reduced in *Clock* mutant mice. *NeuroReport* 10: 613–618.
265. Pickard, G. E., Sollars, P. J., Rinchik, E. M., Nolan, P. M. & Bucan, M. (1995). Mutagenesis and behavioral screening for altered circadian activity identifies the mouse mutant, *Wheels*. *Brain Research* 705: 255–266.
266. Bacon, Y., Ooi, A., Kerr, S., Shaw-Andrews, L., Winchester, L., Breeds, S., Tymoska-Lalanne, Z., Clay, J., Greenfield, A. G. & Nolan, P. M. (2004). Screening for novel ENU-induced rhythm, entrainment and activity mutants. *Genes, Brain and Behavior* 3: 196–205.
267. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D. & Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
268. Bunger, M. K., Wilsbacher, L. D., Moran, S. M., Clendenin, C., Radcliffe, L. A., Hogenesch, J. B., Simon, M. C., Takahashi, J. S. & Bradfield, C. A. (2000). *Mop3* is an essential component of the master circadian pacemaker in mammals. *Cell* 103: 1009–1017.

269. Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P., Dawut, L., Smithies, O., Takahashi, J. S. & Sancar, A. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 282: 1490–1494.
270. Connolly, M. S. & Lynch, C. B. (1983). Classical genetic analysis of circadian body temperature rhythms in mice. *Behavior Genetics* 13: 491–500.
271. Puchalski, W. & Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.
272. Guyomarc'h, C., Lumineau, S. & 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.
273. Onai, K., Okamoto, K., Nishimoto, H., Morioka, C., Hirano, M., Kami-ike, N. & 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.
274. De Castro, J. M. (2001). Heritability of diurnal changes in food intake in free-living humans. *Nutrition* 17: 713–720.
275. Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.
276. Barrett, R. K. & Page, T. L. (1989). Effects of light on circadian pacemaker development. I. The freerunning period. *Journal of Comparative Physiology A* 165: 41–49.
277. Roenneberg, T. & Morse, D. (1993). Two circadian oscillators in one cell. *Nature* 362: 362–364.
278. Morse, D., Hastings, J. W. & Roenneberg, T. (1994). Different phase responses of the two circadian oscillators in *Gonyaulax*. *Journal of Biological Rhythms* 9: 263–274.
279. Swann, J. & 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.
280. Pickard, G. E. & Turek, F. W. (1982). Splitting of the circadian rhythm of activity is abolished by unilateral lesions of the suprachiasmatic nuclei. *Science* 215: 1119–1121.
281. Pickard, G. E., Kahn, R. & Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiology and Behavior* 32: 763–766.
282. Swann, J. M. & Turek, F. W. (1985). Multiple circadian oscillators regulate the timing of behavioral and endocrine rhythms in female golden hamsters. *Science* 228: 898–900.
283. Morin, L. P. (1988). Age-related changes in hamster circadian period, entrainment, and rhythm splitting. *Journal of Biological Rhythms* 3: 237–248.
284. Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *Journal of Biological Rhythms* 8: 189–197.
285. Hallonquist, J., Lindegger, M. & Mrosovsky, N. (1994). Rubidium chloride fuses split circadian activity rhythms in hamsters housed in bright constant light. *Chronobiology International* 11: 65–71.
286. De la Iglesia, H. O., Meyer, J. & 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.
287. Mendoza, J. Y., Dardente, H., Escobar, C., Pevét, P. & Challet, E. (2004). Dark pulse resetting of the suprachiasmatic clock in Syrian hamsters: behavioral phase-shifts and clock gene expression. *Neuroscience* 127: 529–537.
288. Daan, S. & Berde, C. (1978). Two coupled oscillators: simulations of the circadian pacemaker in mammalian activity rhythms. *Journal of Theoretical Biology* 70: 297–313.
289. 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.
290. Oda, G. A. & Friesen, W. O. (2002). A model of "splitting" of running-wheel activity in hamsters. *Journal of Biological Rhythms* 17: 76–88.
291. Cheung, P. W. & McCormack, C. E. (1983). Splitting of the locomotor activity rhythm in rats by exposure to continuous light. *American Journal of Physiology* 244: H573–H576.
292. Schardt, U., Wilhelm, I. & Erkert, H. G. (1989). Splitting of the circadian activity rhythm in common marmosets (*Callithrix j. jacchus*; Primates). *Experientia* 45: 1112–1115.
293. Zlomanczuk, P., Margraf, R. R. & 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.
294. Honma, S. & Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
295. Pohl, H. (1983). Light pulses entrain the circadian activity rhythm of a diurnal rodent (*Ammospermophilus leucurus*). *Comparative Biochemistry and Physiology B* 76: 723–729.
296. Lovegrove, B. G. & 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.
297. Harrington, M. E., Eskes, G. A., Dickson, P. & Rusak, B. (1990). Lesions dorsal to the suprachiasmatic nuclei abolish split activity rhythms of hamsters. *Brain Research Bulletin* 24: 593–597.
298. De la Iglesia, H. O., Meyer, J., Carpino, A. & Schwartz, W. J. (2000). Antiphase oscillation of the left and right suprachiasmatic nuclei. *Science* 290: 799–801.
299. Watanabe, T., Yoshimura, T., McMahon, D. G. & Ebihara, S. (2003). Unimodal circadian rhythm in the suprachiasmatic nucleus of behaviorally splitting mice. *Neuroscience Letters* 345: 49–52.

300. Jagota, A., de la Iglesia, H. O. & Schwartz, W. J. (2000). Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro*. *Nature Neuroscience* 3: 372–376.
301. Burgoon, P. W., Lindberg, P. T. & 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.
302. Grima, B., Chélot, E., Xia, R. & Rouyer, F. (2004). Morning and evening peaks of activity rely on different clock neurons of the *Drosophila* brain. *Nature* 431: 869–873.
303. Mrosovsky, N. & Janik, D. (1993). Behavioral decoupling of circadian rhythms. *Journal of Biological Rhythms* 8: 57–65.
304. Edelstein, K., de la Iglesia, H. O. & Mrosovsky, N. (2003). Period gene expression in the suprachiasmatic nucleus of behaviorally decoupled hamsters. *Molecular Brain Research* 114: 40–45.
305. Gorman, M. R., Yellon, S. M. & Lee, T. M. (2001). Temporal reorganization of the suprachiasmatic nuclei in hamsters with split circadian rhythms. *Journal of Biological Rhythms* 16: 552–563.
306. Gorman, M. R. & Lee, T. M. (2001). Daily novel wheel running reorganizes and splits hamster circadian activity rhythms. *Journal of Biological Rhythms* 16: 541–551.
307. Gorman, M. R. (2001). Exotic photoperiods induce and entrain split circadian activity rhythms in hamsters. *Journal of Comparative Physiology A* 187: 793–800.
308. Gorman, M. R., Elliott, J. A. & Evans, J. A. (2003). Plasticity of hamster circadian entrainment patterns depends on light intensity. *Chronobiology International* 20: 233–248.
309. Gorman, M. R. & 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.
310. Kleitman, N. & 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.
311. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.
312. Moore-Ede, M. C., Kass, D. A. & Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
313. Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sánchez, R., Ríos, C. D., Freitag, W. O., Richardson, G. S. & Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep–wake cycle. *Science* 233: 667–671.
314. Minors, D. S., Waterhouse, J. M., Folkard, S. & 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.
315. Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D. & 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.
316. Dijk, D. J. & 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.
317. Roberts, M. H. & Xie, X. (1996). Phase relationship between ocular and behavioral circadian rhythms in *Bulla gouldiana* exposed to different photoperiods. *Physiology and Behavior* 59: 703–708.
318. Waterhouse, J., Minors, D., Folkard, S., Owens, D., Atkinson, G., Macdonald, I., Reilly, T., Sytnik, N. & Tucker, P. (1998). Light of domestic intensity produces phase shifts of the circadian oscillator in humans. *Neuroscience Letters* 245: 97–100.
319. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A. & 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.
320. Lockley, S. W., Skene, D. J., Butler, L. J. & Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 22: 616–623.
321. Wright, K. P., Hull, J. T. & Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *American Journal of Physiology* 283: R1370–R1377.
322. Hack, L. M., Lockley, S. W., Arendt, J. & 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.
323. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Spontaneous internal desynchronization of circadian rhythms in the squirrel monkey. *Comparative Biochemistry and Physiology A* 58: 63–67.
324. Ruis, J. F., Rietveld, W. J. & 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. Labyak, S. E. & Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
326. Miles, L. E. M., Raynal, D. M. & Wilson, M. A. (1977). Blind man living in normal society has circadian rhythms of 24.9 hours. *Science* 198: 421–423.
327. Zulley, J. & Campbell, S. S. (1985). Napping behavior during "spontaneous internal desynchronization": sleep remains in synchrony with body temperature. *Human Neurobiology* 4: 123–126.
328. Folkard, S., Wever, R. A. & Wildgruber, C. M. (1983). Multi-oscillatory control of circadian rhythms in human performance. *Nature* 305: 223–226.

329. Folkard, S., Hume, K. I., Minors, D. S., Waterhouse, J. M. & Watson, F. L. (1985). Independence of the circadian rhythm in alertness from the sleep/wake cycle. *Nature* 313: 678–679.
330. Green, J., Pollak, C. P. & Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
331. 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.
332. Aschoff, J. (1994). The timing of defecation within sleep–wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
333. Aschoff, J. (1994). Naps as integral parts of the wake time within the human sleep–wake cycle. *Journal of Biological Rhythms* 9: 145–155.
334. 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.
335. Aschoff, J. & Wever, R. (1976). Human circadian rhythms: a multioscillatory system. *Federation Proceedings* 35: 2326–2332.
336. 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.
337. Campbell, S. & Zulley, J. (1988). Napping as a biological rhythm: disentrainment of the human sleep/wake system. In: Koella, W. P., Obál, F., Schultz, H. & Visser, P. (Eds.). *Sleep '86*. Stuttgart: Gustav Fischer, pp. 3–10.
338. Campbell, S. S. & Zulley, J. (1989). Evidence for circadian influence on human slow wave sleep during daytime sleep episodes. *Psychophysiology* 26: 580–585.
339. Daan, S., Beersma, D. G. M. & Borbély, A. A. (1984). Timing of human sleep: recovery process gated by a circadian pacemaker. *American Journal of Physiology* 246: R161–R178.
340. Eastman, C. (1984). Are separate temperature and activity oscillators necessary to explain the phenomena of human circadian rhythms? In: Moore-Ede, M. C. & Czeisler, C. A. (Eds.). *Mathematical Models of the Circadian Sleep-Wake Cycle*. New York: Raven, pp. 81–101.
341. Dunn, J. D., Castro, A. J. & McNulty, J. A. (1977). Effect of suprachiasmatic ablation on the daily temperature rhythm. *Neuroscience Letters* 6: 345–348.
342. Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N. & 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.
343. Powell, E. W., Halberg, F., Pasley, J. N., Lubanovic, W., Ernsberger, P. & Scheving, L. E. (1980). Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *Journal of Thermal Biology* 5: 189–196.
344. Fuller, C. A., Lydic, R., Sulzman, F. M., Albers, H. E., Tepper, B. & 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.
345. Satinoff, E. & 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.
346. Refinetti, R., Kaufman, C. M. & 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.
347. Moritz, R. F. A. & Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.
348. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P. & 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.
349. Mahoney, M., Bult, A. & 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.
350. Glass, J. D., Tardif, S. D., Clements, R. & Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
351. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S. & 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.
352. 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.
353. Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.
354. Sheeba, V., Chandrashekaran, M. K., Joshi, A. & Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *Journal of Insect Physiology* 48: 25–32.
355. Rajaratnam, S. M. W. & Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
356. Oosthuizen, M. K., Cooper, H. M. & 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.

357. Reinberg, A., Halberg, F., Ghata, J. & 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.

358. Pollak, C. P. & Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.

359. Czeisler, C. A., Duffy, J. F, Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M., Silva, E. J., Allan, J. S., Emens, J. S., Dijk, D. J. & Kronauer, R. E. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177–2181.

360. Honma, K., Honma, S. & Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.

361. Sack, R. L., Brandes, R. W., Kendall, A. R. & Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.

362. Weitzman, E. D., Moline, M. L., Czeisler, C. A. & Zimmerman, J. C. (1982). Chronobiology of aging: temperature, sleep–wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.

363. Page, T. L., Mans, C. & Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.

364. Honnebier, M. B. O. M., Jenkins, S. L. & 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.

365. Daan, S. & 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.

366. Rosenberg, R. S., Zee, P. C. & Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.

367. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.

368. Perret, M., Aujard, F., Séguy, M. & 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.

369. 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.

370. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.

371. Von Gall, C., Duffield, G. E., Hastings, M. H., Kopp, M. D. A., Dehghani, F., Korf, H. W. & 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.

372. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S. & 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.

373. Benloucif, S. & 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.

374. Kas, M. J. H. & Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.

375. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences U.S.A.* 59: 414–421.

376. Lindberg, R. G. & Hayden, P. (1974). Thermoperiodic entrainment of arousal from torpor in the little pocket mouse, *Perognathus longimembris*. *Chronobiologia* 1: 356–361.

377. Innicenti, A., Minutini, L. & Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.

378. Hamasaka, Y., Watari, Y., Arai, T., Numata, H. & 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.

379. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S. & 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.

380. Stokes, M. A., Kent, S. & 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.

381. Usui, S., Takahashi, Y. & Okazaki, T. (2000). Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. *American Journal of Physiology* 278: R1148–R1156.

382. Honma, K., von Goetz, C. & Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.

383. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M. & Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.

384. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M. & 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.

385. Begall, S., Daan, S., Burda, H. & Overkamp, G. J. F. (2002). Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83: 153–158.

386. Kramm, K. R. & Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.

7 Photic Environmental Mechanisms

CHAPTER OUTLINE

7.1 Nonparametric Theory of Entrainment
7.2 Photic Parameters
7.3 Synthesis and Models

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 variance from 24.0 hours was presented as a beneficial feature that allows circadian physiologists to feel confident that the periodicity is truly endogenous and not merely induced by undetected environmental cycles. What is beneficial to the circadian physiologist, however, is not necessarily beneficial to the organism. In the real world — where the Sun rises and sets every 24.0 hours — organisms with a circadian period that differs from 24.0 hours can get into serious trouble. For example, 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. 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. 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.

The circadian rhythms of 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 this question can be answered, some terminology and concepts used in circadian physiology must be clarified. For example, how does the free-running period of the circadian pacemaker (symbolized by the Greek lower case letter *tau*, τ) adapt to match the period of an external clock (symbolized by the Roman upper case letter T)? Because the external clock provides the time to the pacemaker, or internal clock, the external clock can be called a "time giver" (or — as originally named in German — a *Zeitgeber*). If the zeitgeber is effective, the internal clock follows it — the pacemaker is *entrained* by the zeitgeber. That is, the entrainment of the circadian pacemaker by a zeitgeber is attained by the modulation of τ towards T. Reworded, this means "the biological clock synchronizes to the outside world by adjusting to a 24-hour day."

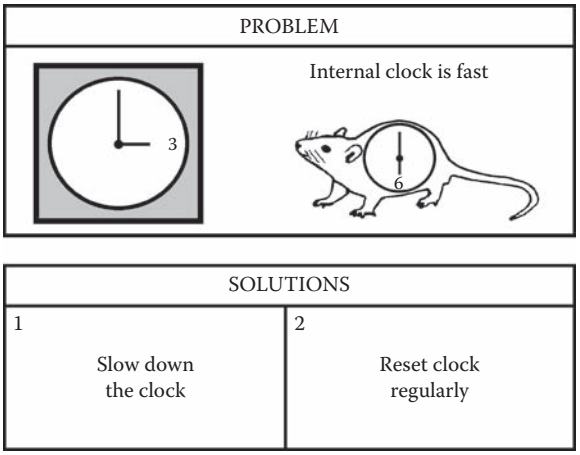


FIGURE 7.1 Two solutions for a problem. The existence of endogenous periodicity with a period different from 24.0 hours presents a synchronization problem. Two solutions are possible: the speed of the internal clock may be corrected or the clock may be reset often.

If the pacemaker is to be *entrained by* the zeitgeber (or, as 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). There are two ways to change the period of a clock (Figure 7.1). The speed of the clock can be changed by making it run faster or slower, or a change in speed can be simulated by resetting the clock each day. If you own an old winding watch (Figure 7.2), then 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. If the watch is fast, you can fix it by moving the arms back (or "delaying" the clock) a little bit each day.

7.1.1 Phase Shifts

Chapter 6 showed that the intensity of constant light can change the period of circadian rhythms (Aschoff's rule), so you know that the speed of the circadian clock can be modified by environmental stimuli. These stimuli also can affect resetting of the circadian clock. Pittendrigh demonstrated this effect in the early 1950s,[1] while studying the pattern of emergence (eclosion) of fruit flies from the pupal stage. He noticed that if the flies were raised from

FIGURE 7.2 A pocket watch from the early 1900s. Old watches like this one need to be reset almost daily to keep the correct time. (Source: Photograph by R. Refinetti.)

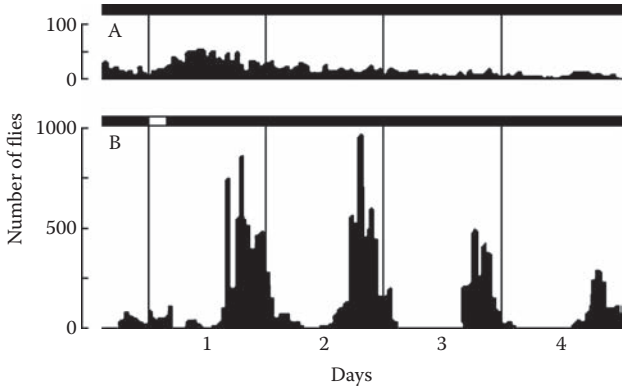


FIGURE 7.3 Flies are born. If eggs of the fruit fly (*Drosophila pseudoobscura*) are maintained in constant darkness, flies emerge from the pupal stage at random times of the day (Panel A). However, if the pupae are briefly exposed to light once, a circadian rhythm of eclosion ensues (Panel B). (Source: Adapted from Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in *Drosophila*. *Proceedings of the National Academy of Sciences U.S.A.* 40: 1018–1029.)

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 effect might occur because the light pulse synchronized the pupae, so that they started to eclode in daily bursts. He was unable to 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] The concept that synchronization could be induced by light implied that light was capable of resetting the circadian clocks of the pupae. Pittendrigh and many other researchers later

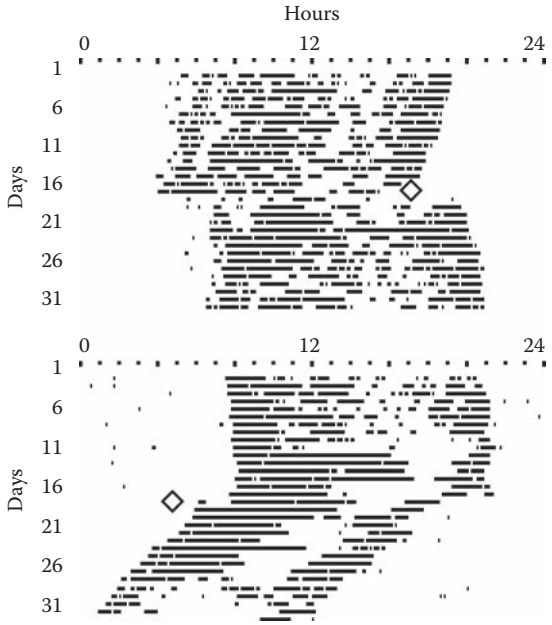


FIGURE 7.4 Shifted by light. The actograms show the running-wheel activity rhythms of two Nile grass rats (*Arvicanthis niloticus*) maintained in constant darkness and presented with single, brief (1 hour) pulses of light (400 lux). A light pulse presented 13 hours after the onset of activity (CT 13) evokes a phase delay of the activity rhythm (top). A light pulse presented 3 hours before the onset of activity (CT 21) evokes a phase advance of the activity rhythm (bottom). (Source: Archives of the Refinetti lab.)

documented the resetting of the circadian clock of individual organisms, using discrete light pulses, in nonanimals and invertebrate animals[1,3–5], reptiles and birds,[6–9] and many mammalian species, including golden hamsters,[10–22] domestic mice,[23–30] field mice,[31–36] laboratory rats,[37–41] humans,[42–53] and other mammals.[54–69] Comprehensive literature reviews on this topic were published in 1965[70] and 1999.[71] 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.[72]

Figure 7.4 shows two examples of resetting of circadian rhythms (*phase shifts*). 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 (top actogram) or 3 hours before the activity onset on the 17th day (bottom actogram). Note that, in the top actogram, the activity onsets shifted 3 hours to the right (i.e., they were *delayed* by 3 hours) after the pulse. In the bottom actogram, 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. (Chapter 5 discussed the concept of *circadian time*. If "circadian time zero" (CT 0) is

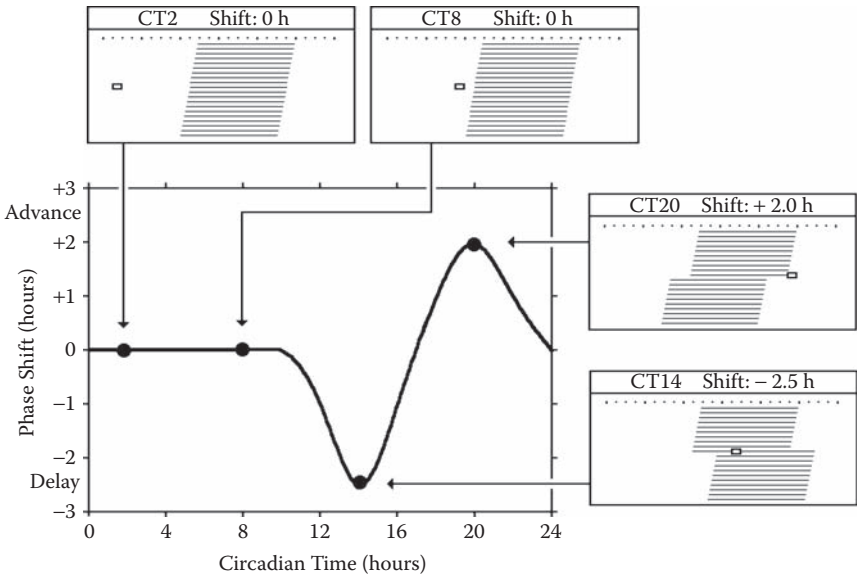


FIGURE 7.5 How to build a PRC. By presenting light pulses at different circadian times and observing the evoked phase shifts, one can build a phase-response curve (PRC). The small rectangles in the actograms indicate the times of the light pulses.

defined 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 several actograms depicting phase shifts (abbreviated as *delta phi*, $\Delta\phi$) of a fictitious animal evoked by light pulses presented at different circadian times. For example, 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 demonstrates that phase shifts can be plotted as a function of circadian time to generate a *phase-response curve* (PRC). Note that, by convention, advances are plotted as positive shifts, while delays are plotted as negative shifts. This fictitious PRC closely resembles real mammalian PRCs in that a "dead zone" is present during most of subjective day (CT 0 to CT 10), a phase-delay region occurs during early subjective night (CT 10 to CT 17), and a phase-advance region is present 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) takes you through the steps of building a PRC.

To prepare a detailed PRC, several data points must be obtained for each of the 24 circadian hours. Typically, the experimental subjects must spend at least 2 weeks under a light–dark cycle to attain stable entrainment before they are released into constant darkness. Another week is then needed to determine the circadian phase before the light pulse, and a fourth week is required to determine the circadian phase after the pulse (Figure 7.6, left). Thus, each single data point for the PRC requires at least a full month of recording. Data must be recorded for 72 months to obtain as few as three data points for each of the 24 circadian hours (or 72 subjects must be recorded simultaneously for 1 month). To reduce the length of time required to collect the necessary data, alternative protocols have been suggested. Aschoff listed six such protocols,[70] although only two have been used regularly by researchers. The *Type I* protocol was 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 7.6, right). A potential problem with using this protocol — as suggested in the figure — is 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 the phase of the entrained state is used as the measure of prepulse onset time, the circadian time will be incorrectly estimated. You could use the activity onset on the first day of constant darkness as your measure of prepulse onset time, but then you would 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.

Figure 7.7 (top) shows a PRC for the Nile grass rat. 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). Note the presence of a zone with rather small phase shifts (the dead zone, from

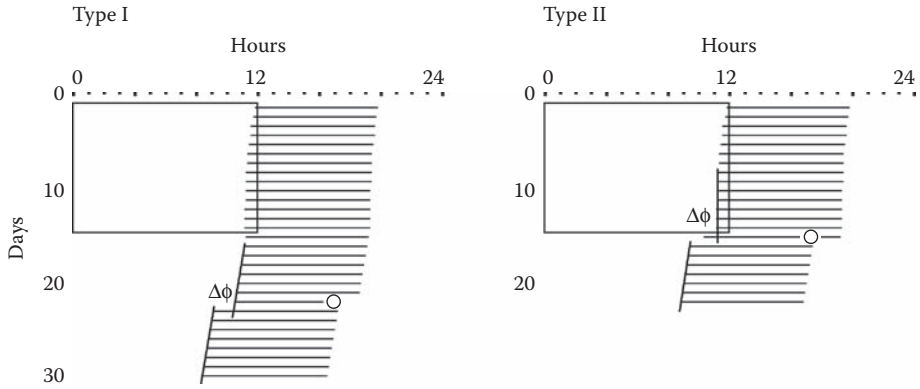


FIGURE 7.6 Protocols for phase shifting. Two protocols have been used often in the study of phase shifts evoked by single pulses. In the Type I protocol (left), the organism is allowed to freerun in constant darkness for several days before and after the pulse (which is indicated by the small open circle). In the Type II protocol (right), 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 make it easier to see the phase shifts (Δϕ).

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). We can also plot the phase shifts in the form of a phase transition curve (PTC), as shown in the bottom panel of Figure 7.7. This type of curve shows the new phase (the phase after the 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 usually is not 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 shown later in this chapter, the PTC can be helpful in the analysis of phase shifts in organisms that exhibit large shifts.

The five PRCs shown in Figure 7.8 illustrate the differences and similarities of PRCs of different species. Although all PRCs have a phase-delay region during early subjective night, the shapes of the curves show considerable interspecies differences. Note that the ordinates (Y-axes) of the graphs have been scaled to accommodate the various PRCs. Thus, for example, the PRC of the domestic mouse has maximal phase advances slightly exceeding 1 hour and phase delays of less than 3 hours, while 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).

It is also instructive to examine differences in PRCs 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

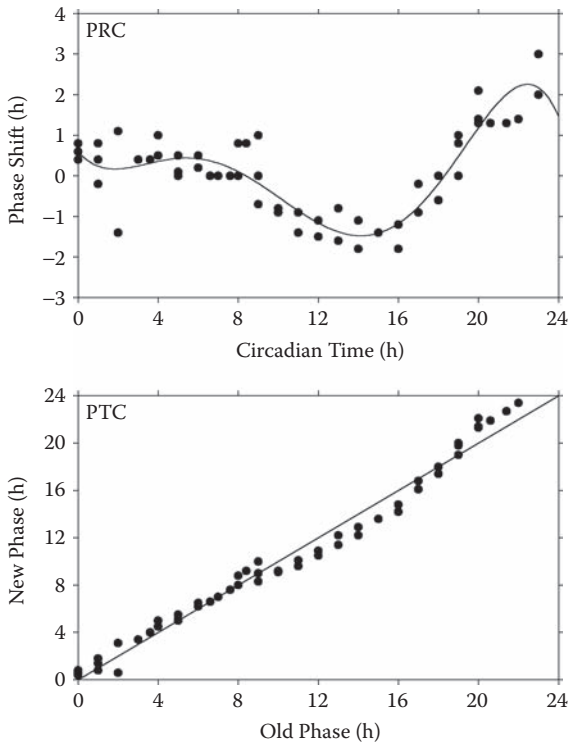


FIGURE 7.7 PRC and PTC. The graphs show phase-shift data plotted as a phase-response curve (PRC) and as a phase transition curve (PTC). The data points correspond to phase shifts of the running-wheel activity rhythms of Nile grass rats (*Arvicanthis niloticus*) evoked by 4-hour light pulses (360 lux) at different circadian times. In the PRC plot, phase advances are plotted as positive shifts and phase delays are plotted as negative shifts. (Source: 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.)

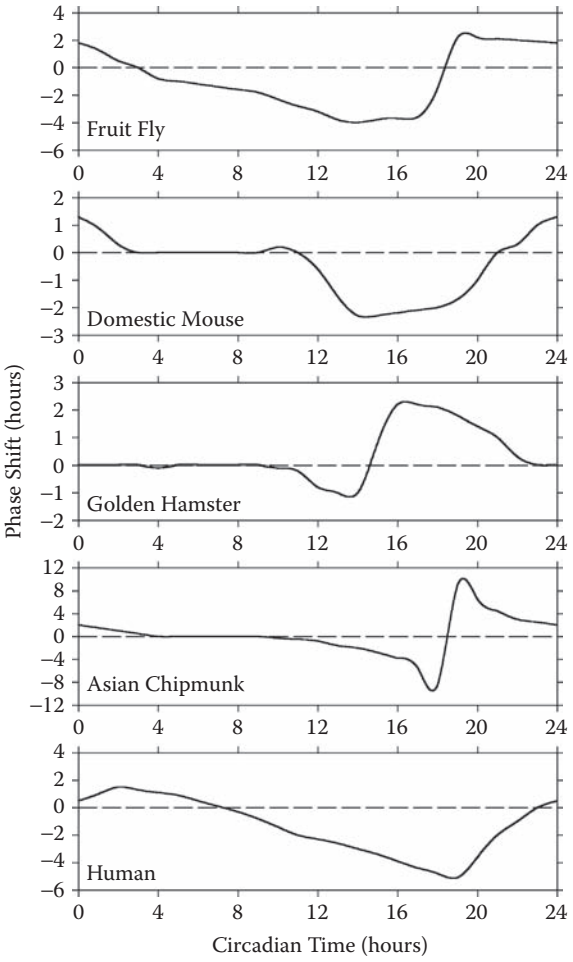


FIGURE 7.8 Interspecies differences in the PRC. The graphs show the phase-response curves (PRCs) that describe the phase-shifting effects of light pulses presented at different circadian times for five animal species. By convention, phase advances are plotted as positive shifts, while phase delays are plotted as negative shifts. (Sources: Rothenfluh, A., Abodeely, M., Price, J. L. & 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; Benloucif, S. & 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; Takahashi, J. S., DeCoursey, P. J., Mauman, L. & Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188; Honma, S. & Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396; Jewett, M. E., Kronauer, R. E. & 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.)

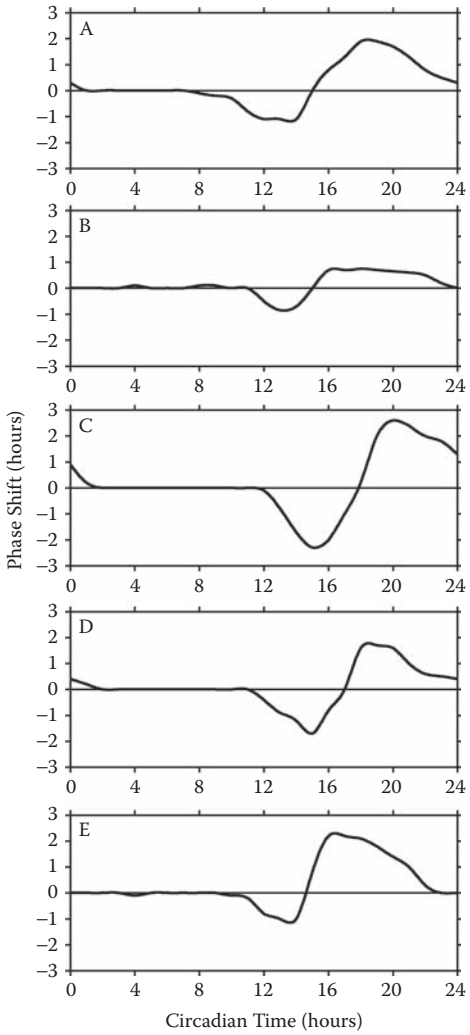


FIGURE 7.9 Intraspecies differences in the PRC. The graphs show the phase-response curves (PRCs) that describe the phase-shifting effects of light pulses presented at different circadian times to golden hamsters (*Mesocricets auratus*) in five different laboratories. Across the five studies, pulse duration varied from 15 to 60 minutes and pulse intensity varied from 150 to 400 lux. By convention, phase advances are plotted as positive shifts, while phase delays are plotted as negative shifts. (Sources: Daan, S. & 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; Grosse, J., Loudon, A. S. I. & 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; Meijer, J. H. & 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; Rosenberg, R. S., Zee, P. C. & Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495; Takahashi, J. S., DeCoursey, P. J., Mauman, L. & Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.)

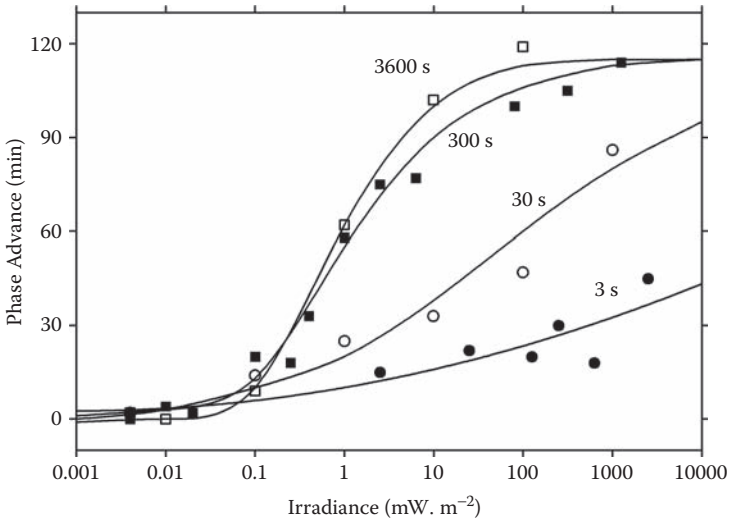


FIGURE 7.10 Temporal summation in the circadian system. In the circadian system of the golden hamster (*Mesocricetus auratus*), intensity and duration of the photic stimulus can be balanced to evoke the same phase shift. For example, a 60-minute phase advance can be evoked either by a 1-hour pulse at 1 mW · m^{-2} or by a 30-second pulse at 100 mW · m^{-2}. (Source: Adapted from Nelson, D. E. & 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.)

the shifts varies more than one might expect from study to study, however, and this variance cannot be accounted for by differences in the duration or intensity of the light pulses used in the different studies. (The five studies used relatively similar durations and intensities of light pulses. Most determinations of PRCs in rodents involve light pulses of 15 to 60 minutes at intensities (illuminance) of 150 to 400 lux.)

Few investigators have examined the effects of large variations in stimulus duration and intensity on the magnitude of the evoked phase shifts. Figure 7.10 shows the results of one very good study conducted on golden hamsters. Pulses of green light (λ = 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. Note that, regardless of stimulus duration, no phase shifts are evoked at intensities below 0.1 mW · m^{-2} (approximately 0.2 lux). Thus, 0.1 mW · m^{-2} (or 0.2 lux) is the threshold intensity for the elicitation of phase shifts.[13] Other studies found similar threshold values (0.1 to 0.3 lux), using pulses of green or white light in hamsters,[73] rats,[38] and mice.[23,34] Studies on humans rely on less-than-ideal methods because subjects refuse to stay in constant darkness for several weeks,[49,74–76] but the results suggest that the threshold is at least two orders of magnitude higher (20 to 100 lux).[49,76] Of course, a higher threshold means lower sensitivity, and it is not surprising that diurnal humans are less sensitive to light than nocturnal rodents.

In Figure 7.10 note also that the circadian system of the hamster exhibits the phenomenon of *temporal summation*, which is well-known in the visual system.[77,78] *Temporal summation* means 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 · m^{-2}) or by a long weak pulse (3600 seconds at 1 mW · 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. In Figure 7.10, a stimulus duration of 1 hour (3600 seconds) yields a saturation level of about 100 mW · m^{-2} (30 lux). Other studies found similar saturation values (30 to 150 lux), using pulses of green or white light in hamsters,[73] rats,[38] and mice.[23,34] In humans, the saturation level seems to be much higher (1000 lux or more).[49,76]

In Figure 7.10 also note 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. These findings mean that the initial seconds of stimulation are more crucial than the later seconds for the elicitation of phase shifts. Figure 7.11 demonstrates this phenomenon 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. Although the curve still is 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

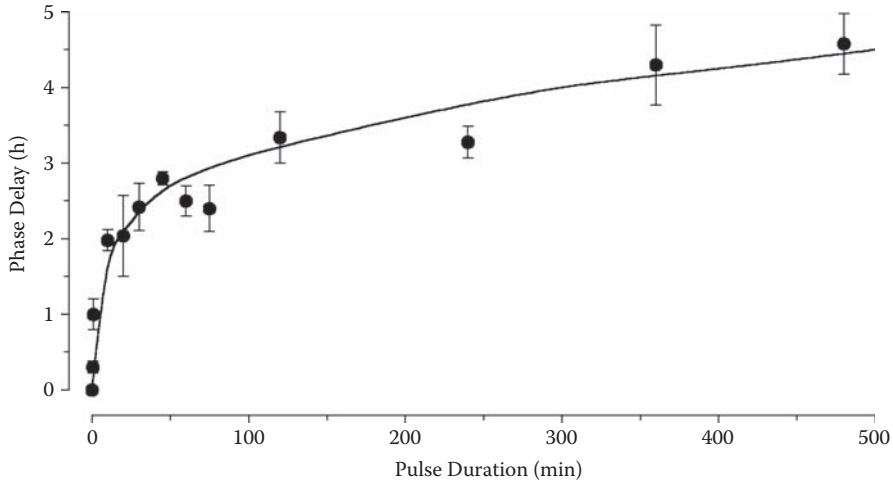


FIGURE 7.11 Endurance of temporal summation. In the circadian system of the domestic mouse (*Mus musculus*), the increase in the magnitude of phase delays, resulting from the increase in the duration of photic stimulation, levels off at durations of about 1 hour but persists through durations of at least 8 hours. In this graph, each point corresponds to the mean (± SE) of five mice. Light pulses of 360 lux were presented starting 4 hours after the onset of activity (CT 16). (Source: Archives of the Refinetti lab.)

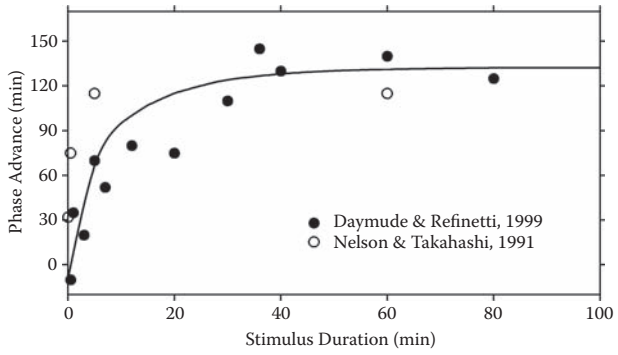


FIGURE 7.12 Ceiling of temporal summation. In the circadian system of the golden hamster (*Mesocricetus auratus*), the increase in the magnitude of phase advances, resulting from the increase in the duration of photic stimulation, levels off at durations of about half an hour. Data from two laboratories, using slightly different photic stimuli and times of stimulation, can be described approximately by the same function. (Sources: Daymude, J. A. & Refinetti, R. (1999). Phase-shifting effects of single and multiple light pulses in the golden hamster. *Biological Rhythm Research* 30: 202–215; Nelson, D. E. & 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.)

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). It is clear, however, that most of the phase shift caused by a light pulse is due to the first hour of stimulation.[13,19,73] Figure 7.12 shows a similar duration–response curve for the golden hamster, based on two independent studies. In this figure the half-maximal response seems to be attained at a stimulus duration of 5 minutes, and response saturation appears to occur at a stimulus duration of about 40 minutes. It is interesting that 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 light is sufficient to evoke a full phase shift if this tiny bit of light is spread over an entire hour (that is, 60 pulses of 20 milliseconds presented at 1-minute intervals).[79] Although the findings are not as impressive, it also has 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, a hundred 3-second pulses alternated with 3 seconds of darkness, or a thousand 0.3-second pulses alternated with 0.3 seconds of darkness.[80] In humans, it has been demonstrated that five 15-minute pulses separated by 1 hour of darkness are just as effective as a single 6.5-hour pulse.[81] 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. Thus, the effects of long pulses can be predicted by reference to the PRC for brief pulses. Assume that the smallest functional unit of time 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 six 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. The pulse is started at CT 16, which might be 6:00 A.M. The pulse causes a 2-hour delay, thus moving

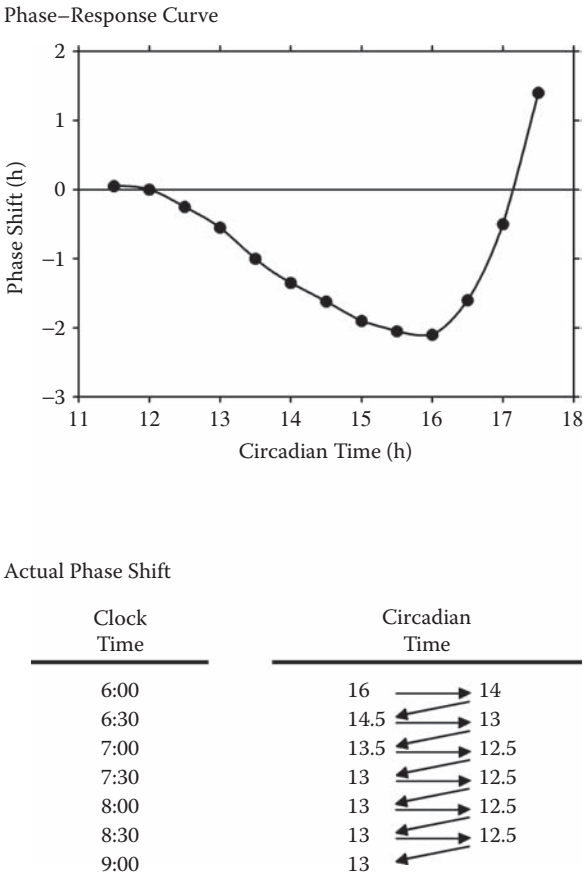


FIGURE 7.13 Temporal integration of the PRC. Using the phase-response curve (PRC) for brief discrete light pulses, one can estimate the phase-shifting effect of longer light pulses. See text for details.

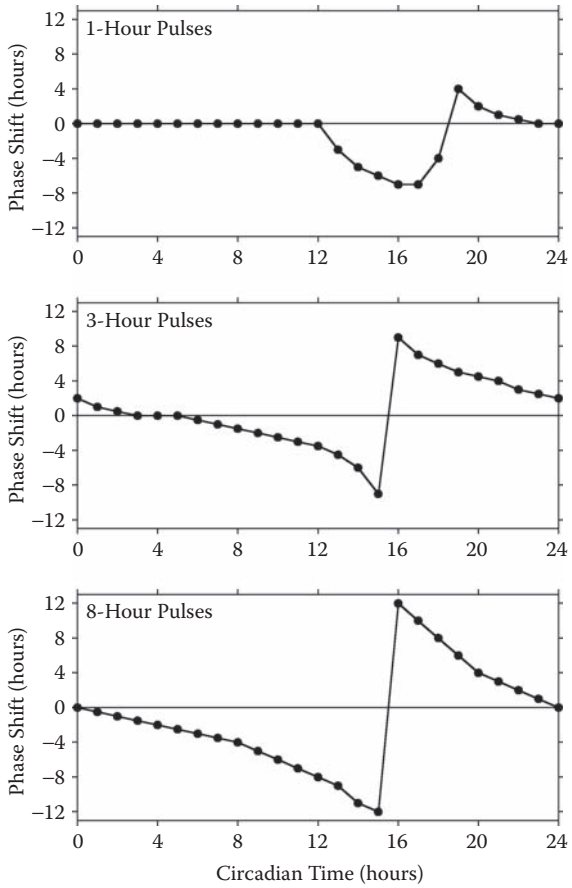


FIGURE 7.14 An awesome shift. In the flesh fly (*Sarcophaga argyrostoma*), increases in the duration of light pulses can lead to maximal phase shifts (full 12 hours), as shown in these three phase-response curves (PRCs). The pulses consisted of white light at approximately 1000 lux. Pupal eclosion was studied. (Source: 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.)

the mouse's clock to CT 14. However, the mouse's clock is still ticking, so that at 6:30 it is 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 then takes it to CT 13.5 at 7:00 A.M. The continuing light pulse evokes a 1-hour delay, thus moving the mouse's clock to CT 12.5. Note that from this time forward, the mouse's clock will lock onto a half-hour cycle of advances and delays, so that no further progression of shifts will occur. When the long light pulse finally ends at 9:00 A.M., the mouse's clock will be at CT 13. Because the pulse started at CT 16, the total light-induced shift will equal 3 circadian hours. Therefore, although a 3-hour pulse is six times as long as a 30-minute pulse, it evokes a phase shift that is only one-and-a-half times as large. For the PRC used here, the maximal phase shift at CT 16 is attained by a 1.5 hour pulse. Any additional light has no phase-shifting effect.

The reasoning used in the previous paragraphs 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 (*Sarcophoga argyrostoma*). Note 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 it 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

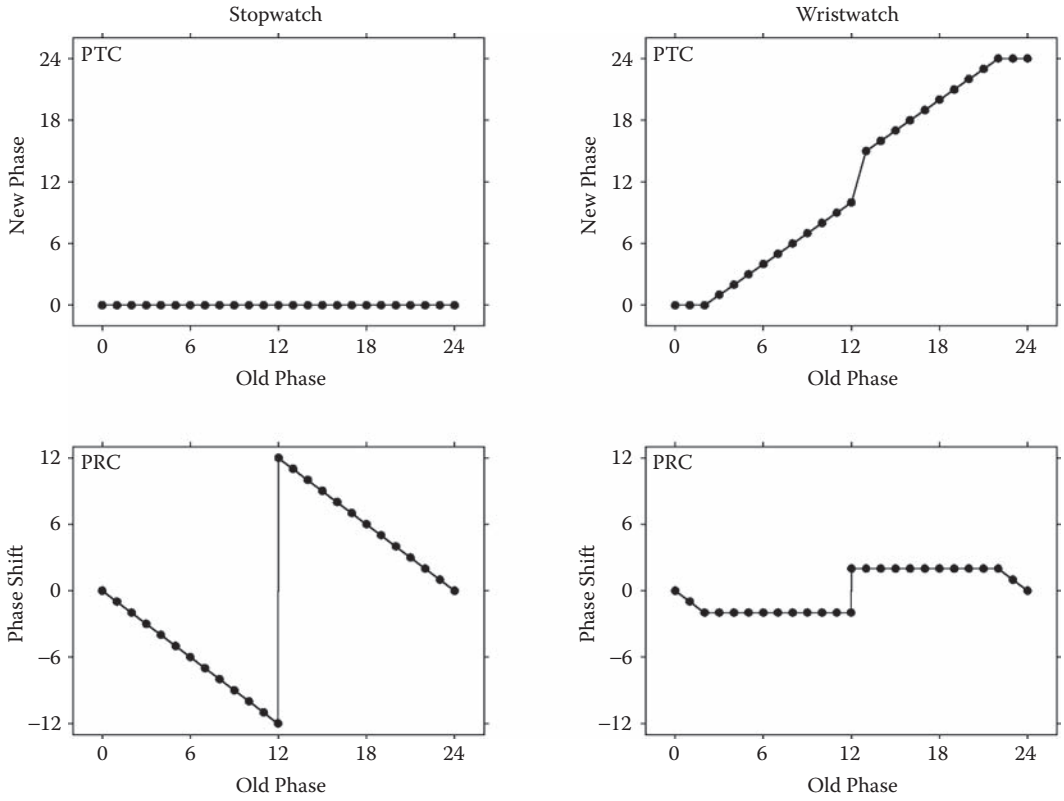


FIGURE 7.15 Type 0 and Type 1 resetting. The graphs show typical phase transition curves (PTCs) and phase-response curves (PRCs) for resetting of a stopwatch (Type 0 resetting) and of a regular wristwatch (Type 1 resetting). See text for details.

hand of the clock can be fully and instantly reset to the starting position by activation of the reset button. Regardless of the location of the arm on the dial, it always returns to the point of origin. Thus, the "new phase" is always the same, regardless of the "old phase," as illustrated in the top left panel of Figure 7.15. If this PTC is converted into a PRC, the result is the bottom left graph in Figure 7.15. In contrast, resetting of a regular wristwatch requires the gradual adjustment of the clock's arms. If the hour arm is moved by, say, a maximum of 2 hours in the direction of midnight, the relationship between "new phase" and "old phase" is similar to the one described in the top right panel of the figure, and the PRC is similar to the one in the bottom right panel. 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.[82] To further explain these concepts, I included a short computer tutorial in the program Entrain (see Exercise 7.3).

While Type 0 PRCs have been described for invertebrates and simpler organisms,[71,83] only Type 1 PRCs have been described in birds and mammals. Large phase shifts with a sharp break point between the phase-delay region and the phase-advance region were documented in both birds and mammals,[7,59] 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,[62] but these results are inconsistent with the PRCs obtained in numerous other laboratories, as previously seen in Figure 7.9. 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 conditions in this particular laboratory. I once observed a 12-hour phase shift in a golden hamster in my own laboratory but was never able to replicate the conditions.

One research team made the controversial claim that Type 0 resetting could be achieved in humans by the use of a multipulse paradigm.[42,50] This unorthodox paradigm involved the presentation of three 5-hour pulses on 3 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,[19,39] which suggested that the human circadian system may be peculiar.[84] 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.[85] Because of the current uncertainty about the exact shape of the human PRC for single pulses, it can be equally

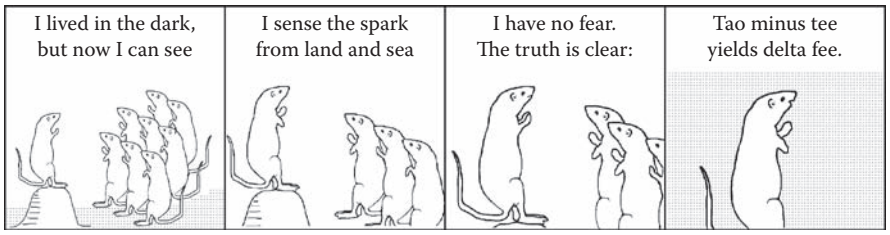


FIGURE 7.16 Entrainment rule: $\tau - T = \Delta\phi$. The wise rat intones rhymes about the basic rule of entrainment.

argued that the 3-pulse paradigm is *not* equivalent to the sum of 3 single pulses.[86] Resolution of this controversy awaits 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 at the beginning of the chapter). Indeed, according to Pittendrigh's *nonparametric theory of entrainment*, entrainment is due to the daily resetting of the pacemaker by the zeitgeber.[87] 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 (a leading Dutch researcher, introduced in Chapter 1) says that Aschoff was a champion of parametric entrainment.[88] I cannot argue with someone who worked closely with both Pittendrigh and Aschoff, but I must quote Aschoff in his last published article: "It seems likely that nonparametric entrainment plays a predominant role, [although] contributions of parametric effects are probably underrated."[89] 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,[90] might be seen as a contradiction to the nonparametric theory of entrainment. However, as Pittendrigh pointed out,[91] and Aschoff recognized,[92] the apparent parametric effect embodied in Aschoff's rule can be simply explained by nonparametric mechanisms. Indeed, continuous illumination stimulates all points of the PRC and, unless the PRC is perfectly symmetrical, it results 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). This issue is discussed further 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 are discussed in Chapter 8.

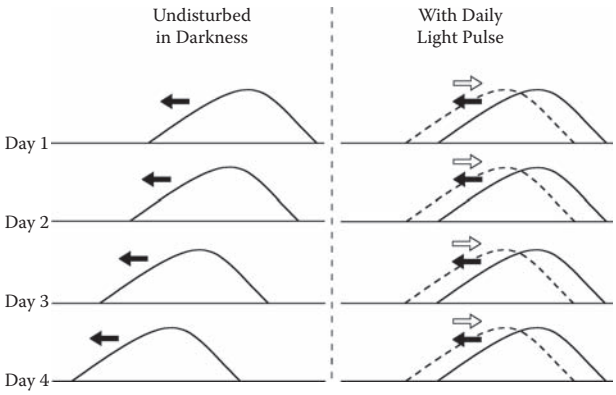


FIGURE 7.17 Entrainment rule: diagram. The free-running rhythm of an organism left undisturbed in darkness drifts slowly from day to day. A light pulse appropriately timed each day can cause a phase shift that offsets the natural drift of the rhythm, so that the expressed rhythm has a period of exactly 24 hours.

Remember that 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 expressed as a simple equation: $\tau - T = \Delta\phi$ ("*tao* minus *tee* equals *delta fee*, Figure 7.16). 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. Suppose 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's clock must delay 36 minutes each day (23.4 – 24.0 = –0.6 hour = –36 minutes). Using the PRC in Figure 7.13, you can see that a 36-minute (0.6-hour) delay is caused by a light pulse at CT 13 (CT 12 representing the onset of activity in a nocturnal animal). Thus, if a light pulse is presented at CT 13 each day, a 0.6-hour delay is caused each day, and the observed period of the activity rhythm is 24 hours (Figure 7.17).

Note that you do not even need to know when CT 13 occurs. If pulses are presented every 24 hours, the animal's rhythm will be advanced initially 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. You may have 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. Note 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 example, at CT 22 in the 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 the light–dark cycle is introduced at the "right" time (CT 13), entrainment will occur right away.

Before I present specific experimental results that corroborate the nonparametric theory of entrainment, I want to examine briefly a potential generalization about the shape of the PRC. A simple way to compare the PRCs of different species is to compute a 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 example, it is reasonable to expect that organisms with relatively short endogenous periods should have large D/A ratios, because they require large delays 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 to attain entrainment. In actuality, no such relationship is found across animal species (Figure 7.18). Evidently, this potential physiological advantage was not selected for 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. In a similar fashion, a long-period organism needs an ample phase-advance region, but it may or may not have a modest phase-delay region.

The nonparametric theory of entrainment states 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). Light pulses can cause phase shifts and phase shifts are theoretically sufficient to account for entrainment. But has this theory actually been tested? Yes it has. Laboratory studies using daily light pulses as entraining agents have confirmed that stable entrainment

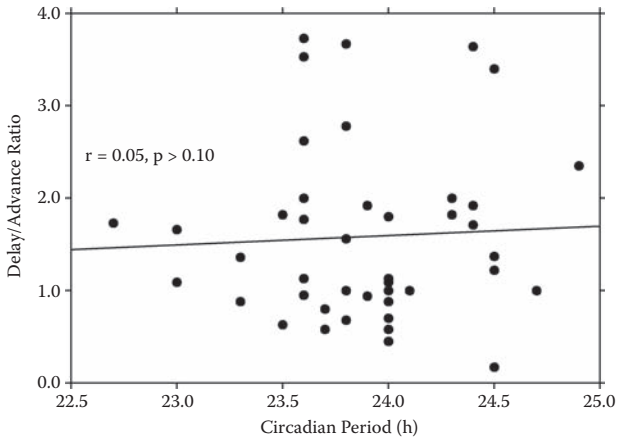


FIGURE 7.18 Relationship between free-running period and PRC. This graph shows the relationship between free-running period (τ) and the ratio of maximal delay and maximal advance (D/A ratio) of the photic phase-response curve (PRC), as determined by various investigators in a variety of vertebrate and invertebrate animal species. No statistically significant relationship exists between $\boldsymbol{\tau}$ and D/A ratio. (Sources: See references 4, 6–12, 14, 16, 17, 21, 26, 28, 29, 32, 36–39, 46, 48, 51, 55–57, 59, 60, 63–69, 79, 86, 234–236 in the *Literature Cited* section.)

can be attained with the presentation of brief daily light pulses.[54,66,69,93–100] Figure 7.19 provides an example. A domestic mouse (*Mus musculus*) was initially kept in constant darkness, and its running-wheel activity was continuously recorded. Starting on the eighth day, a single 15-minute light pulse was presented each day at 08:00 hours (indicated by the small open circles). Note the two hallmarks of entrainment: (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, entrainment could not have been said to have occurred. However, if the animal had exhibited $\tau = 24$ hours from the start, this criterion could not have been used. 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 conceivably could 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 was actually entrained. The phenomenon of *masking* is discussed in detail in Section 7.2.

If the nonparametric theory of entrainment is correct, one should be able to establish entrainment as long as the necessary phase shift is provided — even if light pulses

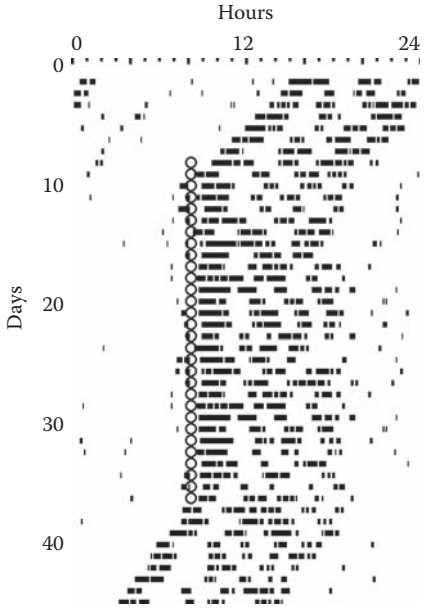


FIGURE 7.19 A pulse a day keeps the freerun away. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained in constant darkness except for a 15-minute light pulse (360 lux) each day, as indicated by the small circles. After a few days of free-running ($\tau < 24$ hours), the mouse reached a point where the phase-delaying effect of the light pulse exactly offset the natural advance of the circadian clock — that is, entrainment was established ($\tau = 24.0$ hours). The free-running resumed when the daily pulses were discontinued. (Source: Archives of the Refinetti lab.)

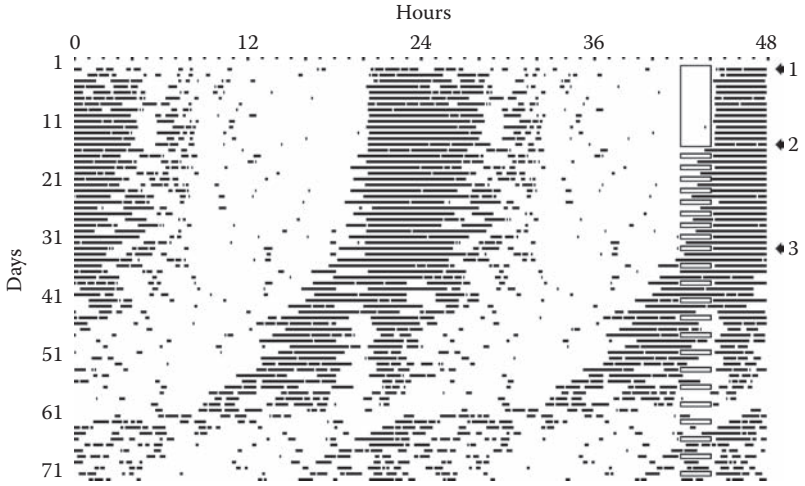


FIGURE 7.20 What if you skip a day or two? This double-plotted actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained in constant darkness and stimulated with a 2-hour light pulse (300 lux) under three different schedules: 1) every day, 2) every second day, and 3) every third day. The times of the pulses are single-plotted as open rectangles. Pulses presented every third day were not capable of maintaining entrainment, as discussed in the text. (Source: Archives of the Refinetti lab.)

are not presented every day. Indeed, as shown in Figure 7.20, 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 delay

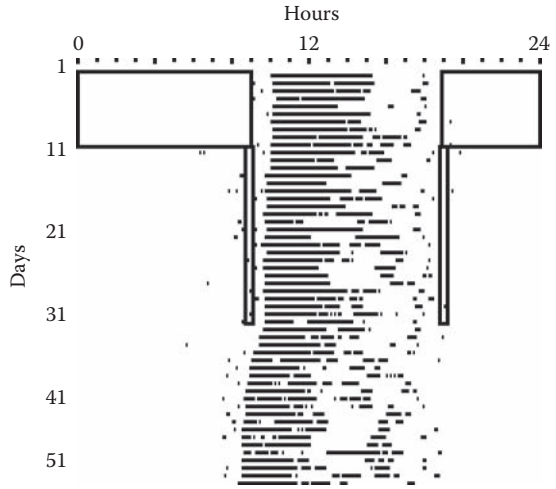


FIGURE 7.21 Dawn and dusk are enough for a hamster. This actogram shows the running-wheel activity rhythm of a male golden hamster (*Mesocricetus auratus*) maintained first under a light–dark cycle (LD 14:10), then under a skeleton photoperiod with half hour of light at dawn and dusk, and then under 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. (Source: Archives of the Refinetti lab.)

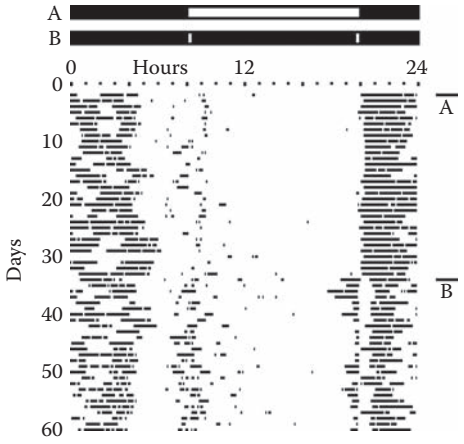


FIGURE 7.22 Dawn and dusk are enough for a mouse. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained first under a light–dark cycle (A) and then under a skeleton photoperiod with half hour of light at dawn and dusk (B). The activity pattern is very similar under the full light–dark cycle and the skeleton photoperiod, but the onsets are slightly earlier and less regular under the skeleton photoperiod. (Source: Archives of the Refinetti lab.)

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. The important finding in this example is that entrainment was maintained in the every-second-day segment.

In the real world, light–dark cycles are not limited to a single short pulse of light every day or every second day. 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,[28,58,60,66,69,96,101–108] even in species whose *visual* system is insensitive to light.[100,109–111] Figure 7.21 shows an example for a sighted animal (the golden hamster). The hamster was maintained initially under an LD 14:10 photoperiod and later under a skeleton photoperiod with a half hour of light at dawn and a half hour of light at dusk, before 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. Figure 7.22 shows a second example, which used a mouse. The pattern of entrainment was similar under a complete photoperiod and a skeleton photoperiod, but early onsets were unmasked in the latter condition. Pittendrigh placed considerable emphasis on skeleton photoperiods,[87] most likely because light pulses at dawn and dusk are needed to modulate his two putative oscillators (discussed in Chapter 6). However, the effect of light pulses at dawn and dusk generally does not differ from that of a single daily pulse (for example, compare Figures 7.19 and 7.20 with Figures 7.21 and 7.22). Organisms with periods shorter than 24 hours need a light pulse at dusk (phase-delay region of the PRC), while 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 rather than a skeleton photoperiod) is present, entrainment is complicated only slightly by the presence of additional, "unnecessary" light. Most of the extra light remains 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 in the stimulated range, as discussed in Section 7.3. Figure 7.23 shows the persistence of entrainment for a representative Siberian hamster (*Phodopus sungorus*) under LD 12:12 for 8 consecutive months, which corresponds to about one-third of the lifespan of most laboratory rodents. A small drift of the onsets occurred during the first few days, as the phase angle of entrainment was slowly established. After that, however, the activity pattern remained stable, subject only

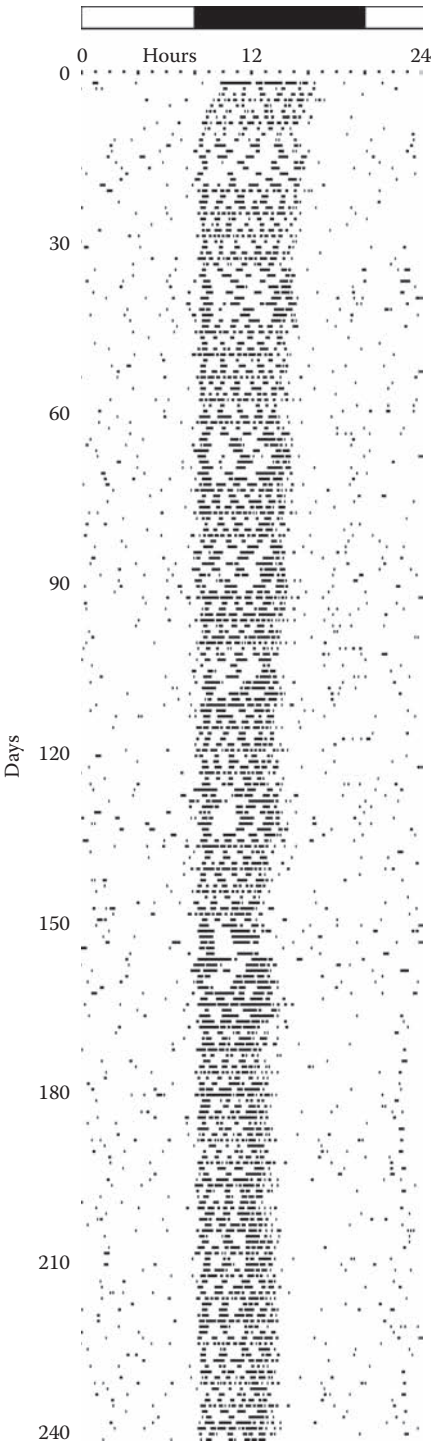


FIGURE 7.23 It keeps running, and running, and running. This actogram shows the running-wheel activity rhythm of a Siberian hamster (*Phodopus sungorus*) maintained under a light–dark cycle (LD 12:12) for 8 consecutive months (i.e., one-third of the animal's life). The rhythm drifted slightly during the first few days until the appropriate phase-angle of entrainment was attained, but the activity pattern remained essentially constant through the recording period. (Source: Archives of the Refinetti lab.)

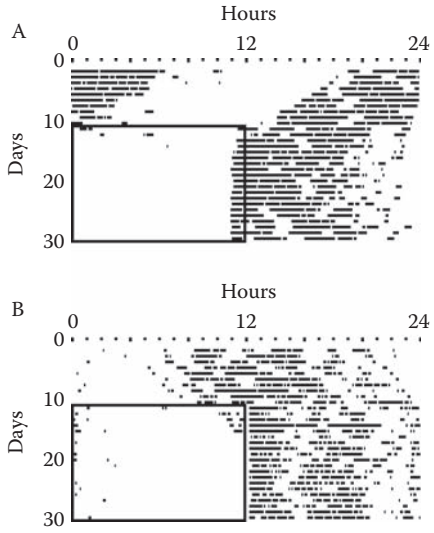


FIGURE 7.24 Different τ, different ψ. The actograms show the rhythms of running-wheel activity of two domestic mice (*Mus musculus*), one with an endogenous period shorter than 24 hours (A) and one with an endogenous period longer than 24 hours (B). The rectangles indicate the duration of the light phase 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. (Source: Archives of the Refinetti lab.)

to the usual irregularities associated with "biological noise."

The phase angle of entrainment (ψ) is dependent on the free-running period of the organism, as predicted by the equation $\Delta\phi = \tau - T$. Consider Figure 7.24, 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). Note that although both mice entrained to the LD cycle, the mouse with $\tau <$ 24 hours started running before dusk each day, while 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. In contrast, 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.

What if T does not equal 24 hours? This situation does not happen in nature, but it 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.[8,9,15,30,100,112–133] Figure 7.25 provides an example. A Nile grass rat was subjected to a T cycle

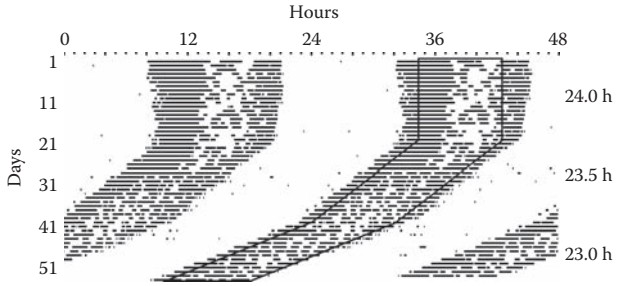


FIGURE 7.25 Moved by the light. This double-plotted actogram shows the running-wheel activity rhythm of a Nile grass rat (*Arvicanthis niloticus*) maintained under a light–dark cycle with 8 hours of light per cycle and a total duration of 24.0, 23.5, or 23.0 hours (as indicated by the single-plotted polygon and the numbers in the right margin). Note how the activity pattern tracks the light–dark cycle. (Source: 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.)

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). Note that the activity pattern tracks the T cycles — that is, 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. Figure 7.26 shows two additional examples (using mice) of entrainment by T cycles. 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 1.5 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

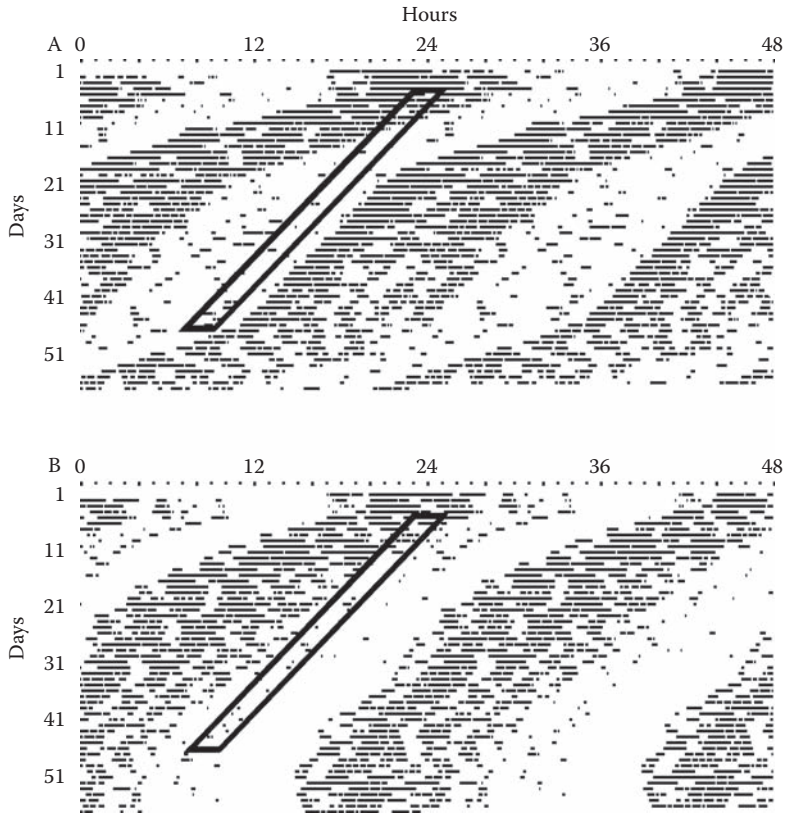


FIGURE 7.26 T cycles. Light–dark cycles with a period different from 24 hours are usually called T cycles. These two double-plotted actograms show the running-wheel activity rhythms of two domestic mice (*Mus musculus*) maintained under a T cycle (period = 23.6 hours, 2 hours of light per cycle), as 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), while the mouse with an endogenous period longer than 23.6 hours ran before the light pulse (B). (Source: Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.)

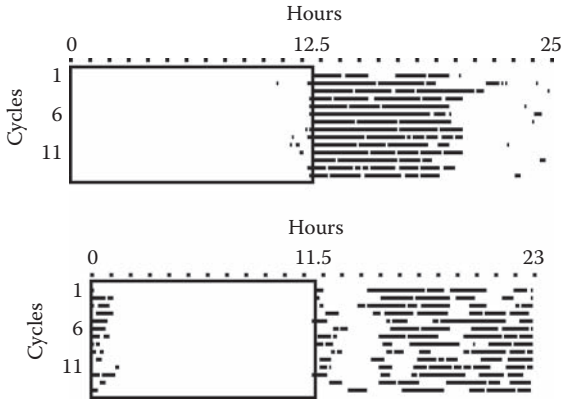


FIGURE 7.27 Different T, different ψ. These actograms show the running-wheel activity rhythms of domestic mice (*Mus musculus*) maintained under a T cycle with a period of 25 hours (top) and a T cycle with a period of 23 hours (bottom). In both cases, half of the cycle was illuminated (360 lux) and the 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. (Source: Archives of the Refinetti lab.)

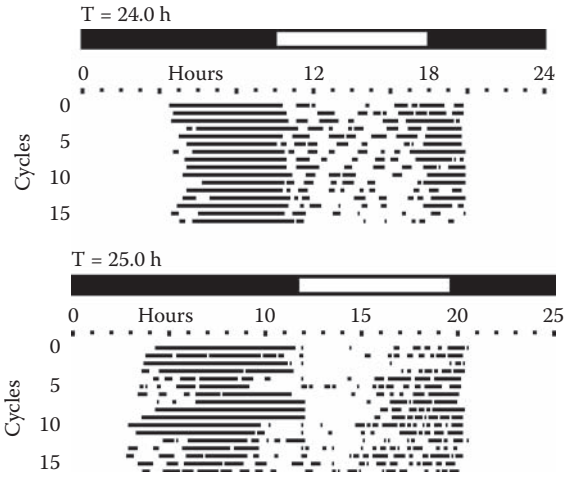


FIGURE 7.28 Different T, different ψ (again). These actograms show the running-wheel activity rhythms of a Nile grass rat (*Arvicanthis niloticus*) maintained under a T cycle with a period of 24 hours (top) and a T cycle with a period of 25 hours (bottom). In both cases, the light phase of the cycle (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. (Source: Archives of the Refinetti lab.)

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, while 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. Figure 7.27 shows actograms of the activity rhythms of mice maintained under T = 25 hours and T = 23 hours (illuminance = 300 lux). The actograms are plotted modulo T to facilitate visual inspection. Note 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 was 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 7.28, 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, while a grass rat maintained under a T cycle of 25 hours starts running earlier, about 8 to 9 hours before lights-on. 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, note 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

The range of periods of an effective zeitgeber is limited. For example, 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. 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 example, 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 24 hours, its range of entrainment can be estimated to comprise periods between 22 and 25 hours. Table 7.1 lists estimates of the range of entrainment for various species. Ranges of entrainment can be as narrow as 23.5 to 24.9 hours for the flying squirrel (*Glaucomys volans*) or as wide as 11.8 to 35.8 hours for the flesh fly (*Sarcophaga argyrostoma*). Of course, estimates of the range of entrainment based on the PRC can only be approximate. 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 organism will receive a single light pulse per day, while 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,[57,106,121,124,128–130,134] although they have rarely examined in detail the relationship with the amplitude of the PRC. Figure 7.29 shows

TABLE 7.1
Predicted Ranges of Entrainment[a]

Species[b]	Lower End (h)	Upper End (h)	Source
Arvicanthis ansorgei	22.9	25.3	55
Arvicanthis niloticus	22.9	24.7	56
	21.6	25.3	57
Callithrix jacchus	21.6	24.8	69
Coturnix coturnix	16.5	33.8	7
	14.5	32.3	9
Drosophila melanogaster	22.3	28.3	234
	22.1	28.3	235
Eutamias sibiricus	14.9	32.4	59
Glaucomys volans	23.5	24.9	60
Gonyaulax polyedra	20.8	25.2	237
	12.5	26.0	3
Homo sapiens	23.0	29.6	86
	22.2	27.3	51
	21.5	28.6	48
	18.5	25.5	46
Leucophaea madarae	18.9	29.3	4
Mesocricetus auratus	23.2	24.9	17
	22.3	25.7	14
	22.1	25.1	11
	21.8	25.0	12
	21.7	25.6	21
	21.7	26.5	10
	21.4	26.3	16
Microcebus murinus	21.2	25.3	68
Mus booduga	22.9	26.3	32
	22.9	25.2	36
Mus musculus	22.8	26.5	11
	22.8	26.5	28
	22.8	25.7	79
	22.3	25.9	29
	22.2	26.4	26
	21.3	26.2	236
Octodon degus	22.9	24.5	63
	21.6	24.7	64
Passer domesticus	16.7	32.7	8
Peromyscus leucopus	23.5	24.9	11
Peromyscus maniculatus	22.5	25.1	11
Phodopus sungorus	22.7	26.2	65
Rattus norvegicus	23.1	26.9	38
	23.0	29.5	39
	23.0	26.8	37
Sarcophaga argyrostoma	11.8	35.8	5
Sceloporus occidentalis	21.6	25.5	6
Sturnus vulgaris	20.6	25.5	7
Tamias striatus	22.9	29.6	66
Tupaia belangeri	22.7	24.5	67

[a] In each case, the maximal delay and maximal advance in the phase-response curve (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, while 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 species names, refer to the *Organisms Used* appendix at the end of the book.

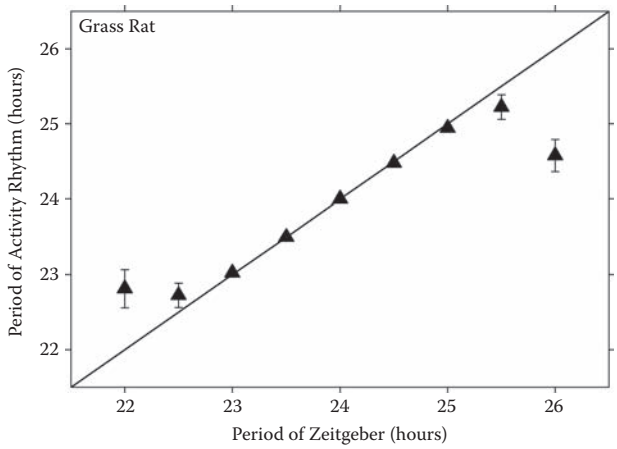


FIGURE 7.29 Range of entrainment. The graph shows the mean period of the rhythm of running-wheel activity of Nile grass rats (*Arvicanthis niloticus*) maintained under T cycles with 8 hours of light per cycle (360 lux). Each point corresponds to the mean (± SE) of eight or nine animals. The periods of 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. (Source: 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.)

TABLE 7.2
Comparison of Predicted and Empirical Ranges of Entrainment

	Predicted			Empirical		
Species	**Lower End (h)**	**Upper End (h)**	**Source**	**Lower End (h)**	**Upper End (h)**	**Source**
Arvicanthis niloticus	21.6	25.3	57	22.5	25.5	57
Homo sapiens	21.5	28.6	48	20.5	29.0	106
Mesocricetus auratus	22.3	25.7	14	22.5	25.5	134
Rattus norvegicus	23.0	29.5	39	23.5	28.5	130

the results from a study on the range of entrainment of the Nile grass rat. All animals exhibited entrainment from T = 23 hours to T = 25 hours. For zeitgeber periods outside this range, some animals continued to exhibit entrainment, but others did not, so that the group mean 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 close agreement.

The idea of range of entrainment may be misleading. Consider Figure 7.30. The two actograms depict running-wheel activity records of mice maintained under light–dark cycles. Entrainment is apparent in both cases. Yet, in one case T = 12 hours (LD 6:6), and in the other case 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 introduces the idea of entrainment by *frequency demultiplication*.

Start by observing some subtle abnormalities in the actograms in Figure 7.30. Note 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, ran 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 was inactive for a long period of time. To understand why the animal acted this way, compare these activity patterns with the activity pattern exhibited under T = 24 hours (LD 12:12). In the top panel in Figure 7.31, 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 2 days and 2 nights every 24 hours. The animal ran during one of these two nights but did not run during the other night 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

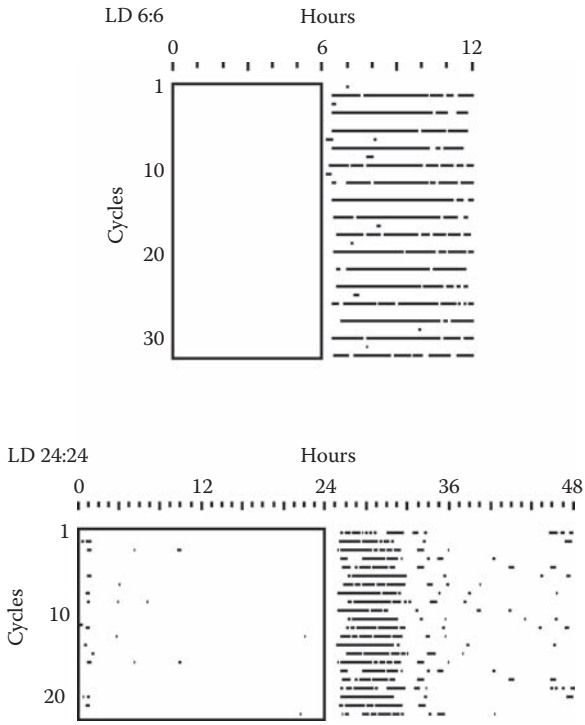


FIGURE 7.30 It looks like normal entrainment. These actograms show the running-wheel activity rhythms of a domestic mouse (*Mus musculus*) maintained under a 12-hour light–dark cycle (top) and a 48-hour cycle (bottom). Normal entrainment is apparent in both cases when the actograms are plotted with hour-axes matching the period of the light–dark cycle (12 and 48 hours, respectively). (Source: Archives of the Refinetti lab.)

cycle. Yet, there is no doubt that the animal was entrained. The period of the zeitgeber (LD 6:6) 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. This explains why activity bands were missing in alternate cycles in Figure 7.30.

In the bottom panel of Figure 7.31, an LD 12:12 was followed by an LD 24:24. Here, the mouse had to *multiply*, rather than 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 (note the missing bands in the lower panel of Figure 7.31). Consequently, the animal runs only every other day. When plotted on a 48-hour axis (as in Figure 7.30), the duration of the activity phase looks rather short, but entrainment appears otherwise normal.

Every species has a limited range of entrainment, and this range may be expanded if the frequency of the zeitgeber is a multiple or submultiple of the circadian frequency. What if the frequency of the zeitgeber is just slightly off the range of entrainment? In this case, one

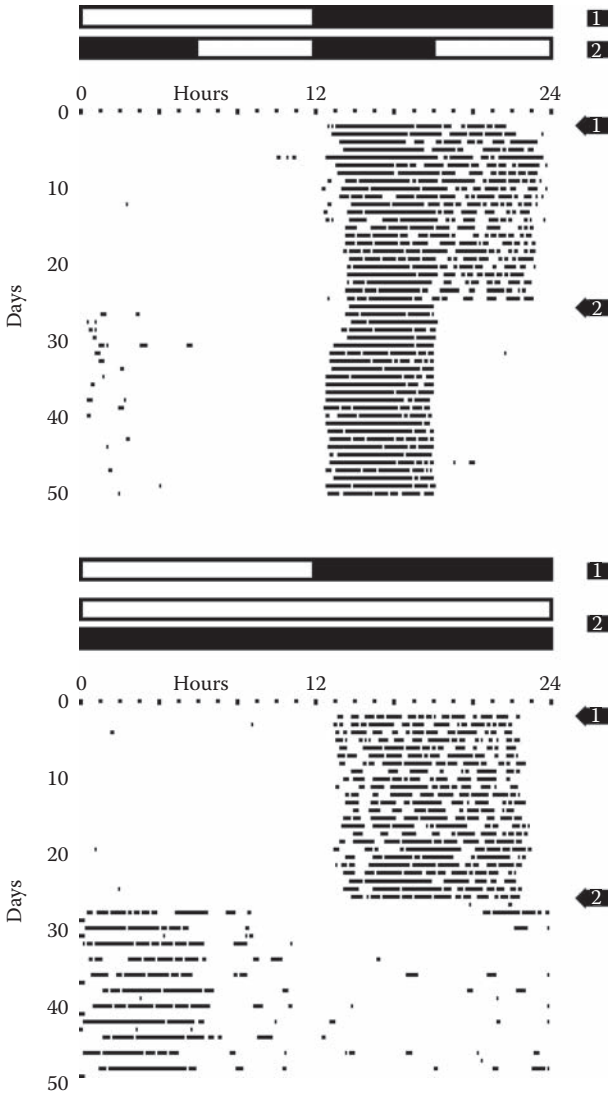


FIGURE 7.31 Entrainment by frequency demultiplication. These actograms use the same data shown in Figure 7.30, but the hour-axes are 24-hours long and additional data are included under a 24-hour light–dark cycle. The timing of the various light–dark cycles is indicated by the horizontal bars above the actograms. These plots clearly demonstrate that the entrainment patterns apparent in Figure 7.30 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 the top actogram), and running activity is exhibited only on alternate days under the LD 24:24 (section 2 in the bottom actogram). (Source: Archives of the Refinetti lab.)

may observe the phenomenon of *relative coordination*.[70,135,136] As exemplified in Figure 7.32, 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

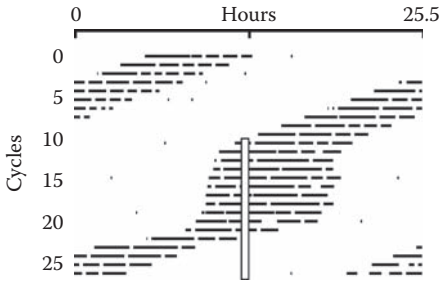


FIGURE 7.32 Relative coordination. A zeitgeber that is unable to entrain a rhythm may still be able to affect it, as shown in this actogram of the running-wheel activity rhythm of a golden hamster (*Mesocricetus auratus*) maintained in constant darkness at first and then exposed to a 25.5-hour T cycle with half hour of light per cycle (vertical rectangle). The zeitgeber temporarily slows the activity rhythm, but the effect is not strong enough to establish stable entrainment. (Source: Archives of the Refinetti lab.)

entrainment. The shift still occurs when the animal is exposed to light in the phase-delay region, which gives the impression that the animal is trying to entrain, but eventually the freerun is resumed (even though the light pulses are still being presented). These considerations imply that records kept over a long period of time are needed to accurately study the phenomenon of entrainment. If analysis is based on short-term data, relative coordination may be erroneously interpreted as evidence of entrainment.

7.1.4 Transients

One aspect of phase shifts and entrainment not yet addressed 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.[18,33,137–140] However, the expressed rhythms shift much more slowly.[14–16,37,68,139] Consider Figure 7.33, 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[141–143] 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. Consider Figure 7.34. 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 appear that entrainment was achieved through phase advances, but the transients

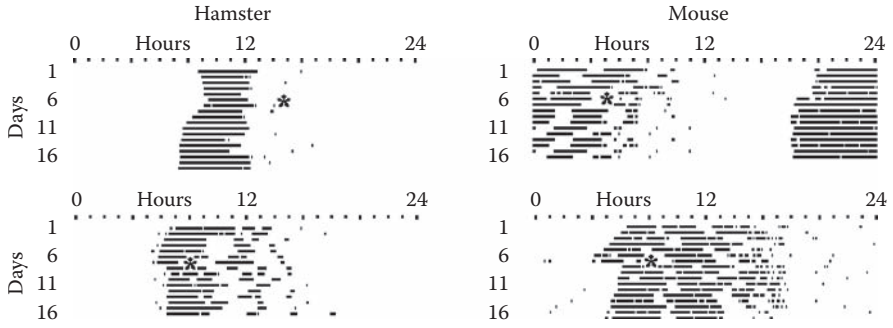


FIGURE 7.33 Transients after single pulses. The actograms show the running-wheel activity rhythms of two golden hamsters (*Mesocricetus auratus*) and two domestic mice (*Mus musculus*) maintained in constant darkness and presented with a single light pulse (360 lux for 1 hour). The times of the light pulses are indicated by stars. The two top actograms show pulse-induced phase advances of the activity rhythms, while the two bottom actograms show pulse-induced phase delays. The phase advance of the golden hamster was achieved through several transient shifts ("transients"). (Source: Adapted from Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.)

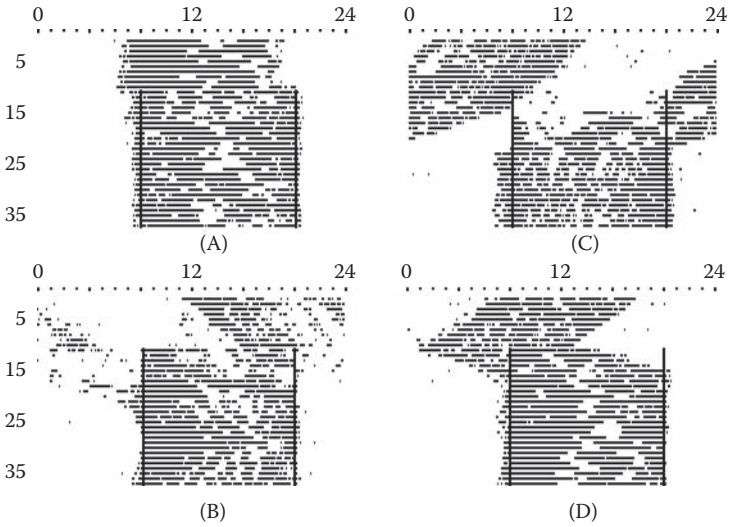


FIGURE 7.34 Transients prior to entrainment. The actograms show the running-wheel activity rhythms of Nile grass rats (*Arvicanthis niloticus*) maintained in constant darkness at first and then subjected to a 24-hour light–dark cycle (LD 12:12, 360 lux). The vertical lines indicate the beginning and end of the light phase of the light–dark cycle. Although all four animals 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. (Source: Archives of the Refinetti lab.)

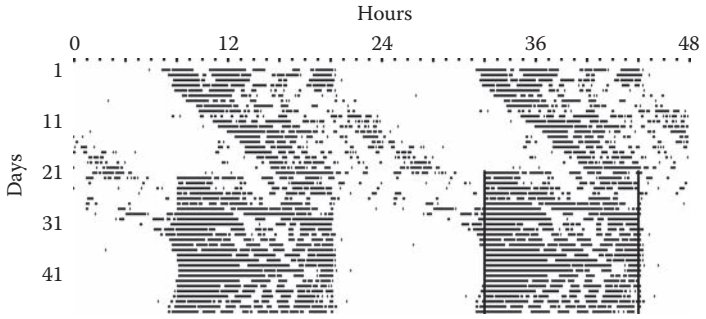


FIGURE 7.35 Advancing or delaying to entrain? The single-plotted actogram shown in Panel B of Figure 7.34 is presented here in double-plot format. In the single-plotted actogram, it seemed that entrainment had been achieved through a rapid advance shift. In the double-plotted actogram, it is evident that entrainment was achieved through several phase-delaying transients.

actually indicate phase-delay transition. This transition is easier to see in the double-plotted actogram redrawn in Figure 7.35.

7.1.5 Photoperiod

Chapter 4 showed that the relative duration of the light and dark phases of a light–dark cycle (*photoperiod*) strongly affects infradian rhythms. Photoperiod also has strong effects on circadian rhythms. Such effects were first described in plants,[144] but they have also been documented in a variety of animals,[145–151] including rodents[20,27,65,66,111,152–171] and other mammals.[68,99,172–176] The most obvious effect of photoperiod on circadian rhythms in animals is the temporal compression or expansion of expressed rhythms. Figure 7.36 shows examples of the running-wheel activity of mice. Note 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). The opposite is true for diurnal animals. In sheep (Figure 7.37), 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 photic stimulation suppresses secretion of the hormone melatonin, 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 this regimen). The fact that melatonin secretion under LD 8:16 starts to decrease *before* the end of

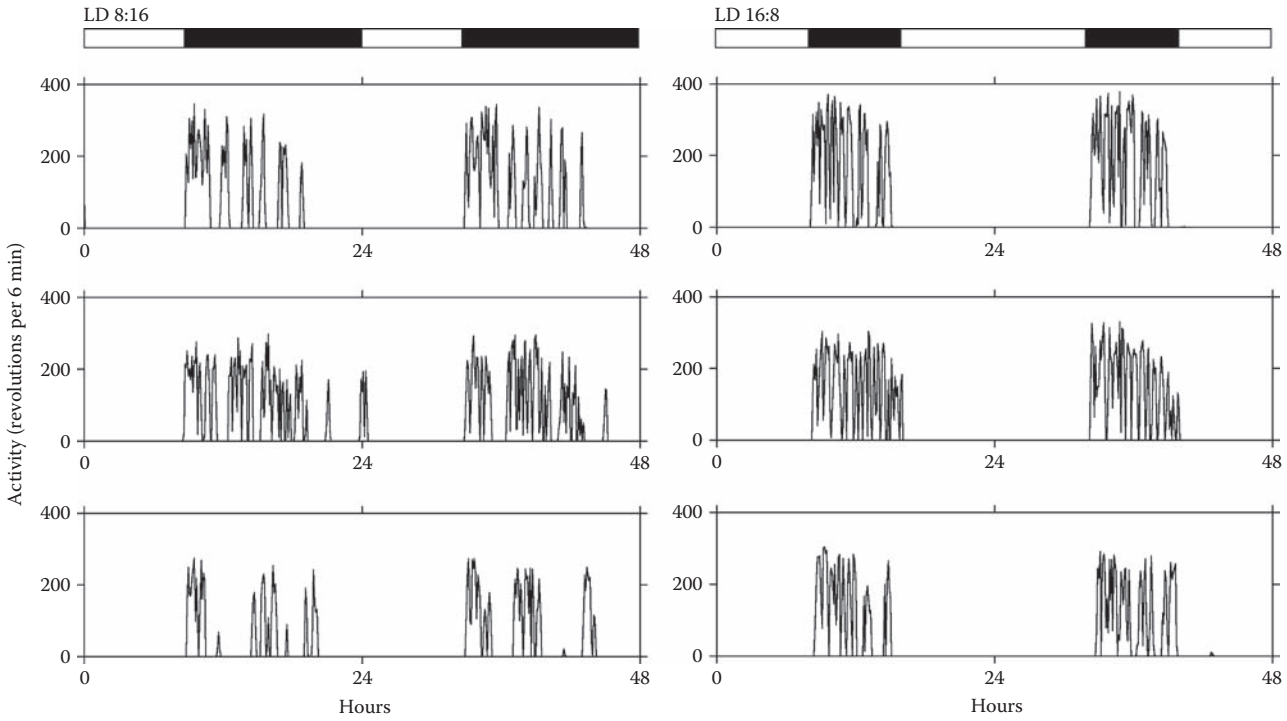


FIGURE 7.36 Photoperiod-induced compression of α in mice. The graphs show 2-day segments of the running-wheel activity records of six domestic mice (*Mus musculus*) maintained under 24-hour light–dark cycles with 16 hours of darkness per day (LD 8:16) or 8 hours of darkness per day (LD 16:8). The duration 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). (Source: Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.)

the scotophase (see the bottom panel in Figure 7.37) 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.[27,173] 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[159] or through the analysis of gene expression.[160,166,169,170,172] 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[157,168] or of the whole organism.[27,65,158] Figure 7.38 shows an example of alterations of the PRC in mice. Although the difference between the three PRCs may not be obvious at first, note 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*.

Chapter 4 showed that photoperiodic organisms use 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 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 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.[144] The rival hypothesis (*internal coincidence*) derives from Pittendrigh's notion of morning and evening oscillators: day length is determined by the phase relationship of the two oscillators.[87] Alterations of the PRC under short days (Figure 7.38) 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

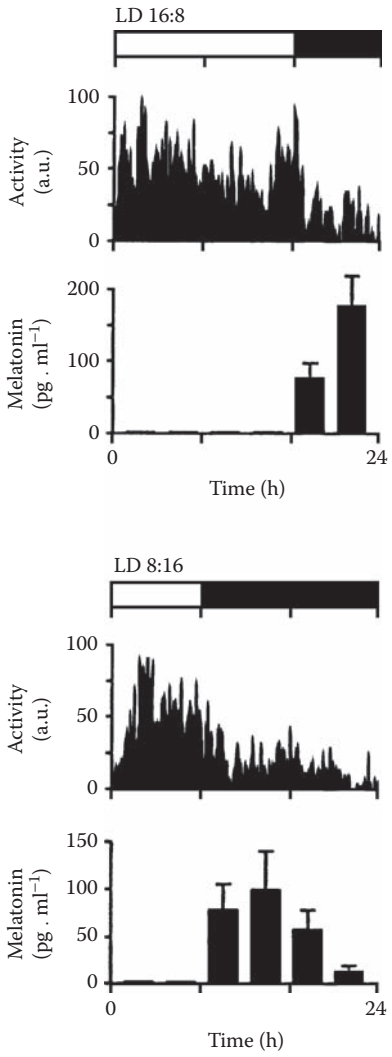


FIGURE 7.37 Photoperiod-induced compression of α in sheep. The graphs show the daily distributions of locomotor activity and melatonin secretion of sheep maintained under 24-hour light–dark cycles with 16 hours of light per day (LD 16:8) or 8 hours of light per day (LD 8:16). The activity data are based on 10 days of recording (with infrared motion sensors) from 28 sheep in each LD group. The melatonin data are means (± SE) of 4 animals per time point in each LD group. Note the compression of α, and expansion of melatonin secretion, under short days (LD 8:16). (Source: Lincoln, G., Messager, S., Andersson, H. & 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 U.S.A.* 99: 13890–13895.)

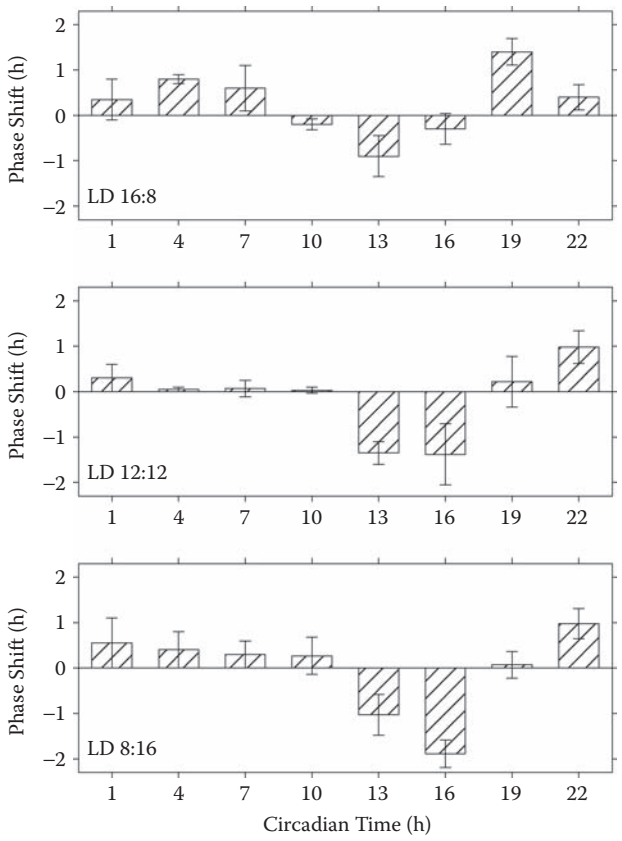


FIGURE 7.38 Previous photoperiod affects PRC. The graphs show the mean phase shifts evoked by single light pulses (1 hour, 300 lux) presented at various circadian times to domestic mice (*Mus musculus*) maintained in constant darkness for 6 days after being exposed to light–dark cycles with different durations of light per day for 4 weeks. Each bar corresponds to the mean (± SE) of four mice. Note 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). (Source: Adapted from Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.)

on the pacemaker. The next section discusses the analysis of the parametric effects of photic stimulation.

7.2 PHOTIC PARAMETERS

At the beginning of this chapter, I noted 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 possibility 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. Light clearly has parametric effects as well as nonparametric effects.

7.2.1 Masking

Perhaps the most ubiquitous parametric effect of light is *masking*.[89,177,178] As mentioned previously, masking refers to the inhibition of expressed rhythms (presumably without an effect on the pacemaker itself). Consider Figure 7.39.

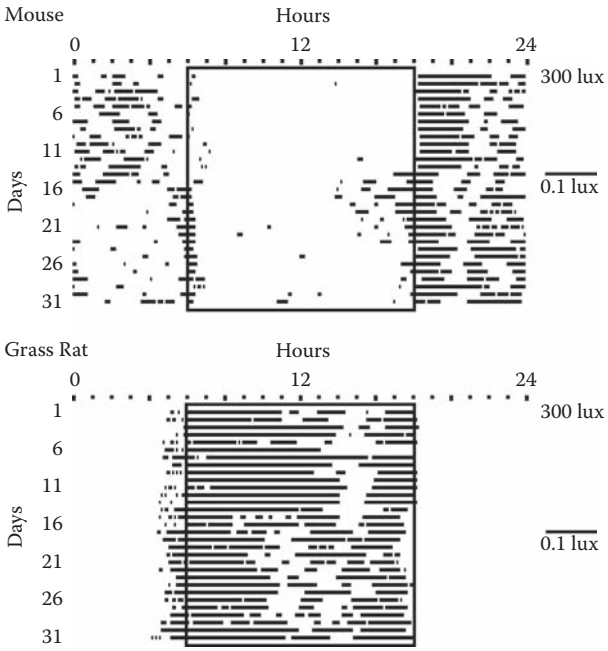


FIGURE 7.39 Brightness and masking. The actograms show the running-wheel activity rhythms of a domestic mouse (*Mus musculus*) and a Nile grass rat (*Arvicanthis niloticus*) maintained under light–dark cycles with 12 hours of light and 12 hours of darkness per day, as indicated by the rectangles. As 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 in the right margin). (Source: Archives of the Refinetti lab.)

The top panel shows the running-wheel activity records 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 masked, although a change in 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 no real change in illuminance occurred during the dark phase. Presumably, the dark phase became subjectively "less dark" when the contrast between light and darkness was reduced.

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,[113,132,145,147,179–183] one researcher has taken the leading role in the study of this phenomenon. Nicholas Mrosovsky (Figure 7.40) is a professor of zoology at the University of Toronto (in Canada). He has conducted numerous studies on masking[184–191] and published a good methodological article on it.[178] His interest in masking derives 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.

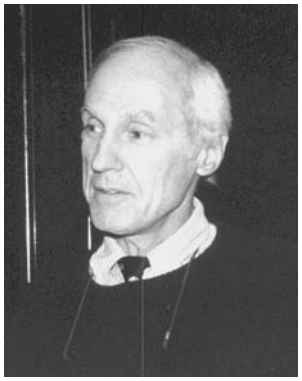


FIGURE 7.40 Nicholas Mrosovsky. This circadian physiologist from the University of Toronto (Canada) is the most active researcher in the mechanism of masking. (Source: Photograph courtesy of Nicholas Mrosovsky.)

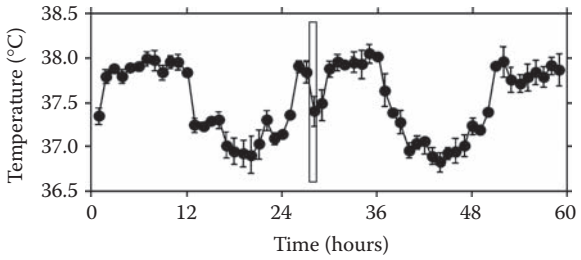


FIGURE 7.41 Masking by brief pulses in laboratory rats. The graph shows a 60-hour segment of the body temperature records of a group of laboratory rats (*Rattus norvegicus*) maintained in constant darkness and exposed to a brief (1 hour) light pulse. The data points represent the means (± SE) of eight rats. A fall of approximately 0.5°C can be observed immediately after the light pulse. (Source: Sei, H. et al. (2003). Prenatal exposure to alcohol alters the light response in postnatal circadian rhythm. *Brain Research* 987: 131–134.)

The acute masking effect of light can be demonstrated much more easily than its chronic effect. Consider Figure 7.41, which shows the mean body temperature of

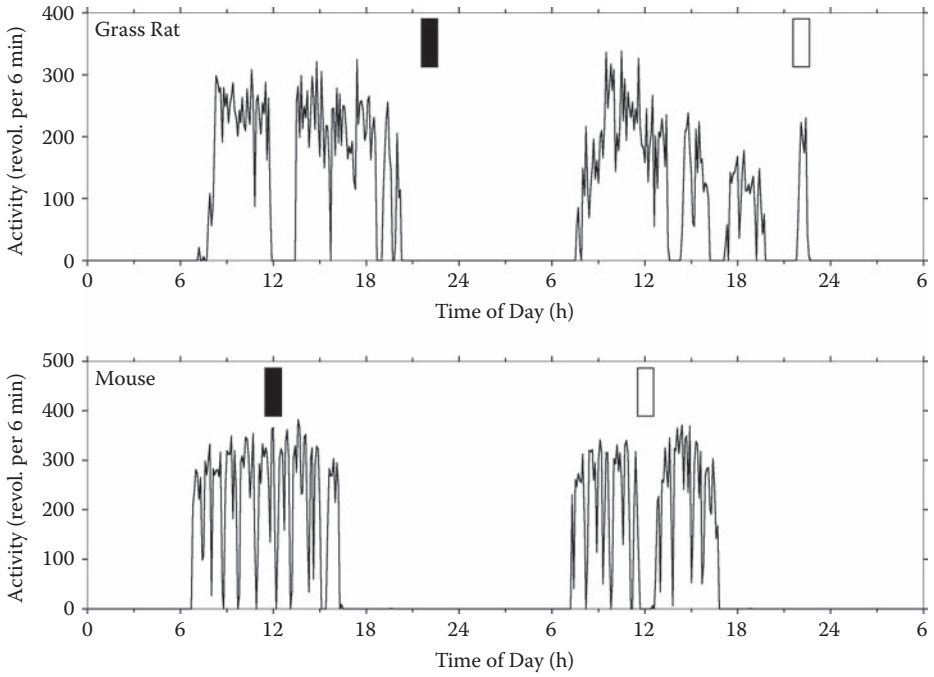


FIGURE 7.42 Masking by brief pulses in grass rats and mice. The graphs show 54-hour segments of the running-wheel activity records of a Nile grass rat (*Arvicanthis niloticus*) and a domestic mouse (*Mus musculus*) maintained in constant darkness and exposed to a brief (1 hour) light pulse. The time 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. Note positive masking by the light pulse in the records of the diurnal grass rat and negative masking in the records of the nocturnal mouse. (Source: Archives of the Refinetti lab.)

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. Note the small but sharp fall in body temperature associated with the light pulse. Next, consider Figure 7.42, which shows the rhythms of 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, while the dark rectangles indicate the corresponding times on the previous day (when no light pulse was presented). The light pulse clearly 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 7.43 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, while light may inhibit activity in nocturnal animals, darkness may inhibit activity in diurnal animals. Figure 7.44 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 at the time of lights-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 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. Consider Figure 7.45. 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 masked by the light. Note that masking restricted activity mostly to

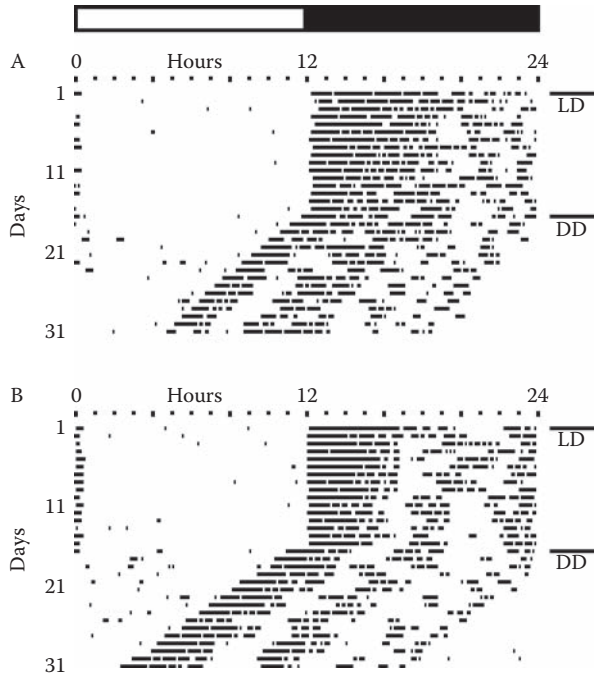


FIGURE 7.43 Masking by light–dark cycle in mice. The actograms show the running-wheel activity rhythms of two mice maintained under a light–dark cycle at first (as indicated by the horizontal bar at the top of the figure) and then in constant darkness (as indicated in the right margin). The animal whose 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. (Source: Archives of the Refinetti lab.)

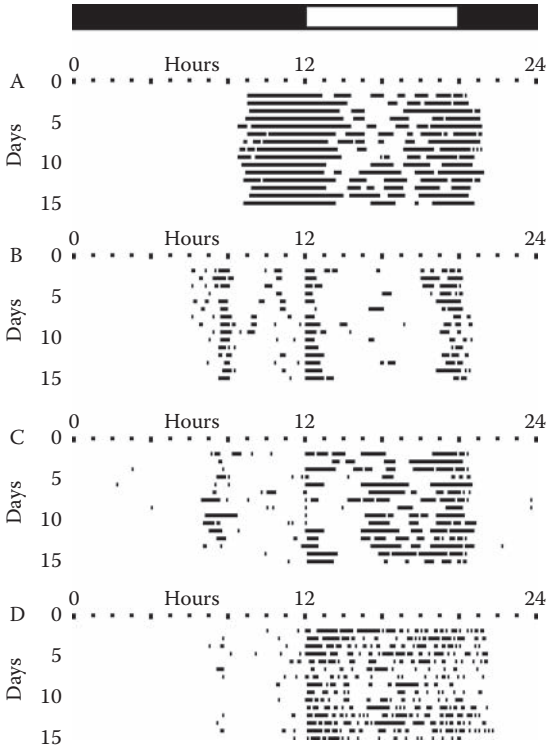


FIGURE 7.44 Masking by light–dark cycle in grass rats. The actograms show the running-wheel activity rhythms of four Nile grass rats (*Arvicanthis niloticus*) maintained under a 24-hour light–dark cycle with 8 hours of light per day (as indicated by the horizontal bar at the top of the figure). Although 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. (Source: Archives of the Refinetti lab.)

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.

Consider just one more case of masking. Figure 7.46 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.[192] 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 finding suggests that the activity pattern exhibited under the full light–dark cycle was the result of masking, not of entrainment.

7.2.2 Aftereffects

Look at Figure 7.4, which I discussed earlier in this chapter. Note that the light pulses caused not only phase shifts of the activity rhythms but also alterations in the free-running periods.The association between phase shifts ($\Delta\phi$) and changes in period ($\Delta\tau$) has been noted by various researchers.[8,16,24,35,40,59,66,193–195] However, the association was often found to be nonsystematic and to involve very small changes in period (under 0.2 hours). In a few studies, in both diurnal[54,59,66,193] and nocturnal[24,35,194] rodents, a systematic association between $\Delta\phi$ and $\Delta\tau$ was found: phase advances were associated with a shortening of period, while 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 (upper panel) lengthened to 24 hours after a phase delay, and the circadian period that was approximately 24 hours (lower panel) shortened considerably after a phase advance. Figure 7.47 shows correlation plots of $\Delta\phi$ and $\Delta\tau$ for three different species. Note that in all three plots 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 they may be large enough to modulate entrainment. The fact that

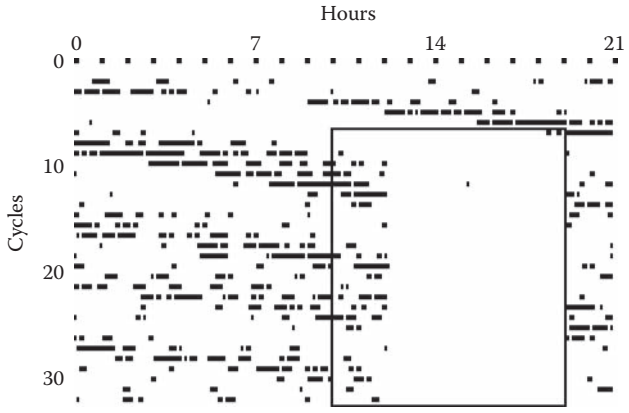


FIGURE 7.45 Masking, not entrainment. This actogram plotted in modulo 21 shows the running-wheel activity rhythm of a domestic mouse ($\tau = 23.6$ hours) exposed to a 21-hour light–dark cycle. The duration of the light phase of the light–dark cycle is indicated by the rectangle. Although the animal 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 activity was negatively masked during the light phase. (Source: Archives of the Refinetti lab.)

phase shifts evoked by a nonphotic stimulus are also associated with changes in τ (although in the opposite direction),[196] 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 example, 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,[4,8,15,121,123,129,131,134,197–201] and an example is provided in Figure 7.48. 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 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.[199] In a study in which the recovery of the free-running period was followed for a longer amount of time, aftereffects were found to last for 3 months or more.[202] The cause of

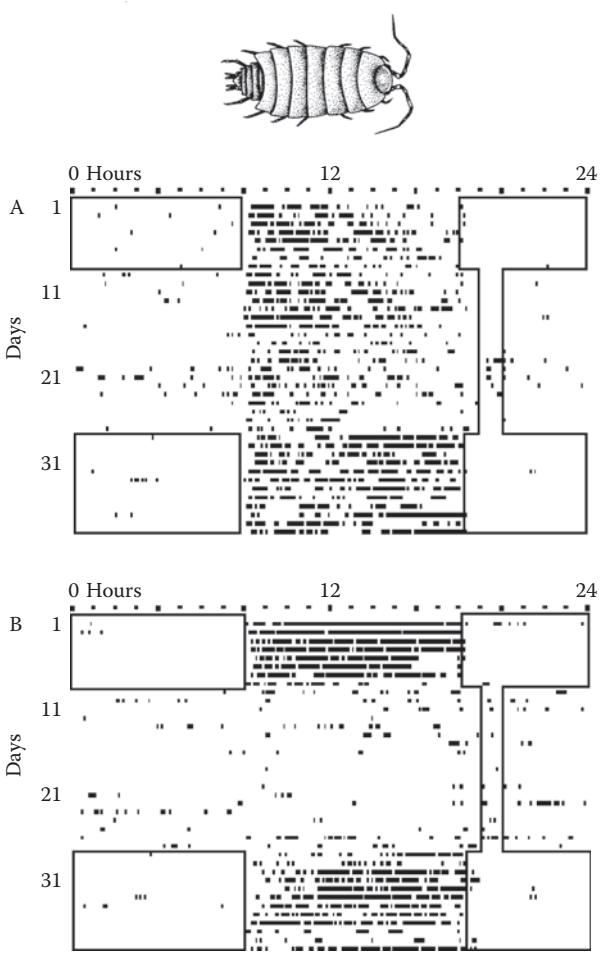


FIGURE 7.46 Bizarre masking in woodlice. The actograms show the rhythms of locomotor activity (measured by infrared motion sensors) of two pill bugs (*Armadillidium vulgare*) maintained under full and skeleton photoperiods (as indicated by the rectangles). The animal whose records appear in Panel A seems 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. (Source: Adapted from Refinetti, R. (2000). Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 73: 575–583.)

aftereffects is not known. Pittendrigh suggested that aftereffects could be due to changes in the phase relationship of his putative morning and evening oscillators,[202] but, at least in cockroaches, this theory was shown to be incorrect.[4]

7.2.3 Dark Adaptation

Dark adaptation is a well known process in the visual system.[77] As you know from your own experience, your vision adapts for optimal performance at different levels of illuminance. When you enter a movie theater in the

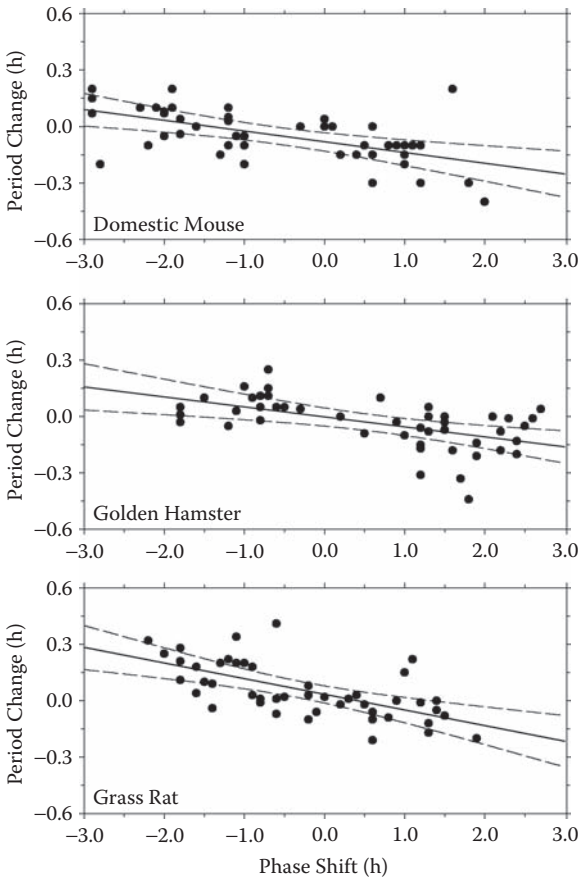


FIGURE 7.47 Phase shifts are accompanied by period changes. Phase shifts of circadian rhythms evoked by single light pulses are often accompanied by small changes in the period of the rhythm, as shown here for three rodent species. Phase shifts of different magnitudes were obtained by light pulses of 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). (Sources: Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107; archives of the Refinetti lab.)

middle of the afternoon, initially you are 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. Your vision adapted to the darkness in the theater. A similar process was described over 30 years ago in the circadian system of the fruit fly (*Drosophila melanogaster*).[203] A researcher 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. 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[15,19] and mice[24,30,204] 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. Figure 7.49 shows data from mice. In the phase-delay region (CT 16) and in the phase-advance region (CT 0), the magnitude of phase shifts evoked by single light pulses is greater the longer the animals are maintained in constant darkness, up to about 3 weeks. Full adaptation is achieved a few days earlier for phase delays than for phase advances. Note 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 to 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).[30]

The limited number of studies conducted so far does not allow interspecies generalizations, but the data shown in Figure 7.50 indicate that considerable variability exists between mouse strains and different rodent species. For example, although dark adaptation is robust in CD-1 mice, it is modest in C57BL/6 mice and virtually nonexistent in Nile grass rats. CD-1 mice are albino, but circadian dark adaptation also is observed in pigmented hamsters.[15,19] When dark adaptation occurs, its effect on 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 7.51), while full adaptation in the circadian system of rodents takes up to 3 weeks.[19,24,30] Two recent studies in humans also suggest that prolonged exposure to darkness or dim light may enhance the suppression of melatonin secretion by nocturnal photic stimulation.[205,206] A study on rats has shown that prolonged exposure to darkness enhances the acute effects of light on sleep.[207]

Little is known about the time course of the reverse process (i.e., *light* adaptation). Preliminary studies in golden hamsters[19] suggest 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.[24] Studies in another laboratory also suggest that light adaptation proceeds rapidly in the circadian system of the mouse.[25] 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 requires additional research. 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. Figure 7.52 illustrates the difference between sensitivity and responsiveness. If one

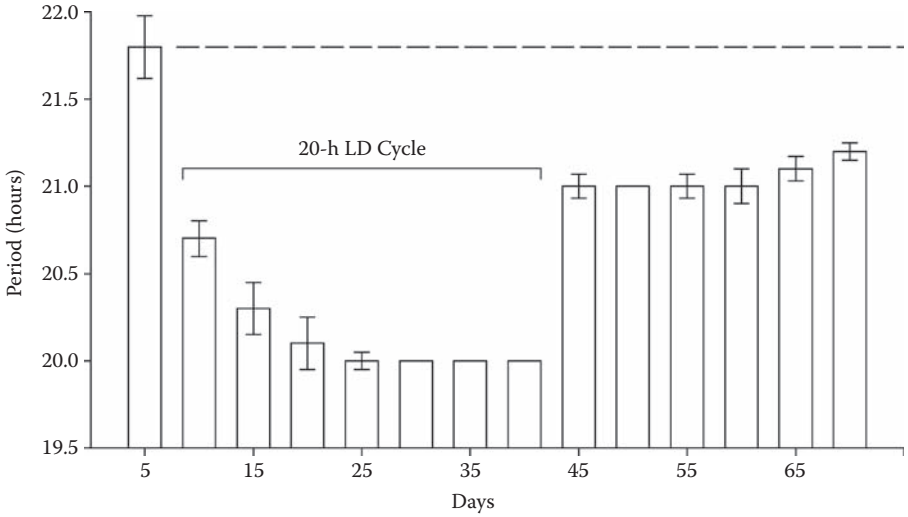


FIGURE 7.48 Aftereffects of entrainment. The period of a zeitgeber to which an organism is exposed prior to being released into constant darkness can have long-lasting effects on the free-running period, as exemplified by these data from a study on *tau*-mutant hamsters. Each bar corresponds to the mean (± SE) of eight 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. (Source: Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.)

measures the response of an organism to various intensities of a stimulus, one routinely finds that the response is related to stimulus intensity according to a sigmoid function (Panel A). This finding 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, compare two processes, such as P1 and P2 in Panel B of Figure 7.52. 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. A third situation can also occur, in which two processes differ in their offsets rather than in their slopes or relative positions along the X-axis (Panel C). In this case, 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, 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 a study that examined data recorded over a short period of time (5 to 60 minutes), a team of researchers found no evidence that previous photic stimulation caused a reduction in photic sensitivity associated with the reduction in responsiveness of the circadian system of the golden hamster.[80] Rather, the researchers 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.[208] This issue requires further study.

The phenomenon of circadian dark adaptation observed in mice and hamsters resembles 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 with rats raised under bright light–dark cycles. This elevation resulted in the conservation of the number of photons that the retina catches each day (photostasis).[209] 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 that it also could be produced in adult rats after

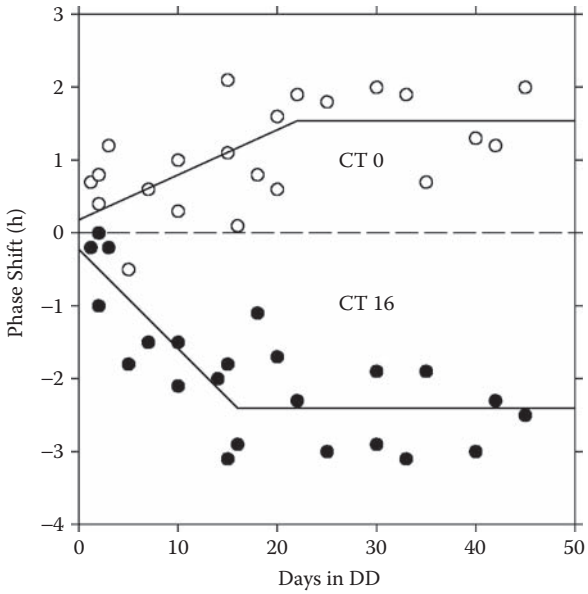


FIGURE 7.49 Dark adaptation in the circadian system of the mouse. The graph shows the phase shifts evoked by 1-hour light pulses presented to domestic mice (*Mus musculus*) maintained in constant darkness for different intervals of time. Both for phase advances (CT 0) and phase delays (CT 16), the magnitude 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. (Source: Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.)

approximately 3 weeks of treatment.[210,211] Thus, circadian dark adaptation might be an expression of photostasis.

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. The regression lines in Figure 7.49 suggest that one should 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 small response would limit the range of entrainment of mice (τ = 23.6 h) to the interval between 23.4 and 23.8 hours. 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.[177] The manner in which the additional light contained in an LD cycle produces the full shift required for entrainment remains to be elucidated.

One can speculate about the evolutionary advantage of dark adaptation in the circadian system. Animals kept in darkness but recently exposed to a natural light–dark cycle are likely to have their circadian pacemakers closely in phase with the zeitgeber, and they will not require large shifts to reestablish entrainment. In contrast, animals kept 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 to reestablish entrainment. Greater responsiveness to light in dark-adapted animals may be an adaptive feature of the circadian system. Additionally, the occurrence of dark adaptation is a *sine qua non* condition for the operation of nonparametric entrainment, as discussed in Section 7.3.

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 no explanations are currently available. Bizarre observations are usually discarded as oddities, but these data on Siberian hamsters (*Phodopus sungorus*) are too robust to be ignored. Some researchers have observed that Siberian hamsters housed under short photoperiods typical of the winter lose their daily rhythms of sleep and become arrhythmic.[164] 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.[212] More impressively, hamsters maintained under a long photoperiod and subjected to two brief light pulses on 2 consecutive nights became arrhythmic.[212] Arrhythmicity (and sometimes freeruns) also was observed in hamsters subjected to a 5-hour delay of the light–dark cycle.[213–215] The Siberian hamster clearly is not an ordinary rodent, and further research on this atypical species may help researchers understand properties of the circadian pacemaker that are not as evident in other species.

7.3 SYNTHESIS AND MODELS

The two previous sections discussed the control of circadian rhythms by the photic environment. Entrainment was demonstrated to be 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.[216] 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 7.53).

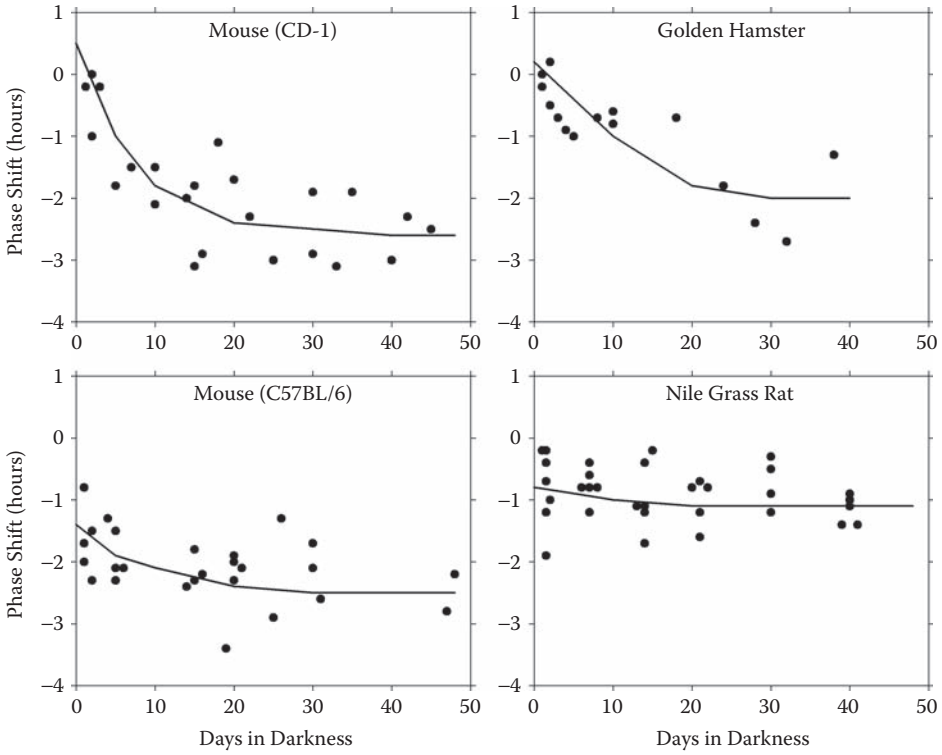


FIGURE 7.50 Dark adaptation in the circadian system of four rodents. The graphs show the phase shifts (delays) evoked by 1-hour light pulses presented to four groups of rodents maintained in constant darkness for different intervals of time. Each data point corresponds to one animal. The magnitude of 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. (Sources: Daymude, J. A. & Refinetti, R. (1999). Phase-shifting effects of single and multiple light pulses in the golden hamster. *Biological Rhythm Research* 30: 202–215; Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440; archives of the Refinetti lab.)

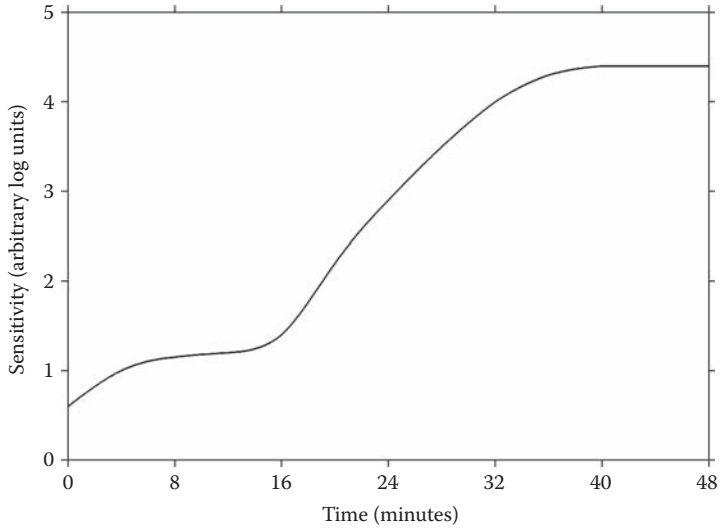


FIGURE 7.51 Dark adaptation in the human visual system. Visual sensitivity increases with time in darkness. Full adaptation is achieved in 40 minutes. (Source: Adapted from Barlow, H. B. & Mollon, J. D. (1982). Psychophysical measurements of visual performance. In: Barlow, H. B. & Mollon, J. D. (Eds.). *The Senses*. New York: Cambridge University Press, pp. 114–132.)

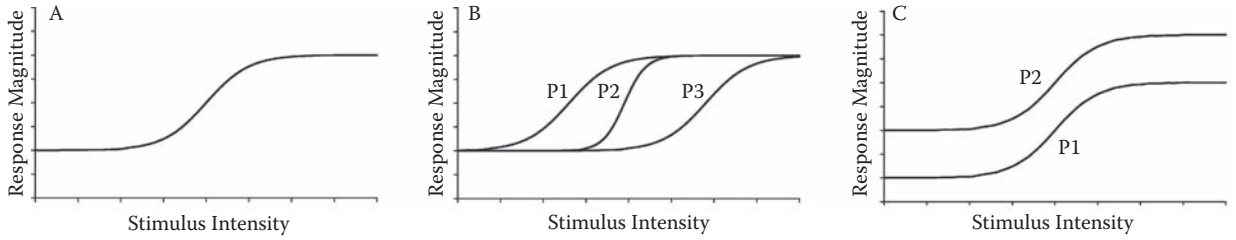


FIGURE 7.52 What is sensitivity? Not all changes in responsiveness are considered to reflect changes in sensitivity. The magnitude of a sensory response is usually a sigmoid function of the intensity of the stimulus (A). Some differences in the function are considered to reflect changes in sensitivity (B) but others are not (C). See text for details.

FIGURE 7.53 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, while other models draw on the pragmatic functional power of computers. (Source: © ArtToday, Tucson, AZ.)

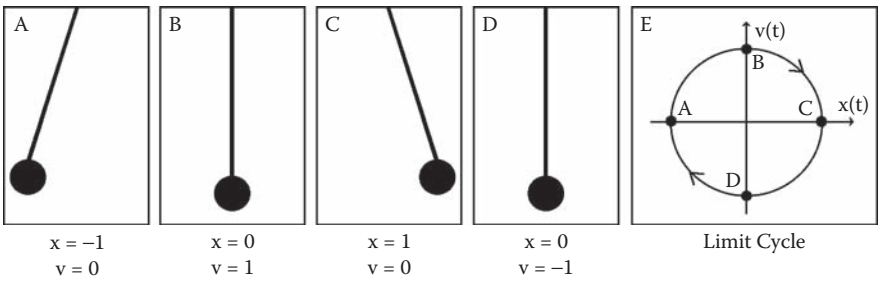


FIGURE 7.54 Defining a limit cycle. If one thinks of the position (x) and velocity (v) of a moving pendulum (A, B, C, and D), then one can easily visualize a limit cycle on a Cartesian plot (E). See text for details.

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 but elegant, well-researched timing device. As diagrammed in Figure 7.54, the movement of the pendulum is 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. Note that if the position and velocity values of the four panels are plotted on a coordinate system (Panel E), a circular pattern that describes the pendulum's movement through eternity (unless the cycle is disturbed) is obtained.

Limit cycles have been used to describe the oscillation of the circadian pacemaker.[217–223] Referring to the circadian pacemaker as a limit cycle oscillator allows, for example, the reconciliation of Type 0 and Type 1 PRCs as evoked by strong and weak stimuli. Type 1 PRCs in invertebrates and nonanimals can become Type 0 PRCs if the intensity or duration of the stimulus is increased. Consider the diagrams in Figure 7.55. Row A illustrates a Type 0 PRC. The example starts with an undisturbed limit cycle (1). Next, a disturbance, such as a light pulse, is introduced in one of the state variables (2). This event disturbs 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

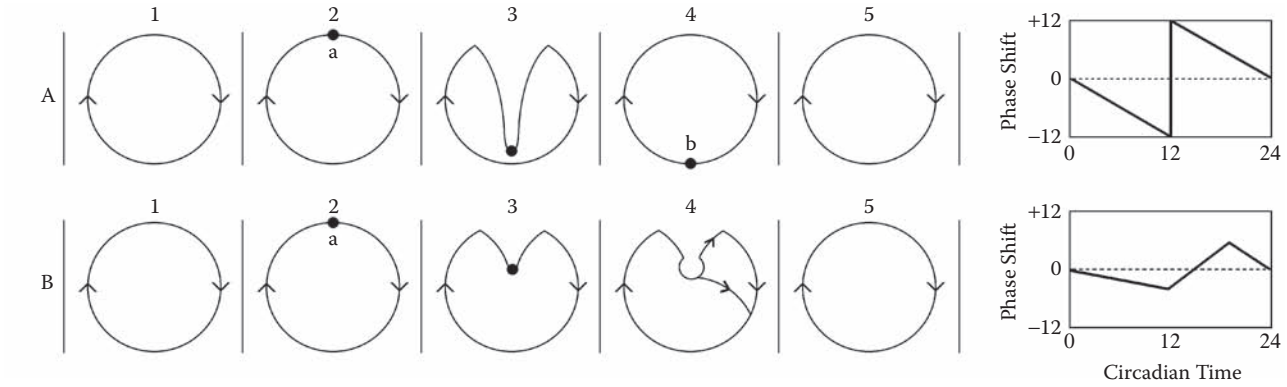


FIGURE 7.55 Type 0 and Type 1 resetting in a limit cycle. If one conceptualizes the circadian pacemaker as a limit cycle oscillator, then one can easily associate a Type 0 PRC with strong resetting stimuli (A) and a Type 1 PRC with weak resetting stimuli (B). See text for details.

12 hours (4). The PRC becomes a Type 0. In contrast, in Row B a small disturbance introduced in the limit cycle causes the cycle to resume at a new phase that is different from the old phase but that does not constitute as large a shift as that in Row A. The PRC becomes a 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, position and velocity were used as variables. In the case of the circadian pacemaker, the two state variables are unknown, so they must be defined arbitrarily.

Chapter 13 examines some complex mathematical models that incorporate what is known about the molecular structure of the circadian clock. A simple limit cycle based on two variables, however, cannot accommodate all the genes that are involved in the system, even if the model's output is mathematically satisfactory.[224] 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) enjoy applying 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. Functional models should avoid the incorporation of assumptions that are not strictly necessary. In particular, assumptions about mathematical properties of the system are restricted to specific functions derived directly from experimental research on actual organisms. To develop a functional model of appropriate complexity, a computer must be used.[225]

To communicate with a computer, you must use *machine language*. If you are familiar with computers, you know that contemporary models use "translation" programs to convert "plain" English into machine language. Consider a simple case such as the addition of two numbers (Figure 7.56). The computer must be sent a precise string of 0s and 1s in an arrangement that the chip inside the computer can read. The sequence shown in Figure 7.56 (Machine Language) is appropriate for a Z-80 chip, which was the main processor in the first personal computers that became popular in the 1980s.[226] Instead of typing 0s and 1s, you could use a shorthand system called *Assembly Language*, which at that time was a great innovation.[227] 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)[228,229] was once the standard programming language provided as a component of the operating systems of all personal computers.[230] After many revisions and upgrades, the language, now known as Microsoft's Visual Basic,[231] is still

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 7.56 How to communicate with a computer. Humans use Machine Language to communicate with computers. Assembly Language, BASIC, and other high-level languages facilitate the use of Machine Language.

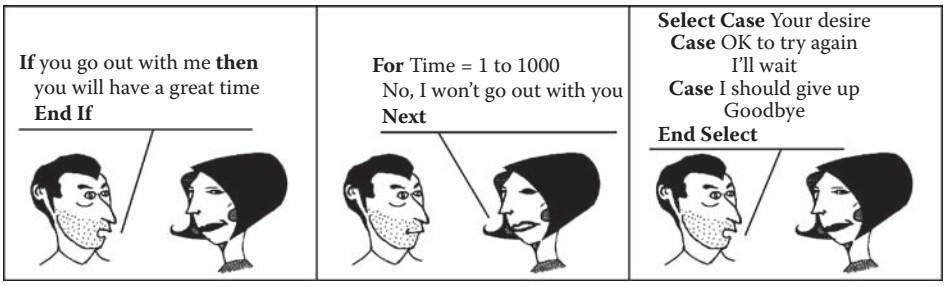


FIGURE 7.57 Speaking BASIC. This conversation structured according to BASIC syntax may sound bizarre, but it can be understood reasonably well by English speakers.

used today and is arguably the world's dominant computer language.[232] In BASIC, the simple and intuitive expression *A = 320 + 430* is all the computer needs to add the two numbers and store the result in memory (bottom of Figure 7.56).

Consider the cartoon in Figure 7.57. The conversation between the two people may be rather strange, but 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, you 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.[233] To model a clock, 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, you 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
    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, you 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:

```
If CT > = 12 and CT < = 20 then Print symbol
```

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 7.58.

The model cannot successfully simulate free-runs in constant light. As shown in Figure 7.59 (left), the model predicts that a mouse placed under constant light will not exhibit any locomotor activity at all, which is incorrect. 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 noted this inconsistency of the nonparametric theory of entrainment and recognized that a provision had to be made for the phenomenon of light adaptation.[91] Strictly speaking, the nonparametric theory of entrainment is incorrect. However, it can be made plausible if the parametric effect of light adaptation is acknowledged. As shown in Section 7.2, the circadian system exhibits dark and light adaptation. If minimal additional code is inserted in the computer program to account for light and dark adaptation, a normal

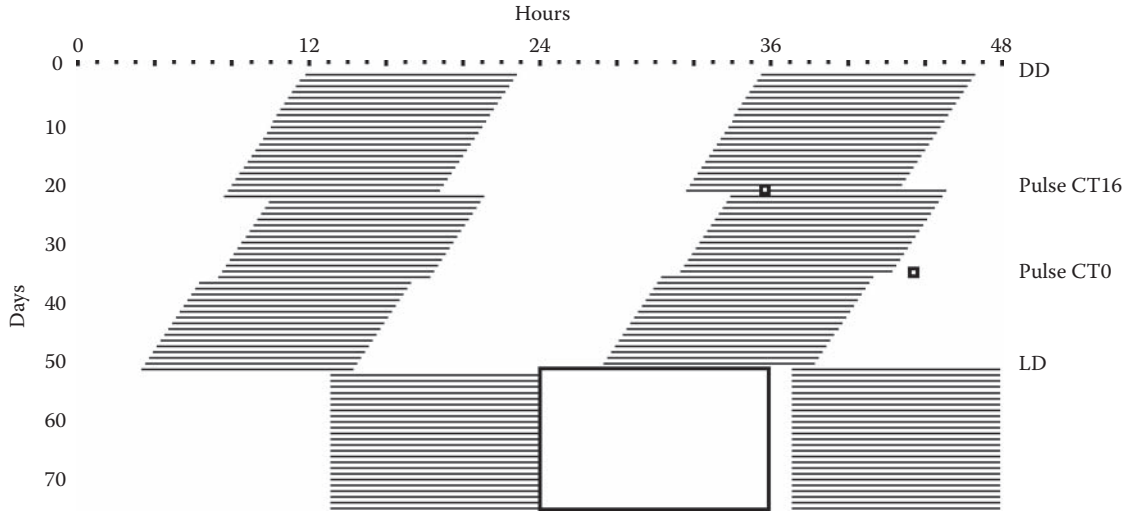


FIGURE 7.58 A computer mouse. This double-plotted actogram shows the simulated activity pattern of a mouse maintained in constant darkness and stimulated with brief light pulses at CT 16 and CT 0 before being subjected to a 24-hour light–dark cycle. The simulated activity pattern resembles the activity pattern of a real mouse relatively well.

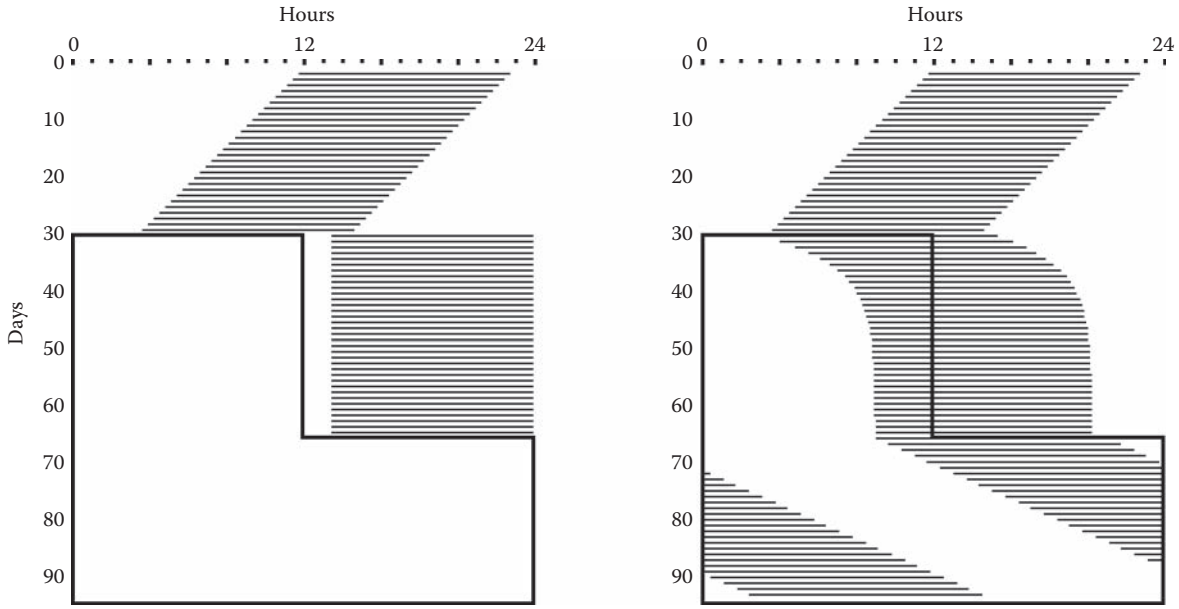


FIGURE 7.59 The need for dark adaptation. A simple computer model of the nonparametric theory of entrainment can simulate the activity pattern of a mouse in constant darkness and under a light–dark cycle but not in constant light (left panel). If the model incorporates a mechanism of dark adaptation, it can simulate activity in constant light, and it provides a better simulation of transients prior to entrainment (right panel).

freerun in constant light — with a period longer than under constant darkness — is observed (Figure 7.59, right). Note that the change in free-running period conforms to Aschoff's rule. In fact, the model confirms the prediction that Aschoff's rule derives from the nonparametric effect of light. Note 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 is included 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

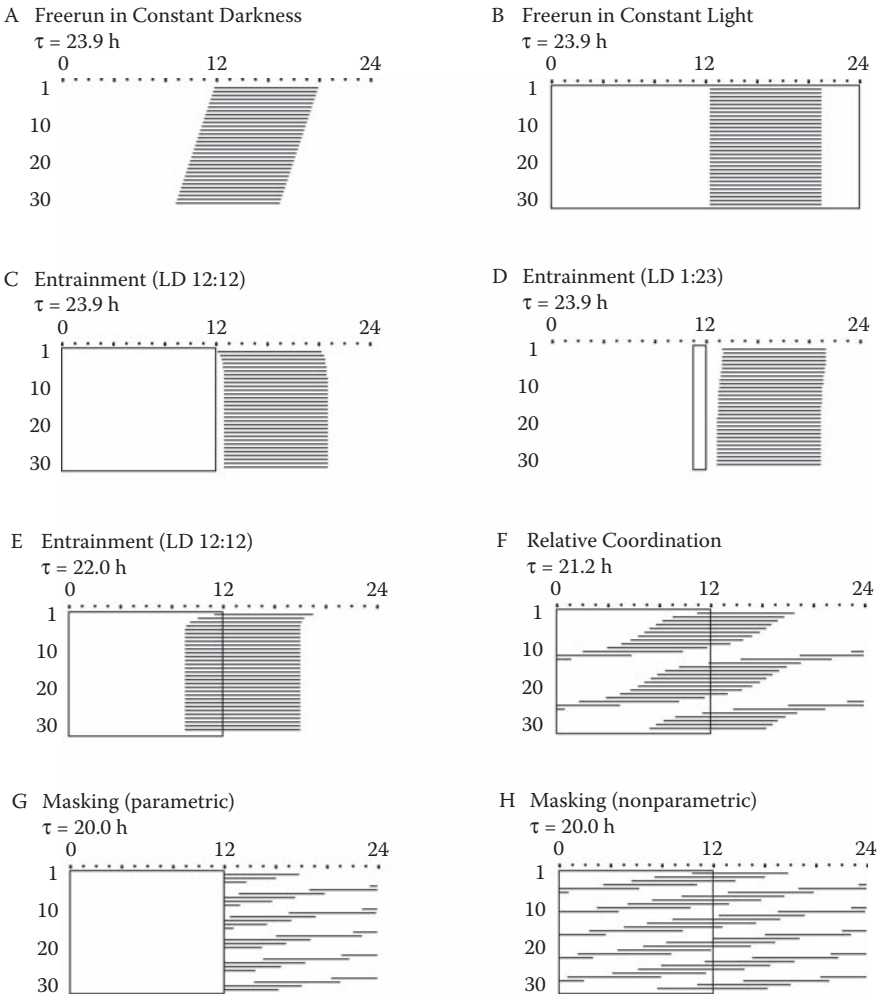


FIGURE 7.60 A good model. The computer model of the nonparametric theory of entrainment that incorporates the mechanism of dark adaptation can produce simulations good enough to summarize most of the photic environmental mechanisms covered in this chapter. See text for details.

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 7.60 shows eight simulations of particular conditions. The top left actogram (A) shows a freerun in constant darkness (DD) of a "hamster" with a circadian period of 23.9 hours. 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). I 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, while 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 7.60 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 from the static PRC. When the animal is exposed to light at all points of the circadian cycle, the actual response to light is produced by a greatly distorted PRC (as each pulse causes a shift, altering the circadian time of the following pulse). Computer simulations allow one to appreciate this difference.

The second row of actograms in Figure 7.60 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 7.60 (G) shows negative masking that easily could 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 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 to indicate masking. Finally, it should be noted that the apparent freerun in 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 change in period 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 expression is not used commonly among circadian physiologists.

The model also is unable to simulate the transients sometimes associated with discrete phase shifts (although it simulates the transients of entrainment) and the aftereffects of entrainment. These failings could be interpreted as a deficiency of the model. However, because the model specifies only pacemaker processes, the failure to simulate the two phenomena also can be interpreted as evidence that single-pulse transients and aftereffects are generated downstream from the pacemaker 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 downstream from the pacemaker. 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 include 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 synthesize dispersed experimental data and elaborate 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

Exercise 7.1 Measuring phase shifts and other effects of photic stimulation

This exercise uses the program Plot to inspect sample data files containing running-wheel activity records of rodents subjected to various forms of photic stimulation.

1. Double-click on the Circadian icon to open the program banner, then click on Plot (the first icon on the left).
2. In the Source panel, double-click on the Data subfolder, 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 (CT 16). Click on the actogram button to see the records.
3. The actogram shows clear circadian rhythmicity with a period shorter than 24.0 hours. A

phase delay of 4 to 5 hours on Day 23 should also be easy-to-see. When you click on an actogram line, the day it represents is identified.

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 pre- 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 pre- 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, change the "No. of days to skip after intervention" to 1, 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. Note that the calculated shift is now –5.0 hours. The value changed because you chose 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 (CT 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 occurred 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). The program displays an error message (above the display window) because it is unable to calculate the shift.
8. The program could not 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. You may not be pleased with the postshift linear regression, however. The program considered the variations in the amount of running, while you may be more concerned about the absolute onsets. A simple compromise 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 view the records. (If your display is in black-and-white mode, make sure to change it to color mode.)
11. 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 that this event occurred. These activity records are an excellent example of *positive masking* by light. Note 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). The yellow and red sections (which indicate higher activity levels) required 8 to 9 days to accomplish the 4-hour phase-advance required by the light–dark cycle (on Day 26, the lights came on at approximately CT 20). Thus, entrainment was attained 8 to 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, lights-off and lights-on times have been indicated in the file (and can be seen as red dots). Click on the actogram button.
13. Note 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.

Exercise 7.2 Viewing PRCs

This exercise uses the program PRC. The 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, when necessary, to comply with the convention that activity onset be designated as CT 12 for nocturnal organisms and as CT 0 for diurnal organisms.

1. Double-click on the Circadian icon to open the program banner, then click on PRC (the sixth icon from the right).
2. Note that you can choose either Photic or Nonphotic stimuli. Use the default choice (Photic) in this exercise. Note also the three utility buttons: one allows you to clear the picture when it gets too crowded, another 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 in a graphics program).
3. Click on the second species name in the list of species (*Arvicanthis ansorgei*). Note 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 estimate 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 *Arvicanthis ansorgei* again. Now you can see 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 see exact values, just click on the curve. For example, if you click on the thick red line at CT 12, a box is displayed that indicates a phase shift of –1.0 hour.
6. Now double-click on the next species (*Arvicanthis niloticus*). The PRC is plotted in green. It is similar but not identical to the curve for *A. ansorgei*.
7. Continue to inspect PRCs. You may need to resize the Y-axis for some species. 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.

Exercise 7.3 Understanding entrainment

This simple exercise uses 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 about 6 minutes to complete and is self-explanatory. Go ahead!

Exercise 7.4 Modeling photic entrainment

This exercise uses the program Model 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 based on photic stimulation of the golden hamster. Details about the program were presented in Section 7.3.

1. Double-click on the Circadian icon to open the program banner, then click on Model (the fifth icon from the right).
2. At the top of the window, you can specify the "endogenous" period of the "organism," and you can choose the number of blocks of simulations to include. 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). Accept the default values listed in the lower panel. Click on Run.
4. Note 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. Note 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 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. Note the difference in the phase angle of entrainment. The onset of activity after entrainment is now more than an hour *after* lights-off. Although the PRC has not changed, the change in period changed the phase angle of entrainment.
6. 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. Note that the pattern of activity did not change much. The actogram under LD 2:22 is essentially the same as under LD 12:12. The similarity is explained by the fact that the important

part of the light–dark cycle is subjective dawn, when phase advances are evoked, and an animal with an endogenous period longer than 24 hours requires phase advances to entrain to a 24-hour light–dark cycle.

7. The result 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. Entrainment was not 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. At least seven "attempts" at entrainment (a slowing down of the clock) occurred, but they were short-lived.
9. Run additional simulations now. Exercise 8.3 (in Chapter 8) further explores the issue of phase shifts induced by single light pulses.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Meijer, J. H. (2001). Photic entrainment of mammals. In: Takahashi, J. S., Turek, F. W., and Moore, R. Y. (Eds.). *Circadian Clocks* (Volume 12 of *Handbook of Behavioral Neurobiology*). 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* (Volume 12 of *Handbook of Behavioral Neurobiology*). 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). Free-running and entrained circadian rhythms. In: Aschoff, J. (Ed.). *Biological Rhythms* (Volume 4 of *Handbook of Behavioral Neurobiology*). New York: Plenum, pp. 81–93. A typical Aschoff article, filled with interesting facts and insightful generalizations. The treatment of entrainment is less in-depth 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* (Volume 4 of *Handbook of Behavioral Neurobiology*). New York: Plenum, pp. 95–124. A classic review article by Pittendrigh, the originator of the nonparametric theory of entrainment. This article is not easy-to-read, but it is worth the effort.

WEB SITES 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 Web Site:
http://www.visionscience.com
Sylvania Lighting International:
http://www.sylvania-lighting.com

LITERATURE CITED

1. Pittendrigh, C. S. (1954). On temperature independence in the clock system controlling emergence time in Drosophila. *Proceedings of the National Academy of Sciences U.S.A.* 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. & 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. & 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. Underwood, H. (1983). Circadian pacemakers in lizards: phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
7. King, V. M., Bentley, G. E. & 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.
8. 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.
9. Zivkovic, B. D., Underwood, H., Steele, C. T. & 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.

10. Stetson, M. H. & Gibson, J. T. (1977). The estrous cycle in golden hamsters: a circadian pacemaker times preovulatory gonadotropin release. *Journal of Experimental Zoology* 201: 289–294.
11. Daan, S. & 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.
12. Takahashi, J. S., DeCoursey, P. J., Mauman, L. & Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.
13. Nelson, D. E. & 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.
14. Rosenberg, R. S., Zee, P. C. & Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.
15. Shimomura, K. & Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
16. Meijer, J. H. & 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.
17. Grosse, J., Loudon, A. S. I. & 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.
18. Best, J. D., Maywood, E. S., Smith, K. L. & Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *Journal of Neuroscience* 19: 828–835.
19. Daymude, J. A. & Refinetti, R. (1999). Phase-shifting effects of single and multiple light pulses in the golden hamster. *Biological Rhythm Research* 30: 202–215.
20. Elliott, J. A. & Tarmarkin, L. (1994). Complex circadian regulation of pineal melatonin and wheel-running in Syrian hamsters. *Journal of Comparative Physiology A* 174: 469–484.
21. Christian, C. A. & 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.
22. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J. & 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.
23. Foster, R. G., Provencio, I., Hudson, D., Fiske, S., De Grip, W. & Menaker, M. (1991). Circadian photoreception in the retinally degenerate mouse (*rd/rd*). *Journal of Comparative Physiology A* 169: 39–50.
24. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
25. Khammanivong, A. & Nelson, D. E. (2000). Light pulses suppress responsiveness within the mouse photic entrainment pathway. *Journal of Biological Rhythms* 15: 393–405.
26. Yoshimura, T., Nishio, M., Goto, M. & 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.
27. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.
28. Kilduff, T. S., Vugrinic, C., Lee, S. L., Milbrandt, J. D., Mikkelsen, J. D., O'Hara, B. F. & 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.
29. Benloucif, S. & 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.
30. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
31. 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.
32. Sharma, V. K., Chandrashekaran, M. K. & 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.
33. Sharma, V. K. & Chandrashekaran, M. K. (1997). Rapid phase resetting of a mammalian circadian rhythm by brief light pulses. *Chronobiology International* 14: 537–548.
34. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
35. 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.
36. Shrama, V. K., Chidambaram, R. & 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.
37. Summer, T. L., Ferraro, J. S. & McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.
38. Bauer, M. S. (1992). Irradiance responsivity and unequivocal type-1 phase responsivity of rat circadian activity rhythms. *American Journal of Physiology* 263: R1110–R1114.
39. Stokes, M. A., Kent, S. & 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.

40. Stokes, M. A., Kent, S. & Armstrong, S. M. (2002). The relationships between phase and period responses to light pulses. *Biological Rhythm Research* 33: 303–317.
41. Stepien, J. M. & Kennaway, D. J. (2001). Phase response relationships between light pulses and melatonin rhythms in rats. *Journal of Biological Rhythms* 16: 234–242.
42. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N. & Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.
43. Frennan, M., Kripke, D. F. & Gillin, J. C. (1989). Bright light can delay human temperature rhythm independent of sleep. *American Journal of Physiology* 257: R136–R141.
44. Buresová, M., Dvoráková, M., Zvolsky, P. & Illnerová, H. (1991). Early morning bright light phase advances the human circadian pacemaker within one day. *Neuroscience Letters* 121: 47–50.
45. Honma, K., Honma, S. & Wada, T. (1987). Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. *Experientia* 43: 1205–1207.
46. Honma, K. & Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167–168.
47. Shanahan, T. L. & 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.
48. Dawson, D., Lack, L. & Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102.
49. Boivin, D. B., Duffy, J. F., Kronauer, R. E. & Czeisler, C. A. (1996). Dose-response relationships for resetting of human circadian clock by light. *Nature* 379: 540–542.
50. Shanahan, T. L., Kronauer, R. E., Duffy, J. F., Williams, G. H. & 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.
51. Khalsa, S. B. S., Jewett, M. E., Cajochen, C. & Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.
52. Warman, V. L., Dijk, D. J., Warman, G. R., Arendt, J. & Skene, D. J. (2003). Phase advancing human circadian rhythms with short wavelength light. *Neuroscience Letters* 342: 37–40.
53. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C. & 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.
54. Pohl, H. (1983). Light pulses entrain the circadian activity rhythm of a diurnal rodent (*Ammospermophilus leucurus*). *Comparative Biochemistry and Physiology B* 76: 723–729.
55. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P. & 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.
56. Mahoney, M., Bult, A. & 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.
57. 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.
58. Tavernier, R. J., Largen, A. L. & Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
59. Honma, S. & Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
60. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
61. DeCoursey, P. J. (1960). Daily light sensitivity rhythm in a rodent. *Science* 131: 33–35.
62. Pohl, H. (1985). The circadian system of the Turkish hamster, *Mesocricetus brandti*: responses to light. *Comparative Biochemistry and Physiology A* 81: 613–618.
63. Lee, T. M. & 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.
64. Kas, M. J. H. & 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.
65. Puchalski, W. & Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.
66. Kramm, K. R. & Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.
67. Meijer, J. H., Daan, S., Overkamp, G. J. F. & 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.
68. Schilling, A., Richard, J. P. & 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.
69. Glass, J. D., Tardif, S. D., Clements, R. & Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
70. Aschoff, J. (1965). Response curves in circadian periodicity. In: Aschoff, J. (Ed.). *Circadian Clocks*. Amsterdam: North-Holland, pp. 95–111.

71. Johnson, C. H. (1999). Forty years of PRCs: what have we learned? *Chronobiology International* 16: 711–743.
72. Colwell, C. S., Kaufman, C. M., Menaker, M. & 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.
73. Meijer, J. H., Rusak, B. & 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.
74. Rimmer, D. W., Boivin, D. B., Shanahan, T. L., Kronauer, R. E., Duffy, J. F. & Czeisler, C. A. (2000). Dynamic resetting of the human circadian pacemaker by intermittent bright light. *American Journal of Physiology* 279: R1574–R1579.
75. Middleton, B., Stone, B. M. & 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.
76. Zeitzer, J. M., Dijk, D. J., Kronauer, R. E., Brown, E. N. & Czeisler, C. A. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *Journal of Physiology* 526: 695–702.
77. Barlow, H. B. & Mollon, J. D. (1982). Psychophysical measurements of visual performance. In: Barlow, H. B. & Mollon, J. D. (Eds.). *The Senses*. New York: Cambridge University Press, pp. 114–132.
78. Goldstein, E. B. (2002). *Sensation and Perception*, 6th Edition. Pacific Grove, CA: Wadsworth.
79. Van den Pol, A. N., Cao, V. & Heller, H. C. (1998). Circadian system of mice integrates brief light stimuli. *American Journal of Physiology* 275: R654–R657.
80. Nelson, D. E. & Takahashi, J. S. (1999). Integration and saturation within the circadian photic entrainment pathway of hamsters. *American Journal of Physiology* 277: R1351–R1361.
81. Gronfier, C., Wright, K. P., Kronauer, R. E., Jewett, M. E. & 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.
82. 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.
83. Shaw, J. & Brody, S. (2000). Circadian rhythms in *Neurospora*: a new measurement, the reset zone. *Journal of Biological Rhythms* 15: 225–240.
84. Strogatz, S. H. (1990). Interpreting the human phase response curve to multiple bright-light exposures. *Journal of Biological Rhythms* 5: 169–174.
85. Beersma, D. G. M. & Daan, S. (1993). Strong or weak phase resetting by light pulses in humans? *Journal of Biological Rhythms* 8: 340–347.
86. Jewett, M. E., Kronauer, R. E. & 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.
87. Pittendrigh, C. S. (1981). Circadian systems: entrainment. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology, Volume 4*). New York: Plenum, pp. 95–124.
88. Daan, S. (2000). Colin Pittendrigh, Jürgen Aschoff, and the natural entrainment of circadian systems. *Journal of Biological Rhythms* 15: 195–207.
89. Aschoff, J. (1999). Masking and parametric effects of high-frequency light-dark cycles. *Japanese Journal of Physiology* 49: 11–18.
90. Aschoff, J. (1979). Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
91. Daan, S. & 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.
92. Aschoff, J. (1990). From temperature regulation to rhythm research. *Chronobiology International* 7: 179–186.
93. Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sánchez, R., Ríos, C. D., Freitag, W. O., Richardson, G. S. & Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* 233: 667–671.
94. Sharma, V. K. & 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.
95. Borbély, A. A. & 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.
96. Rosenwasser, A. M., Boulos, Z. & Terman, M. (1983). Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiology and Behavior* 30: 353–359.
97. Terman, M. & Terman, J. (1985). A circadian pacemaker for visual sensitivity? *Annals of the New York Academy of Sciences* 453: 147–161.
98. Beaulé, C., Barry-Shaw, J. & 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.
99. Sulzman, F. M., Fuller, C. A. & 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.
100. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.

101. DeCoursey, P. J., Krulas, J. R., Mele, G. & Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior* 62: 1099–1108.
102. Hamblen-Coyle, M. J., Wheeler, D. A., Rutila, J. E., Rosbash, M. & 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.
103. Pohl, H. (1976). Proportional effect of light on entrained circadian rhythms of birds and mammals. *Journal of Comparative Physiology* 112: 103–108.
104. Riccio, A. P. & Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
105. Honma, K., Honma, S. & Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
106. Wever, R. A. (1989). Light effects on human circadian rhythms: a review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
107. Fuller, C. A. & 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.
108. Ebihara, S. & Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
109. Lovegrove, B. G., Heldmaier, G. & Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
110. Lovegrove, B. G. & 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.
111. Oosthuizen, M. K., Cooper, H. M. & 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.
112. Häster, L. & 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.
113. Wheeler, D. A., Hamblen-Coyle, M. J., Dushay, M. S. & 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.
114. Waterhouse, J., Minors, D., Folkard, S., Owens, D., Atkinson, G., Macdonald, I., Reilly, T., Sytnik, N. & Tucker, P. (1998). Light of domestic intensity produces phase shifts of the circadian oscillator in humans. *Neuroscience Letters* 245: 97–100.
115. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A. & 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.
116. Carmichael, M. S., Nelson, R. J. & Zucker, I. (1981). Hamster activity and estrous cycles: control by a single versus multiple circadian oscillator(s). *Proceedings of the National Academy of Sciences U.S.A.* 78: 7830–7834.
117. Refinetti, R., Nelson, D. E. & 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.
118. Reebs, S. G. & Doucet, P. (1997). Relationship between circadian period and size of phase shifts in Syrian hamsters. *Physiology and Behavior* 61: 661–666.
119. Shimomura, K., Nelson, D. E., Ihara, N. L. & Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
120. Osiel, S., Golombek, D. A. & 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.
121. Davis, F. C. & Menaker, M. (1981). Development of the mouse circadian pacemaker: independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
122. Perrigo, G., Bryant, W. C. & vom Saal, F. S. (1990). A unique neural timing system prevents male mice from harming their own offspring. *Animal Behaviour* 39: 535–539.
123. Aton, S. J., Block, G. D., Tei, H., Yamazaki, S. & 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.
124. Udo, R., Hamada, T., Horikawa, K., Iwahana, E., Miyakawa, K., Otsuka, K. & Shibata, S. (2004). The role of *Clock* in the plasticity of circadian entrainment. *Biochemical and Biophysical Research Communications* 318: 893–898.
125. Hamasaka, Y., Watari, Y., Arai, T., Numata, H. & 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.
126. Weber, A. L. & Adler, N. T. (1978). Dissociation of persistent estrus and a free-running activity cycle in different light cycles. *Biology of Reproduction* 19: 425–430.
127. Sridaran, R. & 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.

128. Vilaplana, J., Cambras, T., Campuzano, A. & 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.
129. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M. & Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
130. Usui, S., Takahashi, Y. & Okazaki, T. (2000). Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. *American Journal of Physiology* 278: R1148–R1156.
131. Canal-Corretger, M. M., Cambras, T. & Díez-Noguera, A. (2003). Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiology International* 20: 21–35.
132. Cambras, T., Chiesa, J., Araujo, J. & 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.
133. Hut, R. A., Mrosovsky, N. & Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
134. Boulos, Z., Macchi, M. M. & Terman, M. (2002). Twilights widen the range of photic entrainment in hamsters. *Journal of Biological Rhythms* 17: 353–363.
135. Aschoff, J., Gerecke, U. & Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
136. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
137. Liu, C. & Reppert, S. M. (2000). GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron* 25: 123–128.
138. Sharma, V. K., Chidambaram, R. & Chandrashekaran, M. K. (2000). Probing the circadian pacemaker of a mouse using two light pulses. *Journal of Biological Rhythms* 15: 67–73.
139. Watanabe, K., Deboer, T. & 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.
140. Asai, M., Yamaguchi, S., Isejima, H., Janouchi, M., Moriya, T., Shibata, S., Kobayashi, M. & 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.
141. Tapp, W. N. & Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
142. Takamure, M., Murakami, N., Takahashi, K., Huroda, H. & 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.
143. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O. & Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
144. Bünning, E. (1960). Circadian rhythms and the time measurement in photoperiodism. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 249–256.
145. Rieger, D., Stanewsky, R. & 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.
146. Chakraborty, S. C., Ross, L. G. & Ross, B. (1992). The effect of photoperiod on the resting metabolism of carp (*Cyprinus carpio*). *Comparative Biochemistry and Physiology A* 101: 77–82.
147. Herrero, M. J., Madrid, J. A. & Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.
148. Foà, A. & 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.
149. Basco, P. S., Rashotte, M. E. & Stephan, F. K. (1996). Photoperiod duration and energy balance in the pigeon. *Physiology and Behavior* 60: 151–159.
150. Veghte, J. H. (1965). Thermal and metabolic responses of the gray jay to cold stress. *Physiological Zoology* 37: 316–328.
151. Brandstätter, R., Kumar, V., Abraham, U. & 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 U.S.A.* 97: 12324–12328.
152. Haim, A., Yedidia, I., Haim, D. & 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.
153. Haim, A. & 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.
154. Haim, A., McDevitt, R. M. & 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.
155. Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. *Physiology and Behavior* 82: 285–294.

156. Garidou, M. L., Vivien-Roels, B., Pévet, P., Miguez, J. & 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.

157. Vuillez, P., Jacob, N., Teclemariam-Mesbah, R. & 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.

158. Evans, J. A., Elliott, J. A. & Gorman, M. R. (2004). Photoperiod differentially modulates photic and non-photic phase response curves of hamsters. *American Journal of Physiology* 286: R539–R546.

159. Mrugala, M., Zlomanczuk, P., Jagota, A. & Schwartz, W. J. (2000). Rhythmic multi-unit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *American Journal of Physiology* 278: R987–R994.

160. Messager, S., Ross, A. W., Barrett, P. & Morgan, P. J. (1999). Decoding photoperiodic time through *Per1* and ICER gene amplitude. *Proceedings of the National Academy of Sciences U.S.A.* 96: 9938–9943.

161. Pitrosky, B., Kirsch, R., Vivien-Roels, B., Georg-Bentz, I., Canguilhem, B. & 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.

162. Anchordoquy, H. C. & 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.

163. Gorman, M. R. (2003). Independence of circadian entrainment state and responses to melatonin in male Siberian hamsters. *BMC Physiology* 3: art. 10.

164. Deboer, T., Vyazovskiy, V. V. & 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.

165. Larkin, J. E., Freeman, D. A. & 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.

166. Sumová, A., Jác, M., Sládek, M., Sauman, I. & 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.

167. Humlová, M. & 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.

168. Sumová, A., Trávnícková, Z. & Illnerová, H. (1995). Memory on long but not on short days is stored in the rat suprachiasmatic nucleus. *Neuroscience Letters* 200: 191–194.

169. Sumová, A., Trávnícková, Z. & Illnerová, H. (2000). Spontaneous c-Fos rhythm in the rat suprachiasmatic nucleus: location and effect of photoperiod. *American Journal of Physiology* 279: R2262–R2269.

170. Jác, M., Sumová, A. & 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.

171. Haim, A., Downs, C. T. & 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.

172. Lincoln, G., Messager, S., Andersson, H. & 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 U.S.A.* 99: 13890–13895.

173. Alila-Johansson, A., Eriksson, L., Soveri, T. & 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.

174. Génin, F. & 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.

175. Perret, M. & 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.

176. Wehr, T. A., Aeschbach, D. & Duncan, W. C. (2001). Evidence for a biological dawn and dusk in the human circadian timing system. *Journal of Physiology* 535: 937–951.

177. Aschoff, J. (1981). Free-running and entrained circadian rhythms. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology, Volume 4*). New York: Plenum, pp. 81–93.

178. Mrosovsky, N. (1999). Masking: history, definitions, and measurement. *Chronobiology International* 16: 415–429.

179. Aschoff, J. & Von Saint Paul, U. (1976). Brain temperature in the unanesthetized chicken: its circadian rhythm of responsiveness to light. *Brain Research* 101: 1–9.

180. Strubbe, J. H., Spiteri, N. J. & Prins, A. J. A. (1986). Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats. *Physiology and Behavior* 36: 647–651.

181. Lincoln, G. A., Andersson, H. & 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.

182. Cajochen, C., Dijk, D. J. & 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.

183. Sone, Y., Hyun, K., Nishimura, S., Lee, Y. & Tokura, H. (2003). Effects of dim or bright-light exposure during the daytime on human gastrointestinal activity. *Chronobiology International* 20: 123–133.
184. Mrosovsky, N., Foster, R. G. & 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.
185. Redlin, U. & Mrosovsky, N. (1999). Masking of locomotor activity in hamsters. *Journal of Comparative Physiology A* 184: 429–437.
186. Redlin, U. & Mrosovsky, N. (1999). Masking by light in hamsters with SCN lesions. *Journal of Comparative Physiology A* 184: 439–448.
187. Edelstein, K. & Mrosovsky, N. (2001). Behavioral responses to light in mice with dorsal lateral geniculate lesions. *Brain Research* 918: 107–112.
188. Mrosovsky, N. & Hattar, S. (2003). Impaired masking responses to light in melanopsin-knockout mice. *Chronobiology International* 20: 989–999.
189. Mrosovsky, N. (2003). Contribution of classic photoreceptors to entrainment. *Journal of Comparative Physiology A* 189: 69–73.
190. Redlin, U., Cooper, H. M. & Mrosovsky, N. (2003). Increased masking response to light after ablation of the visual cortex in mice. *Brain Research* 965: 1–8.
191. Redlin, U. & Mrosovsky, N. (2004). Nocturnal activity in a diurnal rodent (*Arvicanthis niloticus*): the importance of masking. *Journal of Biological Rhythms* 19: 58–67.
192. Refinetti, R. (2000). Circadian rhythm of locomotor activity in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 73: 575–583.
193. Beersma, D. G. M., Daan, S. & 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.
194. Sharma, V. K. & Daan, S. (2002). Circadian phase and period responses to light stimuli in two nocturnal rodents. *Chronobiology International* 19: 659–670.
195. Stokes, M. A., Kent, S. & 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.
196. Weisgerber, D., Redlin, U. & Mrosovsky, N. (1997). Lengthening of circadian period in hamsters by novelty-induced wheel running. *Physiology and Behavior* 62: 759–765.
197. Page, T. L., Wassmer, G. T., Fletcher, J. & 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.
198. Page, T. L. (2000). A novel mechanism for the control of circadian clock period by light. *Journal of Biological Rhythms* 15: 155–162.
199. Refinetti, R. (1998). Influence of early environment on the circadian period of the tau-mutant hamster. *Behavior Genetics* 28: 153–158.
200. Stewart, K. T., Rosenwasser, A. M., Levine, J. D., McEachron, D. L., Volpicelli, J. R. & Adler, N. T. (1990). Circadian rhythmicity and behavioral depression: II. Effects of lighting schedules. *Physiology and Behavior* 48: 157–164.
201. Endo, T., Honma, S., Hashimoto, S. & Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
202. Pittendrigh, C. S. & 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.
203. Winfree, A. T. (1972). Slow dark-adaptation in *Drosophila*'s circadian clock. *Journal of Comparative Physiology* 77: 418–434.
204. Mistlberger, R. E. & Holmes, M. M. (2000). Behavioral feedback regulation of circadian rhythm phase angle in light-dark entrained mice. *American Journal of Physiology* 279: R813–R821.
205. Hébert, M., Martin, S. K., Lee, C. & 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.
206. Smith, K. A., Schoen, M. W. & Czeisler, C. A. (2004). Adaptation of human pineal melatonin suppression by recent photic history. *Journal of Clinical Endocrinology and Metabolism* 89: 3610–3614.
207. Prichard, J. R., Fahy, J. L., Obermeyer, W. H., Behan, M. & Benca, R. M. (2004). Sleep responses to light and dark are shaped by early experience. *Behavioral Neuroscience* 118: 1262–1273.
208. Shimomura, K., Kornhauser, J. M., Wisor, J. P., Umezu, T., Yamazaki, S., Ihara, N. L., Takahashi, J. S. & 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.
209. Penn, J. S. & 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.
210. Penn, J. S. (1998). Early studies of the photostasis phenomenon. In: Williams, T. P. & Thistle, A. B. (Eds.). *Photostasis and Related Phenomena*. New York: Plenum, pp. 1–16.
211. Williams, T. P., Squitieri, A., Henderson, R. P. & Webbers, J. P. P. (1999). Reciprocity between light intensity and rhodopsin concentration across the rat retina. *Journal of Physiology* 516: 869–874.
212. Klante, G. & 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.
213. Ruby, N. F., Saran, A., Kang, T., Franken, P. & 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.

214. Ruby, N. F., Joshi, N. & Heller, H. C. (2002). Constant darkness restores entrainment to phase-delayed Siberian hamsters. *American Journal of Physiology* 283: R1314–R1320.
215. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P. & Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
216. Klerman, E. B. & Jewett, M. E. (1999). Model building, quantitative testing, and model comparison. *Journal of Biological Rhythms* 14: 621–624.
217. Pavlidis, T. (1981). Mathematical models. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology, Volume 4*). New York: Plenum, pp. 41–54.
218. Lakin-Thomas, P. L., Brody, S. & 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.
219. 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.
220. Pedersen, M. & Johnsson, A. (1994). A study of the singularities in a mathematical model for circadian rhythms. *BioSystems* 33: 193–201.
221. Brown, E. N., Choe, Y., Luithardt, H. & Czeisler, C. A. (2000). A statistical model of the human core-temperature circadian rhythm. *American Journal of Physiology* 279: E669–E683.
222. Kunz, H. & 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.
223. Johnson, C. H., Elliott, J. A. & Foster, R. (2003). Entrainment of circadian programs. *Chronobiology International* 20: 741–774.
224. Forger, D. B. & Kronauer, R. E. (2002). Reconciling mathematical models of biological clocks by averaging on approximate manifolds. *SIAM Journal on Applied Mathematics* 62: 1281–1296.
225. Garfinkel, D. (1980). Computer modeling, complex biological systems, and their simplifications. *American Journal of Physiology* 239: R1–R6.
226. Conlan, R. (Ed.) (1985). *Understanding Computers: Computer Basics*. Alexandria, Va.: Time-Life Books.
227. Miller, A. R. (1981). *The 8080/Z80 Assembly Language: Techniques for Improved Programming*. New York: Wiley.
228. Galbraith, R. (1982). *Professional Programming Techniques Starting with the BASICs*. Blue Ridge Summit, Pa.: TAB Books.
229. Microsoft Corporation (1985). *Microsoft GW-BASIC Interpreter User's Guide*. Seattle, WA: Microsoft Corporation.
230. Crider, B. (Ed.) (1984). *BASIC Program Conversions: How to Convert Programs from One Computer to Another.* Tucson, AZ: HP Books.
231. Balena, F. (2002). *Programming Microsoft Visual Basic .NET.* Seattle, WA: Microsoft Press.
232. Aho, A. V. (2004). Software and the future of programming languages. *Science* 303: 1331–1333.
233. Refinetti, R. (1993). A functional model of the mammalian circadian pacemaker. *International Journal of Biomedical Computing* 32: 45–60.
234. Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A. & Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740.
235. Rothenfluh, A., Abodeely, M., Price, J. L. & 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.
236. 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.
237. Comolli, J., Taylor, W. & 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

CHAPTER OUTLINE

8.1 Nonphotic Entrainment
8.2 A Separate Food-Entrainable Pacemaker

8.1 NONPHOTIC ENTRAINMENT

Chapter 7 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 studied most thoroughly, it certainly is not the only one. As diagrammed in Figure 8.1, stimuli that have been shown to entrain circadian rhythms include light as well as several so-called *nonphotic stimuli* such as ambient temperature, food availability, physical activity (exercise), and social contact. In mammals, light seems to have a stronger influence on the circadian system than any of the other stimuli. These other stimuli can, however, significantly affect circadian rhythms, particularly when a light–dark cycle is not simultaneously present.

Variations in *ambient temperature* can affect behavioral and autonomic processes in living organisms (Figure 8.2). We discuss this matter in detail in Chapter 10. Daily cycles in ambient temperature, however, can *entrain* circadian rhythms even in warm-blooded animals whose core temperature is minimally affected by variations in the temperature of the environment. Figure 8.3 shows an example of entrainment by cycles of ambient temperature. 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 in the study, ambient temperature was raised to 33°C for several hours each day (as indicated by the rectangles). Note 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 this hamster, the possibility that the changes in period and phase were due to *masking*, rather than to entrainment, can be further excluded because running took place during the presumably aversive (hot) phase of the temperature cycle. This exclusion criterion cannot always be satisfied, but convincing evidence of entrainment by ambient temperature cycles has been provided in studies on plants and invertebrates,[1–4] reptiles and birds,[5–7] and mammals.[8–15] In general, ambient temperature is a weak zeitgeber in vertebrates, in that not all animals exposed to the cycle actually entrain and, even in those that entrain, entrainment is often rather unstable.

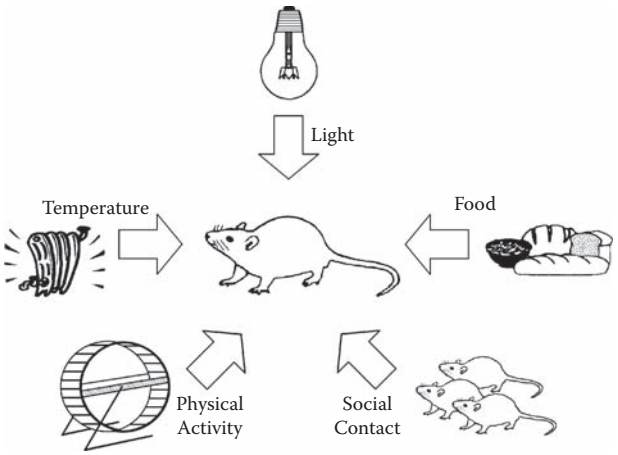


FIGURE 8.1 Many zeitgebers. Several classes of environmental stimuli can synchronize the circadian clock, including light, ambient temperature, food, physical activity, and social contact.

FIGURE 8.2 A cold opossum. Large variations in ambient temperature affect the physiology of living organisms. (Source: © ArtToday, Tucson, AZ.)

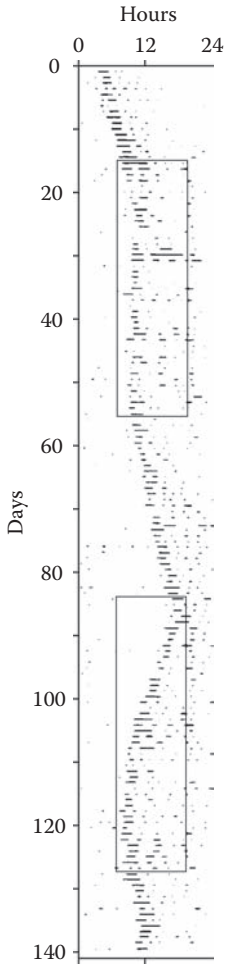


FIGURE 8.3 Cycle of ambient temperature as a zeitgeber. This actogram shows the rhythm of running-wheel activity of a golden hamster (*Mesocricetus auratus*) maintained under constant dim illumination (1 lux) and exposed to cycles of ambient temperature. The rectangles indicate the times of day when 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. (Source: Adapted from Pohl, H. (1998). Temperature cycles as zeitgebers for the circadian clock of two burrowing rodents, the normothermic antelope ground squirrel and the heterothermic Syrian hamster. *Biological Rhythm Research* 29: 311–325.)

It also has been shown that discrete temperature pulses can phase-shift circadian rhythms recorded under otherwise constant conditions.[16–19] This finding suggests that entrainment by temperature, like entrainment by light, is attained by a nonparametric mechanism. The nonparametric action of temperature should be expected, as discussed in Chapter 6, because the circadian clock is temperature compensated (which implies that the period of the clock is not parametrically affected by temperature). Fluctuations in ambient temperature can also *mask* circadian

FIGURE 8.4 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. (Source: © ArtToday, Tucson, AZ.)

rhythms if they are large enough to inhibit locomotor activity or to overtax the thermoregulatory system.

Food availability is another important environmental variable (Figure 8.4), and it also has been shown to entrain circadian rhythms in fish,[20,21] birds,[22,23] and mammals.[24–37] Figure 8.5 shows an example. 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 freely available at all times. At the beginning of the records shown in Figure 8.5, a schedule of food restriction 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). Note 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.

Food availability seems to be a weaker zeitgeber than light. Although food is more essential than light for an animal's survival, light exerts a finer control than food availability over the activity rhythm. In Figure 8.6 two domestic mice (*Mus musculus*) were initially kept under a light–dark cycle (as indicated by the clear rectangles) and later subjected to either a schedule of food restriction (Panel A) or a daily light pulse (Panel B). Note 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

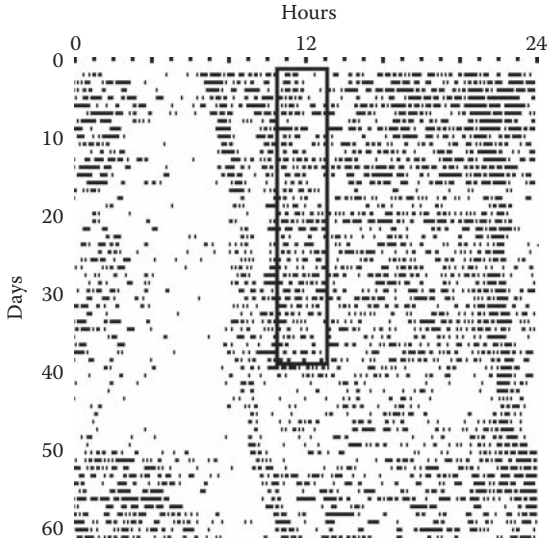


FIGURE 8.5 Feeding schedule as a zeitgeber. This actogram shows the running-wheel activity rhythm of a Nile grass rat (*Arvicanthis niloticus*) maintained under constant illumination (10 lux) and constant ambient temperature (24°C) and exposed to a cycle of food availability. The rectangle indicates the time of day when food was available (90% of the amount of food normally comsuned was delivered at 10:30 A.M. each day, and the animal took approximately 2.5 hours to consume the food). Entrainment was gradually established over several weeks. (Source: Archives of the Refinetti lab.)

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. The difference in the robustness of the rhythms is even more noticeable. The daily onsets of activity were rather variable under the food-restriction 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.

Physical exercise (Figure 8.7) is another stimulus capable of entraining circadian rhythms. Figure 8.8 shows an example. 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.[38–42] In addition, it has been shown that discrete "pulses" of exercise can phase-shift circadian rhythms recorded under otherwise constant conditions.[43–50] These findings suggest 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 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.[51–59] Shifts induced by exercise can counteract shifts induced by photic stimulation.[60–62]

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

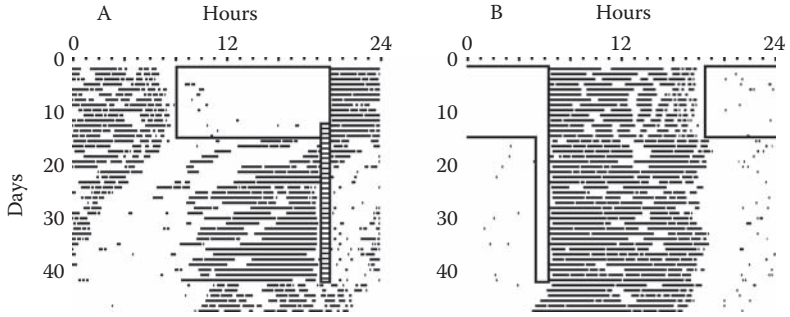


FIGURE 8.6 Comparing food and light as zeitgebers. The actograms show the running-wheel activity rhythms of two domestic mice (*Mus musculus*) maintained first under a light–dark cycle (LD 12:12) and then in constant darkness under either a daily schedule of food availability (A) or a daily schedule 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. (Source: Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.)

FIGURE 8.7 Jogging in the woods. Schedules of physical activity can affect the period and phase of circadian rhythms. (Source: © ArtToday, Tucson, AZ.)

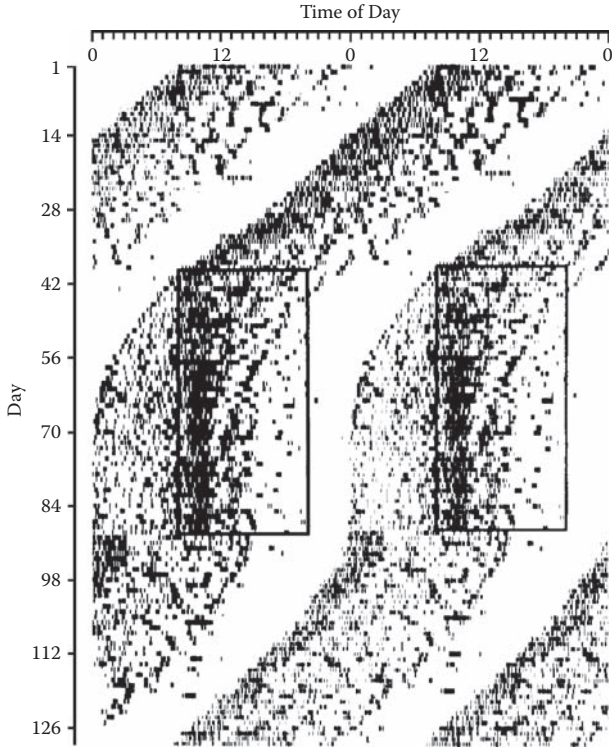


FIGURE 8.8 Exercise schedule as a zeitgeber. This double-plotted actogram shows the rhythm of drinking activity of a domestic mouse (*Mus musculus*) maintained in constant darkness and allowed access to a running wheel for a fraction of each day, as indicated by the rectangles. Entrainment, preceded and followed by free-running, is clearly seen. (Source: Edgar, D. M. & Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933. © American Physiological Society. Reproduced with permission from the publisher and the authors.)

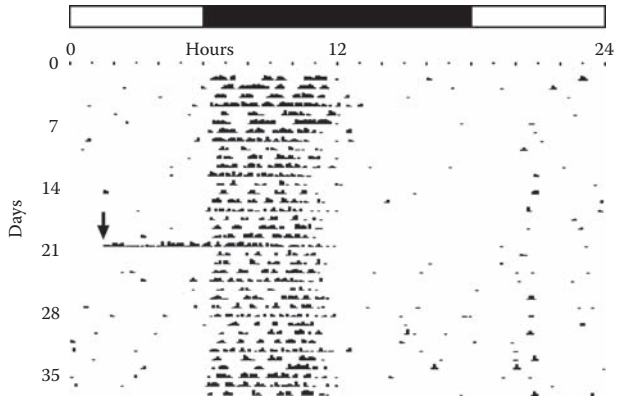


FIGURE 8.9 Please leave my home alone. This actogram shows the running-wheel activity rhythm of a Siberian hamster (*Phodopus sungorus*) maintained under a light–dark cycle, as indicated by the horizontal bar at the top. On Day 21, at the time indicated by the arrow, the animal's cage was replaced with a fresh cage otherwise identical to the old one. The cage change clearly disturbed the animal's activity rhythm. (Source: Archives of the Refinetti lab.)

wheel. Figure 8.9 shows an example for a Siberian hamster (*Phodopus sungorus*). Although the animal ordinarily showed very little activity during the light phase of the light–dark cycle, it showed quite a bit of activity immediately after a cage change during the light phase (arrow). While the disrupted activity pattern seems to last only a few hours when the animals are under a light–dark cycle, cage changes for animals kept in constant darkness can actually phase-shift the circadian clock (presumably because of the exercise "pulse").[63,64]

Figure 8.10 shows a PRC that describes phase shifts evoked by 3-hour pulses of exercise presented at various circadian times. In golden hamsters, exercise pulses can be dispensed easily by transferring the animal to a different cage fitted with a novel running wheel. Hamsters voluntarily exercise for hours under these conditions. Note that large phase advances can be evoked during mid-subjective-day. For comparison, Figure 8.10 also shows a PRC for light pulses (dashed line). As seen in Chapter 7, large phase advances are evoked by light during mid-subjective-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.[65] Chapter 7 showed that photic PRCs display considerable similarity across species. Nonphotic PRCs are much more variable. As shown in Figure 8.11, 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

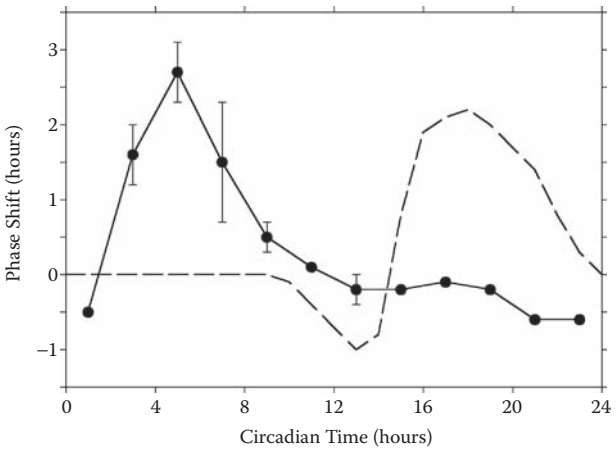


FIGURE 8.10 Comparing the phase-shifting effects of exercise and light. The graph shows phase-response curves (PRCs) for resetting of the circadian clock of the golden hamster (*Mesocricetus auratus*) as determined by phase shifts of the rhythm of running-wheel activity. The circles correspond to the 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. (Sources: Mrosovsky, N., Salmon, P. A., Menaker, M. & Ralph, M. R. (1992). Nonphotic phase shifting in hamster clock mutants. *Journal of Biological Rhythms* 7: 41–49; Takahashi, J. S. et al. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.)

can be found only for restricted groups of stimuli within restricted groups of organisms (especially the golden hamster), as exemplified in Figure 8.12. In this case, the various curves are quite similar.

Many animals, particularly humans, engage in extensive *social interaction* (Figure 8.13), which provides a potential opportunity for social entrainment. In actuality, the experimental evidence that supports the role of social interaction as a zeitgeber is very limited. Some evidence indicates that social stimuli may be effective in the interaction between a mother and her offspring,[66–68] as exemplified in Figure 8.14. Siberian hamster dams previously maintained under a light–dark 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. Note 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. Entrainment of the offspring's rhythms by the mother is perhaps less surprising during gestation, when the fetuses share the mother's circulatory system.[69] However, an ingenious study in sheep showed that environmental light penetrates the mother's abdominal wall and produces a robust light–dark cycle inside the uterus,[70] which means that the circadian clocks of the fetuses could be entrained by the

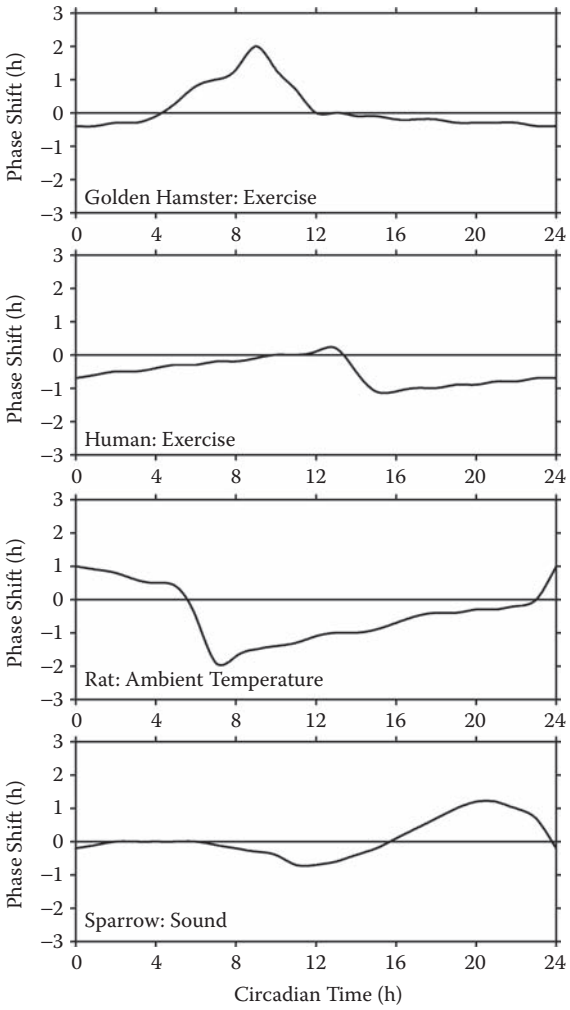


FIGURE 8.11 Nonphotic PRCs. The graphs show phase-response curves (PRCs) for the resetting of circadian rhythms by nonphotic stimuli in four vertebrate species. The various curves share little similarity. (Sources: Wickland, C. R. & Turek, F. W. (1991). Phase-shifting effects of acute increases in activity on circadian locomotor rhythms in hamsters. *American Journal of Physiology* 261: R1109–R1117; Buxton, O. M., Lee, C. W., L'Hermite-Balériaux, M., Turek, F. W. & 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; Francis, A. J. P. & Coleman, G. L. (1997). Phase response curves to ambient temperature pulses in rats. *Physiology and Behavior* 62: 1211–1217; Reebs, S. G. (1989). Acoustical entrainment of circadian activity rhythms in house sparrows: constant light is not necessary. *Ethology* 80: 172–181.)

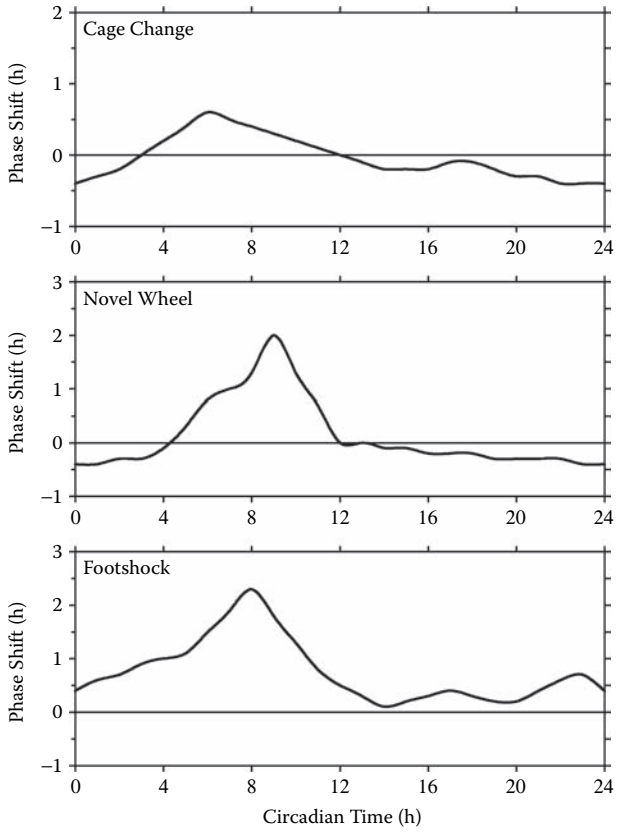


FIGURE 8.12 Consistent nonphotic PRCs. The graphs show phase-response curves (PRCs) for the resetting of circadian rhythms by three nonphotic stimuli in the golden hamster (*Mesocricetus auratus*). In a situation like this — in which a single species and a limited set of stimuli are considered — similarity is present 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). (Sources: Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology* A 162: 35–46; Wickland, C. R. & 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; Cain, S. W., Verwey, M., Hood, S., Leknickas, P., Karatsoreos, I., Yeomans, J. S. & Ralph, M. R. (2004). Reward and aversive stimuli produce similar nonphotic phase shifts. *Behavioral Neuroscience* 118: 131–137.)

FIGURE 8.13 Napping together. Social interaction, such as that between a father and his newborn child, can affect one's daily activity schedule. It is not clear, however, whether the effect corresponds to circadian entrainment or merely masking. (Source: Photograph by K. Z. Refinetti.)

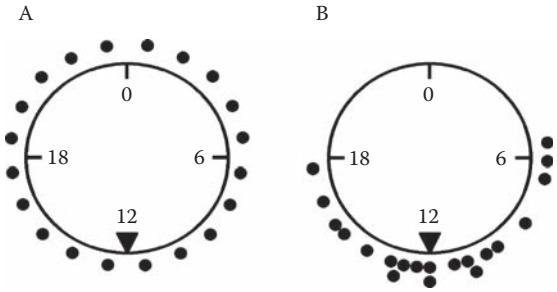


FIGURE 8.14 Maternal entrainment. Female Siberian hamsters (*Phodopus sungorus*) were maintained in constant darkness from the beginning of pregnancy. Shortly after weaning, the pups were given access to running wheels, so that the phase of their circadian clocks could be determined. The small dark circles indicate the times of the onsets of activity of the pups (20 pups from five 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. (Source: Adapted from Duffield, G. E. & 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.)

light–dark cycle rather than by biological rhythms in the mother's body.

Social entrainment in adults remains a controversial concept. There is no doubt that social interaction can transiently disturb circadian rhythms,[71–74] but it is not clear whether it can reset the circadian clock. Some researchers claim to have documented social entrainment,[63,75–80] or at least some form of action of social stimuli on the circadian system.[81–84] In many cases, the data presented as evidence of entrainment can more appropriately be classified as social masking.[85–91] Carefully conducted studies have described various masking effects but have provided no evidence of social entrainment in rodents[92–98] or other mammals.[35,99–103] In birds, socially relevant sounds (conspecific songs) seem to be an effective zeitgeber,[104,105] although their mechanism of action is not known. It is possible that they cause entrainment indirectly by stimulating physical activity.

Figure 8.15 provides an example from a study that revealed no evidence of social entrainment in rodents. Pairs of golden hamsters were housed together under a

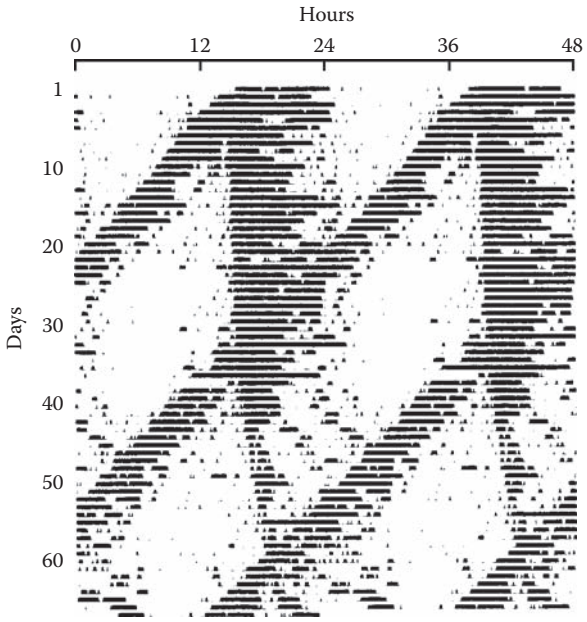


FIGURE 8.15 Lack of social entrainment. This double-plotted actogram shows the combined running-wheel activity rhythms of two male golden hamsters (*Mesocricetus auratus*) housed in a single cage with two running wheels. One of the animals was blinded and the other was exposed to a 23.3-hour light–dark cycle (LD 1:22.3). The blinded animal seems to free-run in total independence from its cage mate (whose circadian period is clearly controlled by the light–dark cycle). (Source: Refinetti, R., Nelson, D. E. & 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.)

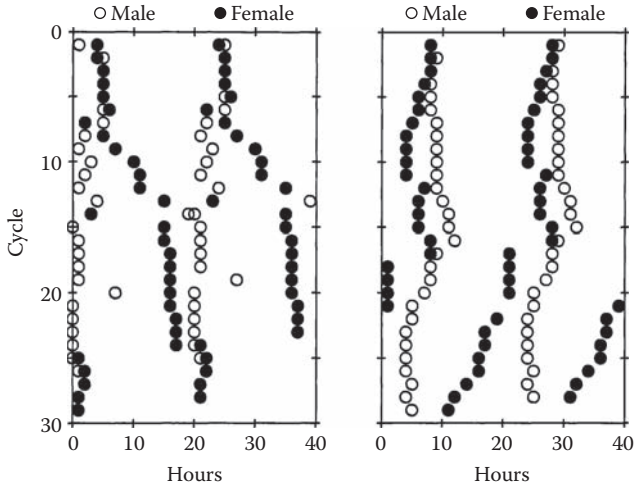


FIGURE 8.16 Lack of social entrainment (again). Male-female pairs of *tau*-mutant hamsters were maintained in constant darkness. The females had been surgically sterilized by tubal ligation to prevent pregnancy. Onsets of activity (as determined by inspection of infrared time-lapse video-photography records) are plotted in double-plot actogram format. Social entrainment is not apparent in either pair of hamsters. (Source: Refinetti, R. et al. (1991). Circadian organization of sex behavior in pairs of tau-mutant hamsters. *FASEB Journal* 5(5): A1126.)

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 evidence 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 ten other pairs of hamsters.[95] One could argue that male golden hamsters are solitary animals that exhibit only aggressive behavior or indifference towards other males. However, as shown in Figure 8.16, 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 filmed continuously 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.

The concept of social entrainment of circadian rhythms is consistent with 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.[106] Several studies claiming to support the idea that social contact can synchronize the reproductive cycle have been conducted in rodents[107,108] and humans.[109–112] All of these studies are flawed, however. None of the studies carefully examined reproductive cycles in individuals over extended periods of time. They relied instead on short-term group-synchrony data. It is impossible, therefore, to distinguish short-term coincidence from actual entrainment. Studies on humans have suffered also from other methodological deficiencies.[113]

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 a temporary interruption of photic stimulation). Figure 8.17 shows an example of a phase shift induced by a dark pulse. A golden hamster was maintained in constant light and received a single 15-minute dark pulse at the time represented by the dark circle. As indicated by the regression lines fit to the pre- 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.[114–123]

Is the PRC for dark pulses a mirror-image of the PRC for light pulses? After all, a brief dark pulse against a background of constant light may be seen as a long light pulse against a background of darkness. One might try to calculate the expected dark-pulse-evoked shift by integrating

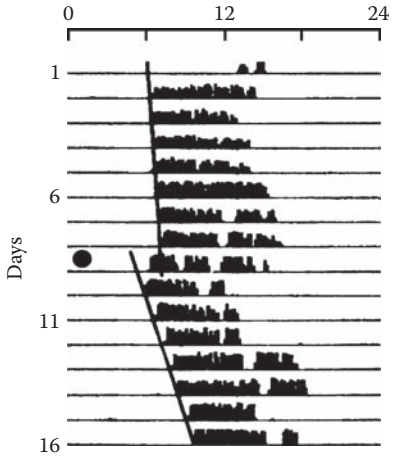


FIGURE 8.17 Dark pulse causes phase shift. The actogram shows the running-wheel activity rhythm of a golden hamster (*Mesocricetus auratus*) maintained in constant light and presented with a single 15-minute pulse of darkness at the time indicated by the dark circle. The oblique lines are linear regressions of the activity onsets that make it easier to see the 2-hour phase advance caused by the dark pulse. (Source: Adapted from Rosenwasser, A. M. & Dwyer, S. M. (2002). Phase shifting the hamster circadian clock by 15-minute dark pulses. *Journal of Biological Rhythms* 17: 238–247.)

the area under the curve of the photic PRC, but assumptions would have to be made about the specific parameters of the integration process. The best approach, at least to start, is to empirically determine the curves for both types of stimuli. Figure 8.18 shows the results of one attempt at this comparison. Mice either were kept in constant darkness and received 1-hour light pulses (150 lux) or were kept in constant light (400 lux) and received 1-hour dark pulses. The resulting PRCs are quite different from each other, and they are not mirror-images. 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.[120] No one has tried to model the dark-pulse curve as a mirror-image of the integrated photic curve.

Another potential zeitgeber is *gravity*. No sizable daily cycle of gravity occurs 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,[124] as shown in Figure 8.19. 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).

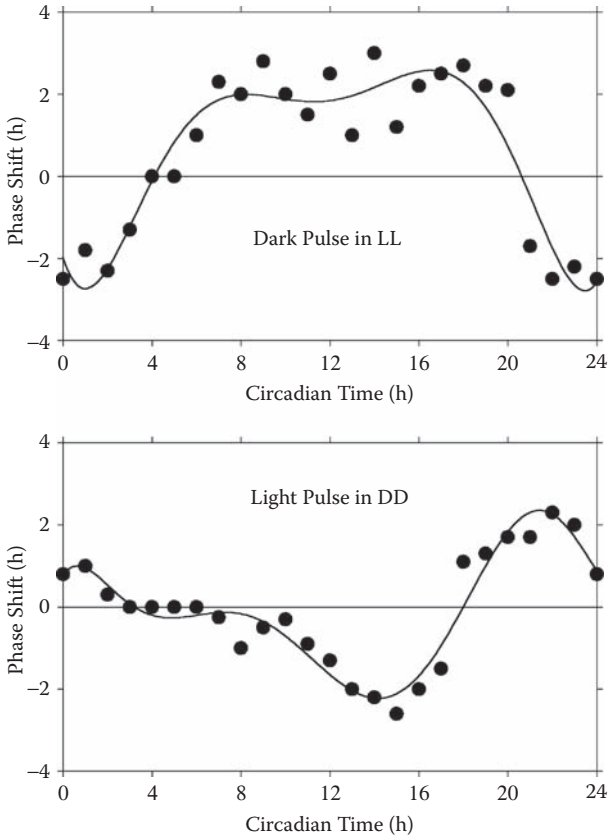


FIGURE 8.18 Dark-pulse PRC. The graphs show the phase-response curves (PRCs) for 1-hour dark pulses presented to mice in constant light (top) and for 1-hour light pulses presented to mice in constant darkness (bottom). The PRCs are very different from each other. (Source: Barbacka-Surowiak, G. (2000). 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.)

Other studies dealing with the effects of gravity on circadian rhythms have investigated only masking.[125–128] Other nonphotic stimuli that have been claimed to reset the circadian pacemaker, but that have not been investigated in detail, include seizures,[129] hypoxia,[130] and sleep.[131,132] As mentioned in Chapter 6, injections of benzodiazepines have been shown consistently to reset the circadian clock.[133–137]

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 8.11) do not suggest the existence of a universal common mechanism. However, the similarities observed in restricted domains (e.g., Figure 8.12) encourage the search for a unifying principle. Ralph Mistlberger (Figure 8.20), a psychology professor at Simon Fraser University (in Canada), has conducted a

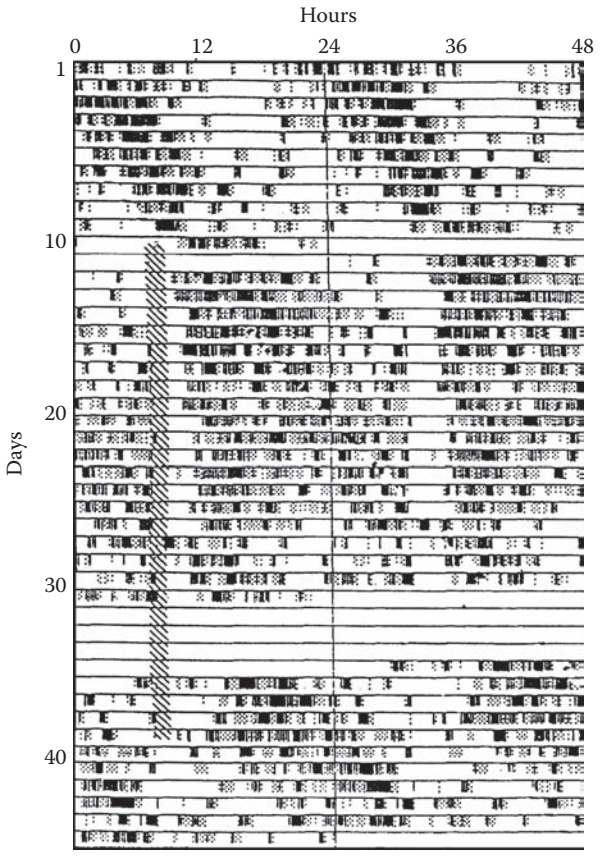


FIGURE 8.19 Entrainment by hypergravity. This double-plotted actogram shows the digitized rhythm of body temperature of a laboratory rat (*Rattus norvegicus*) maintained in constant light (30 lux) for 6 weeks. For 4 weeks, the animal was exposed to 2 *g* gravity (by centrifugation) for 1 hour a day (hatched bar). Note 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.) (Source: Adapted from Demaria-Pesce, V. H., Murakami, D. & 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.)

great deal of research aimed at finding such a principle. He was not the first person to realize that all so-called nonphotic stimuli have one thing in common: they cause *arousal*.[63] However, Mistlberger's studies showed that the notion of arousal is quite complex. 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 of re-entrainment after a phase shift of the light–dark cycle have been obtained by various manipulations intended to increase arousal, such as handling of the animal by the experimenter,[138,139] opportunity for sexual contact,[81] eating after food deprivation,[140] receipt of an electric foot shock,[141] or cage agitation combined with water sprinkling.[142] However, phase shifts were not obtained by other arousing manipulations, such as engagement in a fight leading to defeat,[96] a caffeine injection,[143] a glucocorticoid injection,[144] or exposure to a brief loud noise.[145] In addition, the fact that physical exercise can inhibit phase shifts induced by light[146–148] led researchers to try to inhibit photic phase shifts by manipulations of arousal such as induction of conditioned fear,[149,150] exposure to a strong olfactory stimulus,[151] or sleep deprivation.[152] Mistlberger's studies suggest that arousal alone is not sufficient to evoke nonphotic phase shifts, although arousal is a necessary component of the process.[121,153–155] Figure 8.21 summarizes the results of these studies. The graph shows the mean phase shifts for groups of 12 to 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). Note that arousal alone did not evoke phase shifts larger 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.

FIGURE 8.20 Ralph Mistlberger. This circadian physiologist from Simon Fraser University (Canada) is the most active researcher in the mechanisms underlying nonphotic phase shifts. (Source: Photograph courtesy of Ralph Mistlberger.)

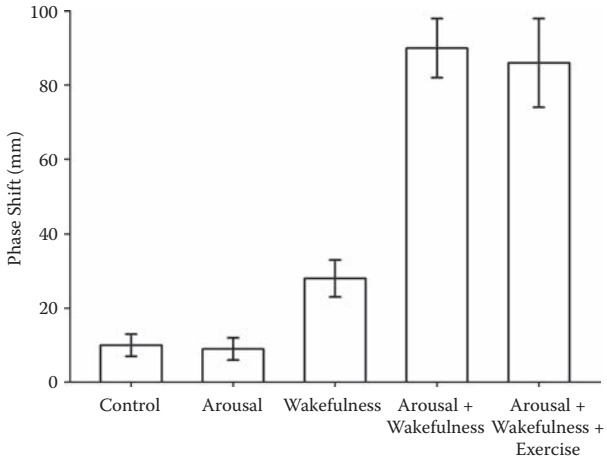


FIGURE 8.21 Dissecting the mechanism of nonphotic phase shifting. The graph shows the mean phase shifts evoked by various stimuli presented for 3 hours at circadian time 6 to male golden hamsters (*Mesocricetus auratus*). Each bar corresponds to the mean (± SE) of 12 to 33 animals. Arousal alone was 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. (Sources: Mistlberger, R. E., Marchant, E. G. & Sinclair, S. V. (1996). Nonphotic phase-shifting and the motivation to run: cold exposure reexamined. *Journal of Biological Rhythms* 11: 208–215; Mistlberger, R. E., Antle, M. C., Webb, I. C., Jones, M., Weinberg, J. & 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.)

The null effect of exercise noted in Figure 8.21 could have been 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). It is clear, however, that neither arousal nor wakefulness alone is sufficient to evoke large phase shifts. Therefore, a mechanism of action common to all nonphotic zeitgebers does not exist. A combination of arousal and wakefulness, perhaps enhanced by exercise, seems to underlie the phase-shifting effects of changes in ambient temperature, food availability, physical activity, social interaction, dark pulses, and other nonphotic zeitgebers. Even if this finding is true for nocturnal animals, however, it cannot apply to diurnal animals. In diurnal animals, as in nocturnal ones, nonphotic stimuli are effective only during subjective day.[42,142] However, nocturnal animals are asleep during most of subjective day, whereas diurnal animals are awake during this time and, therefore, will not be sleep-deprived by the presentation of nonphotic stimuli.

8.2 A SEPARATE FOOD-ENTRAINABLE PACEMAKER

Chapter 9 discusses the possible evolutionary advantages of circadian rhythmicity. The importance of feeding requires no explanation, however. If you do not eat (or obtain nutrients through some form of artificial infusion), you will die. Because feeding is so important, food restriction as a zeitgeber occupies a special place among nonphotic zeitgebers. Section 8.1 presented food restriction as a weaker zeitgeber than light. Yet, animals do not die from lack of light, but they do die from lack of food. Functions that are essential for the survival of an organism need not be essential for the synchronization of circadian rhythms. As a result, conflicts can arise between homeostatic and circadian mechanisms in the body, as discussed in Chapter 10.

In most laboratory studies of circadian rhythms, organisms are fed *ad libitum* (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 usually are not present. It would be wrong, however, to say that the issue of conflicts has escaped the attention of circadian physiologists. A limited but substantial number of studies have examined circadian rhythms of organisms exposed to simultaneous but conflicting cycles of illumination and food availability. Studies have been conducted in fish,[20,156] birds,[157,158] rodents,[33,147,159–169] and other mammals.[25,170,171] An example is shown in Figure 8.22. This double-plotted actogram shows the rhythm of running-wheel activity of a domestic mouse (*Mus musculus*) maintained first under a light–dark cycle with food available *ad libitum*, 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 *ad libitum*. Note the robust pattern of entrainment under the light–dark cycle (LD), which was essentially unaffected by the addition of the cycle of food restriction (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 anthropomorphic terms, the

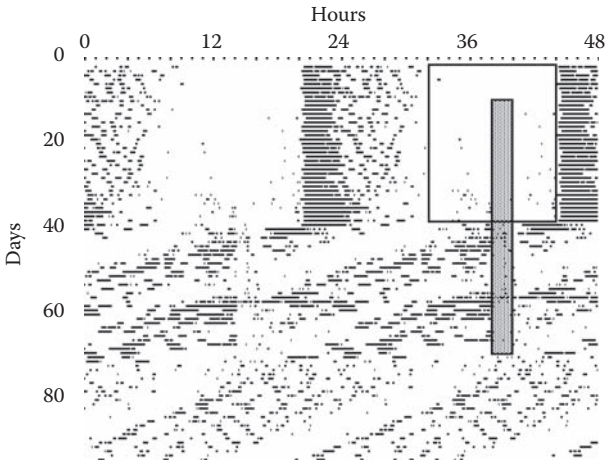


FIGURE 8.22 Conflict of light and food as zeitgebers: light wins. This double-plotted actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle (as indicated by the single-plotted clear rectangle) and a cycle of food availability (as indicated by the stippled rectangle). The cycle of food availability had very little effect on the activity rhythm when presented simultaneously with the light–dark cycle or when presented alone. (Source: Archives of the Refinetti lab.)

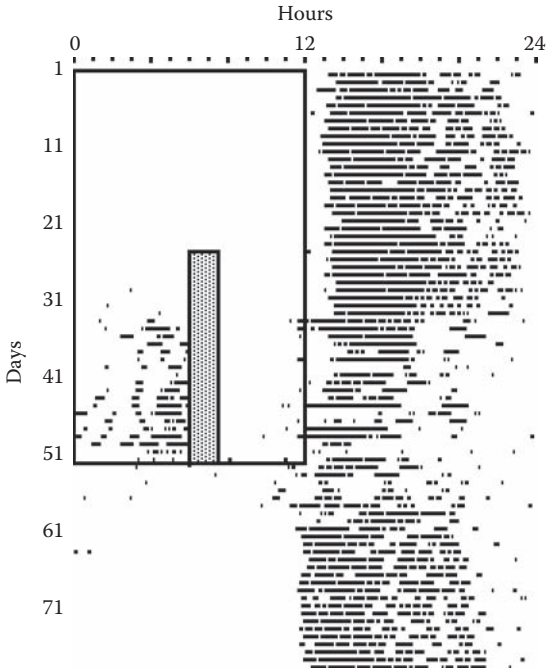


FIGURE 8.23 Conflict of light and food as zeitgebers: food wins. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle (as indicated by the clear rectangle) and a cycle of food availability (as indicated by the stippled rectangle). Although the cycle of food availability did not cause a shift of the activity rhythm (as evidenced by the freerun observed later), it clearly induced extra activity during the light phase. The extra activity preceded the time of food access by several hours. (Source: Archives of the Refinetti lab.)

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 food restriction can be seen in the records shown in Figure 8.23. In this case, a significant change in the activity pattern took place a few days after the food-restriction 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.[25,29,32,33,68,158,161,172–185]

In principle, food anticipatory activity could be a physiological curiosity. By consulting their circadian clocks, animals could literally *anticipate* the delivery of food. In anthropomorphic 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, food anticipatory activity is a much more interesting phenomenon. Consider Figure 8.24. To avoid photic masking of activity (and to reduce the strength of the photic zeitgeber), the light–dark cycle consisted of only 15 minutes of light each day. Note the appropriate control of period and phase by the photic zeitgeber during the first month of recording. When the food-restriction schedule was introduced, anticipatory activity developed rapidly. When both zeitgebers were discontinued (that is, when food became available *ad libitum* 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 entrained previously 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 food anticipatory activity freeran with a period much longer than that of the other component. This dissociation of components has been observed by various research teams.[25,183,186,187] 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 sometimes called the *light-entrainable oscillator* for comparative purposes, even though the master pacemaker is entrainable by various stimuli besides light).

Friedrich Stephan (Figure 8.25), a psychology professor at Florida State University, has spent most of his professional life studying the properties of the food-entrainable oscillator.[188] He would be understandably disappointed if I remarked that the concept of a

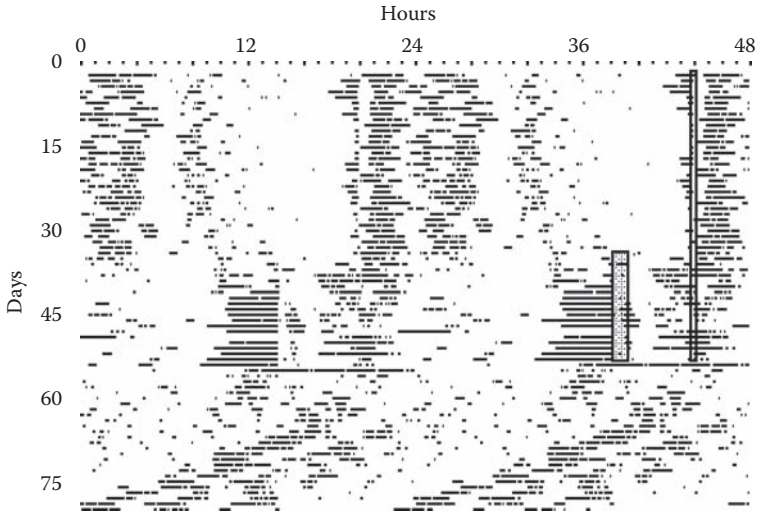


FIGURE 8.24 Conflict of light and food as zeitgebers: two systems revealed. This double-plotted actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) maintained under a skeleton light–dark cycle (as indicated by the narrow, single-plotted clear rectangle) and a cycle of 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). (Source: Archives of the Refinetti lab.)

food-entrainable oscillator, distinct from the master pacemaker, resembles the concept of morning and evening oscillators discredited in Chapter 6. Stephan has nothing to fear, however. His two-oscillator concept has strong empirical bases. The strongest evidence that a food-entrainable oscillator really exists is that food anticipatory activity can be evoked by restricted feeding in animals whose master pacemaker has been destroyed surgically[174,175,183,189–195] or inactivated by genetic engineering.[196] As shown in Figure 8.26, animals with these discrete brain lesions are arrhythmic when kept in constant darkness with food available *ad libitum*. Imposition of a food-restriction schedule evokes anticipatory activity, and this activity pattern is phase-shifted when the time of food access is shifted. Note that if the rat is food-deprived for a few days, 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 solid evidence that the food-entrainable oscillator is a true oscillator — and not just an hourglass mechanism that is restarted daily by food access. Of course, the fact that the food-entrainable oscillator does not express itself under *ad libitum* conditions — and, instead, must be frequently primed by a schedule of food restriction — indicates that it is a damped oscillator, unable to persist indefinitely like the master pacemaker does. Also, 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.[197–199] The food-entrainable oscillator, however, is certainly a genuine oscillator, even if its

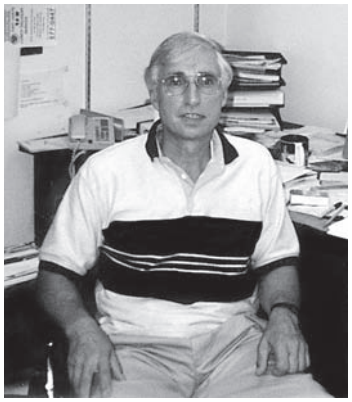


FIGURE 8.25 Friedrich Stephan. This circadian physiologist from Florida State University (Tallahassee) has extensively researched the properties of the food-entrainable pacemaker in rats. (Source: Photograph courtesy of Friedrich Stephan.)

anatomical location has yet to be determined.[200–202] The caloric content of food, rather than just bulk, seems to be the relevant signal for resetting of the oscillator,[203] and glucose seems to be more effective than lipids in this regard[204] (see Figure 8.27).

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 8.28. 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

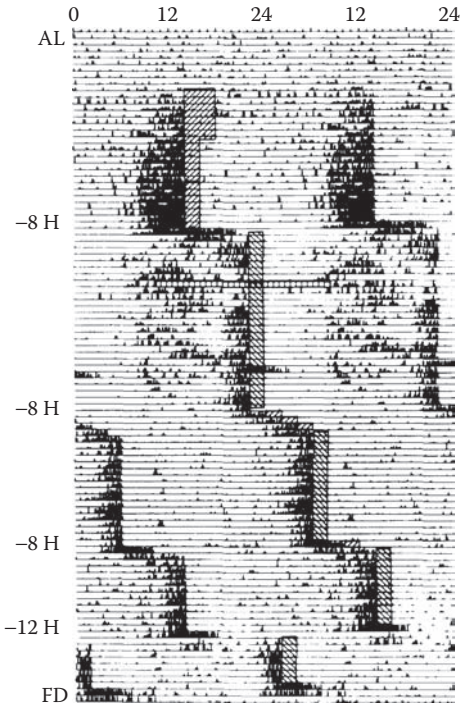


FIGURE 8.26 Confirming the existence of a separate food-entrainable pacemaker. This double-plotted actogram shows the running-wheel activity rhythm of a laboratory rat (*Rattus norvegicus*) rendered arrhythmic by destruction of the master circadian clock in the brain and exposed to cycles of food availability (as indicated by the hatched rectangles). Note the absence of rhythmicity during the initial days under free-feeding conditions (AL, for *ad libitum*) and the presence of clear rhythmicity under the restricted-feeding schedules. Note also the persistence of rhythmicity during the 2 days of total food deprivation (FD). (Source: Stephan, F. K. (1992). Resetting of a feeding-entrainable circadian clock in the rat. *Physiology and Behavior* 52: 985–995. © Elsevier Science Publishers. Reproduced with permission from the publisher and the author.)

the three levels as masking agents, or at the pacemaker levels as zeitgebers. Slave actions probably can feedback on the master pacemaker.

A final reminder is necessary here. A few researchers have been carried away by the concept of the food-entrainable oscillator. From the traditional assumption that food restriction entrains *the* circadian pacemaker, they moved not only to the empirically justified belief that food restriction entrains the food-entrainable pacemaker, but also to the unjustified belief that food restriction does *not* entrain the master pacemaker. As shown in Figure 8.29, food restriction entrains both pacemakers.[176] 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 food anticipatory activity (open arrows) and a light-entrained component (closed arrows) can be seen clearly. When the schedule of food restriction was maintained in constant darkness (DD), the

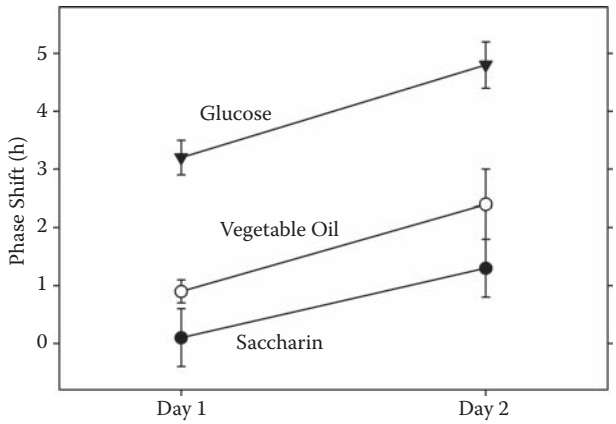


FIGURE 8.27 Dissecting the food-entrainable pacemaker. The graph shows the effects of different nutrients on the pace of re-entrainment of the feeding rhythms of laboratory rats (*Rattus norvegicus*) after an 8-hour delay of the schedule of food restriction. The data points indicate the mean phase shifts (± SE) of seven to eight 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. Note that glucose feeding led to greater phase shifts than the feeding of vegetable oil or saccharin. (Source: Stephan, F. K. & Davidson, A. J. (1998). Glucose, but not fat, phase shifts the feeding-entrained circadian clock. *Physiology and Behavior* 65: 277–288.)

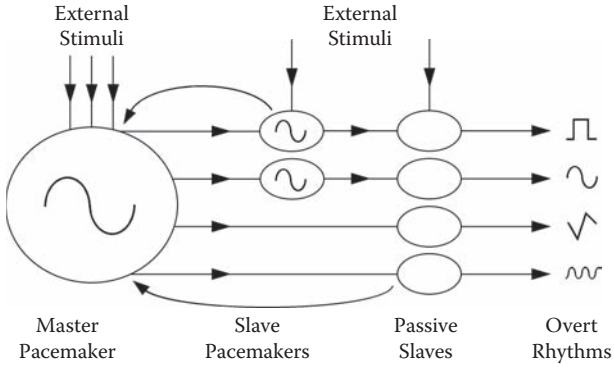


FIGURE 8.28 A hierarchy of pacemakers. The existence of a separate food-entrainable pacemaker requires a reconceptualization of the circadian system as a hierarchy of master and slave pacemakers (and perhaps passive slave pacemakers as well), each of them modulated (or not) by environmental stimuli. (Source: Adapted from Turek, F. W. (1994). Circadian rhythms. *Recent Progress in Hormone Research* 49: 43–90.)

anticipatory-activity component was slightly unmasked but persisted otherwise 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.[176] Thus, the master

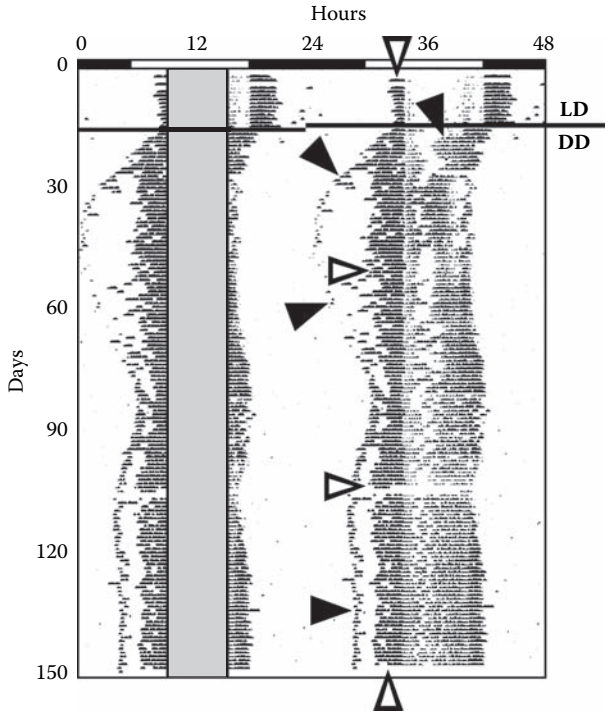


FIGURE 8.29 But let's not get carried away. The "light-entrainable" component of the activity rhythm can definitely be entrained by food restriction, as shown in this double-plot actogram of the running-wheel activity rhythm of a domestic mouse (*Mus musculus*). A 24-hour light–dark cycle (denoted by 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 grey 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 food restriction after the light–dark cycle is eliminated. (Source: Figure courtesy of Abel Bult-Ito, Marina R. Castillo, and Kelly J. Hochstetler, University of Alaska, Fairbanks.)

pacemaker *can* be entrained by a schedule of food restriction.

Chapter 6 discussed endogenous mechanisms of circadian rhythmicity, Chapter 7 covered photic environmental mechanisms, and this chapter focused on nonphotic environmental mechanisms. Chapter 9 discusses the integration of mechanisms.

SUMMARY

1. Although light is the zeitgeber that has been studied most thoroughly, several nonphotic environmental stimuli have been shown to also entrain circadian rhythms. These stimuli include ambient temperature, food availability, physical activity (exercise), and social contact. Nonphotic stimuli appear to have a weaker influence on the circadian system than light.
2. 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

Exercise 8.1 Studying entrainment by food restriction in mice

You can conduct this exercise if you assembled the data acquisition system in Exercise 2.4, or if you have access to a data acquisition system for rodent activity in a researcher's laboratory. In many countries, you also will 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 can complete the necessary paperwork. The goal of the exercise is to provide you with first-hand experience on the process of nonphotic entrainment.

1. At first, you will place mice in individual cages with running-wheels under a light–dark 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 determine 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 grams, or 1.8 ounces). 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 grams (or 0.18 ounces).
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 food restriction is 85% of the free-feeding level. Multiply each mouse's daily consumption by 0.85 to determine the daily ration for food restriction.
4. When you are ready to start the actual study, place the animals in constant darkness for a few days (with food freely available). Make sure to weigh the animals at least once so that you can monitor weight 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 notice an increase in activity several hours before the feeding time. Although food restriction 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 food restriction schedule, you should 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.
7. When the exercise is finished, reinstate the light–dark cycle and make sure that someone is in charge of caring for the animals after you are gone.

Exercise 8.2 Viewing PRCs

This exercise uses the program PRC. 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, when 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. Double-click on the Circadian icon to open the program banner, then click PRC (the sixth icon from the right).
2. Note that you can choose either Photic or Nonphotic stimuli. Click on Nonphotic. Note 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 in a graphics program).
3. The program is easy to use (refer to Exercise 7.2 in Chapter 7 if you need help using it). Explore the nonphotic phase-response curves (PRCs) of the various species. Most curves have small amplitude and fit in the smallest Y-axis scale (from –3 to +3 hours). Note 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).

Exercise 8.3 Building PRCs

This exercise uses the program Model to simulate phase-shifts of the circadian system and build a phase-response curve. Although the PRC used by the program is that for light pulses, the general process is the same as for nonphotic stimuli.

1. Double-click on the Circadian icon to open the program banner, then click on Model (the fifth icon from the right).
2. In the top panel, change Period to 23.5 hours and set the number of Blocks to 2.
3. In 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 light–dark cycle, with lights on at midnight and off at noon, for 14 days).
4. In 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. The animal will be in constant darkness for 28 days and will receive a pulse at CT 20 on the 15th day. CT 20 is 8 circadian hours after the onset of activity (CT 12). 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, click on the onset before the pulse to see the clock time, then click on the onset after the pulse; subtract the two values. 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. Extend an imaginary line from the onsets before the pulse to the first postpulse onset; extend another imaginary line from the onsets after the pulse to the first postpulse onset;

then subtract the onset times on that day. You should obtain a shift of 1.6 hours. (If this step is confusing for you to complete, repeat Exercise 7.1 in Chapter 7.)

7. Because each physical hour is 24/τ 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, it 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, the PRC will be constructed using an animal with a period of 24.0 hours, so that 1 circadian hour is the same as 1 physical hour. Click on Close to return to the main window. In 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. No phase shift (a phase shift of 0.0 circadian hours) should be present. 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, placing circadian time on the abscissa and phase shifts on the ordinate. Note 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 sometimes have a phase-delay region.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Stephan, F. K. (2001). Food-entrainable oscillators in mammals. In: Takahashi, J. S., Turek, F. W., & Moore, R. Y. (Eds.). *Circadian Clocks* (Volume 12 of *Handbook of Behavioral Neurobiology*). New York: Kluwer/Plenum, pp. 223–246. A thorough review of research on the entrainment of circadian rhythms by food restriction.

Mrosovsky, N. (1996). Locomotor activity and nonphotic 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* 8: 250–260. An excellent review article that discusses the relationship between central and peripheral pacemakers, as affected by photic and nonphotic stimuli.

WEB SITES TO EXPLORE

Automated Feeding Systems (Sweeney):
http://www.sweeneyfeeders.com

Directory of Free Electronic Journals:
http://www.doaj.org

Refrigerated Incubators (Revco):
http://www.revco-sci.com

Temperature Control Equipment (Digital Control Systems):
http://www.dcs-inc.net

LITERATURE CITED

1. Sweeney, B. M. & Hastings, J. W. (1960). Effects of temperature upon diurnal rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 87–103.
2. Michael, T. P., Salomé, P. A. & McClung, C. R. (2003). Two *Arabidopsis* circadian oscillators can be distinguished by differential temperature sensitivity. *Proceedings of the National Academy of Sciences U.S.A.* 100: 6878–6883.
3. Page, T. L. (1985). Circadian organization in cockroaches: effects of temperature cycles on locomotor activity. *Journal of Insect Physiology* 31: 235–242.
4. North, R. D. (1993). Entrainment of the circadian rhythm of locomotor activity in wood ants by temperature. *Animal Behaviour* 45: 393–397.
5. Hoffmann, K. (1968). Synchronisation der circadianen Aktivitätsperiodik von Eidechsen durch Temperaturcyclen verschiedener Amplitude. *Zeitschrift für Vergleichende Physiologie* 58: 225–228.
6. Foà, A. & 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.
7. 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.
8. Lindberg, R. G. & Hayden, P. (1974). Thermoperiodic entrainment of arousal from torpor in the little pocket mouse, *Perognathus longimembris*. *Chronobiologia* 1: 356–361.

9. Francis, A. J. P. & 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.
10. Rajaratnam, S. M. W. & Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
11. 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.
12. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
13. Herzog, E. D. & Huckfeldt, R. M. (2003). Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 763–770.
14. Tokura, H. & Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
15. Pálková, M., Sigmund, L. & 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.
16. Webb, H. M., Bennet, M. F., Graves, R. C. & 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.
17. Edery, I., Rutila, J. E. & Rosbash, M. (1994). Phase shifting of the circadian clock by induction of the *Drosophila period* protein. *Science* 263: 237–240.
18. Francis, A. J. P. & Coleman, G. L. (1997). Phase response curves to ambient temperature pulses in rats. *Physiology and Behavior* 62: 1211–1217.
19. Ruby, N. F., Burns, D. E. & 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.
20. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S. & 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.
21. Sánchez-Vásquez, F., Aranda, A. & 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.
22. Hau, M. & Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
23. Hau, M. & 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.
24. Jilge, B. (1991). Restricted feeding: a nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
25. Kennedy, G. A., Coleman, G. J. & Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
26. Kennedy, G. A., Coleman, G. J. & 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.
27. Challet, E., Losee-Olson, S. & Turek, F. W. (1999). Reduced glucose availability attenuates circadian responses to light in mice. *American Journal of Physiology* 276: R1063–R1070.
28. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
29. Edmonds, S. C. & Adler, N. T. (1977). Food and light as entrainers of circadian running activity in the rat. *Physiology and Behavior* 18: 915–919.
30. Coleman, G. J. & 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.
31. Rusak, B., Mistlberger, R. E., Losier, B. & Jones, C. H. (1988). Daily hoarding opportunity entrains the pacemaker for hamster activity rhythms. *Journal of Comparative Physiology A* 164: 165–171.
32. Challet, E., Malan, A. & 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.
33. Holmes, M. M. & Mistlberger, R. E. (2000). Food anticipatory activity and photic entrainment in food-restricted BALB/c mice. *Physiology and Behavior* 68: 655–666.
34. Sharma, V. K., Chidambaram, R., Subbaraj, R. & 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.
35. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
36. Boulos, Z., Frim, D. M., Dewey, L. K. & Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
37. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Feeding time synchronizes primate circadian rhythms. *Physiology and Behavior* 18: 775–779.
38. Reebs, S. G. & 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.
39. 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.
40. Edgar, D. M. & Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.

41. Laemle, L. K. & Ottenweller, J. E. (1999). Nonphotic entrainment of activity and temperature rhythms in anophthalmic mice. *Physiology and Behavior* 66: 461–471.
42. Hut, R. A., Mrosovsky, N. & Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
43. Wickland, C. R. & 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.
44. Mrosovsky, N., Salmon, P. A., Menaker, M. & Ralph, M. R. (1992). Nonphotic phase shifting in hamster clock mutants. *Journal of Biological Rhythms* 7: 41–49.
45. Janik, D. & 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.
46. Gannon, R. L. & Rea, M. A. (1995). Twelve-hour phase shifts of hamster circadian rhythms elicited by voluntary wheel running. *Journal of Biological Rhythms* 10: 196–210.
47. Eastman, C. I., Hoese, E. K., Youngstedt, S. D. & Liu, L. (1995). Phase-shifting human circadian rhythms with exercise during the night shift. *Physiology and Behavior* 58: 1287–1291.
48. Weisgerber, D., Redlin, U. & Mrosovsky, N. (1997). Lengthening of circadian period in hamsters by novelty-induced wheel running. *Physiology and Behavior* 62: 759–765.
49. Buxton, O. M., Lee, C. W., L'Hermite-Balériaux, M., Turek, F. W. & 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.
50. Evans, J. A., Elliott, J. A. & Gorman, M. R. (2004). Photoperiod differentially modulates photic and non-photic phase response curves of hamsters. *American Journal of Physiology* 286: R539–R546.
51. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S. & 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.
52. Edgar, D. M., Martin, C. E. & 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.
53. Edgar, D. M., Kilduff, T. S., Martin, C. E. & 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.
54. Kuroda, H., Fukushima, M., Nakai, M., Katayama, T. & 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.
55. Mistlberger, R. E., Bossert, J. M., Holmes, M. M. & Marchant, E. G. (1998). Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. *Behavioural Brain Research* 96: 93–99.
56. 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.
57. Mistlberger, R. E. & Holmes, M. M. (2000). Behavioral feedback regulation of circadian rhythm phase angle in light-dark entrained mice. *American Journal of Physiology* 279: R813–R821.
58. Deboer, T. & Tobler, I. (2000). Running wheel size influences circadian rhythm period and its phase shift in mice. *Journal of Comparative Physiology A* 186: 969–973.
59. Koteja, P., Swallow, J. G., Carter, P. A. & Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.
60. Mistlberger, R. E. & Antle, M. C. (1998). Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. *Brain Research* 786: 31–38.
61. Christian, C. A. & 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.
62. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J. & 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.
63. Mrosovsky, N. (1988). Phase response curves for social entrainment. *Journal of Comparative Physiology A* 162: 35–46.
64. Pellowski, M. W. & Rosenwasser, A. M. (1996). Circadian phase shifting associated with routine cage maintenance: a retrospective analysis. *Biological Rhythm Research* 27: 130–137.
65. Smith, R. D., Turek, F. W. & 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.
66. Davis, F. C. & 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.
67. Duffield, G. E. & 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.
68. Jilge, B., Kuhnt, B., Landerer, W. & Rest, S. (2001). Circadian temperature rhythms in rabbit pups and in their does. *Laboratory Animals* 35: 364–373.
69. Parraguez, V. H., Valenzuela, G. J., Vergara, M., Ducsay, C. A., Yellon, S. M. & Serón-Ferré, M. (1996). Effect of constant light on fetal and maternal prolactin rhythms in sheep. *Endocrinology* 137: 2355–2361.

70. Parraguez, V. H., Sales, F., Valenzuela, G. J., Vergara, M., Catalán, L. & Serón-Ferré, M. (1998). Diurnal changes in light intensity inside the pregnant uterus in sheep. *Animal Reproduction Science* 52: 123–130.
71. Greco, A. M., Gambardella, P., Sticchi, R., D'Aponte, D., di Renzo, G. & de Franciscis, P. (1989). Effects of individual housing on circadian rhythms of adult rats. *Physiology and Behavior* 45: 363–366.
72. Tornatzky, W. & Miczek, K. A. (1993). Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. *Physiology and Behavior* 53: 983–993.
73. Meerlo, P., De Boer, S. F., Koolhaas, J. M., Daan, S. & 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.
74. Keeney, A. J., Hogg, S. & 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.
75. Wever, R. (1970). Zur Zeitgeber-Stärke eines Licht-Dunkel-Wechsels für die circadiane Periodik des Menschen. *Pflügers Archiv* 321: 133–142.
76. Aschoff, J., Fatranská, M., Giedke, H., Doerr, P., Stamm, D. & Wisser, H. (1971). Human circadian rhythms in continuous darkness: entrainment by social cues. *Science* 171: 213–215.
77. Marimuthu, G., Rajan, S. & 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.
78. Moritz, R. F. A. & Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.
79. Governale, M. M. & 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.
80. Levine, J. D., Funes, P., Dowse, H. B. & Hall, J. C. (2002). Resetting the circadian clock by social experience in *Drosophila melanogaster*. *Science* 298: 2010–2012.
81. Honrado, G. I. & 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.
82. Goel, N. & Lee, T. M. (1995). Social cues accelerate reentrainment of circadian rhythms in diurnal female *Octodon degus* (Rodentia: Octodontidae). *Chronobiology International* 12: 311–323.
83. Goel, N. & 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.
84. Jechura, T. J., Walsh, J. M. & Lee, T. M. (2003). Testosterone suppresses circadian responsiveness to social cues in the diurnal rodent *Octodon degus*. *Journal of Biological Rhythms* 18: 43–50.
85. Kavanau, J. L. (1963). The study of social interaction between small animals. *Animal Behaviour* 11: 263–273.
86. Møller, U. & 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.
87. Regal, P. J. & Connolly, M. S. (1980). Social influences on biological rhythms. *Behaviour* 72: 171–199.
88. Crowley, M. & Bovet, J. (1980). Social synchronization of circadian rhythms in deer mice (*Peromyscus maniculatus*). *Behavioral Ecology and Sociobiology* 7: 99–105.
89. 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.
90. Tornatzky, W., Cole, J. C. & Miczek, K. A. (1998). Recurrent aggressive episodes entrain ultradian heart rate and core temperature rhythms. *Physiology and Behavior* 63: 845–853.
91. Rajaratnam, S. M. W. & Redman, J. R. (1999). Social contact synchronizes free-running activity rhythms of diurnal palm squirrels. *Physiology and Behavior* 66: 21–26.
92. 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.
93. Davis, F. C., Stice, S. & 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.
94. Aschoff, J. & 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.
95. Refinetti, R., Nelson, D. E. & 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.
96. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M. & 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.
97. Gattermann, R. & 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.
98. Meerlo, P. & Daan, S. (1998). Aggressive and sexual social stimuli do not phase shift the circadian temperature rhythm in rats. *Chronobiology International* 15: 231–240.
99. Miles, L. E. M., Raynal, D. M. & Wilson, M. A. (1977). Blind man living in normal society has circadian rhythms of 24.9 hours. *Science* 198: 421–423.

100. 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.
101. Erkert, H. G., Nagel, B. & 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.
102. Kennaway, D. J. & 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.
103. Steel, G. D., Callaway, M., Suedfeld, P. & 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.
104. Gwinner, E. (1966). Entrainment of a circadian rhythm in birds by species-specific song cycles. *Experientia* 22: 765–766.
105. Reebs, S. G. (1989). Acoustical entrainment of circadian activity rhythms in house sparrows: constant light is not necessary. *Ethology* 80: 172–181.
106. McClintock, M. K. (1971). Menstrual synchrony and suppression. *Nature* 229: 244–245.
107. Handelmann, G., Ravizza, R. & Ray, W. J. (1980). Social dominance determines estrous entrainment among female hamsters. *Hormones and Behavior* 14: 107–115.
108. McClintock, M. K. (1984). Estrous synchrony: modulation of ovarian cycle length by female pheromones. *Physiology and Behavior* 32: 701–705.
109. Preti, G., Cutler, W. B., Garcia, C. R., Huggins, G. R. & 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.
110. Weller, A. & Weller, L. (1993). Menstrual synchrony between mothers and daughters and between roommates. *Physiology and Behavior* 53: 943–949.
111. Weller, A. & Weller, L. (1995). The impact of social interaction factors on menstrual synchrony in the workplace. *Psychoneuroendocrinology* 20: 21–31.
112. Stern, K. & McClintock, M. K. (1998). Regulation of ovulation by human pheromones. *Nature* 392: 177–179.
113. Schank, J. C. (2001). Menstrual-cycle synchrony: problems and new directions for research. *Journal of Comparative Psychology* 115: 3–15.
114. Boulos, Z. & Rusak, B. (1982). Circadian phase response curves for dark pulses in the hamster. *Journal of Comparative Physiology A* 146: 411–417.
115. Meijer, J. H., Daan, S., Overkamp, G. J. F. & 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.
116. Van Reeth, O., Zhang, Y., Zee, P. C. & Turek, F. W. (1992). Aging alters feedback effects of the activity-rest cycle on the circadian clock. *American Journal of Physiology* 263: R981–R986.
117. Lee, T. M. & 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.
118. 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.
119. Dwyer, S. M. & 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.
120. Rosenwasser, A. M. & Dwyer, S. M. (2001). Circadian phase shifting: relationships between photic and nonphotic phase-response curves. *Physiology and Behavior* 73: 175–183.
121. Mistlberger, R. E., Belcourt, J. & 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.
122. Rosenwasser, A. M. & Dwyer, S. M. (2002). Phase shifting the hamster circadian clock by 15-minute dark pulses. *Journal of Biological Rhythms* 17: 238–247.
123. Mendoza, J. Y., Dardente, H., Escobar, C., Pevét, P. & Challet, E. (2004). Dark pulse resetting of the suprachiasmatic clock in Syrian hamsters: behavioral phase-shifts and clock gene expression. *Neuroscience* 127: 529–537.
124. Demaria-Pesce, V. H., Murakami, D. & 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.
125. Fuller, C. A., Hoban-Higgins, T. M., Griffin, D. W. & Murakami, D. M. (1994). Influence of gravity on the circadian timing system. *Advances in Space Research* 14(8): 399–408.
126. Murakami, D. M., Tang, I. & 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.
127. Holley, D. C., DeRoshia, C. W., Moran, M. M. & Wade, C. E. (2003). Chronic centrifugation (hypergravity) disrupts the circadian system of the rat. *Journal of Applied Physiology* 95: 1266–1278.
128. Hoban-Higgins, T. M., Alpatov, A. M., Wassmer, G. T., Rietveld, W. J. & 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.
129. Quigg, M., Straume, M., Smith, T., Menaker, M. & Bertram, E. H. (2001). Seizures induce phase shifts of rat circadian rhythms. *Brain Research* 913: 165–169.
130. Jarsky, T. M. & Stephenson, R. (2000). Effects of hypoxia and hypercapnia on circadian rhythms in the golden hamster. *Journal of Applied Physiology* 89: 2130–2138.

131. Goichot, B., Weibel, L., Chapotot, F., Gronfier, C., Piquard, F. & 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.
132. Danilenko, K. V., Cajochen, C. & Wirz-Justice, A. (2003). Is sleep per se a zeitgeber in humans? *Journal of Biological Rhythms* 18: 170–178.
133. Van Reeth, O. & 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.
134. Wickland, C. & 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.
135. Van Reeth, O. & 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.
136. Biello, S. M. & Mrosovsky, N. (1993). Circadian phase-shifts induced by chlordiazepoxide without increased locomotor activity. *Brain Research* 622: 58–62.
137. Vansteensel, M. J., Deboer, T., Dahan, A. & 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.
138. Hastings, M. H., Mead, S. M., Vindlacheruvu, R. R., Ebling, F. J. P., Maywood, E. S. & 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.
139. Antle, M. C. & Mistlberger, R. E. (2000). Circadian clock resetting by sleep deprivation without exercise in the Syrian hamster. *Journal of Neuroscience* 20: 9326–9332.
140. Challet, E., Pévet, P. & 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.
141. Cain, S. W., Verwey, M., Hood, S., Leknickas, P., Karatsoreos, I., Yeomans, J. S. & Ralph, M. R. (2004). Reward and aversive stimuli produce similar nonphotic phase shifts. *Behavioral Neuroscience* 118: 131–137.
142. Glass, J. D., Tardif, S. D., Clements, R. & Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
143. Antle, M. C., Steen, N. M. & Mistlberger, R. E. (2001). Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. *NeuroReport* 12: 2901–2905.
144. Janik, D., Nest, K. J. & Janiga, M. A. (2001). Exogenous corticosteroid and shifts of circadian rhythms in hamsters. *Chronobiology International* 18: 203–213.
145. Vilaplana, J., Cambras, T. & Díez-Noguera, A. (1995). Sound does not entrain motor activity circadian rhythms of rats. *Physiology and Behavior* 58: 975–978.
146. Ralph, M. R. & Mrosovsky, N. (1992). Behavioral inhibition of circadian responses to light. *Journal of Biological Rhythms* 7: 353–359.
147. Mistlberger, R. E. (1991). Scheduled daily exercise or feeding alters the phase of photic entrainment in Syrian hamsters. *Physiology and Behavior* 50: 1257–1260.
148. Sinclair, S. V. & Mistlberger, R. E. (1997). Scheduled activity reorganizes circadian phase of Syrian hamsters under full and skeleton photoperiods. *Behavioural Brain Research* 87: 127–137.
149. Amir, S. & Stewart, J. (1998). Conditioned fear suppresses light-induced resetting of the circadian clock. *Neuroscience* 86: 345–351.
150. Amir, S. & Stewart, J. (1999). The effectiveness of light on the circadian clock is linked to its emotional value. *Neuroscience* 88: 339–345.
151. Amir, S., Cain, S., Sullivan, J., Robinson, B. & 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.
152. Challet, E., Turek, F. W., Laute, M. A. & 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.
153. Mistlberger, R. E., Marchant, E. G. & Sinclair, S. V. (1996). Nonphotic phase-shifting and the motivation to run: cold exposure reexamined. *Journal of Biological Rhythms* 11: 208–215.
154. Mistlberger, R. E., Sinclair, S. V., Marchant, E. G. & Neil, L. (1997). Phase shifts to refeeding in the Syrian hamster mediated by running activity. *Physiology and Behavior* 61: 273–278.
155. Mistlberger, R. E., Antle, M. C., Webb, I. C., Jones, M., Weinberg, J. & 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.
156. Chen, W. M. & Tabata, M. (2002). Individual rainbow trout can learn and anticipate multiple daily feeding times. *Journal of Fish Biology* 61: 1410–1422.
157. Hau, M. & Gwinner, E. (1997). Adjustment of house sparrow circadian rhythms to a simultaneously applied light and food zeitgeber. *Physiology and Behavior* 62: 973–981.
158. Reierth, E. & Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
159. Nelson, W., Scheving, L. & Halberg, F. (1975). Circadian rhythms in mice fed a single daily meal at different stages of lighting regimen. *Journal of Nutrition* 105: 171–184.
160. Hotz, M. M., Connolly, M. S. & 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.

161. Dudley, C. A., Erbel-Sieler, C., Estill, S. J., Reick, M., Franken, P., Pitts, S. & McKnight, S. L. (2003). Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science* 301: 379–383.
162. Shido, O., Sakurada, S. & Nagasaka, T. (1991). Effect of heat acclimation on diurnal changes in body temperature and locomotor activity in rats. *Journal of Physiology* 433: 59–71.
163. Krieger, D. T. (1974). Food and water restriction shifts corticosterone, temperature, activity and brain amine periodicity. *Endocrinology* 95: 1195–1201.
164. Panksepp, J. & Krost, K. (1975). Modification of diurnal feeding patterns by palatability. *Physiology and Behavior* 15: 673–677.
165. Brinkhof, M. W. G., Daan, S. & 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.
166. Lax, P., Zamora, S. & Madrid, J. A. (1999). Food-entrained feeding and locomotor circadian rhythms in rats under different lighting conditions. *Chronobiology International* 16: 281–291.
167. White, W. & Timberlake, W. (1999). Meal-engendered circadian-ensuing activity in rats. *Physiology and Behavior* 65: 625–642.
168. Challet, E., Pévet, P., Vivien-Roels, B. & 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.
169. Challet, E., Pévet, P. & 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.
170. Jilge, B. & 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.
171. Kennedy, G. A., Coleman, G. J. & 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.
172. Reebs, S. G. & Lague, M. (2000). Daily food-anticipatory activity in golden shiners: a test of endogenous timing mechanisms. *Physiology and Behavior* 70: 35–43.
173. Eckerman, D. A. (1999). Scheduling reinforcement about once a day. *Behavioural Processes* 45: 101–114.
174. Paul, M. J., Kauffman, A. S. & Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
175. Abe, H. & 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.
176. Castillo, M. R., Hochstetler, K. J., Tavernier, R. J., Greene, D. M. & Bult-Ito, A. (2004). Entrainment of the master circadian clock by scheduled feeding. *American Journal of Physiology* 287: R551–R555.
177. Crystal, J. D. (2001). Circadian time perception. *Journal of Experimental Psychology: Animal Behavior Processes* 27: 68–78.
178. White, W., Schwartz, G. J. & 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.
179. Díaz-Muñoz, M., Vázquez-Martínez, O., Aguilar-Roblero, R. & 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.
180. Pecoraro, N., Gomez, F., Laugero, K. & Dallman, M. F. (2002). Brief access to sucrose engages food-entrainable rhythms in food-deprived rats. *Behavioral Neuroscience* 116: 757–776.
181. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M. & Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.
182. Mistlberger, R. E. (1992). Anticipatory activity rhythms under daily schedules of water access in the rat. *Journal of Biological Rhythms* 7: 149–160.
183. Clarke, J. D. & Coleman, G. J. (1986). Persistent meal-associated rhythms in SCN-lesioned rats. *Physiology and Behavior* 36: 105–113.
184. Slotten, H. A., Pitrosky, B. & Pévet, P. (1999). Influence of the mode of daily melatonin administration on entrainment of rat circadian rhythms. *Journal of Biological Rhythms* 14: 347–353.
185. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
186. Honma, K., von Goetz, C. & Aschoff, J. (1983). Effects of restricted daily feeding on freerunning circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.
187. Phillips, D. L., Rautenberg, W., Rashotte, M. E. & Stephan, F. K. (1993). Evidence for a separate food-entrainable circadian oscillator in the pigeon. *Physiology and Behavior* 53: 1105–1113.
188. Stephan, F. K. (2001). Food-entrainable oscillators in mammals. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 223–246.
189. Krieger, D. T., Hauser, H. & Krey, L. C. (1977). Suprachiasmatic nuclear lesions do not abolish food-shifted circadian adrenal and temperature rhythmicity. *Science* 197: 398–399.
190. Stephan, F. K., Swann, J. M. & Sisk, C. L. (1979). Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. *Behavioral and Neural Biology* 25: 545–554.

191. Kurumiya, S. & 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.
192. Stephan, F. K. (1992). Resetting of a feeding-entrainable circadian clock in the rat. *Physiology and Behavior* 52: 985-995.
193. Mistlberger, R. E. (1992). Nonphotic entrainment of circadian activity rhythms in suprachiasmatic nuclei ablated hamsters. *Behavioral Neuroscience* 106: 192–202.
194. Mistlberger, R. E. (1993). Circadian properties of anticipatory activity to restricted water access in suprachiasmatic-ablated hamsters. *American Journal of Physiology* 264: R22–R29.
195. Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M. & Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research* 739: 12–18.
196. Pitts, S., Perone, E. & Silver, R. (2003). Food-entrained circadian rhythms are sustained in arrhythmic *Clk*/*Clk* mutant mice. *American Journal of Physiology* 285: R57–R67.
197. Stephan, F. K. (1986). Coupling between feeding- and light-entrainable circadian pacemakers in the rat. *Physiology and Behavior* 38: 537–544.
198. Ottenweller, J. E., Tapp, W. N. & Natelson, B. H. (1990). Phase-shifting the light-dark cycle resets the food-entrainable circadian pacemaker. *American Journal of Physiology* 258: R994–R1000.
199. Rashotte, M. E. & Stephan, F. K. (1996). Coupling between light- and food-entrainable circadian oscillators in pigeons. *Physiology and Behavior* 59: 1005–1010.
200. Mistlberger, R. E. & Rusak, B. (1988). Food-anticipatory circadian rhythms in rats with paraventricular and lateral hypothalamic ablations. *Journal of Biological Rhythms* 3: 277–291.
201. Davidson, A. J. & Stephan, F. K. (1998). Circadian food anticipation persists in capsaicin deafferented rats. *Journal of Biological Rhythms* 13: 422–429.
202. Davidson, A. J., Poole, A. S., Yamazaki, S. & Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain and Behavior* 2: 32–39.
203. Stephan, F. K. (1997). Calories affect zeitgeber properties of the feeding entrained circadian oscillator. *Physiology and Behavior* 62: 995–1002.
204. Stephan, F. K. & Davidson, A. J. (1998). Glucose, but not fat, phase shifts the feeding-entrained circadian clock. *Physiology and Behavior* 65: 277–288.

9 Integration of Mechanisms

CHAPTER OUTLINE

9.1 Internal Order
9.2 Ecology and Evolution
9.3 Lifetime Changes

9.1 INTERNAL ORDER

Previous chapters have shown that rhythmicity occurs in numerous variables, such as locomotor activity, body temperature, melatonin secretion, blood pressure, and so on. Up to this point, circadian rhythms have been discussed as if organisms exhibited only one circadian rhythm at a time — but circadian rhythmicity is exhibited by many variables simultaneously. This fact raises new issues; in particular, how do the multiple rhythms relate to each other — that is, what is the *internal 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 their temporal structures can be compared legitimately? Another issue concerns 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? Circadian physiologists do not have definite answers to these questions, but research has advanced enough so that researchers can at least start to answer them.

Figure 9.1 shows 5-day sections of three variables measured simultaneously in a goat (*Capra hircus*): rectal temperature, plasma concentration of urea, and plasma concentration of cholesterol. Note that the waveforms of the three rhythms are relatively similar (at least at this low temporal resolution of one measurement every 3 hours) but the 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

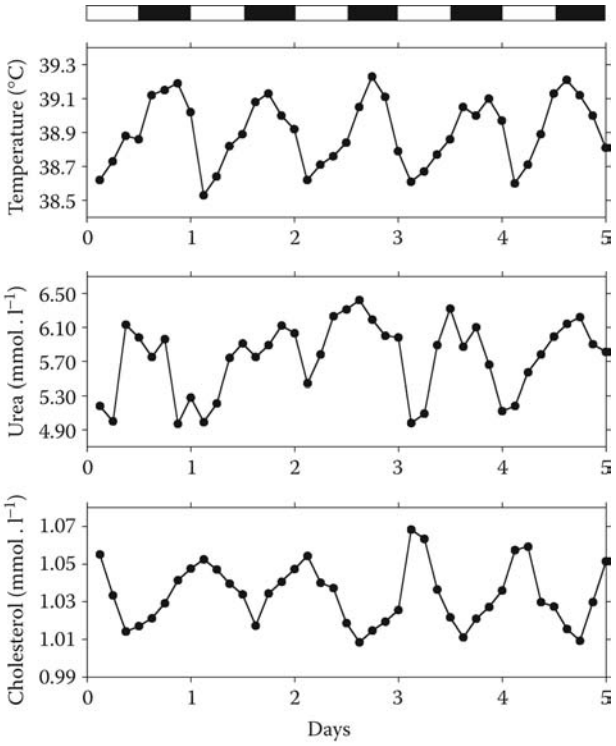


FIGURE 9.1 Three rhythms in a goat. The graphs show 5-day segments of simultaneous records of rectal temperature, plasma urea concentration, and plasma cholesterol concentration of a female goat (*Capra hircus*). The horizontal bars at the top indicate the timing of the light–dark cycle. Note that the 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). (Source: Piccione, G., Caola, G. & 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.)

(*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 (see 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 researchers bother to search, they 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

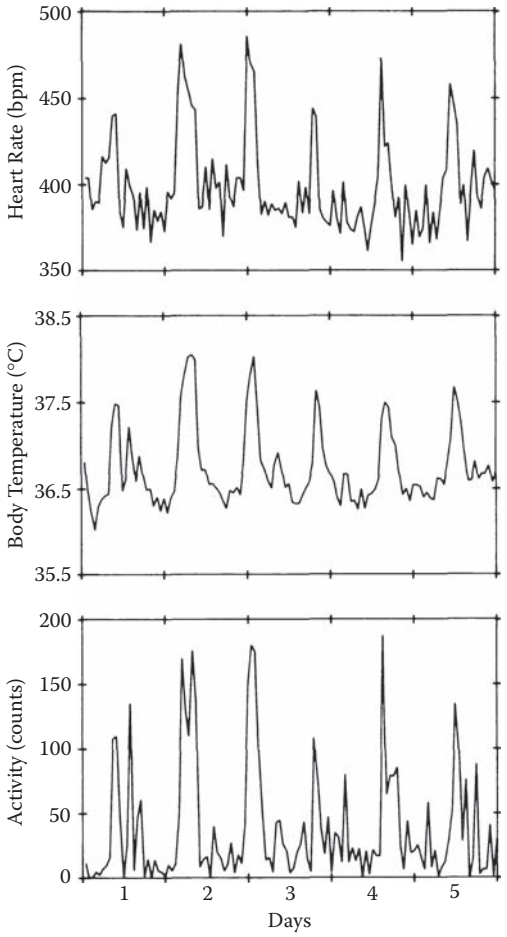


FIGURE 9.2 Three rhythms in a hamster. The graphs show 5-day segments of simultaneous records of heart rate, body temperature, and locomotor activity of a *tau*-mutant hamster (*Mesocricetus auratus*) maintained in constant light (100 lux). The data were collected by telemetry in 6-minute intervals and averaged hourly. Note the close synchronization of the three rhythms. (Source: Refinetti, R. & Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238. © American Physiological Society. Reproduced with permission from the publisher and the authors.)

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. Such monitoring has been done occasionally in laboratory and farm animals[2–9] as well as in humans.[10–17]

When analyzing the phase relationships among various variables that are simultaneously measured, 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 re-entrained to the new light–dark cycle in 4 to 6 days, while the feeding rhythm did so in 7 to 9 days.[18] 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 to 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. Chapter 15 discusses this issue in more detail in relation to *jet lag*.

Before examining the subject of temporal order in greater detail, I should probably point out why the analysis of phase relationships is important. Chapter 3 showed that circadian rhythms can be characterized by six parameters: mean 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 example, in the golden hamster, body temperature oscillates from 36.2 to 37.0°C with a mean of 36.6°C,[19] heart rate oscillates from 370 to 460 beats per minute (bpm) with a mean of 400 bpm,[20] and melatonin content in the pineal gland oscillates from 0 to 700 pg with a mean of 90 pg.[21] 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 be contrasted, 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 considered (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 for digestion to occur before any food is ingested.

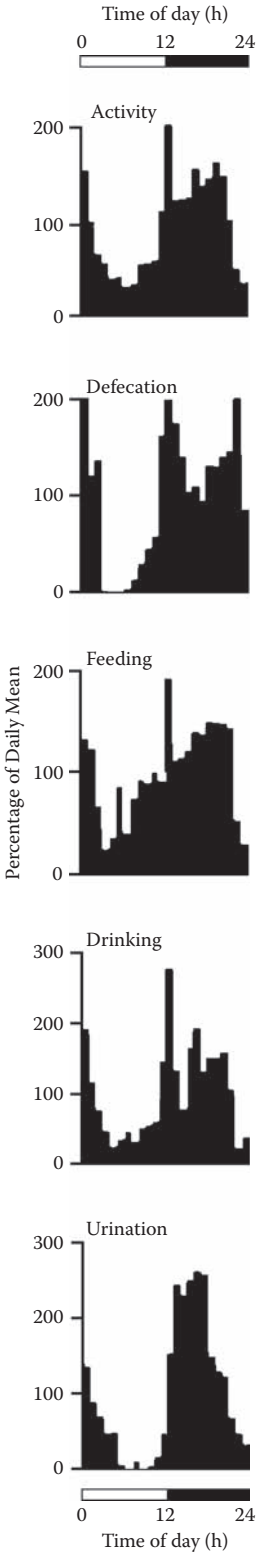


FIGURE 9.3 Five rhythms in a rabbit. The graphs show the daily distribution of values of five behavioral variables recorded from a rabbit (*Oryctolagus cuniculus*). All values are plotted as percentages of the daily mean. Each graph represents the average of 20 consecutive days. The horizontal bars at the top and bottom indicate the timing of the light–dark cycle. Note that all five rhythms peak at night, even though their waveforms vary considerably. (Source: 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.)

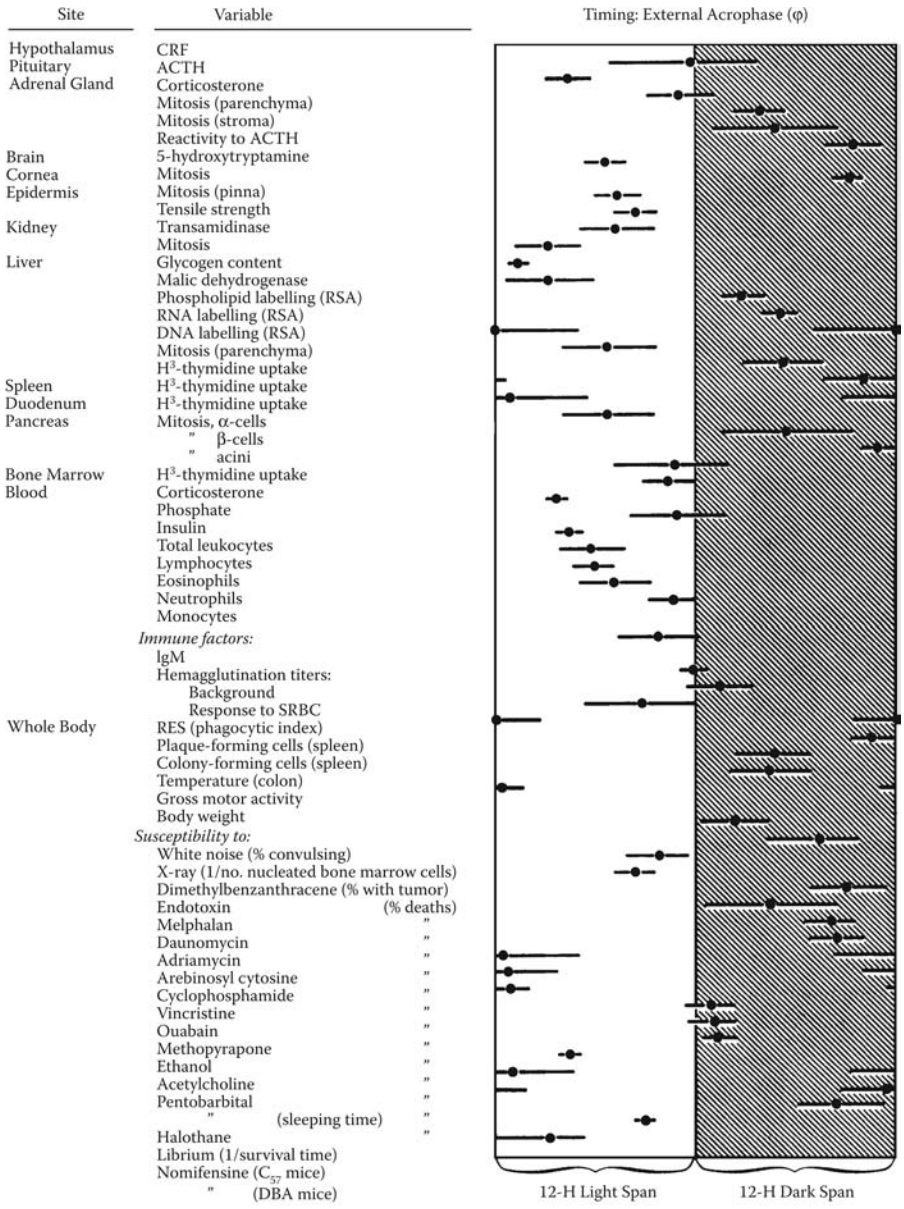


FIGURE 9.4 Sixty rhythms in mice. Shown are the mean acrophases (± SE) of over 60 rhythms measured in groups of mice (*Mus musculus*) maintained under a light–dark cycle (LD 12:12). Note that just as many rhythms peak during the day as during the night in this nocturnal species. (Source: Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N. & 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. © Franz Halberg. Reproduced with permission.)

9.1.1 Relationship between the Body Temperature Rhythm and the Activity Rhythm

Chapter 5 showed 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 monitored simultaneously in many studies on various species.[2,19,22–33] Figure 9.7 shows examples for two species. The temporal courses of the two rhythms are very similar in these 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

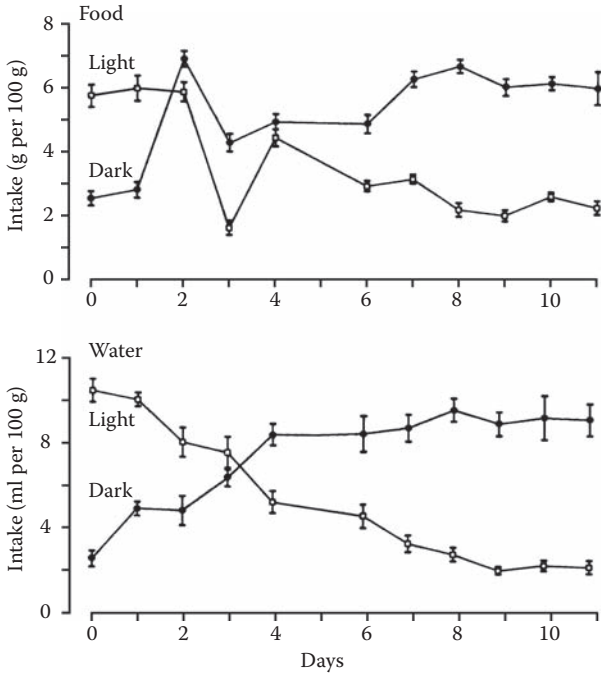


FIGURE 9.5 Jet-lagged rats. The graphs show the daily ingestion of food and water of rats (*Rattus norvegicus*) subjected to a full inversion (12-hour shift) of the light–dark cycle on Day 0. Each data point corresponds to the mean (± SE) of eight rats. Although adaptation to the inverted light–dark 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. (Source: Adapted from Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D. & 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.)

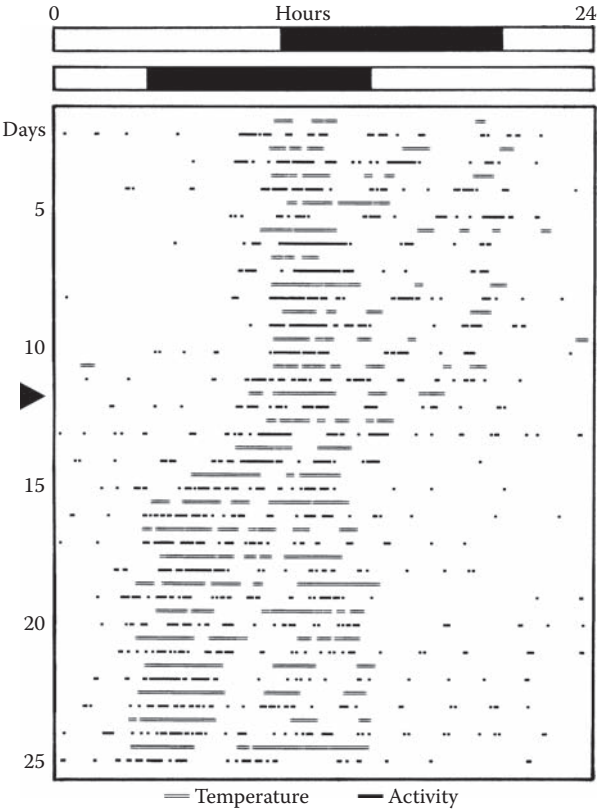


FIGURE 9.6 Jet-lagged hamster. This composite actogram shows the body temperature and locomotor activity rhythms of a golden hamster (*Mesocricetus auratus*) subjected to a 6-hour phase advance of the light–dark cycle. The timing of the light–dark cycles is indicated by the horizontal bars above the 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. (Source: Adapted from Refinetti, R. & Menaker, M. (1993). Effects of imipramine on circadian rhythms in the golden hamster. *Pharmacology, Biochemistry and Behavior* 45: 27–33.)

flying squirrel. Note that body temperature rises when the animal becomes more active and falls when activity is reduced, even though individual bouts of activity may not be correlated with individual rises in body temperature.

In contrast to the data shown in Figure 9.8, the human body temperature rhythm consistently has been reported to start to ascend several hours before awakening.[17,23,34] The relationship between the two rhythms has rarely been analyzed systematically in other animals, but two studies I conducted, one in rats[35] and another in eight different mammalian species,[36] 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 finding suggests that humans and rodents differ on the phase relationship between the rhythms of activity and body temperature. However, the difference is based 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.[36] In Figure 9.9 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 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.[17,23,34] Inspection of published records of temperature and activity rhythms in rodents does not suggest an advance of several hours in the temperature rhythm of

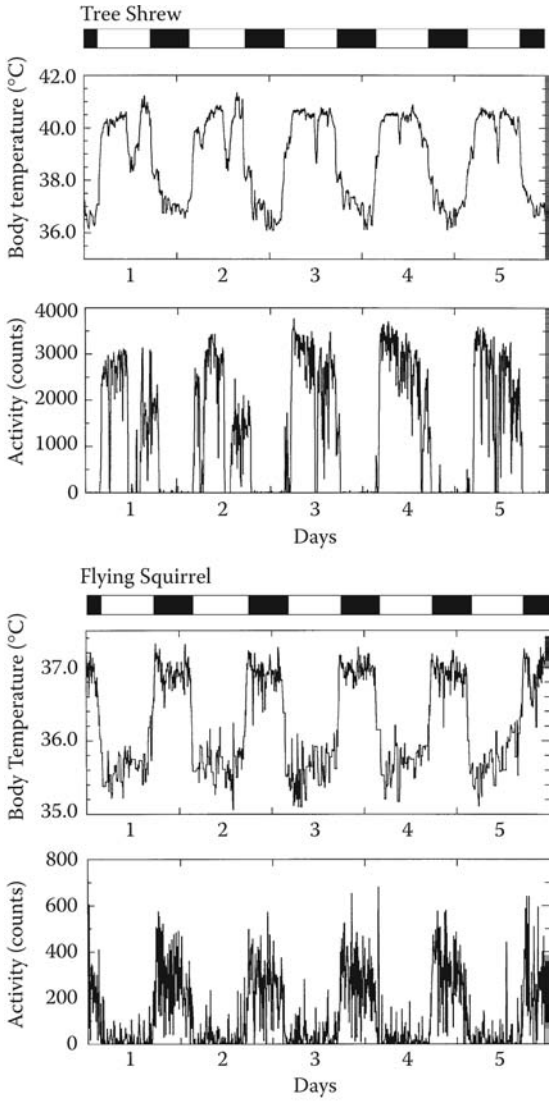


FIGURE 9.7 Synchronization of the rhythms of body temperature and locomotor activity. The graphs show 5-day segments of the records of body temperature and locomotor activity (recorded by telemetry with 6-minute resolution) of a tree shrew (*Tupaia belangeri*) and a flying squirrel (*Glaucomys volans*). The horizontal black and white bars indicate the duration of the dark and light phases of the prevailing light–dark cycle. Note that body temperature and activity level are high during the day in the diurnal tree shrew and high during the night in the nocturnal flying squirrel. (Source: 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.)

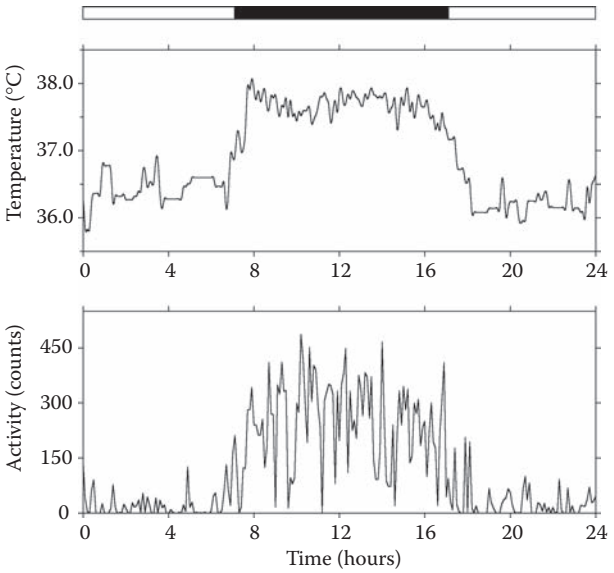


FIGURE 9.8 A closer look at the synchrony of body temperature and activity in the flying squirrel. The graphs show 24-hour segments of the records of body temperature and locomotor activity (recorded by telemetry with 6-minute resolution) of a flying squirrel (*Glaucomys volans*). The horizontal white and dark bars indicate the duration of the light and dark phases of the prevailing light–dark cycle. Note the close temporal relationship of the two variables. (Source: 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.)

animals maintained in constant darkness or constant light.[25,37–39] 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.[35] Figure 9.10 shows the results. The time when the rhythms ascended past the daily mean value was slightly delayed in constant darkness (DD) because the animals were free-running 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.

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 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[40–45] and other vertebrates.[46–52] 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, indicating 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.

To investigate the potential causal link between the activity rhythm and the temperature rhythm, several

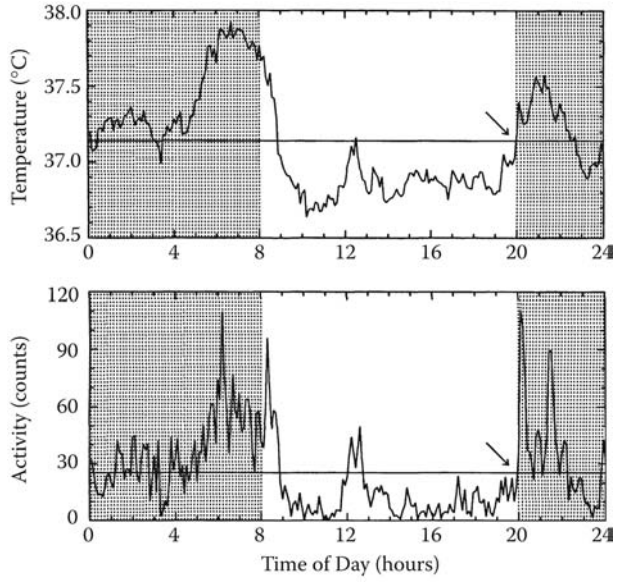


FIGURE 9.9 A closer look at the synchrony of body temperature and activity in the rat. The graphs show the rhythms of body temperature and locomotor activity of a laboratory rat (*Rattus norvegicus*). The data were collected by telemetry every 6 minutes and averaged over 7 consecutive days. The shading 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. (Source: 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.)

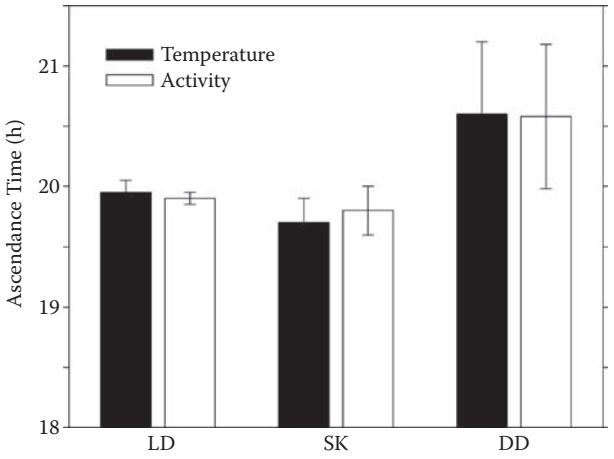


FIGURE 9.10 Persistent synchrony of the rhythms of body temperature and activity. The graph shows the mean time of ascendance past the mean of the rhythms of body temperature and locomotor activity of laboratory rats (*Rattus norvegicus*) maintained under a 24-hour light–dark cycle (LD), a 24-hour skeleton 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. (Source: 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.)

researchers recorded the body temperature rhythm of human subjects maintained in continuous bed rest[53–55] or undergoing a *constant routine* protocol, which involves bed rest as well as sleep deprivation and the ingestion of frequent, equal-size meals.[12,56–58] 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. Bed rest cannot be used with animals — because they do not comply with requests for voluntary rest — but at least two strong pieces of evidence support the argument that the activity rhythm does not cause the temperature rhythm in animals. One piece of evidence 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 more than) the temperature rhythm — especially in small animals with low thermal inertia. Yet, as shown in Figure 9.13, the robustness of the temperature rhythm is greater than the robustness of the activity rhythm in six species of small rodents. Therefore, if the two rhythms shared a causal connection, the temperature rhythm should cause the activity rhythm — and not the other way around.

The second piece of evidence concerns the day–night difference in the correlation between the rhythms of activity and temperature. Although nocturnal animals generally are more active at night than during the day, their body temperature is higher at night regardless of the actual activity level.[25,29,59–61] Conversely, the body temperature of diurnal animals is higher during the *day* regardless of the actual activity level.[36] Figure 9.14 illustrates these relationships. Note that for the nocturnal animals (left column), body temperature is higher at night for all levels of activity, even though activity level has a small effect on body temperature. For the diurnal animals (right column), body temperature is higher during the day for all levels of activity. Thus, the body temperature rhythm in animals, as in humans, is not caused by the activity rhythm.

9.1.2 Other Dependencies

The human body temperature rhythm persists during inactivity and sleep deprivation in the constant routine protocol.[12,56–58] Section 9.1.1 showed that variations in activity can alter ("mask") the temperature rhythm. Can sleep per se (i.e., separate from the obvious association between sleep and inactivity) affect body temperature? Researchers have shown that the occurrence of sleep leads to a lowering of body temperature, even if the magnitude of the temperature change is much smaller than that associated

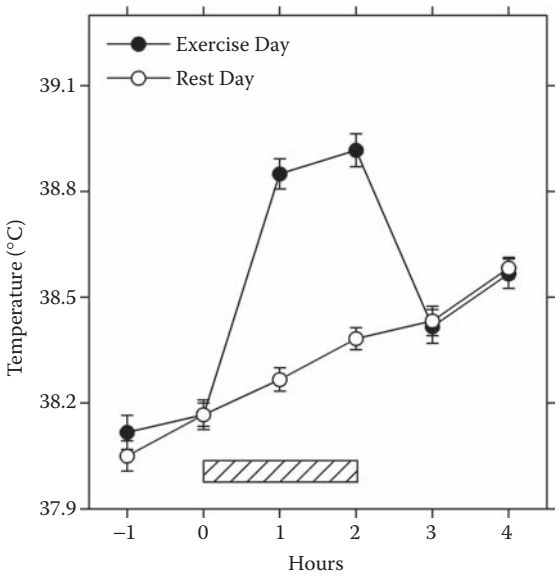


FIGURE 9.11 Exercise causes elevation of body temperature. The graph shows the variation in rectal temperature of horses on a day in which they underwent a 2-hour exercise session and on a day in which they rested in the stables. Each data point corresponds to the mean (± SE) of measurements of six horses. The hatched bar indicates the timing of the exercise session. Note the circadian elevation of body temperature on the rest day and the acute effect of exercise on the exercise day. (Source: Adapted from Piccione, G., Caola, G. & Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.)

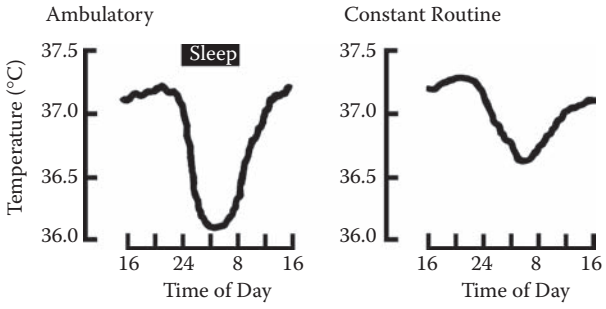


FIGURE 9.12 Activity rhythm does not cause temperature rhythm in humans. The graphs show the average profiles of the body temperature rhythms of healthy young men under ambulatory conditions (with variations in activity) and constant-routine (bed rest, no sleep) conditions. Note that the constant-routine condition reduces the amplitude of the body temperature rhythm but does not eliminate it. (Source: Adapted from Czeisler, C. A. and Dijk, D. J. (2001). Human circadian physiology and sleep-wake regulation. In: Takahashi, J. S. et al. (Eds.). *Circadian Clocks*. New York: Kluwer/Plenum, pp. 531–569.)

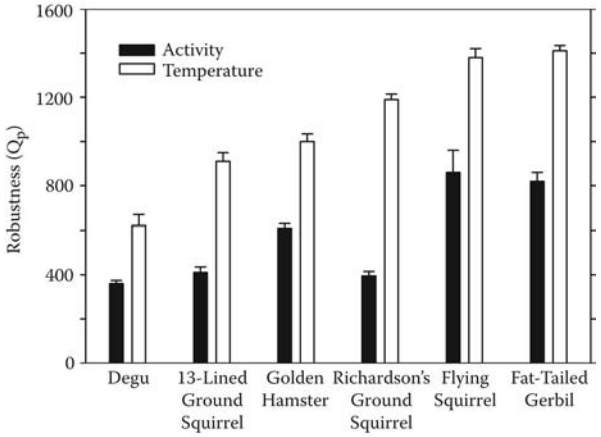


FIGURE 9.13 Activity rhythm is unlikely to cause temperature rhythm in rodents. The graph shows the mean robustness (± SE) of the daily rhythms of locomotor activity and body temperature of three to eight individuals of each of six species of small rodents. Note that the temperature rhythm is consistently more robust than the activity rhythm, which suggests that the activity rhythm cannot be responsible for the temperature rhythm. (Source: Adapted from 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.)

with the normal circadian oscillation.[62,63] The regulation of body temperature is virtually blocked during the REM stage of sleep, so that the body passively cools if ambient temperature is lower than body temperature.[64–77] Photic stimulation prior to sleep, however, can prevent the normal sleep-induced fall in body temperature.[78–82]

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.[65,83–86] 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 9.15, dogs fed a single meal a day still exhibit a clear daily rhythm of body temperature.[87] Humans and animals fed small meals at regular intervals throughout the day also exhibit rhythmicity in body temperature.[12,56–58,88] Animals and humans fed no meal at all (that is, subjected to food deprivation) still show daily rhythmicity in body temperature.[53,89–96] Figure 9.16 provides an example. 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,

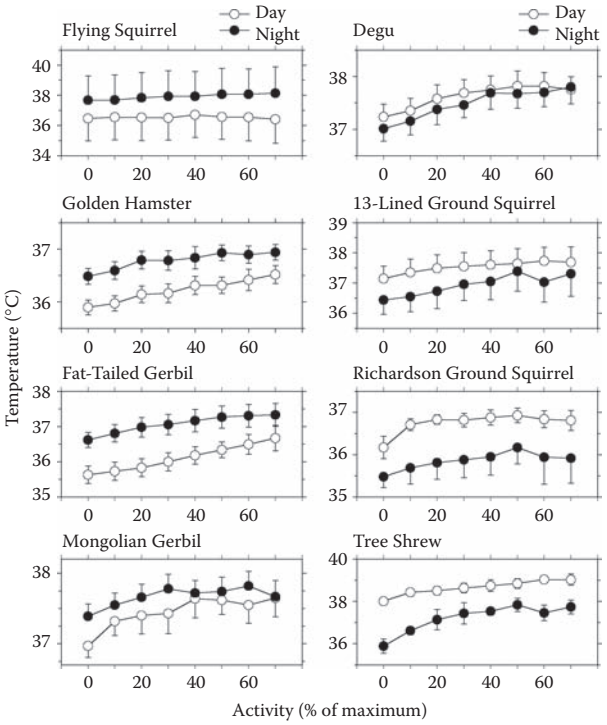


FIGURE 9.14 Activity rhythm does not cause temperature rhythm in small mammals. The graphs show the mean body temperatures associated with different levels of activity, during the day, and at night, in eight species of small mammals. The data points correspond to the means (± SE) of three to eight animals per species. Nocturnal species are represented on the left graphs, and diurnal species are seen on the right graphs. Note 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. (Source: Adapted from 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.)

no food was provided. Food deprivation caused a small decline in body temperature, but rhythmicity was clearly preserved.

Although the feeding rhythm does not cause the temperature rhythm, the feeding rhythm could cause some other rhythm. Figure 9.17 shows that feeding does not cause the secretion rhythm of the hormone leptin in the horse.[97] It also does not cause the rhythm of leptin secretion in the rat.[98] Figure 9.18 shows that feeding also is not responsible for the rhythm of cholesterol production in goats, but it *is* responsible for the maintenance of the rhythm of urea production.[99]

Circadian physiologists have also investigated the possible reliance of the rhythm of heart rate on the rhythm of activity. 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

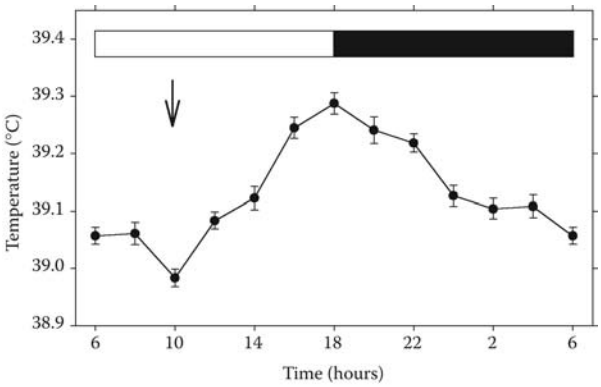


FIGURE 9.15 Body temperature rhythm persists with only one meal per day. The graph shows the mean daily profile of the body temperature rhythm of dogs (*Canis familiaris*) fed one meal a day. The data points correspond to the means (± SE) of seven beagles over 7 consecutive days. The horizontal bar indicates the timing of the light–dark cycle. The arrow indicates the time of feeding. Note that body temperature continues to rise for many hours after the daily meal. (Source: Refinetti, R. & Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.)

rhythm.[12,54,100–102] However, the rhythm of blood pressure may be generated by the activity rhythm.[100]

9.2 ECOLOGY AND EVOLUTION

Internal order is a complex subject. Understanding how organisms adapt to their environment — now and throughout evolution — also is a complicated area of study. Previous chapters discussed several environmental factors that affect circadian rhythms, but these chapters focused on each rhythm individually. This section adds the complexity of interactions among environmental factors to the complexity of the organism's internal order.

9.2.1 Evolution

Circadian rhythmicity is a pervasive characteristic of life on Earth. Circadian rhythmicity is exhibited even by animal species that inhabit subterranean environments and that lost a functional visual system long ago.[51,103–107] These animals are not exposed to daily environmental fluctuations, so they had no selective force to drive the evolution of endogenous circadian clocks. The existence of these clocks means that the animals must have inherited the clocks from nonsubterranean ancestors. 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 9.19). The origins of circadian rhythmicity occurred much further back in time, however, at the beginning of life on Earth, some 4 billion years ago.[108] The Earth was formed 4.5 billion years ago.[109]

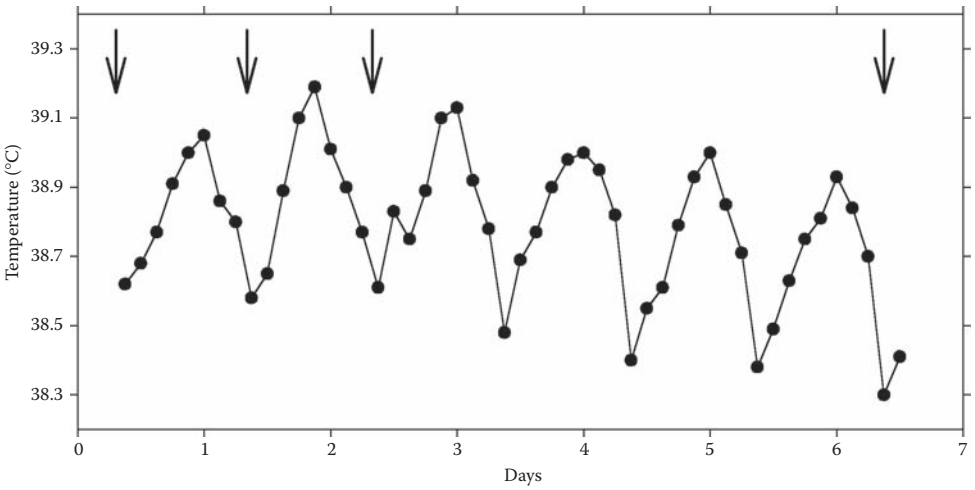


FIGURE 9.16 Body temperature rhythm persists during total food deprivation. The graph shows a segment of the records of rectal temperature of a goat (*Capra hircus*) maintained under a 24-hour light–dark cycle with and without daily meals. The arrows indicate meal times. Note the persistence of the temperature rhythm (with a small fall in the mean level) through 96 hours of food deprivation. (Source: Piccione, G., Caola, G. & 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.)

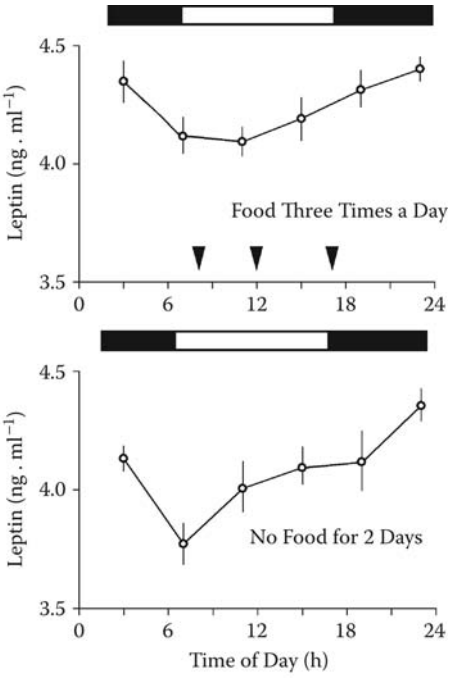


FIGURE 9.17 Rhythm of serum leptin concentration persists during total food deprivation. The graphs show the daily profiles of serum leptin concentration in horses (*Equus caballus*) fed three times a day or maintained without food for 2 days. The data points correspond to the means (± SE) of five horses. The horizontal bars indicate the timing of the light–dark cycle. The inverted triangles indicate feeding times. Note that the daily rhythm persists (and is enhanced) during food deprivation. (Source: Piccione, G., Bertolucci, C., Foà, A. & Caola, G. (2004). Influence of fasting and exercise on the daily rhythm of serum leptin in the horse. *Chronobiology International* 21: 405–417.)

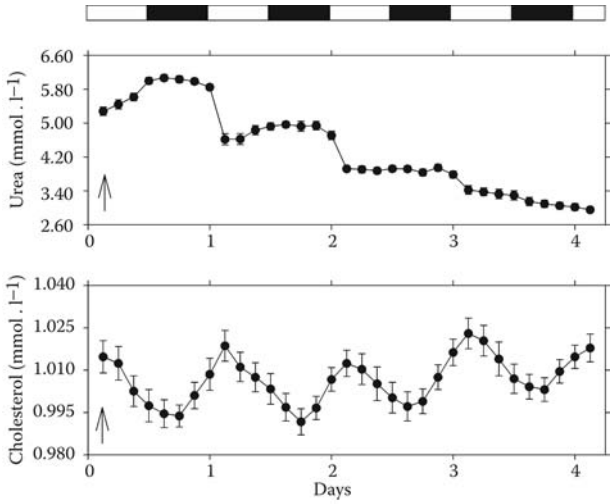


FIGURE 9.18 Rhythm of serum cholesterol concentration persists during total food deprivation, but rhythm of urea concentration does not. The graphs show 4-day segments of the records of serum urea concentration and serum cholesterol concentration of goats (*Capra hircus*) maintained under a light–dark 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. Note that the rhythm of cholesterol concentration persists in the absence of daily meals, but the rhythm of urea concentration gradually vanishes. (Source: Piccione, G., Caola, G. & 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.)

FIGURE 9.19 Raging dinosaur. Dinosaurs, along with many other animals, populated the Earth during the Mesozoic Era. (Source: © ArtToday, Tucson, AZ.)

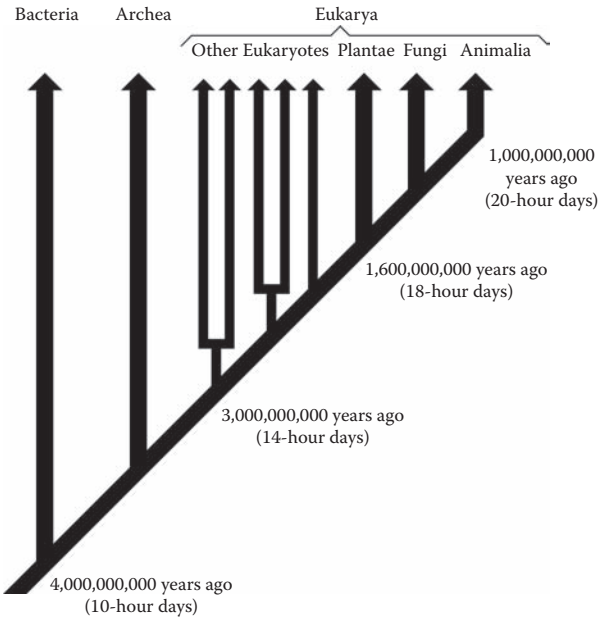


FIGURE 9.20 The tree of life. Life on Earth started not long after the formation of the planet itself, some 4.5 billion years ago. The diagram indicates the approximate time of the divergence of the three domains of life (Bacteria, Archea, and Eukarya) and of the kingdoms of Eukarya. The approximate duration of a day (which was shorter than 24 hours for most of Earth's life) is also indicated at various timepoints. (Sources: Pennisi, E. (2003). A tree of life. *Science* 300: 1694–1695; Benton, M. J. & Ayala, F. J. (2003). Dating the tree of life. *Science* 300: 1698–1700; Meyerowitz, E. M. (2002). Plants compared to animals: the broadest comparative study of development. *Science* 295: 1482–1485; Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science* 284: 2124–2128; Barnett, J. E. (1998). *Time's Pendulum*. New York: Plenum.)

As shown in Figure 9.20, all life forms on Earth can be classified into one of three domains: Bacteria, Archea, and Eukarya.[110] The domain of Bacteria is the oldest branch of the tree of life, having preceded Archea and all eukaryotes (i.e., organisms whose cells contain a nuclear membrane). Because extant bacteria exhibit circadian rhythmicity,[111–115] 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. As discussed in Chapter 12, fundamental differences exist in the molecular machinery of circadian pacemakers among extant organisms of the three domains of life; the existence of these differences supports the hypothesis of multiple evolutionary occurrences. At least one component of the molecular machinery, however, seems to be conserved among animal, plant, and fungal circadian systems,[116] 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.[117–119]

In the strict sense of the term, *circadian* rhythmicity could not possibly have evolved several billion years ago 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 9.20, the duration of a day was only 10 hours 4 billion years ago and only 18 hours 1.5 billion years ago.[120] 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 4 hours. *Tau*-mutant hamsters (animals 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[121] (Figure 9.21). The human species (*Homo sapiens*) appeared only 100,000 years ago[122] and has never experienced a day with a period significantly different from 24 hours.

Mean global temperature has oscillated from 0°C (32°F) to almost 30°C (86°F) since the first vertebrates appeared on Earth (Figure 9.22). If the Earth's geography had remained the same over time, freezing temperatures could have occurred in the 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[123] (Figure 9.23). 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.[124–129] For example, photosynthetic plants could wait for sunlight

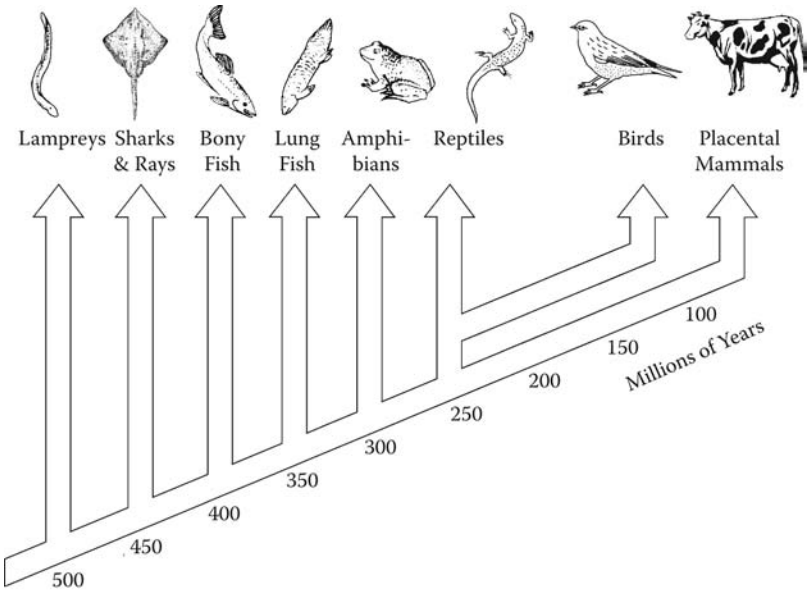


FIGURE 9.21 The vertebrate tree of life. Vertebrates have existed on Earth for 500 million years. The diagram indicates the approximate time of the divergence of several branches of this subphylum of the phylum Chordata. (Sources: Pennisi, E. (2003). A tree of life. *Science* 300: 1694–1695; Benton, M. J. & Ayala, F. J. (2003). Dating the tree of life. *Science* 300: 1698–1700; Stokstad, E. (2001). Exquisite Chinese fossils add new pages to book of life. *Science* 291: 232–236.)

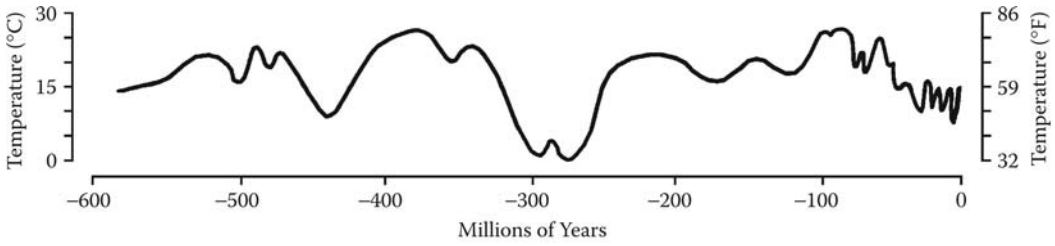


FIGURE 9.22 Ups and downs of Earth's temperature. The graph shows the variation in mean global surface temperature over the last 600 million years. Note the relatively small range of variation during the last 50 million years (when most extant mammalian species evolved). (Sources: Bradley, R. S. (1999). *Paleoclimatology*, 2nd Edition. San Diego, CA: Academic Press; Menne, M. (2000). Global long-term mean land and sea surface temperatures. *Global Surface Temperature Anomalies Report*. National Oceanic and Atmospheric Administration, U.S. Department of Commerce.)

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 with organisms with deficient circadian systems.[114,130–134]

The three main eukaryotic kingdoms (Fungi, Plants, and Animals) diverged between 1 and 1.5 billion years ago[135,136] (Figure 9.20) and started to colonize terrestrial environments soon after that.[137] Mammals did not appear until 300 million years ago and did not evolve a placenta until 100 million years ago[138] (Figure 9.21). Mammals originated in Asia and did not disperse to Europe and North America until 50 million years ago.[139] 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 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 may have been endothermic,[140,141] but it is more likely that they achieved stable body temperature because of the thermal latency associated with their gigantic body sizes.[142–144] Cross-taxa comparisons of basal metabolic

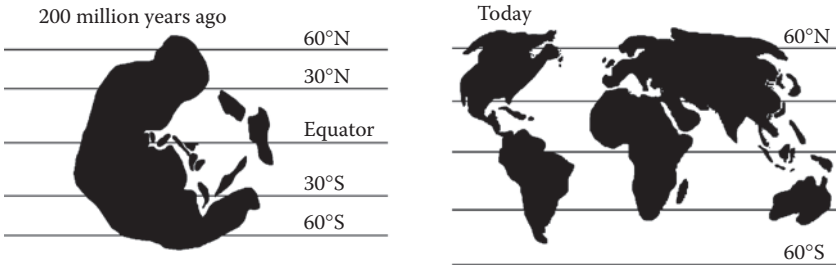


FIGURE 9.23 Geography of ancient earth. Since the early vertebrates evolved (500 million years ago), the climate and the geography of Earth have changed considerably. The diagrams show the organization of continents 200 million years ago and today. (Source: Adapted from Torsvik, T. H. (2003). The Rodinia jigsaw puzzle. *Science* 300: 1379–1381.)

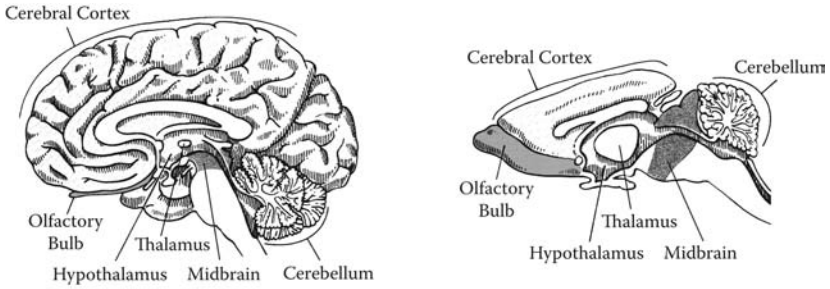


FIGURE 9.24 Of man and rat. These diagrams of midsaggital views of the human brain (left) and the rat brain (right) emphasize similarities and differences in the nervous systems of the two species. Differences are particularly evident in the relative sizes of the cerebral cortex, olfactory bulb, and thalamus. (Source: Adapted from Rosenzweig, M. R. & Leiman, A. L. (1982). *Physiological Psychology.* Lexington, MA: D. C. Heath.)

rate of extant mammalian species suggests that endothermy did not appear until 70 million years ago,[145] and perhaps not until 10 million years ago.[146] A sharp fall of about 20°C in global temperature between 50 and 20 million years ago[147] (Figure 9.22) may have provided a thermal environment that favored mammalian species with high metabolic rates.[146,148]

Five to fifty million species exist on Earth, although only 1.5 million species have been identified and classified.[149] These species all share the property of being alive, but close neighbors in a taxonomic branch share much more. The nervous systems of vertebrates — and particularly of mammals — share many anatomical and physiological characteristics.[150] As shown in Figure 9.24, the human brain and the rat brain share 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, yet obscure, ecological issue in circadian physiology is an organism's adoption of a *nocturnal* niche or a *diurnal* niche (Figure 9.25). In terms of evolution, researchers are 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,[151,152] then the alternation of day and night on Earth's surface would have been of very little importance. Eventually, 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.[153] 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.[154] 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,[155] but one can only speculate whether endothermy evolved in a nocturnal niche[146] or in a diurnal one.[148]

Although many animals today can be classified as either diurnal (day-active) or nocturnal (night-active), many others defy classification. Figure 9.26 shows a few

FIGURE 9.25 Birds of a feather. Some birds, such as the hawk (left), are active during the day; others, such as the owl (right), are active at night. (Source: National Image Library, U.S. Fish and Wildlife Service.)

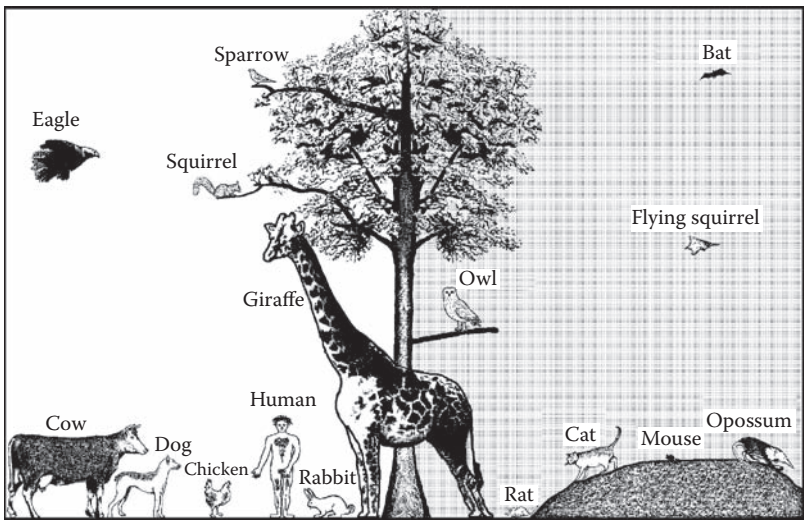


FIGURE 9.26 Temporal niches. The diagram depicts some mammals and birds with diurnal habits (left side), nocturnal habits (right side), or crepuscular habits (giraffe and rabbit).

examples. 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. 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 light–dark cycle are maintained constant. The activity pattern of a golden hamster (*Mesocricetus auratus*), recorded by telemetry under controlled conditions in the laboratory, is illustrated in Figure 9.27. Note 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 9.28 illustrates the activity pattern of a horse (*Equus caballus*) recorded by actigraphy in an indoor stall. Although activity is 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 9.29 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).

You may remember from previous chapters that the most common waveform of the activity rhythm of animals is a bimodal one,[156] which means that enhanced activity at dawn 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, while nocturnal animals fill the space between the peaks at dusk and dawn. Crepuscular

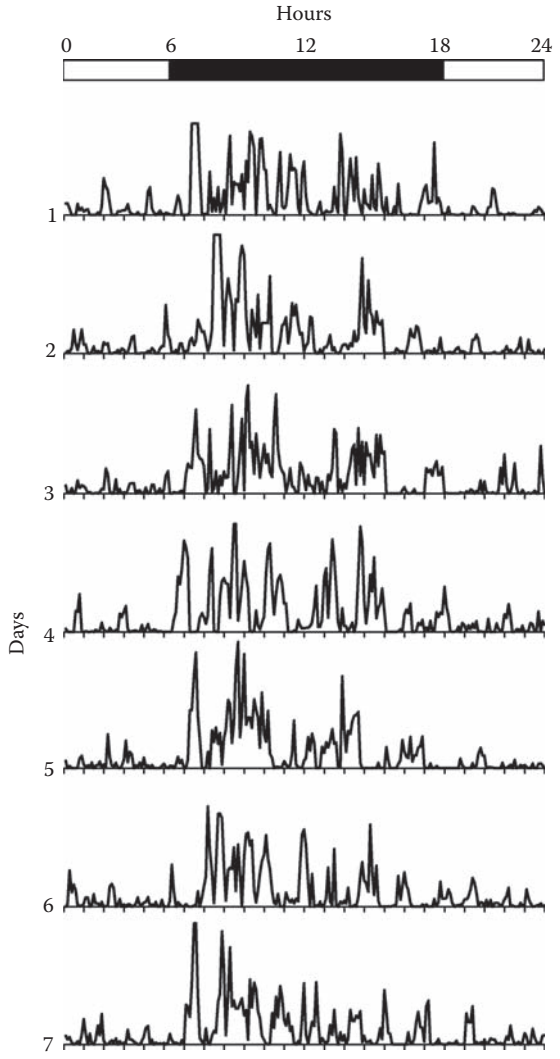


FIGURE 9.27 Golden hamsters are nocturnal animals. This actogram-style plot of a 7-day section of the locomotor activity records of a golden hamster (*Mesocricetus auratus*) shows that golden hamsters are clearly nocturnal and exhibit very little activity during the light phase of the light–dark cycle. 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). (Source: Archives of the Refinetti lab.)

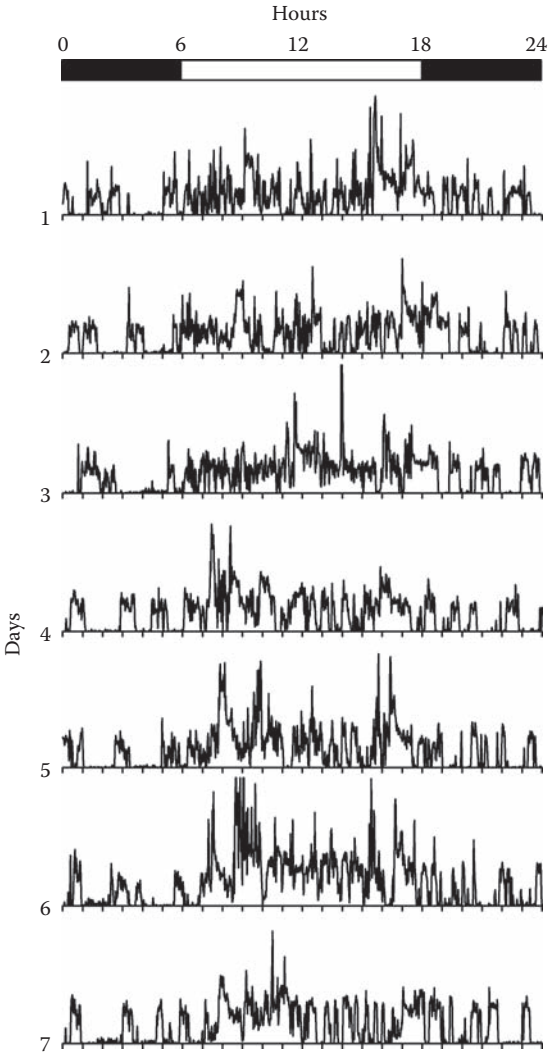


FIGURE 9.28 Horses are diurnal animals — or are they not? This actogram-style plot of a 7-day section of the locomotor activity records of a horse (*Equus caballus*) shows that horses are slightly more active during the light phase of the light–dark cycle, but some activity is evident throughout the day. Activity was recorded at 1-minute intervals with a data logger. The animal was kept in an individual stall under a natural light–dark cycle (indicated by the horizontal bar at the top). (Source: Archives of the Refinetti lab.)

animals do not fill either space preferentially — so that their rhythms are less robust.[36,157,158] 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.[159]

Even in species that exhibit clearly diurnal or clearly nocturnal activity patterns, the distinction between diurnality and nocturnality is not always straightforward. For example, in goldfish (*Carassius auratus*), about 80% of individuals tested in the laboratory are diurnal, while 10% are nocturnal, and 10% are just "messy."[160] In carpenter ants (*Camponotus compressus*), approximately 70% of individually tested animals are nocturnal, while 30% are diurnal.[161] Similarly, in subterranean molerats of various species, some members of the species are diurnal and some are nocturnal.[107,162] Although inter-individual differ-

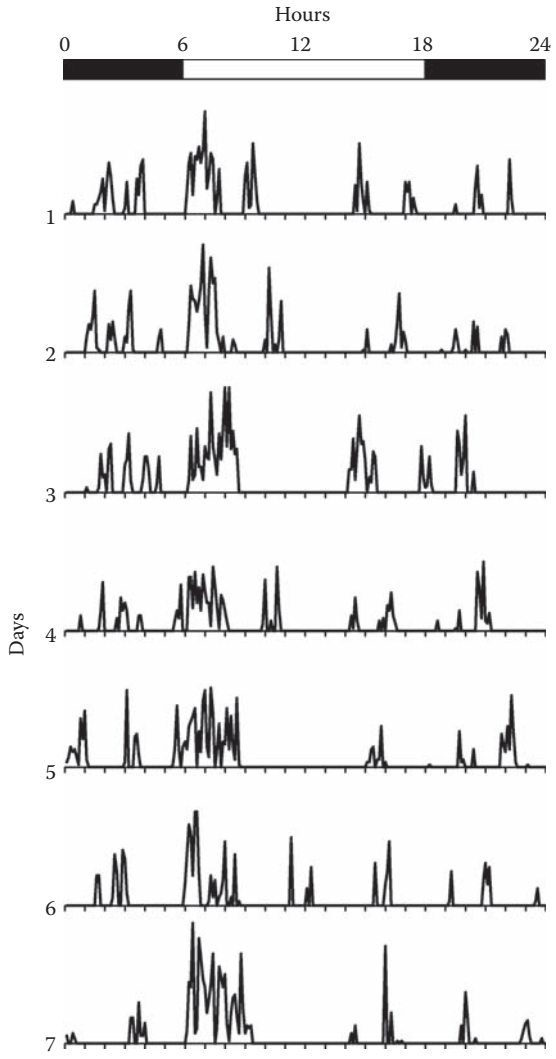


FIGURE 9.29 Mongolian gerbils are … This actogram-style plot of a 7-day section of the locomotor activity records of a Mongolian gerbil (*Meriones unguiculatus*) shows that gerbils are not clearly nocturnal or diurnal. Activity was monitored in a running wheel and recorded at 6-minute intervals. The 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. (Source: Archives of the Refinetti lab.)

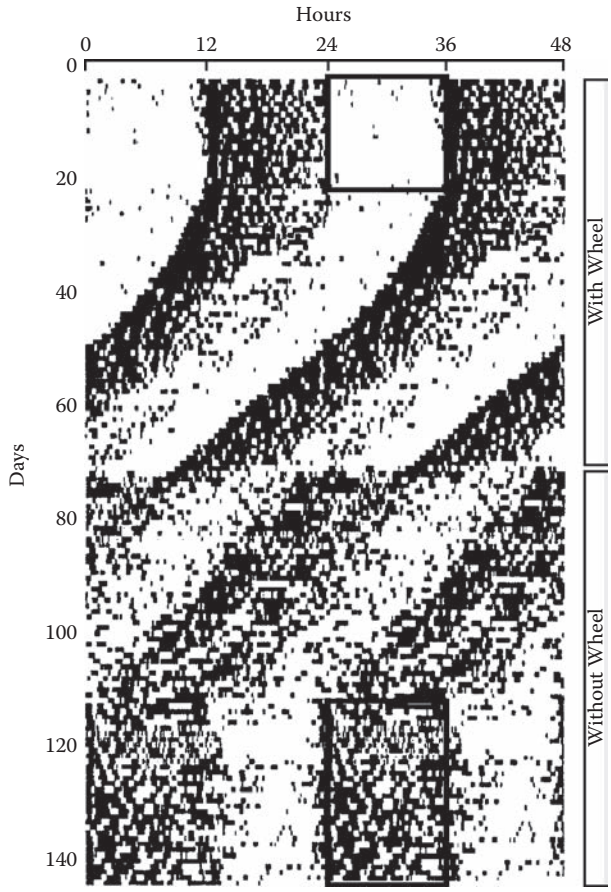


FIGURE 9.30 Degus can be diurnal or nocturnal. This double-plotted actogram shows the rhythm of body temperature of a degu (*Octodon degus*) maintained in constant darkness or under an LD 12:12 cycle (as indicated by the single-plotted rectangles). Plotted time bins correspond to values of body temperature above a 72-hour running mean; values below the mean are not plotted. Note 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. (Source: Adapted from Kas, M. J. H. & Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.)

ences 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. The mechanism also must be quite flexible — because, in some species, the same individual may be diurnal under some circumstances and nocturnal under other circumstances. For example, wolves (*Canis lupus*) are normally nocturnal; however, when traveling over long distances, they move during the day.[163] Conversely, migratory birds are normally diurnal, but they do most of their migratory flight at night.[164,165]

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,[166] can instantly become nocturnal in the laboratory simply by gaining access to a running wheel.[167] Figure 9.30 provides an example. 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

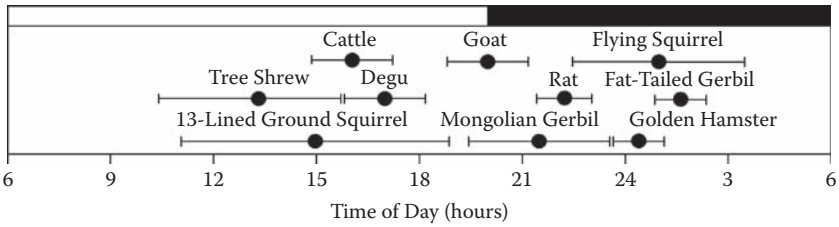


FIGURE 9.31 Where is your acrophase? The graph shows the mean acrophase (and 99% confidence interval) of the body temperature rhythm of 10 mammalian species maintained under similar environmental conditions (ambient temperature around 24°C, summer-like photoperiod). The thick horizontal bar at the top 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. (Sources: Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70; 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; Piccione, G., Caola, G. & 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; Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.)

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.[167] A similar phenomenon — but limited to only a subgroup of animals — has been reported in the Nile grass rat (*Arvicanthis niloticus*).[168] As previously discussed in Chapter 5, the grass rats 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.[169,170] A third research team observed increased crepuscular activity in animals housed with wheels, but the activity pattern was still predominantly diurnal.[171]

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, while golden spiny mice are diurnal. However, if the common spiny mice are removed from the area, the golden spiny mice become nocturnal,[172] suggesting that the golden spiny mice are normally pushed into the diurnal niche by the competition for resources. When golden spiny mice are trapped in the field and immediately tested individually in the laboratory, they exhibit a nocturnal pattern of activity.[173] 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.

What makes diurnal animals behave differently from nocturnal animals remains a mystery. 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 seen 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. Consider Figure 9.31, which shows the acrophases (times of the daily peak) of the body temperature rhythms of ten mammalian species, as 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 that the acrophases of diurnal animals are concentrated in the second half of the light phase, while 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 to be 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.

Aschoff suggested that nocturnal animals tend to have free-running periods shorter than 24 hours, while diurnal animals tend to have free-running periods longer than 24 hours.[174] However, the comparison of free-running periods (τ) of 50 species (Figure 9.32) reveals no significant difference between diurnal and nocturnal animals. Also, no

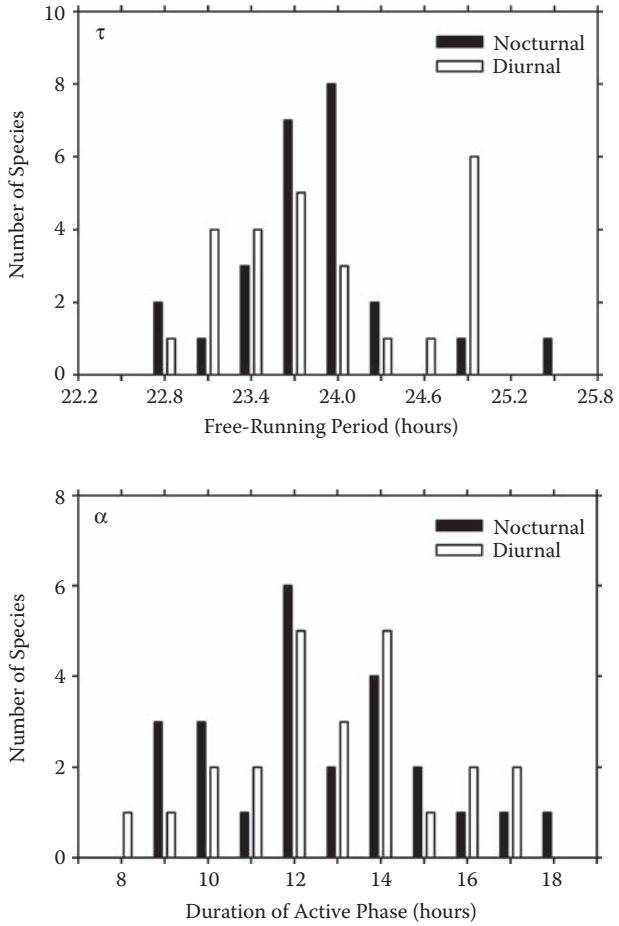


FIGURE 9.32 Free-running periods and duration of active phase of diurnal and nocturnal organisms. The graphs show the frequency distributions of free-running periods (τ) and durations of the active phase (α) of many nocturnal and diurnal species as reported in the literature. The graphs include vertebrate and invertebrate animals, as well as nonanimals. Kolmogorov-Smirnov tests reveal no significant difference between the distributions of diurnal and nocturnal organisms for τ ($D = 0.167$, $p > 0.05$) or α ($D = 0.113$, $p > 0.05$). (Sources: See references 5, 13, 23, 34, 39, 104, 106, 107, 160, 167, 185, 187, 194, 202, 208, 233, 234, 236, 245, 287, 291, 307, 321, 323, 324, 382, 388, 413–501, 501 in the *Literature Cited* section.)

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 generally have found that no clear difference exists between diurnal and nocturnal organisms except for the obvious difference in the phase angles of entrainment.[175–180] 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 9.33). 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.[181–183]

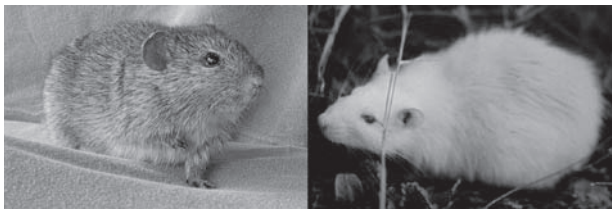


FIGURE 9.33 Furry friends. Some rodents, such as the Nile grass rat (left), are active during the day; others, such as the laboratory rat (right) are active at night. (Source: Photographs by R. Refinetti.)

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 9.34 shows the photic PRCs for the domestic mouse (which is nocturnal) and the Nile grass rat. In the top panel, phase shifts are plotted as a function of hours since the onset of activity. The two curves are very different, confirming that the mouse is nocturnal and the grass rat is diurnal. In the bottom panel, 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 appear very similar. The mouse attains larger phase delays than does the grass rat, but the general characteristics of the curves are essentially identical. Figure 9.35 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 9.34, the PRC for the diurnal Nile grass rat has a dead zone during subjective night and a shallower phase-delay region than 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.[184,185] Studies of PRCs for *nonphotic* resetting have also failed to identify any fundamental difference between nocturnal and diurnal animals.[186,187]

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 two recent studies in Nile grass rats suggest that there may be differences in the operation of the pacemaker during subjective day.[188,189] Because the differences are small and have been identified in the comparison of only two species (the Nile grass rat and the golden hamster), further studies are necessary before any generalizations can be made.

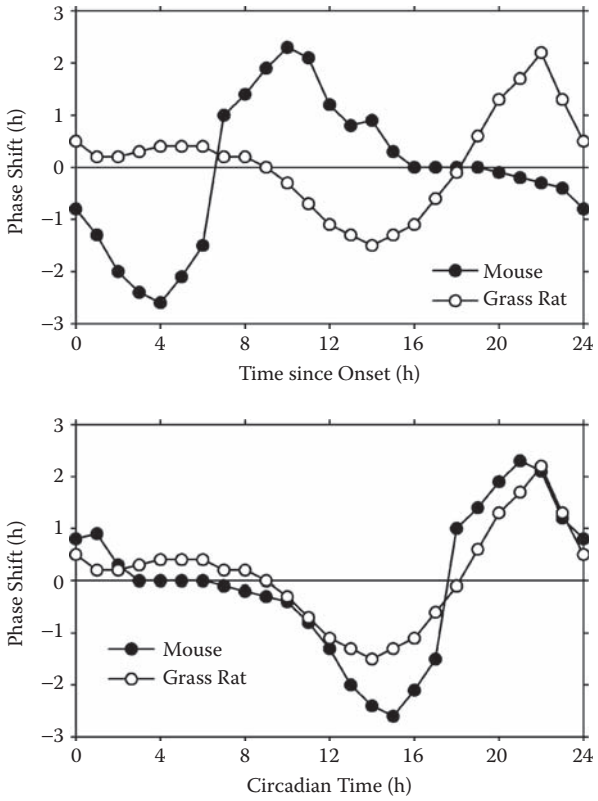


FIGURE 9.34 Timing of light-induced phase shifts in a diurnal and nocturnal rodent. If the phase-response curves (PRCs) for photic resetting of the diurnal Nile grass rat (*Arvicanthis niloticus*) and the nocturnal domestic mouse (*Mus musculus*) are plotted according to hours since activity onset, the curves reflect the difference in activity timing of the two species (top). However, if the PRCs are plotted according to circadian time (with activity onset represented by CT 0 for diurnal animals and CT 12 for nocturnal animals), the two curves are almost identical (bottom). (Sources: 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; 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.)

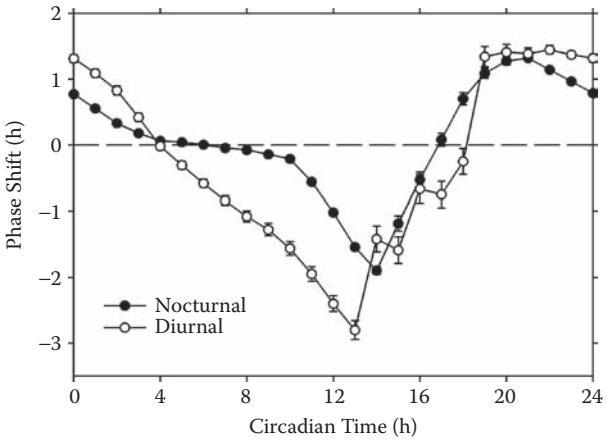


FIGURE 9.35 Timing of light-induced phase shifts in diurnal and nocturnal organisms. The graph shows the average photic phase-response curve (PRC) for 51 species; species are organized into diurnal and nocturnal groups. The graph includes vertebrate and invertebrate animals, as well as nonanimals. The 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. Note that the main difference between the two average PRCs is the absence of a "dead zone" during subjective day (CT 4 through CT 10) in the PRC for diurnal organisms. (Sources: See references 169, 184, 185, 187, 196, 234, 290, 308, 321, 382, 421, 425, 433, 457, 464, 476, 478, 483, 489, 491, 497, 498, 500, 502–524 in the *Literature Cited* section.)

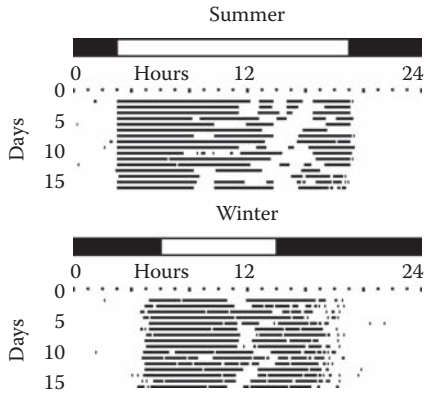


FIGURE 9.36 Like summer, like winter. The actograms show the running-wheel activity rhythms of a Nile grass rat (*Arvicanthis niloticus*) maintained under a summer-like long photoperiod (LD 16:8) and under a winter-like short photoperiod (LD 8:16). The horizontal bars above the actograms indicate the durations of the light and dark phases of the prevailing light–dark cycles. Note that activity occurs for several hours into the night under the winter-like photoperiod. (Source: Archives of the Refinetti lab.)

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 9.36, 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 (top panel), as its activity is almost entirely concentrated in the light phase of the light–dark cycle. However, in the winter (bottom panel), the light phase is so short that, even though the daily duration of activity is compressed, considerable activity takes place during the dark phase.

The duration of photic stimulation received by an organism each day depends on the available hours of light. However, in many cases, organisms are exposed to only a small fraction of the available light. For example, most

humans in urban areas stay indoors most of the time and are exposed to bright light for only a few hours each day. In one study, ten people in San Diego (California) were asked to wear photocells on the forehead continuously, so that 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.[190] 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) were exposed to about 2.5 hours of sunlight in the summer but less than half an hour in the winter.[191] Written questionnaires given to 500 volunteers in Germany and Switzerland indicated that 90 minutes were spent outdoors on workdays and about 4 hours on weekends.[192]

Nocturnal animals, by necessity, are exposed to very little light each day. As discussed in Chapter 7, this lack of light does not adversely affect 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. Many small rodents spend the daylight hours in burrows or nests that shield them from light. Thus, the typical pattern of entrainment of nocturnal rodents found in the laboratory (Panel A in Figure 9.37) can be expected *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 occasional exposure to light would result in a zigzagging pattern of activity consistent with entrainment by frequency demultiplication (Panel B in Figure 9.37). Another possibility is that the animals actually wake up well before dusk and sample the outside illumination several times, but they do not leave the burrow until after sunset. This activity pattern would result in a pattern 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 study 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. Figure 9.38 shows representative results from a study conducted with domestic mice. 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.[170] Only minor disturbances of the activity pattern were observed in studies on golden hamsters.[193–195]

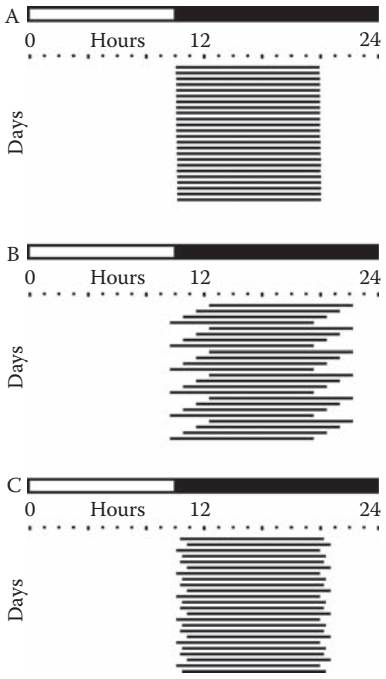


FIGURE 9.37 Hiding from light. These actogram-like diagrams depict three potential patterns of entrainment for a nocturnal animal. Standard entrainment in the presence of an inescapable light–dark cycle is shown in A. When the light–dark cycle can be mostly avoided by use of a shelter, entrainment may be attained by frequency demultiplication (B) or by a slightly disrupted standard process (C), depending on how often the animal exposes itself to light.

These findings seem to suggest that the hypothetical pattern depicted in Panel C of Figure 9.37 is closer to reality than the one depicted in Panel B. Results from a study on flying squirrels (*Glaucomys volans*) often have been presented as evidence of entrainment by frequency demultiplication (the pattern in Panel B),[196] but the data do not justify the claims. As shown in Figure 9.39, 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.

The small effect of nest-box availability may be combined with the effect of changes in photoperiod. Figure 9.40 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. Frequent light sampling clearly seems to provide 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 9.41, mice enter and exit the nest box repeatedly throughout the night and also during the day. Although they spend more time inside

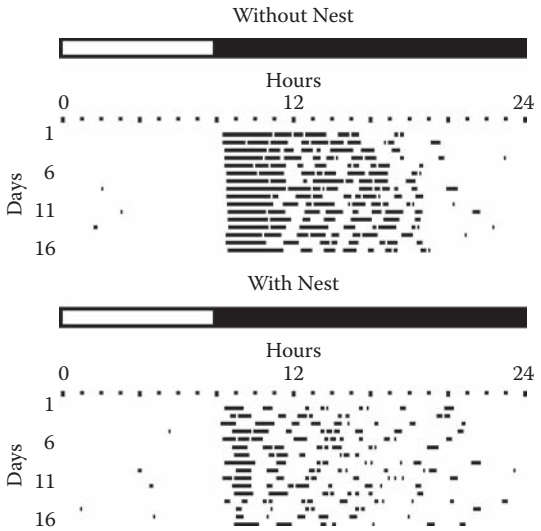


FIGURE 9.38 No zigzagging: mouse. The actograms show the running-wheel activity rhythms of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle (LD 8:16) with and without access to a light-tight nest box. When the nest box is available, the daily onsets of activity are slightly less regular and the running pattern is sparser. However, onsets do not zigzag, as is characteristic of entrainment by frequency demultiplication. (Source: Adapted from Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a semi-natural environment. *Physiology and Behavior* 82: 285–294.)

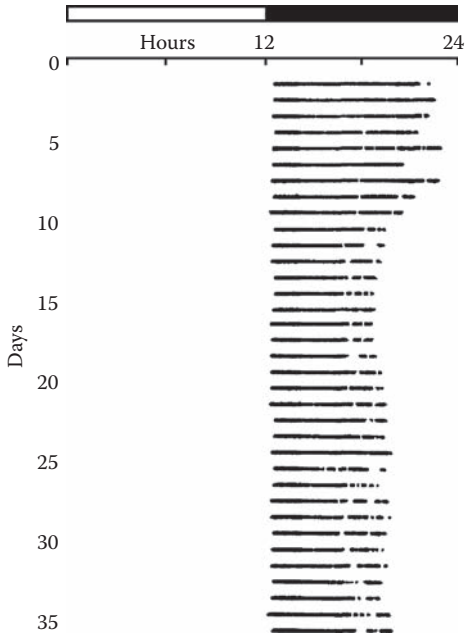


FIGURE 9.39 No zigzagging: flying squirrel. The actogram shows the running-wheel activity rhythm of a flying squirrel (*Glaucomys volans*) maintained under a light–dark cycle (LD 12:12) with access to a light-tight nest box. The daily onsets of activity are very regular and do not exhibit the zigzagging pattern characteristic of entrainment by frequency demultiplication. (Source: Adapted from DeCoursey, P. J. (1986). Light-sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.)

the nest box during the day than during the night, they are constantly sampling the light outside (Figure 9.42, left panel). The reverse is true for the diurnal Nile grass rat (right panel). Extensive light sampling has also been observed in bats[197] and dunnarts.[198]

As discussed in 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 remaining interval of 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.

It has been reported that the European ground squirrel (*Spermophilus citellus*) restricts its above-ground activity to a few hours in the middle of the day, thus avoiding photic stimulation at dawn *and* dusk.[199,200] A diagram depicting the activity pattern under long and short photoperiods is shown in Figure 9.43 (left panels). For comparison, the figure also shows diagrams for the Nile grass rat — which, like other diurnal rodents,[133,166,201] is above ground throughout the light phase (right panels). Under a long photoperiod at least, the European ground squirrel seems to not experience either dawn or dusk, which should prevent it from attaining entrainment. The explanation for this apparent anomaly can be found in 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, which means that α is not a good estimator of subjective day in this species. If the 3 missing hours occur 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. Only small differences have been found,[32,194] so I consider the issue to be unresolved. The range of entrainment (for zeitgeber periods different from

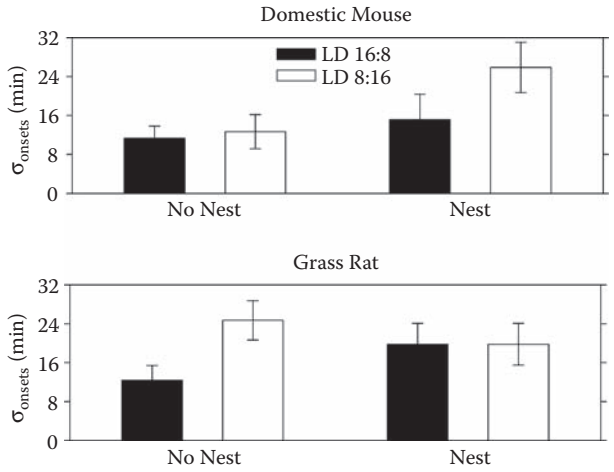


FIGURE 9.40 Variability of onsets. The graphs show the mean variability of onsets (standard deviation of ten consecutive daily onsets) for groups of domestic mice (*Mus musculus*) and Nile grass rats (*Arvicanthis niloticus*) with and without access to light-tight nest boxes. The bars represent the means (± 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 seen less clearly. (Source: Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a semi-natural environment. *Physiology and Behavior* 82: 285–294.)

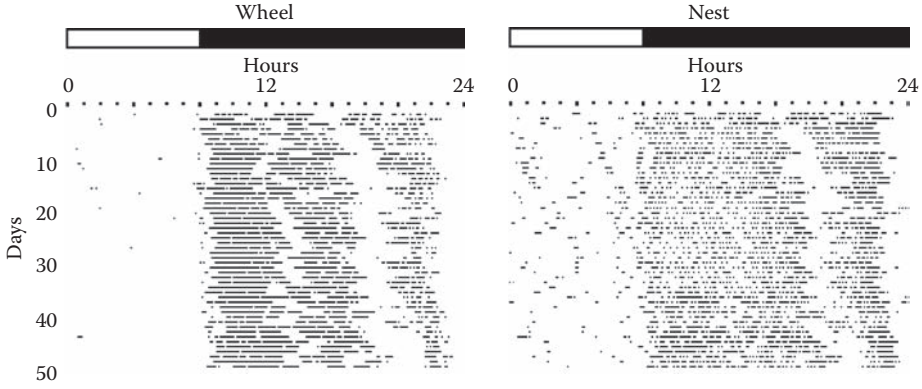


FIGURE 9.41 Lots of light probing. The actograms show the rhythms of running-wheel activity and nest-box visitation of a domestic mouse (*Mus musculus*) maintained under a light–dark cycle (LD 8:16) with access to a light-tight nest box. The animal visited the nest box quite often during the dark phase of the light–dark cycle, when it was most active in the running wheel. The animal moved in and out of the nest box less frequently during the light phase — but frequently enough to provide many occasions for light probing each day. (Source: Adapted from Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a semi-natural environment. *Physiology and Behavior* 82: 285–294.)

24 hours), however, seems to be about an hour or two wider when twilight-inclusive light–dark cycles are used, as compared with standard square-wave cycles.[195,202,203]

One aspect of photic stimulation that is rarely 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.[204–208] Nocturnal rodents turn the lights on only occasionally and essentially remain in darkness.[204–206] Figure 9.44 provides an example. 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 after that. 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

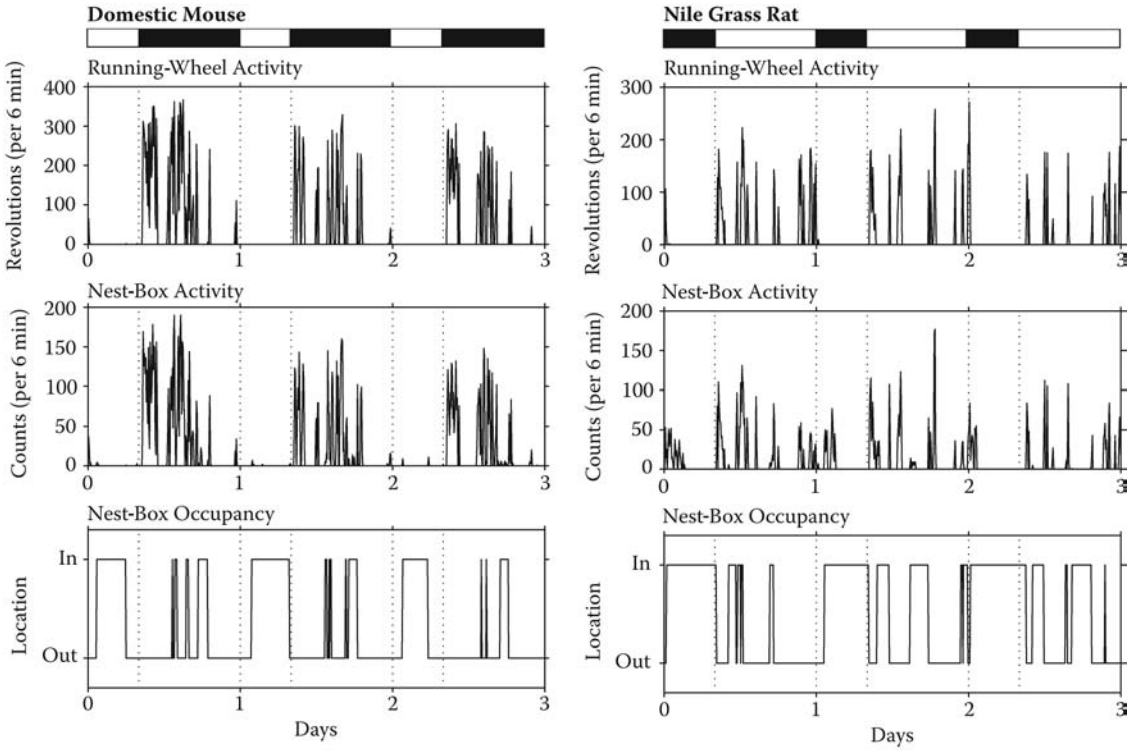


FIGURE 9.42 Staying home. The graphs show 3-day segments of the records of running-wheel activity, movement in and out of the nest box, and nest-box occupancy of a domestic mouse (*Mus musculus*) and a Nile grass rat (*Arvicanthis niloticus*). Both animals were maintained under a light–dark cycle (LD 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. (Source: Archives of the Refinetti lab.)

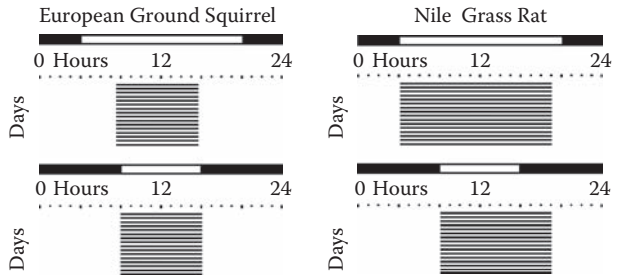


FIGURE 9.43 The difference between ground squirrels and grass rats. These actogram-type diagrams illustrate the different patterns of activity described for the European ground squirrel (*Spermophilus citellus*) in a natural environment and for the Nile grass rat (*Arvicanthis niloticus*) in a seminatural laboratory environment. While 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.

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 choose to expose themselves to light occasionally, but no consistent effect on the circadian system results from it.

9.2.4 Environmental Conflicts

The investigation of the combined effects of multiple environmental stimuli on the operation of the circadian system is a new frontier in the experimental study of circadian rhythms. Previous chapters showed 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, they did not discuss more than one variable at a time. To understand the behavior of animals (including humans) in the real world, one 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.[209] Many laboratory studies have shown, for example, that thermal comfort[210–212]

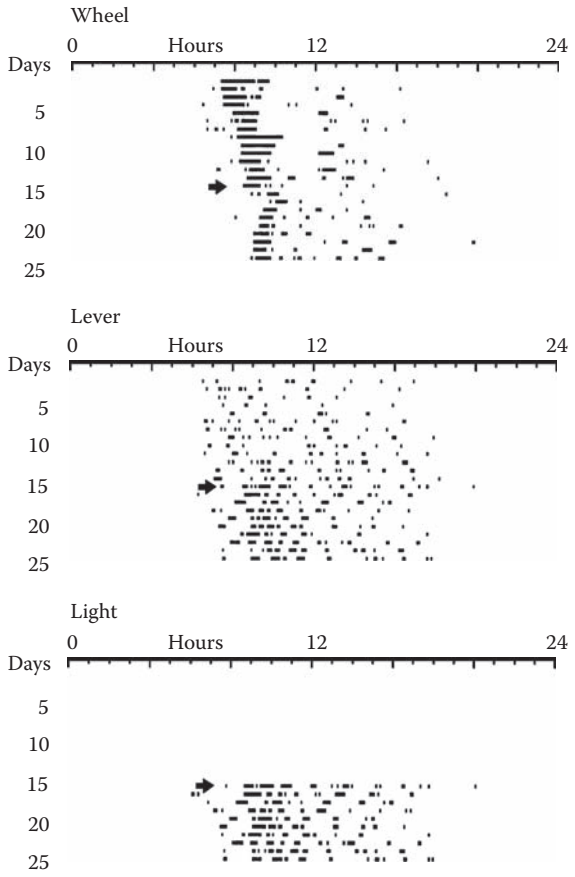


FIGURE 9.44 Self-selected illumination. The actograms show the rhythms of running-wheel activity, lever pressing, and light exposure of a golden hamster (*Mesocricetus auratus*) maintained in constant darkness and allowed to turn on lights (50 lux) by pressing the lever. For the first 2 weeks, lever pressing did not activate the lights. Starting on Day 15 (arrows), the lights were on whenever the hamster pressed the lever and held it down. Note 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. (Source: Adapted from Refinetti, R. (1997). Circadian rhythm of self-exposure to light in the golden hamster. *Behavioural Processes* 40: 107–111.)

or even food[213–215] 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[216,217] or thermal comfort and food availability.[218–222] None of these studies, however, has addressed the issue of entrainment. One study in golden hamsters showed that the animals 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.[223] The field is wide open for new investigations, and I am certainly one of those who look forward to exploring it in the very near future.

9.3 LIFETIME CHANGES

The characteristics of circadian rhythms discussed in this and previous chapters pertained to generic adult organisms. However, life consists of more than adulthood. At the minimum, development and aging must be considered. Also, circadian rhythms in adulthood are affected by reproductive and seasonal cycles. In addition, circadian processes in adult organisms potentially can be modified by experience (*learning*). This section of the book discusses these issues. It also addresses the issue of sexual dimorphism in circadian rhythms.

9.3.1 Intra- and Inter-Individual Variability

Basic research on circadian rhythms, like basic research on other physiological processes, is 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 limited to the statistical requirement of establishing the significance of differences between means. This practice rests on the assumption that intra-individual and inter-individual variabilities are numerically equivalent. However, studies that investigated intra- and inter-individual variabilities in various physiological processes revealed that the two variabilities are often different.[224–228] This difference in variabilities has important implications for the management of individual needs, such as in clinical practice in veterinary or human medicine. If inter-individual variability is greater than intra-individual variability, treatments designed for an "average" patient may turn out to be too weak — or too strong — for a patient who is not "average." If intra-individual variability is greater than inter-individual variability, however, 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.

Few researchers have compared intra- and inter-individual variabilities of circadian rhythms. I am aware of only three studies that conducted such comparison for the rhythms of body temperature,[229] melatonin secretion,[230] and cortisol secretion.[231] A fourth 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.[232] This finding indicates extreme inter-individual 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 9.45 summarizes the results from a well-controlled study of the body temperature rhythm in animals. To avoid the potential bias of selection of an idiosyncratic species, four different species were used: the laboratory rat, the thirteen-lined ground squirrel, the domestic dog, and the horse. All animals exhibited daily

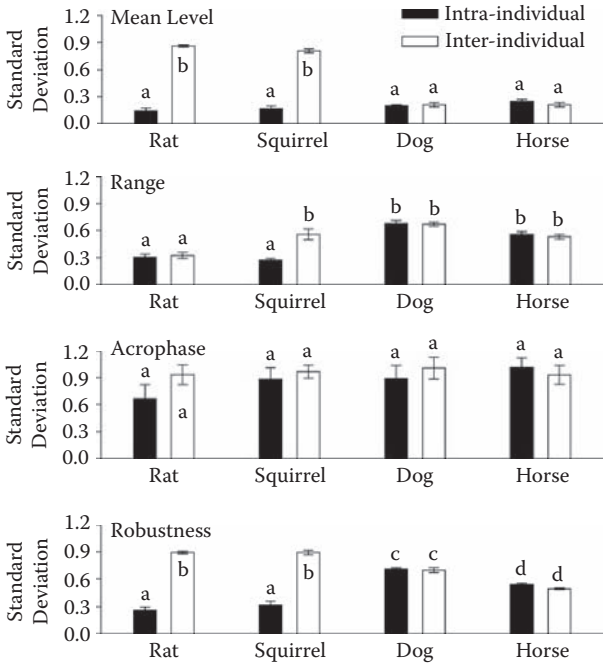


FIGURE 9.45 Intra- and inter-individual variability: parameters of body temperature rhythm. The graphs compare the intra-individual and inter-individual variabilities of four parameters of the body temperature rhythm of laboratory rats, thirteen-lined ground squirrels, beagles, and British thoroughbred 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, b, etc.) are not significantly different from each other. (Source: Refinetti, R. & 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.)

rhythmicity. Intra- and inter-individual variabilities were computed for the mean level, range of oscillation, acrophase, and robustness of the rhythm. Note that the variabilities differ in different parameters of the rhythm and in different species but that — whenever there is a difference between inter-individual variability and intra-individual 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. Inter-individual variability is consistently larger than intra-individual 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 — inter-individual variability is statistically indistinguishable from intra-individual variability in all four species. This fact justifies the practice of ignoring individual differences.

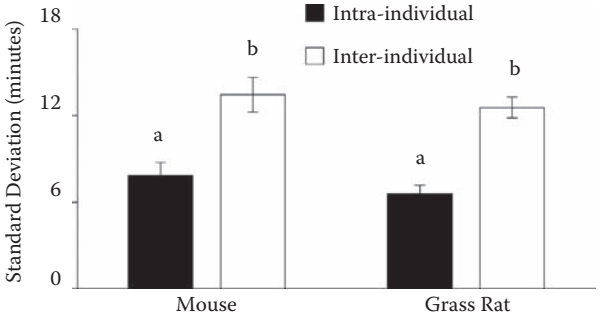


FIGURE 9.46 Intra- and inter-individual variability: free-running period. The graph compares the intra-individual and inter-individual variabilities of the free-running period of laboratory mice and Nile grass rats, as determined by analysis of the running-wheel activity rhythm in constant darkness. All computations of variability (standard deviation of the mean) are based on measurements conducted on seven individuals for seven consecutive blocks of 10 days. The bars show the mean variability (± SE) for each condition. Means sharing the same letter (a or b) are not significantly different from each other. Note that intra-individual variability is smaller than inter-individual variability both in mice and grass rats. (Source: Archives of the Refinetti lab.)

A few investigators also have examined intra- and inter-individual variabilities of the free-running period of the activity rhythm. They found that intra-individual variability was consistently smaller than inter-individual variability,[233–236] as seen in Figure 9.46 for the domestic mouse and the Nile grass rat. In absolute values, a standard deviation of 6 to 8 minutes (intra-individual 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 9.47). Circadian rhythms, like many other processes, must undergo *development*. Developmental processes are controlled both by genetic inheritance (*nature*) and environmental factors (*nurture*), although, as discussed in Chapter 6, the free-running period exhibited by adult rodents does not depend on the period of the light–dark cycle experienced during development.[237,238] In this case, development 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.[239–243] Fruit flies also display rhythmicity despite being raised in constant darkness or constant light, although the lighting conditions during development may affect the free-running periods of the adults in a more complex way.[244,245]

FIGURE 9.47 It's a girl! Babies are born with immature circadian systems. Development of mature circadian rhythms requires many months. (Source: Photograph by R. Refinetti.)

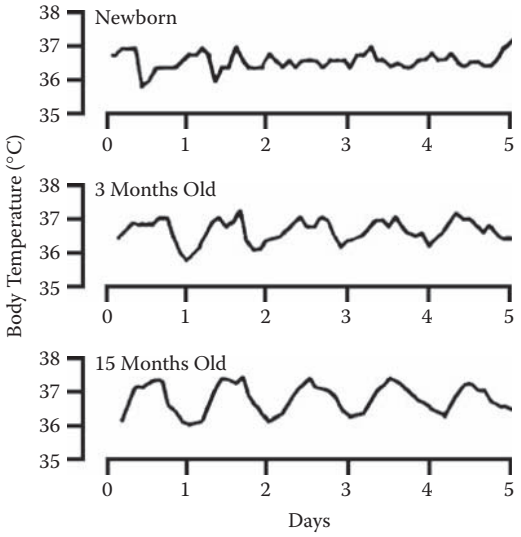


FIGURE 9.48 Maturation of body temperature rhythm in human infants. The graphs show 5-day segments of the body temperature records of human infants at three stages of development of daily rhythmicity. The adult amplitude of the rhythm (1°C) is attained at approximately 15 months of age. (Source: Adapted from Kleitman, N., Titelbaum, S. & Hoffmann, H. (1937). The establishment of the diurnal temperature cycle. *American Journal of Physiology* 119: 48–54.)

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. For example, researchers know that newborn human babies do not have a daily rhythm of melatonin secretion. Rhythmicity is not apparent until 3 months of age.[246,247] Human babies also do not have a rhythm of body temperature. As shown in Figure 9.48, 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.[248,249] 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.[250] Also questionable were reports that daily rhythmicity of activity can be observed as early as a few weeks after birth.[251,252] In the latter studies, activity was recorded by actigraphs worn by the babies, which means that the activity patterns of parents and hospital staff caring for the babies were reflected in the recordings. In other words, the actigraphs correctly detected daily rhythmicity of activity, but the recorded activity was most likely that of adults handling the newborns rather than that of the newborns themselves. Human babies do seem to exhibit daily rhythmicity in adrenal cortisol secretion at the time of birth.[253]

In rats, a rhythm of body temperature with a range of oscillation of 2 to 4°C is observed on the day after birth, but it seems to vanish by 15 to 20 days of age.[254–259] Incipient rhythmicity appears again at 25 days of age and attains the adult range (1.6°C) at 45 days of age,[260] which is approximately the age when the rhythm of corticosterone secretion reaches the adult pattern.[261] 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 probably is not a true precursor of the adult rhythm of body temperature but rather is a form of cold-induced torpor.[256,262] In rabbits, temperature rhythmicity can be observed as early as 4 or 5 days after birth in pups allowed to remain with the doe[263,264] but not in pups kept in isolation with continuous intra-esophageal feeding.[257] Daily rhythmicity of activity in rabbits can be detected 6 days after birth, although the adult pattern is not attained until 2 months later.[265]

Newborn calves (Figure 9.49) lack daily rhythmicity of body temperature. Daily rhythms comparable with those of adults are not observed until 2 months after birth.[266] One research group reported the presence of rhythmicity 2 weeks after birth,[267] but the calves they studied 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 9.50), 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 and foals also develop daily rhythms of body temperature during early life, although adult rhythms seem to be attained earlier than in calves.[268] As shown in Figure 9.51, a stable dusk–dawn difference is achieved about 1 month after birth. Evidently, different species

FIGURE 9.49 My mom is a cow! Minutes after giving birth, a young cow attends to her newborn calf. (Source: Photograph by Scott Bauer, Agricultural Research Service, U.S. Department of Agriculture.)

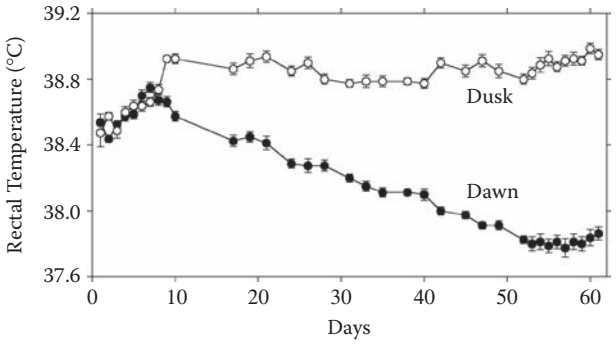


FIGURE 9.50 Maturation of body temperature rhythm in bovine infants. The graph shows the mean rectal temperature (± SE) of eight calves (*Bos taurus*), as measured at dawn and dusk for 2 months following birth. Day 0 is the day of birth. The adult amplitude of the rhythm (1°C) is attained at approximately 50 days of age. (Source: Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.)

develop the body temperature rhythm at different rates. The same is true for other rhythms. For example, the rhythm of melatonin secretion is present immediately after birth in seals,[269] 2 weeks after birth in hamsters and rats,[270] and 3 months after birth in humans.[246,247] The delay in the expression of rhythms is most likely due to the development of mechanisms downstream from the circadian pacemaker. In rats and sheep, the pacemaker has been shown to already be oscillating before birth![271–275] The circadian clock is already functional during embryonic development in zebrafish as well.[276]

Development progresses all the way through adolescence, and a few studies have examined changes in the circadian system in this time period. In humans, children

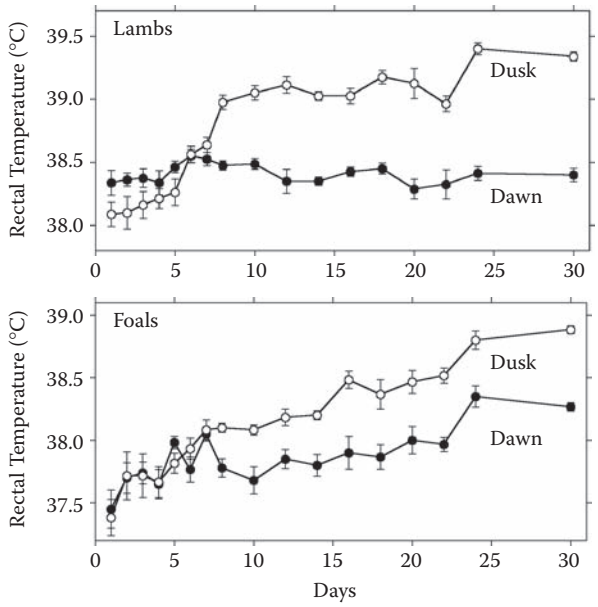


FIGURE 9.51 Maturation of body temperature rhythm in ovine and equine infants. The graphs show the mean rectal temperature (± SE) of eight lambs (*Ovis aries*) and six foals (*Equus caballus*), as measured at dawn and dusk for a month following birth. Day 0 is the day of birth. The adult amplitudes of the rhythms are attained at the end of the first month of life. (Source: Piccione, G., Caola, G., & Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.)

tend to prefer activities early in the day (at least earlier than most adults), and this preference does not vanish until the ninth or tenth grade (14 years of age).[277–279] 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[280] (which corresponds to approximately 18 years of age in humans).

9.3.3 Rhythmic Interactions

Chapter 4 described various ultradian and infradian rhythms. This section addresses the interactions that potentially can occur between ultradian/infradian rhythms and circadian rhythms. The discussion starts with *tidal* rhythms, about which relatively little is known. 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.[281–284] Figure 9.52 shows one example. 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

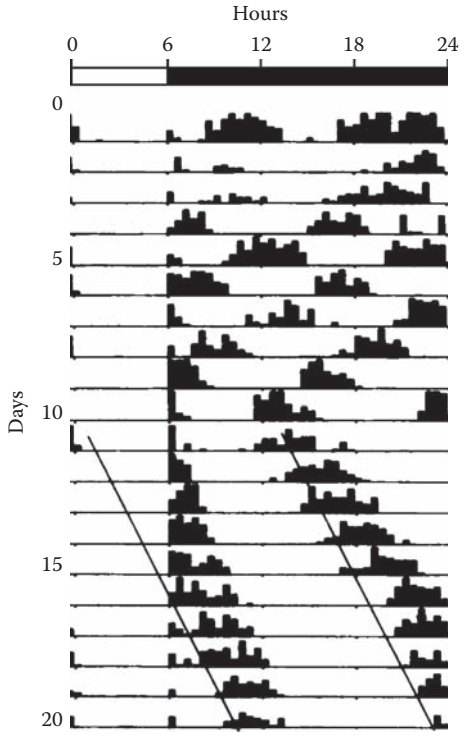


FIGURE 9.52 Circadian or circatidal rhythm? This actogram shows the rhythm of swimming activity of a zooplankton (*Dimorphostylis asiatica*) maintained under a 24-hour light–dark cycle (as indicated by the horizontal white and dark bar at the top). Starting on Day 11, a 12.5-hour cycle of hydrostatic pressure was superposed on the light–dark cycle. The diagonal lines indicate the times of maximum hydrostatic pressure. Note that the light–dark cycle produced masking only, while the cycle of hydrostatic pressure produced entrainment. (Source: Adapted from 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.)

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 it was expressed only during the dark phase of the light–dark cycle. This photic masking of activity occurred also 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,[282] simultaneous presence of both components,[285,286] alternation of circadian and circatidal components in the same individual,[284,287] and absence of both circadian and circatidal endogenous rhythmicity.[281] Some of the differences in results may be due to genuine differences between species, but many more studies are needed to elucidate the issue.

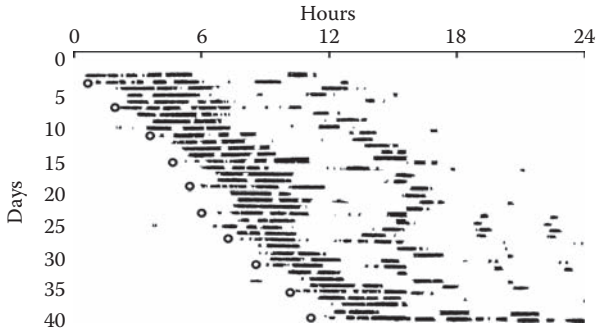


FIGURE 9.53 Dependence of estrous cycle on circadian rhythm in a mammal? This actogram shows the rhythm of running-wheel activity of a female golden hamster (*Mesocricetus auratus*) maintained under constant dim light. The circles denote the onsets of sexual receptivity (which happen every 4 days under a 24-hour light–dark cycle). Note that the estrous cycle freeruns with a period that is a quadruple multiple of the activity rhythm. (Source: Adapted from Fitzgerald, K. M. & Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences U.S.A.* 73: 2923–2927.)

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). In the late 1960s, John Alleva and colleagues at the Food and Drug Administration (in Washington, D.C.) decided to investigate whether estrous cycles, like circadian rhythms, would freerun in constant environmental conditions. Chapter 4 showed that estrous cycles had been studied for quite some time, and Alleva knew 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. Because 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. Alleva and his colleagues conducted the study[288] and found that the estrous cycle freeran when the animals were housed in constant light, but, to their surprise, they also observed that the estrous cycle freeran together 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,[289–293] and an example is shown in Figure 9.53. 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.[294–297] 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 the timing of follicle maturation seemed to be 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 9.53, 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 this difference great enough? No researcher would expect the free-running estrous period to be exactly 96.0 hours, just as no researcher would expect the circadian period to be exactly 24.0 hours. Is it possible that the idea that the estrous period is a multiple of the circadian period is 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 four-cycle multiple should have a period of 80 hours, which is considerably shorter than 96 hours. When this study was actually conducted, an estrous period of 100 hours was found.[298] Of course, 100 is a multiple of 20 — but it is a five-cycle multiple, not a four-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. For example, 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.[296,299–304] Thus, the notion that the circadian system gates the operation of the ovaries (through pituitary–hypothalamic hormones) is not controversial. 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.

A study on the common marmoset (*Callithrix jacchus*), whose estrous cycle — like that of humans — lasts 28 days, used light–dark cycles with periods of 23 or 26 hours. 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.[305] This finding is in direct contradiction with the 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.[306] Data from studies on fowl are far more dramatic. Figure 9.54 shows one example. A quail (*Coturnix coturnix*) was maintained first under a 24-hour light–dark cycle (top panel) and later in constant darkness (bottom panel). Oviposition (egg laying) and the feeding rhythm were monitored. Both rhythms were entrained by the light–dark cycle, but they freeran with visibly different periods in constant darkness. Such a difference in free-running periods can be explained only by the existence of two separate pacemakers. 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).[307] Similar results were obtained in other studies on quail,[308,309] chicken,[310,311] and turkey,[312] 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 9.55). In addition, endogenous circannual rhythms can affect the operation of circadian rhythms, and vice versa.

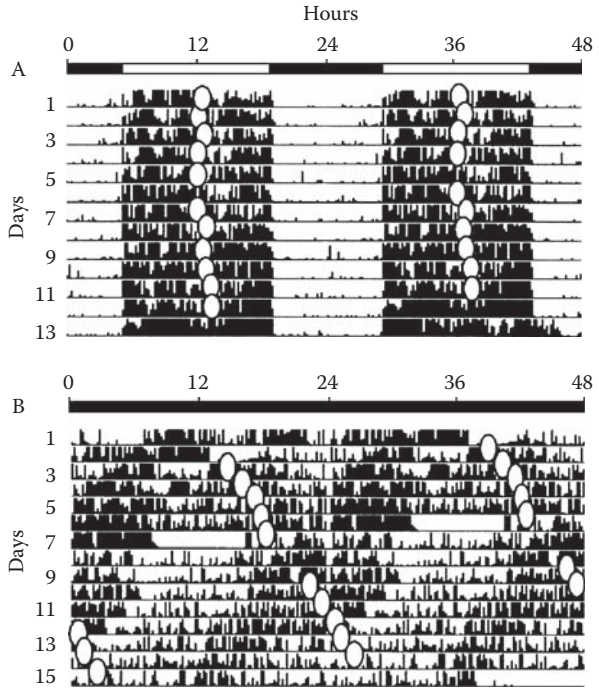


FIGURE 9.54 Independence of circadian rhythm and estrous cycle in a bird. These two double-plotted actograms show the rhythms of feeding activity and egg laying of quails (*Coturnix coturnix*) maintained under a light–dark cycle (A) or in constant darkness (B). The white ovals indicate the time of oviposition. Note that entrainment of both rhythms is apparent under the light–dark cycle (A), but the feeding rhythm freeruns with a period shorter than 24 hours while the egg-laying rhythm freeruns with a period longer than 24 hours in constant darkness (B). (Source: Adapted from Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.)

FIGURE 9.55 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. (Source: Yellowstone National Park Wildlife Graphics, U.S. National Park Service.)

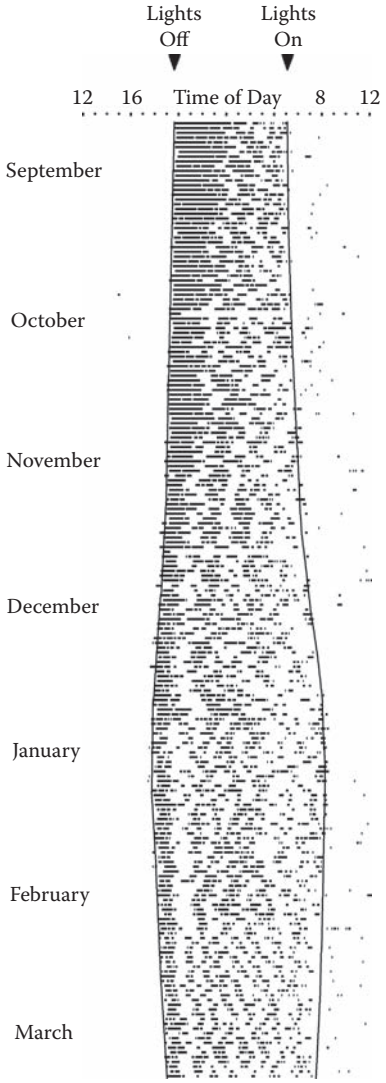


FIGURE 9.56 Following the seasons. This actogram shows the running-wheel activity rhythm of a domestic mouse (*Mus musculus*) subjected to a variable 24-hour light–dark cycle that simulated the natural variation in photoperiod at a latitude of 30° North. The vertical lines indicate the times of lights-off and lights-on. Note the gradual expansion of the active phase of the daily cycle (α) as the nights become longer in the winter. (Source: Archives of the Refinetti lab.)

Chapter 7 mentioned briefly that the seasonal variation in photoperiod causes a temporal compression or expansion of circadian rhythms. This compression or expansion has been observed in natural settings[313–318] as well as in the laboratory (where only the photoperiod is changed).[319–324] Figure 9.56 shows locomotor activity data from a mouse maintained in the laboratory under a simulated natural photoperiod. Note the gradual expansion of the daily active phase (α) as the nights get longer throughout the autumn and first part of winter. Note also that α is compressed as the nights get shorter in the late winter and early spring. Figure 9.57 shows waveform plots of the

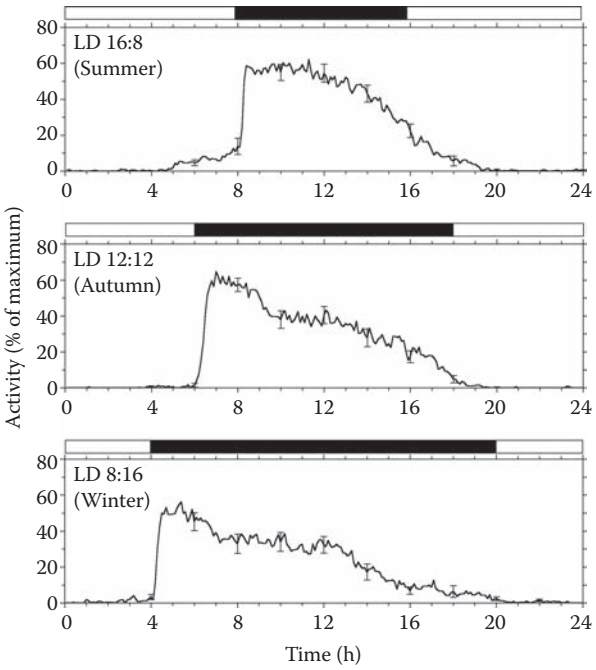


FIGURE 9.57 Modulation of α by photoperiod in mice. The graphs show the mean daily activity patterns of laboratory mice (*Mus musculus*) maintained under three different photoperiods. The curves reflect the means of 30 mice per group (averaged over 6 days for each animal) at 6-minute intervals. Standard errors (SE) are plotted in 2-hour intervals to prevent cluttering of the figures. Note 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). (Source: Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.)

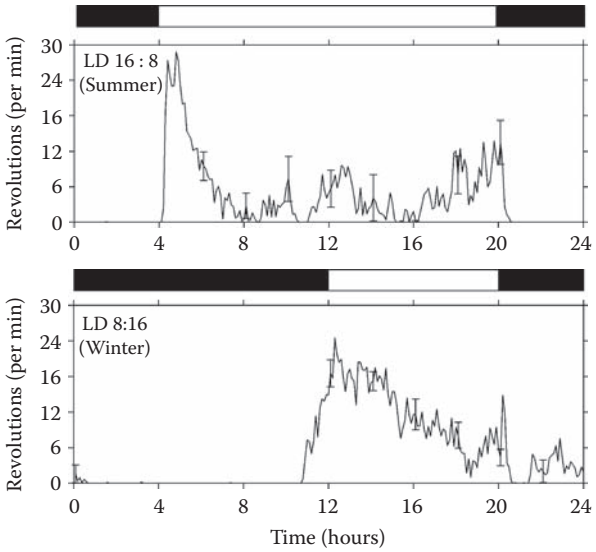


FIGURE 9.58 Modulation of α by photoperiod in grass rats. The graphs show the mean daily activity patterns of Nile grass rats (*Arvicanthis niloticus*) maintained under two different photoperiods, as indicated by the horizontal bars. The curves reflect the means of seven grass rats (averaged over 10 days for each animal) at 6-minute intervals. Standard errors (SE) are plotted in 2-hour intervals to prevent cluttering of the figures. Note the compression of the active phase of the daily cycle (α) when the photophase is short (lower panel). (Source: Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a semi-natural environment. *Physiology and Behavior* 82: 285–294.)

activity rhythms of groups of mice under simulated summer, autumn, and winter photoperiods. For comparison, waveform plots for the activity rhythms of Nile grass rats under simulated summer and winter photoperiods are shown in Figure 9.58. As nocturnal animals, mice expand α in the winter; as diurnal animals, grass rats compress α in the winter. Note that compression of α is not complete, so that mice are active for a few hours after dusk 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).

Compression and expansion of α could, however, be artifacts of laboratory conditions. This possibility was refuted by a study on sheep. Mouflon sheep (*Ovis musimon*) (Figure 9.59) were housed outdoors all year round, and their feeding rhythm was studied. As shown in Figure 9.60, 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, while summertime is associated with rhythm expansion in diurnal

FIGURE 9.59 Wild sheep. Mouflon sheep (*Ovis musimon*) are ruminants indigenous to southern Europe. (Source: Photograph courtesy of Rickey Hunt, Avery, TX.)

animals and rhythm compression in nocturnal animals. Badgers (*Meles meles*) seem to be an exception to this 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 that time of the year, so that the badgers must spend more time foraging.[325] Humans also can be considered an exception to the rule because

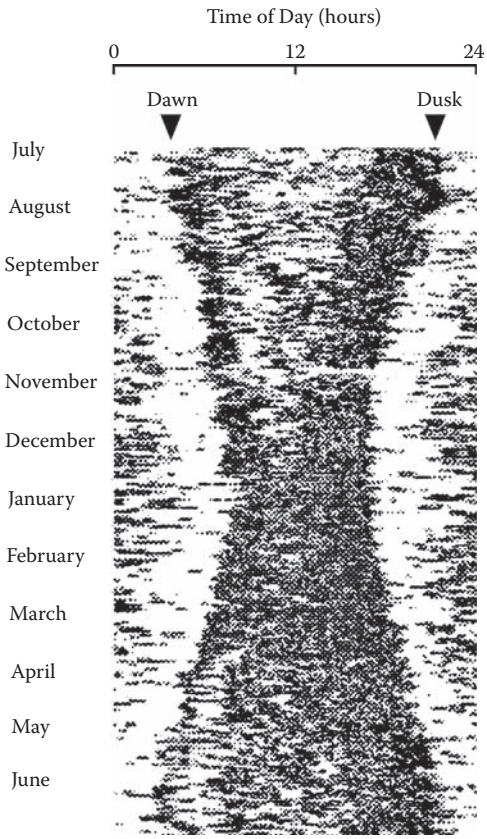


FIGURE 9.60 Modulation of α by photoperiod in mouflon sheep. This actogram shows the feeding rhythm of a mouflon sheep (*Ovis musimon*) maintained outdoors in Germany for a full year. Note the gradual contraction (and later expansion) of the feeding rhythm as the days become shorter in the winter (and longer again in the summer). (Source: Adapted from Berger, A., Scheibe, K. M., Michaelis, S. & 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.)

social work conventions force people to be active for the same number of hours each day regardless of season.[326,327]

Seasonal variations have also been documented in other parameters of circadian rhythms, such as phase, amplitude, and period.[91,173,328–336] An interesting seasonal modulation of rhythm *amplitude* is observed in beavers (*Castor canadensis*). During the winter, in Canada and the northern United States, beavers remain sequestered in their lodges or underneath the ice cover, so that their daily rhythm of activity is almost flat, while robust rhythmicity is present in the summer.[337–339] Seasonal modulation of the *phase* of circadian rhythms is exemplified by the activity pattern of the golden-mantled ground squirrel (*Spermophilus lateralis*), as shown in Figure 9.61. 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

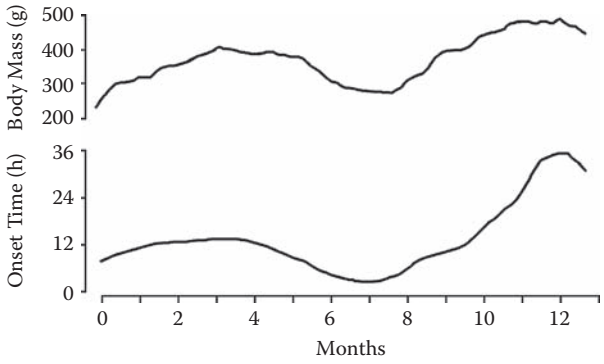


FIGURE 9.61 Circannual modulation of circadian period? The graphs show the annual oscillations of body mass and of circadian activity onset of a golden-mantled ground squirrel (*Spermophilus lateralis*) maintained in the laboratory under constant ambient temperature (22°C) and constant illumination (LL). Note that activity onset oscillates in synchrony with body mass, which suggests a possible circannual modulation of circadian period. (Source: Adapted from Mrosovsky, N., Boshes, M., Hallonquist, J. D. & Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.)

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 animal was the only one for which reliable activity data could be obtained.[329] Therefore, it is impossible to ascertain whether the conclusion can be generalized to other members of the species, much less to other species.

Annual rhythms affect circadian rhythms, but circadian rhythms can also affect annual rhythms. Perhaps the best example of this interaction is the circadian modulation of entry into and arousal from hibernation. Consider Figure 9.62. 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 tight, it is not random in three of the four variables. For shallow torpor, most entries occurred between midnight and 10 A.M., while 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., while the arousals were scattered all over the day. Studies in this and other species of hibernators agree 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. Some investigators have found that arousal is restricted to

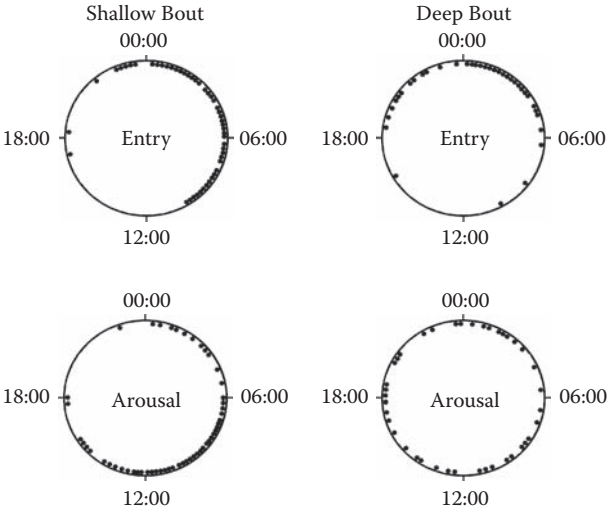


FIGURE 9.62 Circadian modulation of hibernation. The graphs show the distributions of the times of entry into and arousal from hibernation of eight European hamsters (*Cricetus cricetus*) maintained in the laboratory under simulated winter conditions (8°C, LD 8:16). The data are plotted separately for shallow and deep torpor bouts. Note that for entry into shallow or deep torpor, the distributions are more or less clustered around the late evening and early morning. (Source: Waßmer, T. & Wollnik, F. (1997). Timing of torpor bouts during hibernation in European hamsters. *Journal of Comparative Physiology B* 167: 270–279.)

a narrow segment of the day,[332,340–342] while others have not found this to be true.[333,343–345] Because the conflicting findings have been obtained in different species, they may be explained by species differences. The central question is whether the circadian system remains functional during hibernation, since a functional clock is required for the timing of arousal. Some researchers have observed circadian rhythmicity of body temperature (with very small amplitude) during hibernation,[328,332,342,346] while others have not observed rhythmicity.[91,333,344] A study of metabolic activity of various brain areas identified high activity in the site of the master circadian pacemaker during hibernation.[347] Thus, there is at least some evidence that the circadian system remains functional during hibernation.

9.3.4 Aging

Although most people do not like to think about it, everyone gets old and dies. 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 3 days. The fish *Notobranchius furzeri* (Figure 9.63) 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 age of 190 years. Most humans do not celebrate their

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	Lagomorth (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) and Primate Info Net (University of Wisconsin, Madison).

FIGURE 9.63 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. (Source: Photograph by Julian Haffegee, British Killfish Association.)

80th birthday. As shown in Figure 9.64, improvements in life style have raised human expected longevity in the last 1.5 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 people will be dead before 80 years of age.

Aging brings the decline of many functions well before death. One decline is in the frequency of sexual

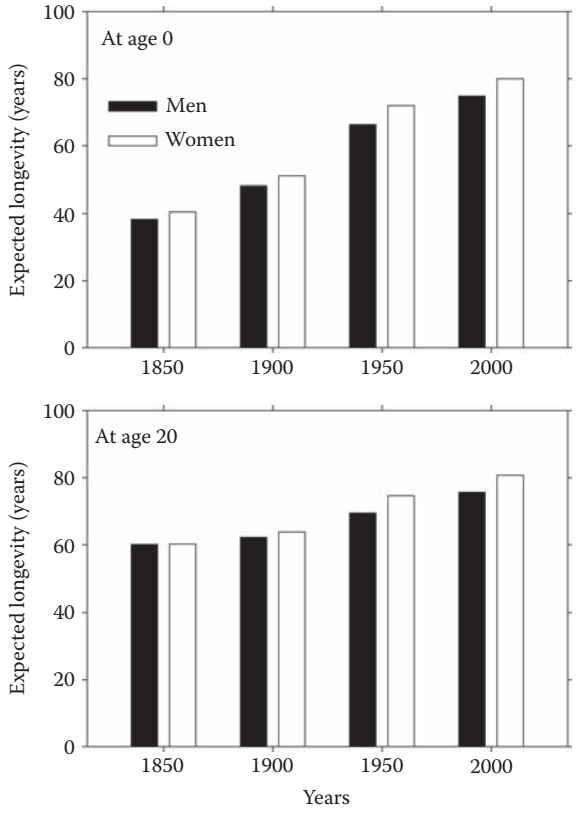


FIGURE 9.64 How long will you live? The graphs show the expected longevity of U.S. residents at birth and at 20 years of age, by gender and by half-century. The greatest improvement in longevity in the last 150 years was a reduction in childhood mortality. An average 20-year-old American alive today will be dead before his or her 80th birthday. (Source: National Center for Health Statistics, U.S. Centers for Disease Control and Prevention.)

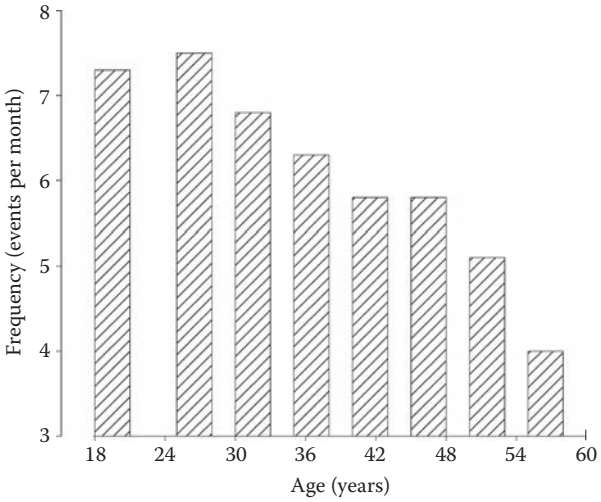


FIGURE 9.65 How often do you have sex? According to a survey of 1200 American adults who had had one or more sexual partners during the preceding year, the frequency of sex declines with age. The average American has sex twice a week in the early 20s and once a week in the late 50s. (Source: Laumann, E. O. et al. (1994). *The Social Organization of Sexuality.* Chicago: University of Chicago Press.)

behavior. As shown in Figure 9.65, young American adults have sex about twice a week on average, while people in their 50s have sex about once a week.

Generation after generation, people are born, age, and die. Therefore, one might be tempted to refer to one's life cycle as a biological rhythm. I do not think that the usage is appropriate. The standard use of *biological rhythm* refers to a repetitive process in a biological system. "Biological system" usually refers to an organism (such as a tree, a mouse, or a human organism). For each organism, however, life is not a repetitive process. Humans are born and die only once. So, although an individual 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. The biological system could be considered 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 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.[348]

The best defined change in the circadian system related to aging is a reduction in the *amplitude* of circadian rhythms.[349–351] Figure 9.66 provides an example for the rhythm of body temperature in two rats. Note that the rhythm of the young rat has an amplitude of almost 2°C, while 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[34,352–354] as well as in various rodent species.[355–364] Generally, the level of locomotor activity is greatly reduced in old age, so that the amplitude of the activity rhythm is also reduced.[356,359,362,363,365–370] An example is shown in Figure 9.67. 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. In general, the 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.[56,370–374]

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

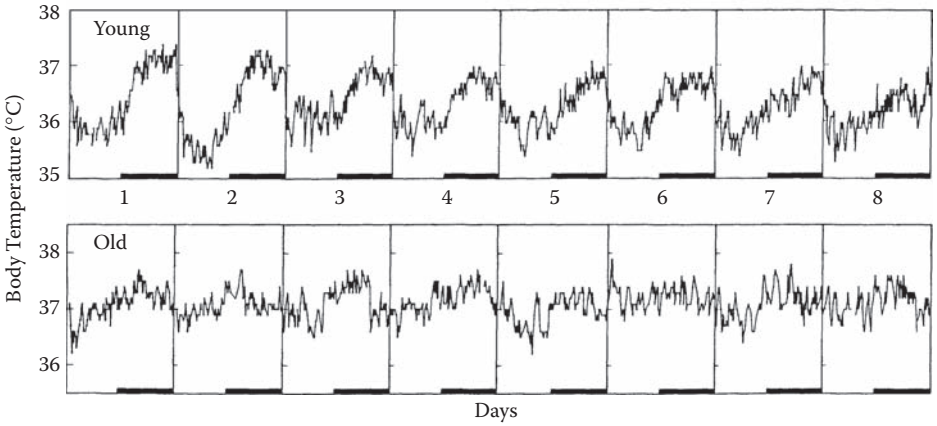


FIGURE 9.66 Reduction in rhythm amplitude with age. The graphs show 8-day segments of the body temperature records of two representative rats (*Rattus norvegicus*) at a young age (2 months) and at a very old age (29 months). The thin dark horizontal bars indicate the timing of the dark phase of the prevailing light–dark cycle (LD 12:12). The amplitude of the temperature rhythm is clearly reduced in the old animal. (Source: Refinetti, R., Ma, H. & Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543. © Elsevier Science Publishers. Reproduced with permission from Elsevier.)

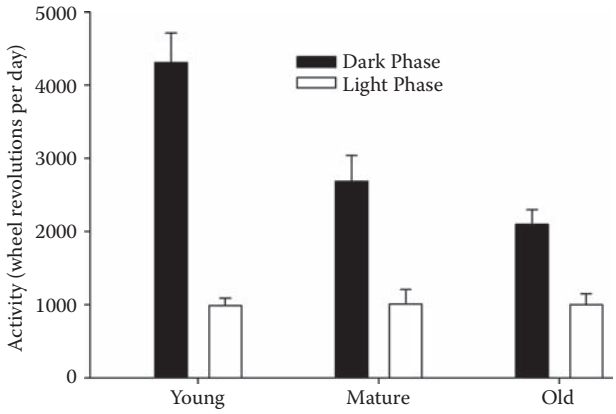


FIGURE 9.67 Reduction in mean level of rhythm with age. The graph shows the variation in the mean number of wheel revolutions (± SE) of eight mice (*Mus musculus*) during the dark and light phases of the light–dark cycle as they aged. Mice of a fast-aging strain (SAMP8) were used. Note that running activity during the dark phase, but not during the light phase, is considerably reduced as the animals get older. (Source: Pang, K. C. H., Miller, J. P. & McAuley, J. D. (2004). Circadian rhythms in SAMP8: a longitudinal study of the effects of age and experience. *Neurobiology of Aging* 25: 111–123.)

tissue from the brain region that contains the master circadian pacemaker.[375] The brain grafts not only restored the amplitude of the activity rhythm but also increased the longevity of the animals. I suspect that attempts to replicate these findings 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[375] corresponds to an increase of about 12 years in human longevity. I am confident that 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 angle of entrainment in old age has been documented in humans[354,376–379] and rodents.[357,360,367] 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 rise-time in old people suggests the occurrence of an age-dependent lengthening of the circadian period. That is, old people wake up earlier to compensate for a lengthening of their circadian period. However, an earlier rise-time in nocturnal rodents with endogenous period shorter than 24 hours implies a shortening of the circadian period.

Is there direct evidence of a change in period associated with aging? Yes, there is — although the results are conflicting. Figure 9.68 shows the results from one study on golden hamsters. 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 entire lives, it is not possible to dissociate the effects of aging from the effects of prolonged exposure to darkness. Studies on golden hamsters, however, generally agree that circadian period is shortened in old age.[380–386] Shortening of circadian period was also observed in deer mice[380] and laboratory rats.[387] However, lengthening of circadian period was observed in aging domestic mice[237,388] and canaries.[389] I am aware of only

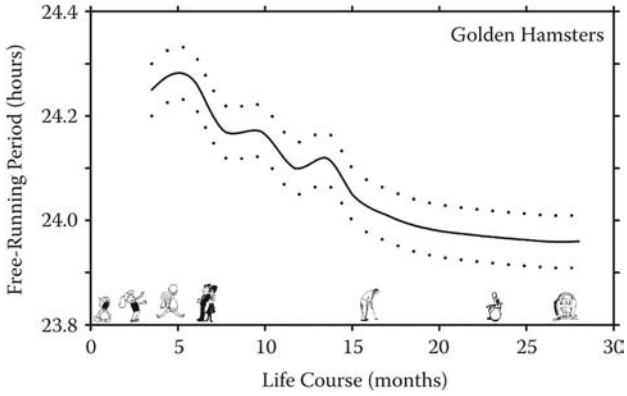


FIGURE 9.68 Shortening of free-running period with age. The graph shows the mean free-running period of golden hamsters (*Mesocricetus auratus*) maintained in constant darkness (by blinding) from 1 month of age to death. The solid line corresponds to the mean of approximately 15 hamsters (dotted lines: ± 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. (Source: Morin, L. P. (1988). Age-related changes in hamster circadian period, entrainment, and rhythm splitting. *Journal of Biological Rhythms* 3: 237–248.)

two studies in humans. One found no difference between the free-running periods of young and old subjects,[208] while the other found shorter periods in older subjects.[34] It seems that circadian period does change with age in at least some species, but the changes in period are not always consistent with the changes in phase angle of entrainment.

9.3.5 Learning

Previous chapters alluded to the instinctual nature of circadian rhythms. For example, the free-running period of a species is genetically determined (inherited) and does not rely on learned experiences. Entrainment of circadian rhythms also is established by reflexive phase-shifts evoked by environmental stimuli. Chapter 5 showed that the circadian system can affect the ability to learn. Can the ability to learn affect the circadian system?

The phase of a rhythm can be changed by phase-shifting environmental stimuli, but this response is reflexive, 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 honey bees 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.[390] This phenomenon implies that the bees can learn an association between a time and a place.

Figure 9.69 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

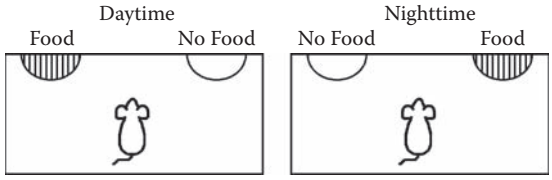


FIGURE 9.69 The time-place paradigm. In this simple time-learning paradigm, food is consistently found in one place at one time of the day and in another place at another time of the day. A starved animal quickly learns to choose the right place at the right time.

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 and afternoon times or early night and late night times, so that the animal must use circadian time as the discriminative stimulus. Figure 9.70 shows an example of results obtained in this paradigm. Rats were trained to feed at one feeder early in the night and at another feeder late in the night for an entire month. The data shown refer to the average of 4 days after the 1-month training. Note 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,[391,392] rats,[393,394] birds,[395,396] and fishes.[397,398]

Studies on rats have raised controversies that have not been fully resolved. Some researchers have suggested that time–place learning occurs only under special protocols,[399] only in certain strains of rats,[400] and only if food restriction is involved.[401] One study suggested that rats may never learn a true time-place association.[402] After the animals learned to choose the correct place at the correct time of day (morning *versus* afternoon), the researchers occasionally started to 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 finding 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.[403] 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

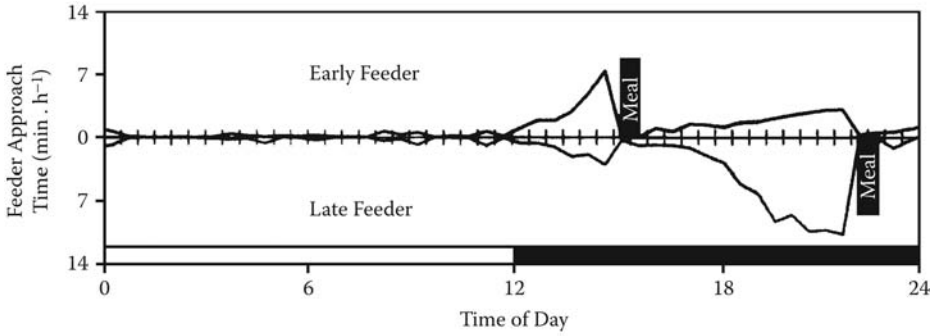


FIGURE 9.70 Time-place learning in rats. The graph shows the mean time spent by rats (*Rattus norvegicus*) close to a feeder previously associated with meals early at night and close to a feeder previously associated with meals late at night. The curves correspond to the means of 16 rats, each averaged over 4 days. Training had been underway for a month. Note 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. (Source: Aragona, B. J., Curtis, J. T., Davidson, A. J., Wang, Z. & Stephan, F. K. (2002). Behavioral and neurochemical investigation of circadian time-place learning in the rat. *Journal of Biological Rhythms* 17: 330–344.)

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 may not involve temporal learning at all.

The issue of learning in the circadian system can be taken a step further. Few researchers 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 process 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. Consider Figure 9.71. 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 eventually will 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 is 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 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 and did not publish their results. One group of investigators, however, reported that air puffs previously paired with light pulses can cause phase shifts in rats.[404] 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 (CT) 15. The results are shown in Figure 9.72. 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 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 evoked a phase shift. The data clearly suggest that the phase-shifting response can be classically conditioned. The same research team later reported success when using light as the unconditional stimulus and circadian time as the conditional stimulus.[405]

The data in Figure 9.72 bother me because the number of animals used was extremely small (namely, 3 rats per group). One of probably many research teams that failed to replicate the findings eventually published a report.[406] 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.[407] Both groups also used skeleton photoperiods (with white light only), and one claimed to obtain conditioning[408] while the other still found nothing significant.[406] Therefore, the conditions necessary for successful conditioning of the phase-shifting response remain rather obscure.[409] The only thing that is very clear is that 9 years after the publication of the original article,[404] no independent group has confirmed the occurrence of classical conditioning in the circadian system.

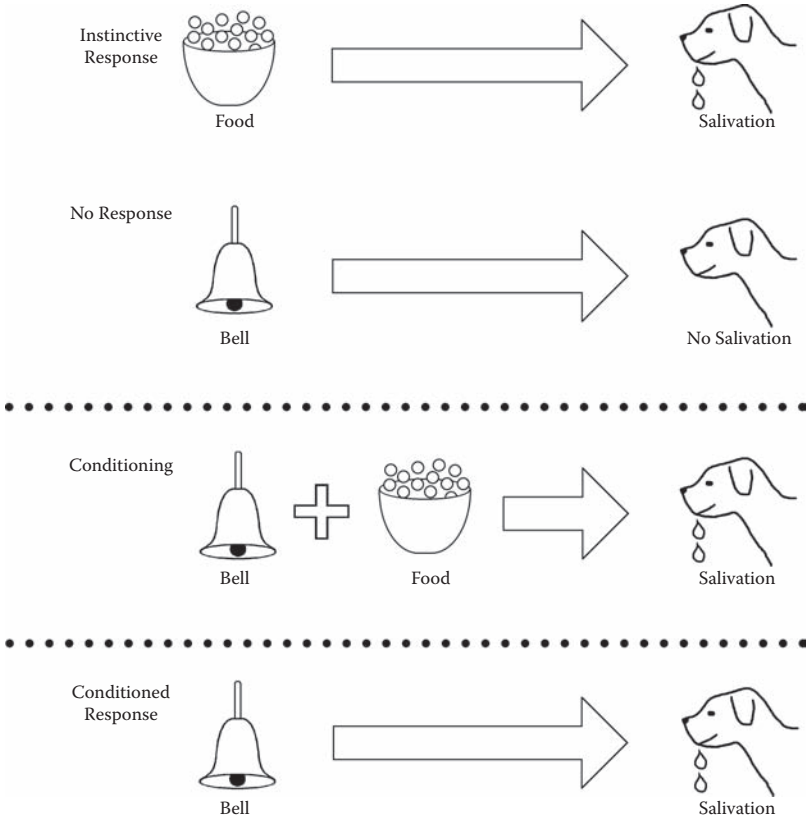


FIGURE 9.71 Classical conditioning. Associative learning by classical conditioning relies on a stimulus that unconditionally elicits a response (such as food) and an originally neutral stimulus (such as the sound of a bell). During conditioning, the neutral stimulus is paired with the unconditional stimulus. After several trials, the neutral stimulus acquires the ability to elicit the response and becomes a conditional (or conditioned) stimulus.

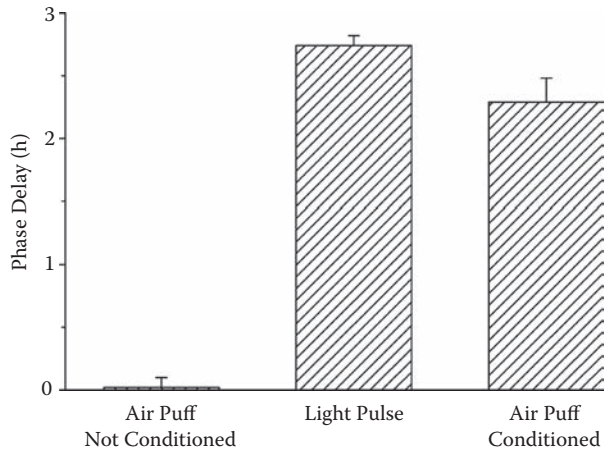


FIGURE 9.72 Classical conditioning in the circadian system. The graph shows the mean phase delays of the locomotor activity rhythms of rats (*Rattus norvegicus*) exposed to brief pulses of a neutral stimulus (an air puff), an unconditional stimulus (a light pulse), or a conditioned stimulus (an air puff previously paired with a light pulse). Each bar represents the mean (± SE) of three rats. Note that the conditioned stimulus elicited a mean phase delay almost as large as that elicited by the unconditional stimulus. (Source: Amir, S. & Stewart, J. (1996). Resetting of the circadian clock by a conditioned stimulus. *Nature* 379: 542–545.)

9.3.6 Sexual Dimorphism

Physical differences between the sexes are generally small but 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, while women have vaginas, uteruses, ovaries, and breasts. Psychological differences between men and women are often downplayed, but they are also significant (Figure 9.73). When it comes to personal relationships, men tend to emphasize practical aspects, while women tend to emphasize romantic aspects.[410] This final subsection of the chapter looks at sexual differences in the circadian system. Very little research has been systematically conducted on the topic of sexual dimorphism of circadian rhythms.

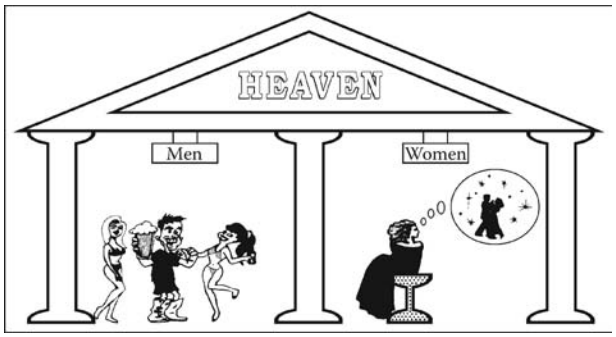


FIGURE 9.73 Being in Heaven. This cartoon presents a humorous view of sex differences in cognitive processes.

Figure 9.74 compares male and female mice using three variables. Male mice are larger than female mice, but they are also less active. Regarding free-running period, the two sexes do not differ.[411] Few other studies have systematically compared the free-running periods of males and females. In golden hamsters, the two sexes were found not to differ;[366,412] in rats, the free-running period was found to be slightly shorter in females than in males.[413] Female hamsters were found to exhibit greater variation than males in circadian phase.[414] Except for a rise during proestrus, the daily rhythm of body temperature of female rats was found to be equivalent to that of males.[415] 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.[416] In humans adults, females were found to be consistently more predisposed to early rising than males,[192,417,418] but no sex difference was found in children.[278]

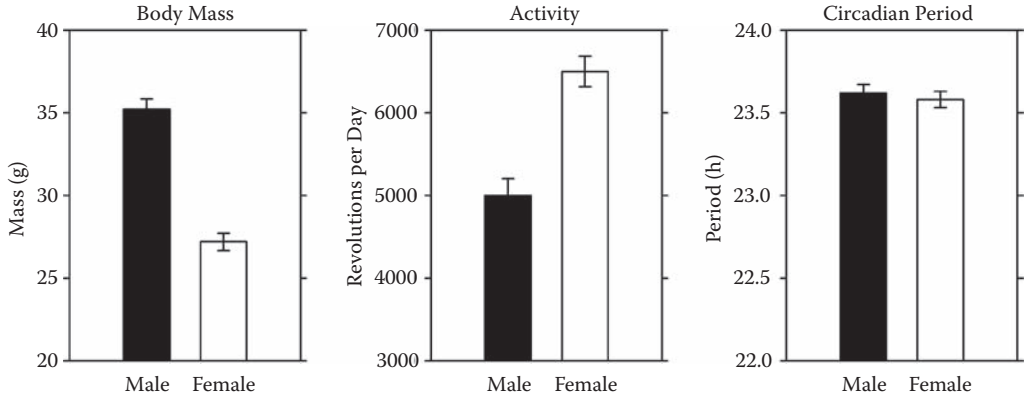


FIGURE 9.74 Sex differences in mice. The graphs show the mean body masses, mean running-wheel activity levels, and mean free-running periods in constant darkness of male and female domestic mice (*Mus musculus*). Each bar corresponds to the mean (± SE) of 24 mice. Significant sex differences are present in body mass and activity level but not in circadian period. (Source: Koteja, P., Swallow, J. G., Carter, P. A. & Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.)

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. The rhythm of body temperature, however, is *not* caused either by the rhythm of activity or by the rhythm of feeding.
2. Circadian rhythmicity is an evolutionarily old process found in all domains of life today. Some organisms are diurnal, some are nocturnal, and some are crepuscular. 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.

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

Exercise 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 record your own rhythms or those of a friend or colleague. Rhythms that can be conveniently measured include body temperature, heart rate, blood pressure, amount of food ingested, urinary excretion, and general sleepiness.

1. Refer to Exercises 1.3 and 5.1 for instructions on measuring body temperature and heart rate.
2. To measure blood pressure, use a standard sphygmomanometer with an inflatable cuff around your arm. Record systolic and diastolic pressures as two separate variables, or record mean arterial blood pressure (the mean of the systolic and diastolic measurements).
3. To measure the amount of food ingested, record the time and weight of any food you eat. Weigh food on 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. 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.
4. To record urinary volume, measure the time and volume of urine every time you empty your bladder. To measure the volume of urine, simply collect it in a calibrated beaker. To measure general sleepiness, use a simple subjective scale with scores ranging from 0 (very awake) to 5 (very sleepy).
5. Another interesting variable that you can record is reaction time (that is, how fast a person can react to the presentation of a stimulus). The CD-ROM enclosed 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 Reaction.exe in the folder where the other programs are located ("\Program Files\Circadian"). The program is quite intuitive and easy to use.
6. If you or someone you know has the facilities for measuring hormones, you can also measure the concentration of hormones in your saliva or urine. Salivary melatonin would be an especially interesting variable to measure because the rhythm of melatonin concentration peaks at night, not during the day like most other variables you will record.
7. If possible, measure each variable every hour. If you cannot record the values every hour, then record them every 2 or 3 hours. 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). It will be difficult to measure variables at night while you are sleeping. If you are using a friend as the subject, you may be able to measure some variables while he or she sleeps. If you are your own subject, you may occasionally use an alarm clock to wake yourself up in the middle of the night to measure variables, but do not do this too often — otherwise, you may disturb your body's clock. Food intake and urinary excretion will be recorded as 0s during sleep time (unless the subject is a sleep walker or a bed wetter). Sleepiness can be assumed to be maximal when the person is asleep. Reaction time is "off-the-chart" during sleep.
8. When you finish collecting data, 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. Data analysis can highlight various aspects of daily rhythmicity. First, 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). Third, you may observe 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 to be able to compare their acrophases. Fourth, you can speculate about the dependence of some rhythms on others. For example, 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, stay awake all night and record urine output. You can then compare the measurements of urinary excretion on a typical night with those taken on the night that you stayed awake. Note that to make this a fair comparison, you will have to withhold liquid intake during the entire night that you stay awake.

Exercise 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. You did not use invasive research methods, however, or conduct the investigation under controlled environmental conditions. If you have access to a properly equipped laboratory, have the approval of a supervisor, and are supervised by an experienced animal researcher, you can conduct the following exercise. The species you use is not a critical factor. In most countries, you do not need an approved animal research protocol to conduct research on invertebrates, although not too many rhythms can be recorded in an invertebrate. Among the vertebrates, laboratory rats are probably the most convenient species to use. You can 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.

1. If the data collection procedures are automated, conduct the study for at least a week to ensure that you accumulate enough data points for analysis. Some variables can be sampled every minute, while others cannot be sampled more often than once every hour or two hours. If you cannot record any particular variable more often than every 3 or 4 hours, do not record it at all.
2. If possible, 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). You can then compare the relative phase angles of the various rhythms between the entrained state and the free-running state.
3. When you finish collecting data, 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. Use a computer program to prepare the graphs. Data analysis can highlight various aspects of daily rhythmicity. First, 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). Third, you may observe 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 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 freerun state? Does a rhythm that peaked earlier in the day than another rhythm now peak after it?

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Futuyma, D. J. (1998). *Evolutionary Biology* (3rd Edition). Sunderland, MA: Sinauer. A good undergraduate textbook on the biology of evolution.

Lincoln, R. J., Boxshall, G. A. & Clark, P. F. (1998). *A Dictionary of Ecology, Evolution and Systematics* (2nd Edition). New York: Cambridge University Press. Not a book to be read from cover to cover, but a very helpful resource for dealing with evolutionary issues.

Gilbert, S. F. (2003). *Developmental Biology* (7th Edition). Sunderland, MA: Sinauer. A good undergraduate textbook on developmental biology.

Ekerdt, D. J. (Ed.). (2002). *Encyclopedia of Aging*. New York: Macmillan. This four-volume set contains more than 400 entries on aging-related topics accessible to high-school and college students.

WEB SITES TO EXPLORE

BBC's Evolution Web Site:
http://www.bbc.co.uk/education/darwin/index.shtml

Paleoclimatology Program of the 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

U.S. National Institute on Aging:
http://www.nia.nih.gov

Virtual Library of Developmental Biology:
http://www.sdbonline.org/Other/VL_DB.html

LITERATURE CITED

1. Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N. & 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. Meinrath, M. & D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
3. 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.
4. Fischette, C. T., Edinger, H. M. & Siegel, A. (1981). Temporary desynchronization among circadian rhythms with lateral fornix ablation. *Brain Research* 229: 85–101.
5. Honnebier, M. B. O. M., Jenkins, S. L. & 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.
6. Robinson, E. L. & Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
7. Moore-Ede, M. C., Kass, D. A. & Herd, J. A. (1977). Transient circadian internal desynchronization after light–dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
8. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L. & 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.
9. Jilge, B. (1991). Restricted feeding: a nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
10. Scales, W. E., Vander, A. J., Brown, M. B. & Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
11. Ghata, J., Reinberg, A., Lagoguey, M. & 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.
12. Kräuchi, K. & 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.
13. Kennaway, D. J. & 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.
14. Kraemer, S., Danker-Hopfe, H., Dorn, H., Schmidt, A., Ehlert, I. & 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.
15. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J. & 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.
16. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M. & 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.
17. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F. & Pauly, J. E. (Eds.). *Chronobiology.* Tokyo: Igaku Shoin, pp. 451–459.
18. Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D. & 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.
19. Refinetti, R. & Menaker, M. (1992). The circadian rhythm of body temperature of normal and *tau*-mutant golden hamsters. *Journal of Thermal Biology* 17: 129–133.
20. Refinetti, R. & Menaker, M. (1993). Independence of heart rate and circadian period in the golden hamster. *American Journal of Physiology* 264: R235–R238.
21. Pitrosky, B., Kirsch, R., Vivien-Roels, B., Georg-Bentz, I., Canguilhem, B. & 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.
22. 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.
23. Aschoff, J., Gerecke, U. & Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
24. Aschoff, J. & von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
25. Honma, K. & Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
26. Pickard, G. E., Kahn, R. & Silver, R. (1984). Splitting of the circadian rhythm of body temperature in the golden hamster. *Physiology and Behavior* 32: 763–766.
27. Kittrell, E. M. W. & Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.

28. Tapp, W. N. & Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
29. Brown, C. M. & Refinetti, R. (1996). Daily rhythms of metabolic heat production, body temperature, and locomotor activity in golden hamsters. *Journal of Thermal Biology* 21: 227–230.
30. Weinert, D. & Waterhouse, J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. *Physiology and Behavior* 63: 837–843.
31. DeCoursey, P. J., Pius, S., Sandlin, C., Wethey, D. & Schull, J. (1998). Relationship of circadian temperature and activity rhythms in two rodent species. *Physiology and Behavior* 65: 457–463.
32. Tang, I., Murakami, D. M. & 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.
33. Weinert, D., Nevill, A., Weinandy, R. & 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.
34. Weitzman, E. D., Moline, M. L., Czeisler, C. A. & Zimmerman, J. C. (1982). Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
35. 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.
36. 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.
37. Eastman, C. & Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
38. Oshima, I., Yamada, H., Goto, M., Sato, K. & 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.
39. Refinetti, R. & Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
40. Davy, J. (1845). On the temperature of man. *Philosophical Transactions of the Royal Society of London* 135: 319–333.
41. Saltin, B., Gagge, A. P. & Stolwijk, J. A. J. (1970). Body temperature and sweat during thermal transients caused by exercise. *Journal of Applied Physiology* 28: 318–327.
42. Cabanac, M., Cunningham, D. J. & Stolwijk, J. A. J. (1971). Thermoregulatory set point during exercise: a behavioral approach. *Journal of Comparative and Physiological Psychology* 76: 94–102.
43. Webb, P. (1993). Daily activity and body temperature. *European Journal of Applied Physiology* 66: 174–177.
44. Deschenes, M. R., Kraemer, W. J., Bush, J. A., Doughty, T. A., Kim, D., Mullen, K. M. & Ramsey, K. (1998). Biorhythmic influences on functional capacity of human muscle and physiological responses. *Medicine and Science in Sports and Exercise* 30: 1399–1407.
45. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C. & 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.
46. Simpson, S. & 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.
47. Arnold, J., LeBlanc, J., Cote, J., Lalonde, J. & Richard, D. (1986). Exercise suppression of thermoregulatory thermogenesis in warm- and cold-acclimated rats. *Canadian Journal of Physiology and Pharmacology* 64: 922–926.
48. Yousef, M. K., Robertson, W. D., Dill, D. B. & Johnson, H. D. (1973). Energetic cost of running in the antelope ground squirrel *Ammospermophilus leucurus*. *Physiological Zoology* 46: 139–147.
49. Zerba, E. & Walsberg, G. E. (1992). Exercise-generated heat contributes to thermoregulation by Gambel's quail in the cold. *Journal of Experimental Biology* 171: 409–422.
50. Golombek, D. A., Ortega, G. & Cardinali, D. P. (1993). Wheel running raises body temperature and changes the daily cycle in golden hamsters. *Physiology and Behavior* 53: 1049–1054.
51. Riccio, A. P. & Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
52. Piccione, G., Caola, G. & Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
53. Marotte, H. & Timbal, J. (1981). Circadian rhythm of temperature in man: comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
54. Gander, P. H., Connell, L. J. & 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.
55. Golja, P., Eiken, O., Rodman, S., Sirok, B. & 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.
56. Monk, T. H., Buysse, D. J., Reynolds, C. F., Kupfer, D. J. & Houck, P. R. (1996). Subjective alertness rhythms in elderly people. *Journal of Biological Rhythms* 11: 268–276.

57. Carrier, J. & Monk, T. H. (1997). Estimating the endogenous circadian temperature rhythm without keeping people awake. *Journal of Biological Rhythms* 12: 266–277.
58. Murray, G., Allen, N. B. & Trinder, J. (2002). Mood and the circadian system: investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
59. Bolles, R. C., Duncan, P. M., Grossen, N. E. & Matter, C. F. (1968). Relationship between activity level and body temperature in the rat. *Psychological Reports* 23: 991–994.
60. 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.
61. Gordon, C. J. & 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.
62. Obál Jr., F., Rubicsek, G., Alföldi, P., Sáry, G. & 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.
63. Barrett, J., Lack, L. & Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
64. Heller, H. C., Graf, R. & Rautenberg, W. (1983). Circadian and arousal state influences on thermoregulation in the pigeon. *American Journal of Physiology* 245: R321–R328.
65. Abrams, R. & Hammel, H. T. (1964). Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping. *American Journal of Physiology* 206: 641–646.
66. Glotzbach, S. F. & Heller, H. C. (1976). Central nervous regulation of body temperature during sleep. *Science* 194: 537–539.
67. Glotzbach, S. F. & Heller, H. C. (1984). Changes in the thermal characteristics of hypothalamic neurons during sleep and wakefulness. *Brain Research* 309: 17–26.
68. Schmidek, W. R., Zachariassen, K. E. & 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.
69. Walker, J. M., Walker, L. E., Harris, D. V. & Berger, R. J. (1983). Cessation of thermoregulation during REM sleep. *American Journal of Physiology* 244: R114–R118.
70. Parmeggiani, P. L. & 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.
71. Hendricks, J. C. (1982). Absence of shivering in the cat during paradoxical sleep without atonia. *Experimental Neurology* 75: 700–710.
72. Amoros, C., Sagot, J. C., Libert, J. P. & Candas, V. (1986). Sweat gland response to local heating during sleep in man. *Journal de Physiology* 81: 209–215.
73. Shapiro, C. M., Moore, A. T., Mitchell, D. & Yodaiken, M. L. (1974). How well does man thermoregulate during sleep? *Experientia* 30: 1279–1281.
74. Sagot, J. C., Amoros, C., Candas, V. & Libert, J. P. (1987). Sweating responses and body temperatures during nocturnal sleep in humans. *American Journal of Physiology* 252: R462–R470.
75. Muzet, A., Libert, J. P. & Candas, V. (1984). Ambient temperature and human sleep. *Experientia* 40: 425–429.
76. Dewasmes, G., Bothorel, B., Candas, V. & 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.
77. Tayefeh, F., Plattnet, O., Sessler, D. I., Ikeda, T. & Marder, D. (1998). Circadian changes in the sweating-to-vasoconstriction interthreshold range. *Pflügers Archiv* 435: 402–406.
78. Dijk, D. J., Cajochen, C. & 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.
79. Myers, B. L. & Badia, P. (1993). Immediate effects of different light intensities on body temperature and alertness. *Physiology and Behavior* 54: 199–202.
80. Cagnacci, A., Soldani, R. & 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.
81. Lavoie, S., Paquet, J., Selmaoui, B., Rufiange, M. & Dumont, M. (2003). Vigilance levels during and after bright light exposure in the first half of the night. *Chronobiology International* 20: 1019–1038.
82. Rüger, M., Gordijn, M. C. M., Beersma, D. G. M., de Vries, B. & Daan, S. (2003). Acute and phase-shifting effects of ocular and extraocular light in human circadian physiology. *Journal of Biological Rhythms* 18: 409–419.
83. Ingram, D. L. & 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.
84. Wilson, H. R., Mather, F. B., Brigmon, R. L., Besch, E. L., Dugan, V. P. & Boulos, N. Z. (1989). Feeding time and body temperature interactions in broiler breeders. *Poultry Science* 68: 608–616.
85. Mohr, E. G. & Krzywanek, H. (1995). Endogenous oscillator and regulatory mechanisms of body temperature in sheep. *Physiology and Behavior* 57: 339–347.
86. Miyazaki, H., Yoshida, M., Samura, K., Matsumoto, H., Ikemoto, F. & 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.
87. Refinetti, R. & Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.
88. Piccione, G., Caola, G. & Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.

89. Bolles, R. C. & Duncan, P. M. (1969). Daily course of activity and subcutaneous body temperature in hungry and thirsty rats. *Physiology and Behavior* 4: 87–89.
90. Ingram, D. L. & 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.
91. Fowler, P. A. & 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.
92. Hohtola, E., Hissa, R., Pyörnilä, A., Rintamäki, H. & Saarela, S. (1991). Nocturnal hypothermia in fasting Japanese quail: the effect of ambient temperature. *Physiology and Behavior* 49: 563–567.
93. Shido, O., Sakurada, S., Kohda, W. & Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
94. Rashotte, M. E., Pastukhov, I. F., Poliakov, E. L. & 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.
95. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T. & Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *Journal of Physiology* 526: 417–424.
96. Piccione, G., Caola, G. & Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
97. Piccione, G., Bertolucci, C., Foà, A. & Caola, G. (2004). Influence of fasting and exercise on the daily rhythm of serum leptin in the horse. *Chronobiology International* 21: 405–417.
98. Kalsbeek, A., Fliers, E., Romijn, J. A., la Fleur, S. E., Wortel, J., Bakker, O., Endert, E. & Buijs, R. M. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685.
99. Piccione, G., Caola, G. & 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.
100. Kerkhof, G. A., Van Dongen, H. P. A. & Bobbert, A. C. (1998). Absence of endogenous circadian rhythmicity in blood pressure? *American Journal of Hypertension* 11: 373–377.
101. Scheer, F. A. J. L., van Doornen, L. J. P. & 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.
102. Viola, A. U., Simon, C., Ehrhart, J., Geny, B., Piquard, F., Muzet, A. & 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.
103. Lovegrove, B. G., Heldmaier, G. & Ruf, T. (1993). Circadian activity rhythms in colonies of "blind" molerats, *Cryptomys damarensis* (Bathyergidae). *South African Journal of Zoology* 28: 46–55.
104. Lovegrove, B. G. & 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.
105. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
106. Begall, S., Daan, S., Burda, H. & Overkamp, G. J. F. (2002). Activity patterns in a subterranean social rodent, *Spalacopus cyanus* (Octodontidae). *Journal of Mammalogy* 83: 153–158.
107. Oosthuizen, M. K., Cooper, H. M. & 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.
108. Meyerowitz, E. M. (2002). Plants compared to animals: the broadest comparative study of development. *Science* 295: 1482–1485.
109. Jacobsen, S. B. (2003). How old is planet Earth? *Science* 300: 1513–1514.
110. Doolittle, W. F. (1999). Phylogenetic classification and the universal tree. *Science* 284: 2124–2128.
111. Rogers, L. A. & Greenbank, G. R. (1930). The intermittent growth of bacterial cultures. *Journal of Bacteriology* 19: 181–190.
112. Halberg, F. & 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.
113. Kondo, T., Mori, T., Lebedeva, N. V., Aoki, S., Ishiura, M. & Golden, S. S. (1997). Circadian rhythms in rapidly dividing cyanobacteria. *Science* 275: 224–227.
114. Ouyang, Y., Andersson, C. R., Kondo, T., Golden, S. S. & Johnson, C. H. (1998). Resonating circadian clocks enhance fitness in cyanobacteria. *Proceedings of the National Academy of Sciences U.S.A.* 95: 8660–8664.
115. Dvomyk, V., Vinogradova, O. & Nevo, E. (2003). Origin and evolution of circadian clock genes in prokaryotes. *Proceedings of the National Academy of Sciences U.S.A.* 100: 2495–2500.
116. Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M. & Allada, R. (2002). A role for casein kinase 2-α in the *Drosophila* circadian clock. *Nature* 420: 816–820.
117. Van Gelder, R. N., Herzog, E. D., Schwartz, W. J. & Taghert, P. H. (2003). Circadian rhythms: in the loop at last. *Science* 300: 1534–1535.
118. Chang, D. C., McWatters, H. G., Williams, J. A., Gotter, A. L., Levine, J. D. & Reppert, S. M. (2003). Constructing a feedback loop with circadian clock molecules from the silkmoth, *Antheraea pernyi*. *Journal of Biological Chemistry* 278: 38149–38158.

119. Constance, C. M., Green, C. B., Tei, H. & Block, G. D. (2002). *Bulla gouldiana period* exhibits unique regulation at the mRNA and protein levels. *Journal of Biological Rhythms* 17: 413–427.
120. Barnett, J. E. (1998). *Time's Pendulum: From Sundials to Atomic Clocks*. New York: Plenum.
121. Murphy, W. J., Eizirik, E., O'Brien, S. J., Madsen, O., Scally, M., Douady, C. J., Teeling, E., Ryder, O. A., Stanhope, M. J., de Jong, W. W. & Springer, M. S. (2001). Resolution of the early placental mammal radiation using Bayesian phylogenetics. *Science* 294: 2348–2351.
122. Stringer, C. (2002). Modern human origins: progress and prospects. *Philosophical Transactions of the Royal Society of London B* 357: 563–579.
123. Torsvik, T. H. (2003). The Rodinia jigsaw puzzle. *Science* 300: 1379–1381.
124. Halberg, F. (1953). Some physiological and clinical aspects of 24-hour periodicity. *Journal-Lancet* 73: 20–32.
125. Pittendrigh, C. S. (1967). Circadian rhythms, space research and manned space flight. *Life Sciences and Space Research* 5: 122–134.
126. Engelmann, W. (1988). Evolution and selective advantage of circadian rhythms. *Acta Physiologica Polonica* 39: 345–356.
127. Marques, M. D. & Waterhouse, J. M. (1994). Masking and the evolution of circadian rhythmicity. *Chronobiology International* 11: 146–155.
128. Roenneberg, T. & Merrow, M. (2002). Life before the clock: modeling circadian evolution. *Journal of Biological Rhythms* 17: 495–505.
129. Rutter, J., Reick, M. & McKnight, S. L. (2002). Metabolism and the control of circadian rhythms. *Annual Review of Biochemistry* 71: 307–331.
130. Green, R. M., Tingay, S., Wang, Z. Y. & Tobin, E. M. (2002). Circadian rhythms confer a higher level of fitness to Arabidopsis plants. *Plant Physiology* 129: 576–584.
131. Michael, T. P., Salomé, P. A., Yu, H. J., Spencer, T. R., Sharp, E. L., McPeek, M. A., Alonso, J. M., Ecker, J. R. & McClung, C. R. (2003). Enhanced fitness conferred by naturally occurring variation in the circadian clock. *Science* 302: 1049–1053.
132. Beaver, L. M., Gvakharia, B. O., Vollintine, T. S., Hege, D. M., Stanewsky, R. & Giebultowicz, J. M. (2002). Loss of circadian clock function decreases reproductive fitness in males of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences U.S.A.* 99: 2134–2139.
133. DeCoursey, P. J., Krulas, J. R., Mele, G. & Holley, D. C. (1997). Circadian performance of suprachiasmatic nuclei (SCN)-lesioned antelope ground squirrels in a desert enclosure. *Physiology and Behavior* 62: 1099–1108.
134. DeCoursey, P. J., Walker, J. K. & Smith, S. A. (2000). A circadian pacemaker in free-living chipmunks: essential for survival? *Journal of Comparative Physiology A* 186: 169–180.
135. Field, K. G., Olsen, G. J., Lane, D. J., Giovannoni, S. J., Ghiselin, M. T., Raff, E. C., Pace, N. R. & Raff, R. A. (1988). Molecular phylogeny of the animal kingdom. *Science* 239: 748–753.
136. Doolittle, R. F., Feng, D. F., Tsang, S., Cho, G. & Little, E. (1996). Determining divergence times of the major kingdoms of living organisms with a protein clock. *Science* 271: 470–477.
137. Heckman, D. S., Geiser, D. M., Eidell, B. R., Stauffer, R. L., Kardos, N. L. & Hedges, S. B. (2001). Molecular evidence for the early colonization of land by fungi and plants. *Science* 293: 1129–1133.
138. Benton, M. J. & Ayala, F. J. (2003). Dating the tree of life. *Science* 300: 1698–1700.
139. Bowen, G. J., Clyde, W. C., Koch, P. L., Ting, S., Alroy, J., Tsubamoto, T., Wang, Y. & Wang, Y. (2002). Mammalian dispersal at the Paleocene/Eocene boundary. *Science* 295: 2062–2065.
140. Bakker, R. T. (1972). Anatomical and ecological evidence of endothermy in dinosaurs. *Nature* 238: 81–85.
141. Barrick, R. E. & Showers, W. J. (1994). Thermophysiology of *Tyrannosaurus rex*: evidence from oxygen isotopes. *Science* 265: 222–224.
142. Thulborn, R. A. (1973). Thermoregulation in dinosaurs. *Nature* 245: 51–52.
143. Paladino, F. V., O'Connor, M. P. & Spotila, J. R. (1990). Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* 344: 858–860.
144. Ruben, J. A., Hillenius, W. J., Geist, N. R., Leitch, A., Jones, T. D., Currie, P. J., Horner, J. R. & Espe, G. (1996). The metabolic status of some late Cretaceous dinosaurs. *Science* 273: 1204–1207.
145. Dawson, T. J. (1972). Primitive mammals and patterns in the evolution of thermoregulation. In: Bligh, J. & Moore, R. E. (Eds.). *Essays on Temperature Regulation*. Amsterdam: North-Holland, pp. 1–18.
146. Hammel, H. T. (1983). Phylogeny of regulatory mechanisms in temperature regulation. *Journal of Thermal Biology* 8: 37–42.
147. Crowley, T. J., Short, D. A., Mengel, J. G. & North, G. R. (1986). Role of seasonality in the evolution of climate during the last 100 million years. *Science* 231: 579–584.
148. Crompton, A. W., Taylor, C. R. & Jagger, J. A. (1978). Evolution of homeothermy in mammals. *Nature* 272: 333–336.
149. May, R. M. (1988). How many species are there on Earth? *Science* 241: 1441–1449.
150. Sarnat, H. B. & Netsky, M. G. (1981). *Evolution of the Nervous System*, 2nd Edition. New York: Oxford University Press.
151. Amend, J. P. & Shock, E. L. (1998). Energetics of amino acid synthesis in hydrothermal ecosystems. *Science* 281: 1659–1662.
152. Cody, G. D., Boctor, N. Z., Filley, T. R., Hazen, R. M., Scott, J. H., Sharma, A. & Yoder, H. S. (2000). Primordial carbonylated iron-sulfur compounds and the sythesis of pyruvate. *Science* 289: 1337–1340.
153. Gehring, W. & Rosbash, M. (2003). The coevolution of blue-light photoreception and circadian rhythms. *Journal of Molecular Evolution* 57: S286–S289.

154. Cloudsley-Thompson, J. L. (1960). Adaptive functions of circadian rhythms. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 345–355.
155. Daan, S. (1981). Adaptive daily strategies in behavior. In: Aschoff, J. (Ed.). *Biological Rhythms* (*Handbook of Behavioral Neurobiology, Volume 4*). New York: Plenum, pp. 275–298.
156. Aschoff, J. (1966). Circadian activity pattern with two peaks. *Ecology* 47: 657–662.
157. Kas, M. J. H. & Edgar, D. M. (1998). Crepuscular rhythms of EEG sleep-wake in a hystricomorph rodent, *Octodon degus*. *Journal of Biological Rhythms* 13: 9–17.
158. Verhagen, L. A. W., Pévet, P., Saboureau, M., Sicard, B., Nesme, B., Claustrat, B., Buijs, R. M. & 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.
159. Kurumiya, S. & Kawamura, H. (1988). Circadian oscillation of the multiple unit activity in the guinea pig suprachiasmatic nucleus. *Journal of Comparative Physiology A* 162: 301–308.
160. Iigo, M. & Tabata, M. (1996). Circadian rhythms of locomotor activity in the goldfish *Carassius auratus*. *Physiology and Behavior* 60: 775–781.
161. Sharma, V. K., Lone, S. R., Mathew, D., Goel, A. & 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.
162. Oster, H., Avivi, A., Joel, A., Albrecht, U. & 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.
163. Merrill, S. B. & Mech, L. D. (2003). The usefulness of GPS telemetry to study wolf circadian and social activity. *Wildlife Society Bulletin* 31: 947–960.
164. Rattenborg, N. C., Mandt, B. H., Obermeyer, W. H., Winsauer, P. J., Huber, R., Wikelski, M. & Benca, R. M. (2004). Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLOS Biology* 2: 924–936.
165. Cochran, W. W. (1987). Orientation and other migratory behaviours of a Swainson's thrush followed for 1500 km. *Animal Behaviour* 35: 927–928.
166. Kenagy, G. J., Nespolo, R. F., Vásquez, R. A. & Bozinovic, F. (2002). Daily and seasonal limits of time and temperature to activity of degus. *Revista Chilena de Historia Natural* 75: 567–581.
167. Kas, M. J. H. & Edgar, D. M. (1999). A nonphotic stimulus inverts the diurnal-nocturnal phase preference in *Octodon degus*. *Journal of Neuroscience* 19: 328–333.
168. Blanchong, J. A., McElhinny, T. L., Mahoney, M. M. & Smale, L. (1999). Nocturnal and diurnal rhythms in the unstriped Nile rate, *Arvicanthis niloticus*. *Journal of Biological Rhythms* 14: 364–377.
169. 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.
170. Refinetti, R. (2004). Daily activity patterns of a nocturnal and a diurnal rodent in a seminatural environment. *Physiology and Behavior* 82: 285–294.
171. Redlin, U. & Mrosovsky, N. (2004). Nocturnal activity in a diurnal rodent (*Arvicanthis niloticus*): the importance of masking. *Journal of Biological Rhythms* 19: 58–67.
172. Shkolnik, A. (1971). Diurnal activity in a small desert rodent. *International Journal of Biometeorology* 15: 115–120.
173. Kronfeld-Schor, N., Dayan, T., Elvert, R., Haim, A., Zisapel, N. & Heldmaier, G. (2001). On the use of the time axis for ecological separation: diel rhythms as an evolutionary constraint. *American Naturalist* 158: 451–457.
174. Aschoff, J. (1979). Circadian rhythms: influences of internal and external factors on the period measured in constant conditions. *Zeitschrift für Tierpsychologie* 49: 225–249.
175. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
176. Lincoln, G., Messager, S., Andersson, H. & 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 U.S.A.* 99: 13890–13895.
177. Fidler, A. E. & 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.
178. Mrosovsky, N. (2003). Beyond the suprachiasmatic nucleus. *Chronobiology International* 20: 1–8.
179. Dardente, H., Menet, J. S., Challet, E., Tournier, B. B., Pévet, P. & 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.
180. Smale, L., Lee, T. & Nunez, A. A. (2003). Mammalian diurnality: some facts and gaps. *Journal of Biological Rhythms* 18: 356–366.
181. Nunez, A. A., Bult, A., McElhinny, T. L. & 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.
182. Novak, C. M., Harris, J. A., Smale, L. & 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.
183. Schwartz, M. D., Nunez, A. A. & Smale, L. (2004). Differences in the suprachiasmatic nucleus and lower subparaventricular zone of diurnal and nocturnal rodents. *Neuroscience* 127: 13–23.

184. Kas, M. J. H. & 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.
185. Mahoney, M., Bult, A. & 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.
186. Hut, R. A., Mrosovsky, N. & Daan, S. (1999). Nonphotic entrainment in a diurnal mammal, the European ground squirrel (*Spermophilus citellus*). *Journal of Biological Rhythms* 14: 409–419.
187. Glass, J. D., Tardif, S. D., Clements, R. & Mrosovsky, N. (2001). Photic and nonphotic circadian phase resetting in a diurnal primate, the common marmoset. *American Journal of Physiology* 280: R191–R197.
188. Novak, C. M. & 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.
189. Novak, C. M. & 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.
190. Savides, T. J., Messin, S., Senger, C. & Kripke, D. F. (1986). Natural light exposure of young adults. *Physiology and Behavior* 38: 571–574.
191. Cole, R. J., Kripke, D. F., Wisbey, J., Mason, W. J., Gruen, W., Hauri, P. J. & Juarez, S. (1995). Seasonal variation in human illumination exposure at two different latitudes. *Journal of Biological Rhythms* 10: 324–334.
192. Roenneberg, T., Wirz-Justice, A. & Merrow, M. (2003). Life between clocks: daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
193. Pratt, B. L. & Goldman, B. D. (1986). Activity rhythms and photoperiodism of Syrian hamsters in a simulated burrow system. *Physiology and Behavior* 36: 83–89.
194. Boulos, Z., Macchi, M., Houpt, T. A. & Terman, M. (1996). Photic entrainment in hamsters: effects of simulated twilights and nest box availability. *Journal of Biological Rhythms* 11: 216–233.
195. Boulos, Z., Macchi, M. & Terman, M. (1996). Twilight transitions promote circadian entrainment to lengthening light–dark cycles. *American Journal of Physiology* 271: R813–R818.
196. DeCoursey, P. J. (1986). Light sampling behavior in photoentrainment of a rodent circadian rhythm. *Journal of Comparative Physiology A* 159: 161–169.
197. Marimuthu, G., Rajan, S. & 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.
198. Kennedy, G. A., Coleman, G. J. & 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.
199. Hut, R. A., van Oort, B. E. H. & 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.
200. Everts, L. G., Strijkstra, A. M., Hut, R. A., Hoffmann, I. E. & Millesi, E. (2004). Seasonal variation in daily activity patterns of free-ranging European ground squirrels (*Spermophilus citellus*). *Chronobiology International* 21: 57–71.
201. Blanchong, J. A. & Smale, L. (2000). Temporal patterns of activity of the unstriped Nile rat, *Arvicanthis niloticus*. *Journal of Mammalogy* 81: 595–599.
202. Usui, S., Takahashi, Y. & Okazaki, T. (2000). Range of entrainment of rat circadian rhythms to sinusoidal light-intensity cycles. *American Journal of Physiology* 278: R1148–R1156.
203. Boulos, Z., Macchi, M. M. & Terman, M. (2002). Twilights widen the range of photic entrainment in hamsters. *Journal of Biological Rhythms* 17: 353–363.
204. Thor, D. H. & Hoats, D. L. (1968). A circadian variable in self-exposure to light by the rat. *Psychonomic Science* 12: 1–2.
205. Fishman, R. & Roffwarg, H. P. (1970). Free choice of lighting and sleep-waking behavior in the laboratory rat. *Psychophysiology* 7: 304–305.
206. Refinetti, R. (1997). Circadian rhythm of self-exposure to light in the golden hamster. *Behavioural Processes* 40: 107–111.
207. Sevenster, P., Feuth de Bruijn, E. & Huisman, J. J. (1995). Temporal structure in stickleback behaviour. *Behaviour* 132: 1267–1284.
208. Czeisler, C. A., Duffy, J. F, Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., Ronda, J. M., Silva, E. J., Allan, J. S., Emens, J. S., Dijk, D. J. & Kronauer, R. E. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284: 2177–2181.
209. Krebs, J. R. & Davies, N. B. (1993). *An Introduction to Behavioural Ecology*, 3rd Edition. London: Blackwell Scientific.
210. Adair, E. R. & Wright, B. A. (1976). Behavioral thermoregulation in the squirrel monkey when response effort is varied. *Journal of Comparative and Physiological Psychology* 90: 179–184.
211. Refinetti, R. (1982). Custo da resposta termoregulatória de pressão à barra. *Ciência e Cultura* 34(S): 896–897.
212. Refinetti, R. & 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.
213. Rashotte, M. E. & 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.
214. Collier, G. H., Johnson, D. F., Naveira, J. & Cybulski, K. A. (1989). Ambient temperature and food costs: effects on behavior patterns in rats. *American Journal of Physiology* 257: R1328–R1334.

215. Sherwin, C. M. & Nicol, C. J. (1996). Reorganization of behaviour in laboratory mice, *Mus musculus*, with varying cost of access to resources. *Animal Behaviour* 51: 1087–1093.
216. Sullivan, C. M. & Fisher, K. C. (1954). The effects of light on temperature selection in speckled trout *Salvelinus fontinalis* (Mitchill). *Biological Bulletin* 107: 278–288.
217. Refinetti, R. (1984). Behavioral temperature regulation in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 47: 29–43.
218. Rautenberg, W., May, B. & Arabin, G. (1980). Behavioral and autonomic temperature regulation in competition with food intake and water balance of pigeons. *Pflügers Archiv* 384: 253–260.
219. Munson, B. H., McCormick, J. H. & 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.
220. Cabanac, M. & Johnson, K. G. (1983). Analysis of a conflict between palatability and cold exposure in rats. *Physiology and Behavior* 31: 249–253.
221. Refinetti, R. (1983). Conflito entre alimentação e termorregulação no peixinho dourado. *Ciência e Cultura* 35(S): 820.
222. Balasko, M. & 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.
223. Reebs, S. G. & Maillet, D. (2003). Effect of cage enrichment on the daily use of running wheels by Syrian hamsters. *Chronobiology International* 20: 9–20.
224. Refinetti, R. & Horvath, S. M. (1989). Thermopreferendum of the rat: inter- and intra-subject variability. *Behavioral and Neural Biology* 52: 87–94.
225. Refinetti, R. (1989). Magnitude estimation of warmth: intra- and intersubject variability. *Perception and Psychophysics* 46: 81–84.
226. van Vonderen, I. K., Kooistra, H. S. & 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.
227. Gidal, B. E., Radulovic, L. L., Kruger, S., Rutecki, P., Pitterle, M. & Bockbrader, H.N. (2000). Inter- and intra-subject variability in gabapentin absorpotion and absolute bioavailability. *Epilepsy Research* 40: 123–127.
228. Symanski, E., Bergamaschi, E. & 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.
229. Refinetti, R. & Piccione, G. (2005). Intra- and interindividual variability in the circadian rhythm of body temperature of rats, squirrels, dogs, and horses. *Journal of Thermal Biology* 30: 139–146.
230. Barassin, S., Saboureau, M., Kalsbeek, A., Bothorel, B., Vivien-Roels, B., Malan, A. Buijs, R. M., Guardiola-Lemaitre, B. & Pévet, P. (1999). Interindividual differences in the pattern of melatonin secretion of the Wistar rat. *Journal of Pineal Research* 27: 193–201.
231. Selmaoui, B. & 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.
232. Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D. & 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.
233. DeCoursey, P. (1960). Phase control of activity in a rodent. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 49–55.
234. 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.
235. Pohl, H. (1983). Light pulses entrain the circadian activity rhythm of a diurnal rodent (*Ammospermophilus leucurus*). *Comparative Biochemistry and Physiology B* 76: 723–729.
236. 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.
237. Davis, F. C. & Menaker, M. (1981). Development of the mouse circadian pacemaker: independence from environmental cycles. *Journal of Comparative Physiology A* 143: 527–539.
238. Refinetti, R. (1998). Influence of early environment on the circadian period of the *tau*-mutant hamster. *Behavior Genetics* 28: 153–158.
239. Duffield, G. E. & 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.
240. Canal-Corretger, M. M., Cambras, T., Vilaplana, J. & 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.
241. Yamazaki, S., Alones, V. & Menaker, M. (2002). Interaction of the retina with suprachiasmatic pacemakers in the control of circadian behavior. *Journal of Biological Rhythms* 17: 315–329.
242. Canal-Corretger, M. M., Cambras, T. & Díez-Noguera, A. (2003). Effect of light during lactation on the phasic and tonic responses of the rat pacemaker. *Chronobiology International* 20: 21–35.
243. Aton, S. J., Block, G. D., Tei, H., Yamazaki, S. & 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.
244. Tomioka, K., Uwozumi, K. & Matsumoto, N. (1997). Light cycles given during development affect free-running period of circadian locomotor rhythm of *period* mutants in *Drosophila melanogaster*. *Journal of Insect Physiology* 43: 297–305.

245. Sheeba, V., Chandrashekaran, M. K., Joshi, A. & Sharma, V. K. (2002). Developmental plasticity of the locomotor activity rhythm of *Drosophila melanogaster*. *Journal of Insect Physiology* 48: 25–32.
246. Attanasio, A., Rager, K. & Gupta, D. (1986). Ontogeny of circadian rhythmicity for melatonin, serotonin, and N-acetylserotonin in humans. *Journal of Pineal Research* 3: 251–256.
247. Ardura, J., Gutierrez, R., Andres, J. & Agapito, T. (2003). Emergence and evolution of the circadian rhythm of melatonin in children. *Hormone Research* 59: 66–72.
248. Kleitman, N., Titelbaum, S. & Hoffmann, H. (1937). The establishment of the diurnal temperature cycle. *American Journal of Physiology* 119: 48–54.
249. Abe, K. & Fukui, S. (1979). The individual development of circadian temperature rhythm in infants. *Journal of Interdisciplinary Cycle Research* 10: 227–232.
250. Updike, P. A., Accurso, F. J. & Jones, R. H. (1985). Physiologic circadian rhythmicity in preterm infants. *Nursing Research* 34: 160–163.
251. Rivkees, S. A., Mayes, L., Jacobs, H. & Gross, I. (2004). Rest-activity patterns of premature infants are regulated by cycled lighting. *Pediatrics* 113: 833–839.
252. Korte, J., Hoehn, T. & Siegmund, R. (2004). Actigraphic recordings of activity-rest rhythms of neonates born by different delivery modes. *Chronobiology International* 21: 95–106.
253. Serón-Ferré, M., Riffo, R., Valenzuela, G. J. & 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.
254. Schmidt, I., Barone, A. & Carlisle, H. J. (1986). Diurnal cycle of core temperature in huddling, week-old rat pups. *Physiology and Behavior* 37: 105–109.
255. Spiers, D. E. (1988). Nocturnal shifts in thermal and metabolic responses of the immature rat. *Journal of Applied Physiology* 64: 2119–2124.
256. Nuesslein, B. & Schmidt, I. (1990). Development of circadian cycle of core temperature in juvenile rats. *American Journal of Physiology* 259: R270–R276.
257. Nuesslein-Hildesheim, B., Imai-Matsumura, K., Döring, H. & 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.
258. Imai-Matsumura, K., Kaul, R. & Schmidt, I. (1995). Juvenile circadian core temperature rhythm in Wistar and Lean (Fa/–) Zucker rat pups. *Physiology and Behavior* 57: 135–139.
259. Seifert, E. L. & 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.
260. Kittrell, E. M. W. & Satinoff, E. (1986). Development of the circadian rhythm of body temperature in rats. *Physiology and Behavior* 38: 99–104.
261. Allen, C. & Kendall, J. W. (1967). Maturation of the circadian rhythm of plasma corticosterone in the rat. *Endocrinology* 80: 926–930.
262. Nuesslein-Hildesheim, B. & 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.
263. Jilge, B., Kuhnt, B., Landerer, W. & Rest, S. (2000). Circadian thermoregulation in suckling rabbit pups. *Journal of Biological Rhythms* 15: 329–335.
264. Jilge, B., Kuhnt, B., Landerer, W. & Rest, S. (2001). Circadian temperature rhythms in rabbit pups and in their does. *Laboratory Animals* 35: 364–373.
265. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
266. Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
267. Macaulay, A. S., Hahn, G. L., Clark, D. H. & Sisson, D. V. (1995). Comparison of calf housing types and tympanic temperature rhythms in Holstein calves. *Journal of Dairy Science* 78: 856–862.
268. Piccione, G. Caola, G. & Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.
269. Aarseth, J. J., Van't Hof, T. J. & Stokkan, K. A. (2003). Melatonin is rhythmic in newborn seals exposed to continuous light. *Journal of Comparative Physiology B* 173: 37–42.
270. Tamarkin, L., Reppert, S. M., Orloff, D. J., Klein, D. C., Yellon, S. M. & 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.
271. Reppert, S. M. & 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.
272. Shibata, S. & Moore, R. Y. (1987). Development of neuronal activity in the rat suprachiasmatic nucleus. *Brain Research* 431: 311–315.
273. Bendová, Z., Sumová, A. & Illnerová, H. (2004). Development of circadian rhythmicity and photoperiodic response in subdivisions of the rat suprachiasmatic nucleus. *Developmental Brain Research* 148: 105–112.
274. Houghton, D. C., Young, I. R. & 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.
275. Parraguez, V. H., Valenzuela, G. J., Vergara, M., Ducsay, C. A., Yellon, S. M. & Serón-Ferré, M. (1996). Effect of constant light on fetal and maternal prolactin rhythms in sheep. *Endocrinology* 137: 2355–2361.
276. Delaunay, F., Thisse, C., Marchand, O., Laudet, V. & Thisse, B. (2000). An inherited functional circadian clock in zebrafish embryos. *Science* 289: 297–300.
277. Shinkoda, H., Matsumoto, K., Park, Y. M. & Nagashima, H. (2000). Sleep-wake habits of schoolchildren according to grade. *Psychiatry and Clinical Neurosciences* 54: 287-289.

278. Kim, S., Dueker, G. L., Hasher, L. & Goldstein, D. (2002). Children's time of day preference: age, gender and ethnic differences. *Personality and Individual Differences* 33: 1083–1090.
279. Giannotti, F., Cortesi, F., Sebastiani, T. & Ottaviano, S. (2002). Circadian preference, sleep and daytime behaviour in adolescence. *Journal of Sleep Research* 11: 191–199.
280. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K. & 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.
281. 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.
282. de la Iglesia, H. O., Rodríguez, E. M. & Dezi, R. E. (1994). Burrow plugging in the crab *Uca uruguayensis* and its synchronization with photoperiod and tides. *Physiology and Behavior* 55: 913–919.
283. Wikelski, M. & Hau, M. (1995). Is there an endogenous tidal foraging rhythm in marine iguanas? *Journal of Biological Rhythms* 10: 335–350.
284. 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.
285. Palmer, J. D. (1967). Daily and tidal components in the persistent rhythmic activity of the crab, *Sesarma*. *Nature* 215: 64–66.
286. Cummings, S. M. & Morgan, E. (2001). Time-keeping system of the eel pout, *Zoarces viviparus*. *Chronobiology International* 18: 27–46.
287. Akiyama, T. (1997). Tidal adaptation of a circadian clock controlling a crustacean swimming behavior. *Zoological Science* 14: 901–906.
288. Alleva, J. J., Waleski, M. V. & Alleva, F. R. (1971). A biological clock controlling the estrous cycle of the hamster. *Endocrinology* 88: 1368–1379.
289. Fitzgerald, K. M. & Zucker, I. (1976). Circadian organization of the estrous cycle of the golden hamster. *Proceedings of the National Academy of Sciences U.S.A.* 73: 2923–2927.
290. Stetson, M. H. & Gibson, J. T. (1977). The estrous cycle in golden hamsters: a circadian pacemaker times preovulatory gonadotropin release. *Journal of Experimental Zoology* 201: 289–294.
291. Sridaran, R. & 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.
292. Carmichael, M. S., Nelson, R. J. & Zucker, I. (1981). Hamster activity and estrous cycles: control by a single versus multiple circadian oscillator(s). *Proceedings of the National Academy of Sciences U.S.A.* 78: 7830–7834.
293. Swann, J. & 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.
294. Stetson, M. H. & Watson-Whitmyre, M. (1976). Nucleus suprachiasmaticus: the biological clock in the hamster? *Science* 191: 197–199.
295. Dunn, J. D., Castro, A. J. & McNulty, J. A. (1977). Effect of suprachiasmatic ablation on the daily temperature rhythm. *Neuroscience Letters* 6: 345–348.
296. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N. & Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
297. Miller, B. H., Olson, S. L., Turek, F. W., Levine, J. E., Horton, T. H. & Takahashi, J. S. (2004). Circadian *Clock* mutation disrupts estrous ciclicity and maintenance of pregnancy. *Current Biology* 14: 1367–1373.
298. Refinetti, R. & Menaker, M. (1992). Evidence for separate control of estrous and circadian periodicity in the golden hamster. *Behavioral and Neural Biology* 58: 27–36.
299. Neill, J. D. (1972). Sexual differences in the hypothalamic regulation of prolactin secretion. *Endocrinology* 90: 1154–1159.
300. Seegal, R. F. & Goldman, B. D. (1975). Effects of photoperiod on cyclicity and serum gonadotropins in the Syrian hamster. *Biology of Reproduction* 12: 223–231.
301. Stetson, M. H., Watson-Whitmyre, M., DiPinto, M. N. & Smith, S. G. (1981). Daily luteinizing hormone release in ovariectomized hamsters: effect of barbiturate blockade. *Biology of Reproduction* 24: 139–144.
302. Swann, J. M. & Turek, F. W. (1985). Multiple circadian oscillators regulate the timing of behavioral and endocrine rhythms in female golden hamsters. *Science* 228: 898–900.
303. Lucas, R. J., Stirland, J. A., Darrow, J. M., Menaker, M. & 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.
304. Palm, I. F., van der Beek, E. M., Swarts, H. J. M., van der Vliet, J., Wiegant, V. M., Buijs, R. M. & Kalsbeek, A. (2001). Control of the estradiol-induced prolactin surge by the suprachiasmatic nucleus. *Endocrinology* 142: 2296–2302.
305. Häster, L. & 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.
306. Rauth-Widmann, B., Fuchs, E. & 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.
307. Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.

308. Zivkovic, B. D., Underwood, H., Steele, C. T. & 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.

309. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.

310. Bobr, L. W. & 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.

311. Kadono, H., Besch, E. L. & Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.

312. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W. & 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.

313. 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.

314. Bakko, E. B., Porter, W. P. & Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.

315. Halle, S. (1995). Diel pattern of locomotor activity in populations of root voles, *Microtus oeconomus*. *Journal of Biological Rhythms* 10: 211–224.

316. Scheibe, K. M., Berher, A., Langbein, J., Streich, W. J. & Eichhorn, K. (1999). Comparative analysis of ultradian and circadian behavioural rhythms for diagnosis of biorhythmic state of animals. *Biological Rhythm Research* 30: 216–233.

317. Rezende, E. L., Cortés, A., Bacigalupe, L. D., Nespolo, R. F. & Bozinovic, F. (2003). Ambient temperature limits above-ground activity of the subterranean rodent *Spalacopus cyanus*. *Journal of Arid Environments* 55: 63–74.

318. Berger, A., Scheibe, K. M., Michaelis, S. & 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.

319. Haim, A., Yedidia, I., Haim, D. & 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.

320. Basco, P. S., Rashotte, M. E. & Stephan, F. K. (1996). Photoperiod duration and energy balance in the pigeon. *Physiology and Behavior* 60: 151–159.

321. Schilling, A., Richard, J. P. & 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.

322. Anchordoquy, H. C. & 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.

323. Perret, M. & 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.

324. Refinetti, R. (2002). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.

325. Kowalczyk, R., Jedrzejewska, B. & 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.

326. Binkley, S., Tome, M. B., Crawford, D. & Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.

327. Yoneyama, S., Hashimoto, S. & Honma, K. (1999). Seasonal changes of human circadian rhythms in Antarctica. *American Journal of Physiology* 277: R1091–R1097.

328. 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.

329. Mrosovsky, N., Boshes, M., Hallonquist, J. D. & Lang, K. (1976). Circannual cycle of circadian cycles in a golden-mantled ground squirrel. *Naturwissenschaften* 63: 298–299.

330. Cisse, F., Martineaud, R. & 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.

331. Honma, K., Honma, S., Kohsaka, M. & Fukuda, N. (1992). Seasonal variation in the human circadian rhythm: dissociation between sleep and temperature rhythm. *American Journal of Physiology* 262: R885–R891.

332. Grahn, D. A., Miller, J. D., Houng, V. S. & Heller, H. C. (1994). Persistence of circadian rhythmicity in hibernating ground squirrels. *American Journal of Physiology* 266: R1251–R1258.

333. Wollnik, F. & 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.

334. Lee, T. M. & Zucker, I. (1995). Seasonal variations in circadian rhythms persist in gonadectomized golden-mantled ground squirrels. *Journal of Biological Rhythms* 10: 188–195.

335. van Houwelingen, C. A. J. & 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.
336. Bertolucci, C., Foà, A. & Van't Hof, T. J. (2002). Seasonal variations in circadian rhythms of plasma melatonin in ruin lizards. *Hormones and Behavior* 41: 414–419.
337. Bovet, J. & Oertli, E. F. (1974). Free-running circadian activity rhythms in free-living beaver (*Castor canadensis*). *Journal of Comparative Physiology* 92: 1–10.
338. Lancia, R. A., Dodge, W. E. & Larson, J. S. (1982). Winter activity patterns of two radio-marked beaver colonies. *Journal of Mammalogy* 63: 598–606.
339. Dyck, A. P. & MacArthur, R. A. (1992). Seasonal patterns of body temperature in free-ranging beaver (*Castor canadensis*). *Canadian Journal of Zoology* 70: 1668–1672.
340. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.
341. Körtner, G., Song, X. & 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.
342. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. & Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
343. Waßmer, T. & Wollnik, F. (1997). Timing of torpor bouts during hibernation in European hamsters (*Cricetus cricetus* L.). *Journal of Comparative Physiology B* 167: 270–279.
344. Hut, R. A., Barnes, B. M. & 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.
345. Oklejewicz, M., Daan, S. & Strijkstra, A. M. (2001). Temporal organisation of hibernation in wild-type and *tau* mutant Syrian hamsters. *Journal of Comparative Physiology B* 171: 431–439.
346. Larkin, J. E., Franken, P. & Heller, H. C. (2002). Loss of circadian organization of sleep and wakefulness during hibernation. *American Journal of Physiology* 282: R1086–R1095.
347. Kilduff, T. S., Miller, J. D., Radeke, C. M., Sharp, F. R. & 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.
348. Hayflick, L. (1989). Antecedents of cell aging research. *Experimental Gerontology* 24: 355–365.
349. Brock, M. A. (1985). Biological clocks and aging. *Review of Biological Research in Aging* 2: 445–462.
350. Adan, A. (1997). Diferencias individuales en las variaciones diurnas fisiologicas y comportamentales. *Revista Latinoamericana de Psicologia* 29: 81–114.
351. Turek, F. W., Scarbrough, K., Penev, P., Labyak, S., Valentinuzzi, V. S. & Van Reeth, O. (2001). Aging of the mammalian circadian system. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 291–317.
352. Vitiello, M. V., Smallwood, R. G., Avery, D. H., Pascualy, R. A., Martin, D. C. & Prinz, P. N. (1986). Circadian temperature rhythms in young adult and aged men. *Neurobiology of Aging* 7: 97–100.
353. Touitou, Y., Reinberg, A., Bogdan, A., Auzéby, A., Beck, H. & 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.
354. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T. & Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
355. Yunis, E. J., Fernandes, G., Nelson, W. & Halberg, F. (1974). Circadian temperature rhythms and aging in rodents. In: Scheving, L. E., Halberg, F. & Pauly, J. E. (Eds.). *Chronobiology.* Tokyo: Igaku Shoin, pp. 358–363.
356. Sacher, G. A. & Duffy, P. H. (1978). Age changes in rhythms of energy metabolism, activity, and body temperature in *Mus* and *Peromyscus*. In: Samis, H. V. & Capobianco, S. (Eds.). *Aging and Biological Rhythms*. New York: Plenum, pp. 105–124.
357. Halberg, J., Halberg, E., Regal, P. & Halberg, F. (1981). Changes with age characterize circadian rhythm in telemetered core temperature of stroke-prone rats. *Journal of Gerontology* 36: 28–30.
358. Refinetti, R., Ma, H. & Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
359. Koster-van-Hoffen, G. C., Mirmiran, M., Bos, N. P. A., Witting, W., Delagrande, P. & 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.
360. Li, H. & Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
361. McDonald, R. B., Hoban-Higgins, T. M., Ruhe, R. C., Fuller, C. A. & Horwitz, B. A. (1999). Alterations in endogenous circadian rhythm of core temperature in senescent Fischer 344 rats. *American Journal of Physiology* 276: R824–R830.
362. Irizarry, R. A., Tankersley, C., Frank, R. & Flanders, S. (2001). Assessing homeostasis through circadian patterns. *Biometrics* 57: 1228–1237.
363. Benstaali, C., Bogdan, A. & 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.

364. Frank, R. & Tankersley, C. (2002). Air pollution and daily mortality: a hypothesis concerning the role of impaired homeostasis. *Environmental Health Perspectives* 110: 61–65.

365. Bourlière, F. (1947). Quelques caractéristiques physiologiques de la sénescence chez le rat. *Revue Canadienne de Biologie* 6: 245–259.

366. Duffy, J. F., Viswanathan, N. & Davis, F. C. (1999). Free-running circadian period does not shorten with age in female Syrian hamsters. *Neuroscience Letters* 271: 77–80.

367. Weinert, D. & Waterhouse, J. (1999). Daily activity and body temperature rhythms do not change simultaneously with age in laboratory mice. *Physiology and Behavior* 66: 605–612.

368. Siwak, C. T., Tapp, P. D., Zicker, S. C., Murphey, H. L., Muggenburg, B. A., Head, E. & Cotman, C. W. (2003). Locomotor activity rhythms in dogs vary with age and cognitive status. *Behavioral Neuroscience* 117: 813–824.

369. Pang, K. C. H., Miller, J. P. & McAuley, J. D. (2004). Circadian rhythms in SAMP8: a longitudinal study of the effects of age and experience. *Neurobiology of Aging* 25: 111–123.

370. Tankersley, C. G., Irizarry, R., Flanders, S. E., Rabold, R. & Frank, R. (2003). Unstable heart rate and temperature regulation predict mortality in AKR/J mice. *American Journal of Physiology* 284: R742–R750.

371. Atwood, C. S., James, I. R., Keil, U., Roberts, N. K. & Hartmann, P. E. (1991). Circadian changes in salivary constituents and conductivity in women and men. *Chronobiologia* 18: 125–140.

372. O'Sullivan, C., Duggan, J., Atkins, N. & 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.

373. Zhao, Z. Y., Xie, Y., Fu, Y. R., Bogdan, A. & 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.

374. Winocur, G. & 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.

375. Hurd, M. W. & Ralph, M. R. (1998). The significance of circadian organization for longevity in the golden hamster. *Journal of Biological Rhythms* 13: 430–436.

376. May, C. P., Hasher, L. & Stoltzfus, E. R. (1993). Optimal time of day and the magnitude of age differences in memory. *Psychological Science* 4: 326–330.

377. Baehr, E. K., Revelle, W. & 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.

378. Klerman, E. B., Duffy, J. F., Dijk, D. J. & Czeisler, C. A. (2001). Circadian phase resetting in older people by ocular bright light exposure. *Journal of Investigative Medicine* 49: 30–40.

379. Robilliard, D. L., Archer, S. N., Arendt, J., Lockley, S. W., Hack, L. M., English, J., Leger, D., Smits, M. G., Williams, A., Skene, D. J. & von Schantz, M. (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.

380. Pittendrigh, C. S. & Daan, S. (1974). Circadian oscillations in rodents: a systematic increase of their frequency with age. *Science* 186: 548–550.

381. Morin, L. P. (1988). Age-related changes in hamster circadian period, entrainment, and rhythm splitting. *Journal of Biological Rhythms* 3: 237–248.

382. Rosenberg, R. S., Zee, P. C. & Turek, F. W. (1991). Phase response curves to light in young and old hamsters. *American Journal of Physiology* 261: R491–R495.

383. Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *Journal of Biological Rhythms* 8: 189–197.

384. Viswanathan, N. & Davis, F. C. (1995). Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. *Brain Research* 686: 10–16.

385. Oklejewicz, M., Hut, R. A., Daan, S, Loudon, A. S. I. & Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.

386. Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H. & Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *Journal of Biological Rhythms* 18: 159–169.

387. Witting, W., Mirmiran, M., Bos, N. P. A. & Swaab, D. F. (1994). The effect of old age on the free-running period of circadian rhythms in rat. *Chronobiology International* 11: 103–112.

388. Possidente, B., McEldowney, S. & Pabon, A. (1995). Aging lengthens circadian period for wheel-running activity in C57BL mice. *Physiology and Behavior* 57: 575–579.

389. Pohl, H. (1993). Does aging affect the period of the circadian pacemaker in vertebrates? *Naturwissenschaften* 80: 478–481.

390. Moore, D. (2001). Honey bee circadian clocks: behavioral control from individual workers to whole-colony rhythms. *Journal of Insect Physiology* 47: 843–857.

391. Ralph, M. R., Ko, C. H., Antoniadia, E. A., Seco, P., Irani, F., Presta, C. & 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.

392. Cain, S. W., Chou, T. & Ralph, M. R. (2004). Circadian modulation of performance on an aversion-based place learning task in hamsters. *Behavioural Brain Research* 150: 201–205.

393. Mistlberger, R. E., de Groot, M. H. M., Bossert, J. M. & Marchant, E. G. (1996). Discrimination of circadian phase in intact and suprachiasmatic nuclei-ablated rats. *Brain Research* 739: 12–18.

394. Aragona, B. J., Curtis, J. T., Davidson, A. J., Wang, Z. & Stephan, F. K. (2002). Behavioral and neurochemical investigation of circadian time-place learning in the rat. *Journal of Biological Rhythms* 17: 330–344.

395. Biebach, H., Gordijn, M. & Krebs, J. (1989). Time-place learning by garden warblers, *Sylvia borin*. *Animal Behaviour* 37: 353–360.

396. Saksida, L. M. & Wilkie, D. M. (1994). Time-of-day discrimination by pigeons, *Columba livia*. *Animal Learning and Behavior* 22: 143–154.

397. Reebs, S. G. (1996). Time-place learning in golden shiners (Pisces: Cyprinidae). *Behavioural Processes* 36: 253–262.

398. 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.

399. Thorpe, C. M., Bates, M. E. & Wilkie, D. M. (2003). Rats have trouble associating all three parts of the time-place-event memory code. *Behavioural Processes* 63: 95–110.

400. Cain, S. W., Ko, C. H., Chalmers, J. A. & 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.

401. Lukoyanov, N. V., Pereira, P. A., Mesquita, R. M. & Andrade, J. P. (2002). Restricted feeding facilitates time-place learning in adult rats. *Behavioural Brain Research* 134: 283–290.

402. Carr, J. A. R. & Wilkie, D. M. (1999). Rats are reluctant to use circadian timing in a daily time-place task. *Behavioural Processes* 44: 287–299.

403. Pizzo, M. J. & Crystal, J. D. (2004). Evidence for an alternation strategy in time-place learning. *Behavioural Processes* 67: 533–537.

404. Amir, S. & Stewart, J. (1996). Resetting of the circadian clock by a conditioned stimulus. *Nature* 379: 542–545.

405. Arvanitogiannis, A. & Amir, S. (2001). A novel, associative process modulating photic resetting of the circadian clock. *Neuroscience* 104: 615–618.

406. De Groot, M. H. M. & Rusak, B. (2000). Responses of the circadian system of rats to conditioned and unconditioned stimuli. *Journal of Biological Rhythms* 15: 277–291.

407. Arvanitogiannis, A., Stewart, J. & Amir, S. (2000). Conditioned stimulus control in the circadian system: two tales tell one story. *Journal of Biological Rhythms* 15: 292–293.

408. Arvanitogiannis, A. & Amir, S. (1999). Conditioned stimulus control in the rat circadian system depends on clock resetting during conditioning. *Behavioral Neuroscience* 113: 1297–1300.

409. Rusak, B. & de Groot, M. H. M. (2000). Requirements for conditioning of the rat circadian system remain elusive. *Journal of Biological Rhythms* 15: 294–295.

410. Buss, D. M. (1989). Sex differences in human mate preferences: evolutionary hypotheses tested in 37 cultures. *Behavioral and Brain Sciences* 12: 1–49.

411. Koteja, P., Swallow, J. G., Carter, P. A. & Garland, T. (2003). Different effects of intensity and duration of locomotor activity on circadian period. *Journal of Biological Rhythms* 18: 491–501.

412. Zucker, I., Fitzgerald, K. M. & Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.

413. Francis, A. J. P. & 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.

414. Daan, S. & Oklejewicz, M. (2003). The precision of circadian clocks: assessment and analysis in Syrian hamsters. *Chronobiology International* 20: 209–221.

415. Yang, Y. & 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.

416. Schmidt, F., Yoshimura, Y., Ni, R. X., Kneesel, S. & 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.

417. Campbell, S. S., Gillin, J. C., Kripke, D. F., Erikson, P. & Clopton, P. (1989). Gender differences in the circadian temperature rhythms of healthy elderly subjects: relationships to sleep quality. *Sleep* 12: 529–536.

418. Adan, A. & Natale, V. (2002). Gender differences in morningness-eveningness preference. *Chronobiology International* 19: 709–720.

419. Hoban, T. M., Levine, A. H., Shane, R. B. & Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.

420. Moritz, R. F. A. & Sakofski, F. (1991). The role of the queen in circadian rhythms of honeybees (*Apis mellifera* L.). *Behavioral Ecology and Sociobiology* 29: 361–365.

421. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P. & 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.

422. Sánchez-Vázquez, F. J., Madrid, J. A., Zamora, S. & 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.

423. Stupfel, M., Gourlet, V., Perramon, A., Mérat, P., Putet, G. & Court, L. (1995). Comparison of ultradian and circadian oscillations of carbon dioxide production by various endotherms. *American Journal of Physiology* 268: R253–R265.

424. 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.

425. Tavernier, R. J., Largen, A. L. & Bult-Ito, A. (2004). Circadian organization of a subarctic rodent, the northern red-backed vole (*Clethrionomys rutilus*). *Journal of Biological Rhythms* 19: 238–247.
426. Oshima, I. & 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.
427. Guyomarc'h, C., Lumineau, S. & 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.
428. Lumineau, S. & Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
429. Hurd, M. W., Debruyne, J., Straume, M. & Cahill, G. M. (1998). Circadian rhythms of locomotor activity in zebrafish. *Physiology and Behavior* 65: 465–472.
430. Kennedy, G. A., Coleman, G. J. & Armstrong, S. M. (1991). Restricted feeding entrains circadian wheel-running activity rhythms of the kowari. *American Journal of Physiology* 261: R819–R827.
431. Lowe, C. H., Hinds, D. S., Lardner, P. J. & Justice, K. E. (1967). Natural free-running period in vertebrate animal populations. *Science* 156: 531–534.
432. Yoshii, T., Funada, Y., Ibuki-Ishibashi, T., Matsumoto, A., Tanimura, T. & 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.
433. Honma, S. & Honma, K. (1999). Light-induced uncoupling of multioscillatory circadian system in a diurnal rodent, Asian chipmunk. *American Journal of Physiology* 276: R1390–R1396.
434. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., & 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.
435. Rajaratnam, S. M. W. & Redman, J. R. (1998). Entrainment of activity rhythms to temperature cycles in diurnal palm squirrels. *Physiology and Behavior* 63: 271–277.
436. Refinetti, R. & Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.
437. Koilraj, A. J., Sharma, V. K., Marimuthu, G. & 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.
438. Chandrashekaran, M. K., Marimuthu, G. & 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.
439. Reinberg, A., Halberg, F., Ghata, J. & 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.
440. Pollak, C. P. & Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
441. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C. & Knauer, R. S. (1980). Human sleep: its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.
442. Green, J., Pollak, C. P. & Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
443. Steel, G. D., Callaway, M., Suedfeld, P. & 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.
444. Aschoff, J. (1994). The timing of defecation within sleep-wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
445. Campbell, S. S., Dawson, D. & Zulley, J. (1993). When the human circadian system is caught napping: evidence for endogenous rhythms close to 24 hours. *Sleep* 16: 638–640.
446. Aschoff, J. (1994). Naps as integral parts of the wake time within the human sleep-wake cycle. *Journal of Biological Rhythms* 9: 145–155.
447. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A. & 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.
448. Wever, R. A. (1989). Light effects on human circadian rhythms: a review of recent Andechs experiments. *Journal of Biological Rhythms* 4: 161–185.
449. Honma, K., Honma, S. & Wada, T. (1987). Entrainment of human circadian rhythms by artificial bright light cycles. *Experientia* 43: 572–574.
450. Lockley, S. W., Skene, D. J., Butler, L. J. & Arendt, J. (1999). Sleep and activity rhythms are related to circadian phase in the blind. *Sleep* 22: 616–623.
451. Endo, T., Honma, S., Hashimoto, S. & Honma, K. (1999). After-effect of entrainment on the period of human circadian system. *Japanese Journal of Physiology* 49: 425–430.
452. Sack, R. L., Brandes, R. W., Kendall, A. R. & Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
453. Hack, L. M., Lockley, S. W., Arendt, J. & 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.
454. Tosini, G. & 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.
455. Tosini, G. & Menaker, M. (1995). Circadian rhythm of body temperature in an ectotherm (*Iguana iguana*). *Journal of Biological Rhythms* 10: 248–255.

456. Bartell, P. A., Miranda-Anaya, M. & Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
457. Page, T. L., Mans, C. & Griffeth, G. (2001). History dependence of circadian pacemaker period in the cockroach. *Journal of Insect Physiology* 47: 1085–1093.
458. 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.
459. Tokura, H. & Aschoff, J. (1983). Effects of temperature on the circadian rhythm of pig-tailed macaques *Macaca nemestrina*. *American Journal of Physiology* 245: R800–R804.
460. Shimomura, K., Nelson, D. E., Ihara, N. L. & Menaker, M. (1997). Photoperiodic time measurement in *tau* mutant hamsters. *Journal of Biological Rhythms* 12: 423–430.
461. Weinert, D., Fritzche, P. & Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International* 18: 921–932.
462. Ralph, M. R. & Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
463. Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
464. Daan, S. & 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.
465. Refinetti, R. (2001). Dark adaptation in the circadian system of the mouse. *Physiology and Behavior* 74: 101–107.
466. Refinetti, R., Nelson, D. E. & 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.
467. Perret, M., Aujard, F., Séguy, M. & 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.
468. Gerkema, M. P., Groos, G. A. & 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.
469. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & Subbaraj, R. (1999). Relationship between light intensity and phase resetting in a mammalian circadian system. *Journal of Experimental Zoology* 283: 181–185.
470. Edgar, D. M., Kilduff, T. S., Martin, C. E. & 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.
471. Edgar, D. M., Martin, C. E. & 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.
472. Von Gall, C., Duffield, G. E., Hastings, M. H., Kopp, M. D. A., Dehghani, F., Korf, H. W. & 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.
473. Abe, H., Honma, S., Honma, K., Suzuki, T. & 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.
474. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S. & 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.
475. Edgar, D. M. & Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
476. Benloucif, S. & 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.
477. Labyak, S. E. & Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
478. Lee, T. M. & 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.
479. Kennedy, G. A., Hudson, R. & Armstrong, S. M. (1994). Circadian wheel running activity rhythms in two strains of domestic rabbit. *Physiology and Behavior* 55: 385–389.
480. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences U.S.A.* 59: 414–421.
481. Hau, M. & Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
482. Lindberg, R. G. & Hayden, P. (1974). Thermoperiodic entrainment of arousal from torpor in the little pocket mouse, *Perognathus longimembris*. *Chronobiologia* 1: 356–361.
483. Puchalski, W. & Lynch, G. R. (1991). Circadian characteristics of Djungarian hamsters: effects of photoperiodic pretreament and artificial selection. *American Journal of Physiology* 261: R670–R676.
484. Innicenti, A., Minutini, L. & Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.

485. Hamasaka, Y., Watari, Y., Arai, T., Numata, H. & 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.

486. Borbély, A. A. & 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.

487. Ikeda, M. & 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.

488. Yamada, N., Shimoda, K., Ohi, K., Takahashi, S. & 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.

489. Summer, T. L., Ferraro, J. S. & McCormack, C. E. (1984). Phase-response and Aschoff illuminance curves for locomotor activity rhythm of the rat. *American Journal of Physiology* 246: R299–R304.

490. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.

491. Stokes, M. A., Kent, S. & 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.

492. Canal-Corretger, M. M., Vilaplana, J., Cambras, T. & 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.

493. Honma, K., von Goetz, C. & Aschoff, J. (1983). Effects of restricted daily feeding on free-running circadian rhythms in rats. *Physiology and Behavior* 30: 905–913.

494. Mendoza, J. Y., Aguilar-Roblero, R., Díaz-Muñoz, M. & Escobar, C. (2003). Daily epinephrine but not norepinephrine administration produces anticipatory drinking behavior in rats. *Biological Rhythm Research* 34: 73–90.

495. Meerlo, P., van den Hoofdakker, R. H., Koolhaas, J. M. & 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.

496. Fuller, C. A. & 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.

497. Underwood, H. (1983). Circadian pacemakers in lizards: phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.

498. King, V. M., Bentley, G. E. & 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.

499. Ebihara, S. & Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.

500. Kramm, K. R. & Kramm, D. A. (1980). Photoperiodic control of circadian activity rhythms in diurnal rodents. *International Journal of Biometeorology* 24: 65–76.

501. Herrero, M. J., Madrid, J. A. & Sánchez-Vázquez, F. J. (2003). Entrainment to light of circadian activity rhythms in tench (*Tinca tinca*). *Chronobiology International* 20: 1001–1017.

502. Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A. & Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740.

503. Rothenfluh, A., Abodeely, M., Price, J. L. & 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.

504. Comolli, J., Taylor, W. & 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.

505. Morse, D., Hastings, J. W. & Roenneberg, T. (1994). Different phase responses of the two circadian oscillators in *Gonyaulax*. *Journal of Biological Rhythms* 9: 263–274.

506. Khalsa, S. B. S., Jewett, M. E., Cajochen, C. & Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.

507. Jewett, M. E., Kronauer, R. E. & 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.

508. Dawson, D., Lack, L. & Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102.

509. Honma, K. & Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167–168.

510. Christian, C. A. & 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.

511. Grosse, J., Loudon, A. S. I. & 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.

512. Meijer, J. H. & 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.

513. Takahashi, J. S., DeCoursey, P. J., Mauman, L. & Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.

514. Shimomura, K. & Menaker, M. (1994). Light-induced phase shifts in *tau* mutant hamsters. *Journal of Biological Rhythms* 9: 97–110.
515. Sharma, V. K., Chandrashekaran, M. K. & 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.
516. Shrama, V. K., Chidambaram, R. & 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.
517. 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.
518. Kilduff, T. S., Vugrinic, C., Lee, S. L., Milbrandt, J. D., Mikkelsen, J. D., O'Hara, B. F. & 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.
519. Yoshimura, T., Nishio, M., Goto, M. & 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.
520. Van den Pol, A. N., Cao, V. & Heller, H. C. (1998). Circadian system of mice integrates brief light stimuli. *American Journal of Physiology* 275: R654–R657.
521. Sharma, V. K. & Daan, S. (2002). Circadian phase and period responses to light stimuli in two nocturnal rodents. *Chronobiology International* 19: 659–670.
522. Bauer, M. S. (1992). Irradiance responsivity and unequivocal type-1 phase responsivity of rat circadian activity rhythms. *American Journal of Physiology* 263: R1110–R1114.
523. 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.
524. Meijer, J. H., Daan, S., Overkamp, G. J. F. & 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.

10 Homeostasis and Circadian Rhythmicity

CHAPTER OUTLINE

10.1 Temperature Regulation
10.2 Sleep, Feeding, and Energy Expenditure

10.1 TEMPERATURE REGULATION

The concept of *homeostasis* is perhaps the most important concept in physiology. From its origins in the work of Claude Bernard (Figure 10.1) in the 1800s,[1] this concept has been refined over decades[2] and is considered an essential part of cultural literacy.[3] As defined by the Commission for Thermal Physiology of the International Union of Physiological Sciences, homeostasis characterizes "the relative constancy of physicochemical properties of the internal environment of an organism as being maintained by regulation."[4]

Although homeostasis applies to numerous processes in the body — such as those required for proper electrolyte balance, sufficient supply of nutrients, and adequate secretion of hormones — temperature regulation is possibly the most intuitive form of homeostasis. Consider Figure 10.2. To remain warm in the winter, the house must have a temperature sensor, which sends a signal to a thermostat, which controls a heater (Panel A). Temperature regulation in a living organism can be illustrated using the same diagram with just a small rearrangement of the components to make the terms more explicit (Panel B). 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. 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 entire cycle is repeated as needed.

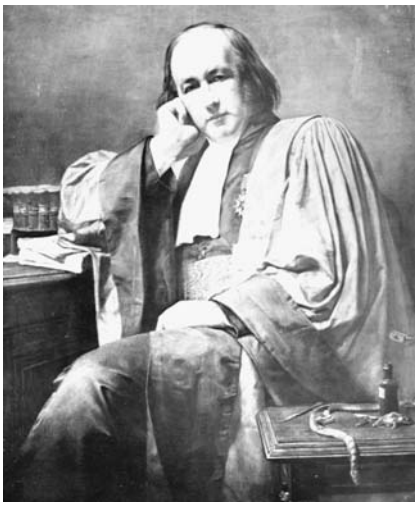


FIGURE 10.1 Claude Bernard (1813–1878). This French physiologist developed the concept of homeostasis. (Source: Bibliothèque Interuniversitaire de Médecine, Paris, France.)

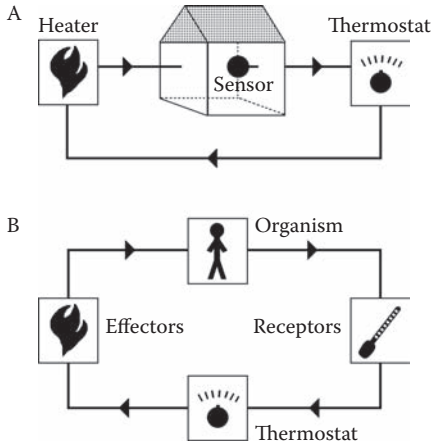


FIGURE 10.2 Homeostasis. The concept of homeostasis (regulation by negative feedback) is illustrated in a diagram of the heating system of a house (A) and in a diagram of the thermoregulatory system of organisms (B).

As shown in the preceding chapters of this book, physiological variables are not maintained constant in the absence of environmental perturbations but instead display regular circadian rhythmicity. Although circadian rhythmicity clearly interferes with the constancy of the internal environment, only during the last 20 years or so have researchers systematically studied the integration of homeostatic and circadian processes of physiological regulation. In the case of body temperature regulation, thermal physiologists and circadian physiologists ignored each other's work for most of the 20th century. In the next

sections, I review the accumulated knowledge on the homeostatic control of body temperature (the process of *temperature regulation*), and then I relate it to the accumulated knowledge on the *circadian* control of body temperature.

10.1.1 Homeostatic Control of Body Temperature

Chapter 6 alluded to the fact 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 two-fold change in the reaction rate.[5,6] 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 was previously. Considering that the mean air temperature on Earth varies from far 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, organisms clearly must adapt to the thermal environment 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 body temperature. Most organisms do not regulate body temperature — or they regulate it in a limited fashion — but they are well adapted to the thermal environment. For example, many Antarctic fishes spend their entire life cycle at subfreezing body temperatures,[7] and some Archea survive and grow at temperatures up to 120°C.[8] Lower vertebrates (reptiles, amphibians, and fishes) use antifreeze proteins to avoid freezing in the winter.[9,10]

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. (Source: © ArtToday, Tucson, AZ.)

Traditionally, organisms that do not regulate body temperature (and, therefore, are usually cold to the touch) are called *cold-blooded*, while organisms that regulate body temperature (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. If you lived in a very warm place (with ambient temperature occasionally exceeding 33°C or 91°F), however, you may have noticed that frogs feel warm to the touch. The terms *cold-blooded* and *warm-blooded* are inaccurate. 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. Frogs are poikilothermic. Because the skin temperature of the human hand is approximately 33°C, objects feel warm to the touch when their temperature exceeds 33°C.[11–15] A frog living in a warm environment feels warm to the touch. A dog, which is homeothermic and has a body temperature similar to that of a human, would feel cold to your touch if you had a very high fever.

Core temperature generally is regulated around 35°C in marsupial mammals, 37.5°C in placental mammals, and 41°C in birds (see Table 5.3 in Chapter 5). Some researchers have suggested that the core temperature of terrestrial homeotherms must be set within the range of 35 to 45°C because only this range provides a proper balance between the costs of energy conservation in the cold and the costs of water conservation in the heat.[16]

Many years after kindergarten, you also must have learned that things in this world are rarely as simple as they first appear to be. Consider the distinction between poikilothermy and homeothermy. The distinction works well if organisms are kept in small cells at constant temperatures in the laboratory, but it breaks down very quickly if organisms are allowed to move freely in their natural environments. Consider Figure 10.4. The top graph shows that the body temperature of an alligator housed in a chamber in the laboratory follows ambient temperature very closely. Thus, the alligator is a poikilotherm. However, the middle panel shows that in the field — with freedom to warm itself by basking in the sun and cool itself by entering the water — the alligator maintains a constant body temperature independently of air temperature. Now the alligator is a homeotherm. When allowed to use behavioral responses, the alligator maintains its body temperature almost as precisely as a "true" homeotherm — the mouse (bottom panel). Evidently, poikilothermic organisms can exhibit sophisticated temperature regulation through behavioral means. Thus, the distinction between poikilothermy and homeothermy is too broad for most uses. A finer distinction must be made to account for thermoregulatory differences limited

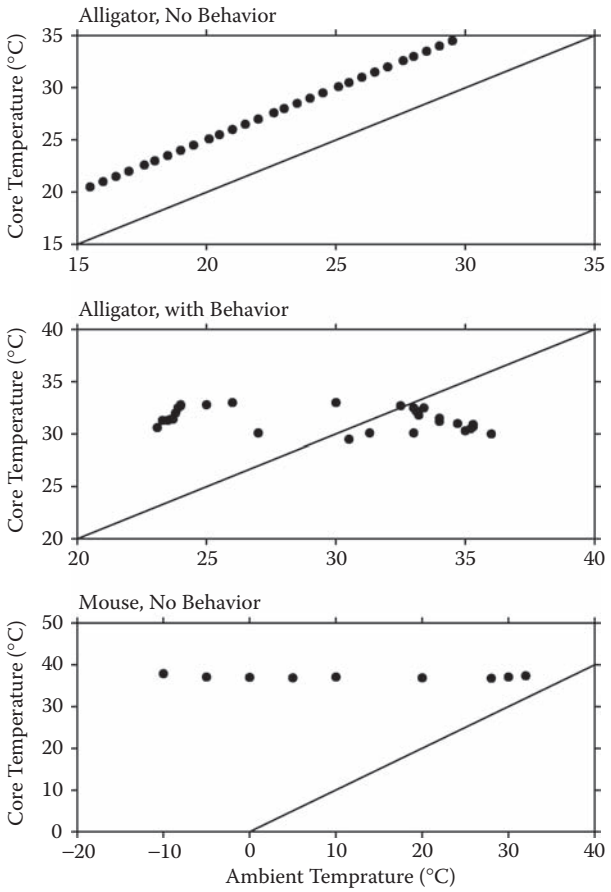


FIGURE 10.4 Poikilotherms, homeotherms, and other therms. The body core temperature of an alligator held in a chamber in the laboratory closely follows air temperature. However, if the alligator is allowed to thermoregulate behaviorally, its core body temperature is much less dependent on air temperature. In mice, body core temperature is independent of ambient temperature (within limits) even in the absence of behavioral responses. In all three graphs, the diagonal lines indicate identity between core temperature and ambient temperature. (Sources: Smith, E. N. (1975). Thermoregulation of the American alligator, *Alligator mississippiensis*. *Physiological Zoology* 48: 177–194; Oufara, S., Barré, H., Rouanet, J. L. & Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *American Journal of Physiology* 253: R39–R45.)

to autonomic processes. Organisms that can generate enough heat endogenously to warm their bodies above ambient temperature without resorting to behavior are called *endothermic* organisms; conversely, organisms that require external sources to warm their bodies above ambient temperature are called *ectothermic* organisms. The resting metabolic rate of endothermic animals is four to five times higher than that of ectothermic animals of similar body size tested at the same body temperature. Endothermic animals have relatively larger internal organs, the organs have a greater proportion of mitochondria (the cell organelles responsible for metabolic oxidation), and the mitochondria have a greater relative membrane surface.[17,18]

Mammals and birds are generally endothermic, while all other life forms on Earth are ectothermic. Exceptions exist, however. Several species of tuna fish can maintain body temperature relatively constant at 25°C while swimming in water ranging from 3 to 31°C.[19–21] Some beetles can maintain a constant thoracic temperature of 38°C while air temperature varies from 13 to 24°C.[22] An even more impressive finding is that several groups of plants can raise metabolic heat production when blooming, causing dramatic elevations in the temperature of the inflorescence. Temperatures of 15 to 20°C were recorded in eastern skunk cabbage plants kept at ambient temperatures from –15 to 5°C.[23] In philodendrons, temperatures of 38 to 46°C were recorded in plants kept at ambient temperatures from 4 to 39°C.[24,25] Temperatures of 30 to 35°C were recorded in sacred lotus plants kept at ambient temperatures from 10 to 30°C.[26,27] Early in the 1970s, the chemical responsible for increased thermogenesis (the *calorigen*) was isolated in voodoo lilies,[28] and the isolate was later identified as salicylic acid.[29] It is ironic that the substance that produces a "fever" in plants is a major antipyretic in mammals.[30]

Exceptions exist also among mammals and birds. As discussed in Chapter 4, animals in the hibernating state allow body temperature to fall along with ambient temperature and do not take corrective measures unless body temperature 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 (*Spermophilus tereticaudus*) exhibit a different form of heterothermy: even when fully active, they allow body temperature to fall more than 5°C when exposed briefly to a moderately cold environment.[31,32] Thirteen-lined ground squirrels (Figure 10.5) also allow body temperature to fluctuate with ambient temperature during the nonhibernating season.[33] The Mashona molerat (*Cryptomys hottentotus*) can maintain a stable, low body temperature of 33°C only in the narrow range of ambient temperatures between 26 and 32°C.[34] As an extreme case, the body temperature of the naked molerat (*Heterocephalus glaber*) follows ambient temperature throughout the range from 12 to 37°C.[35] In contrast, a true homeotherm can maintain body temperature within a very narrow range despite large fluctuations in ambient temperature. For example, during an entire year in an outdoor paddock, a sheep (*Ovis aries*) maintained its core temperature between 37 and 39°C, while ambient temperature varied from –10 to 20°C.[36]

Engineering models have often been used to describe homeothermic temperature regulation. A very simple model is diagrammed in the top panel of Figure 10.6. (Figure 10.2 showed the same diagram.) A more detailed

FIGURE 10.5 A heterothermic animal. The thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) is a facultative homeotherm. It often allows its body temperature to fluctuate in cold environments, and it hibernates in the winter. (Source: Photograph by Mark F. Wallner. All rights reserved.)

model is diagrammed in the middle panel. Note that the single Receptors box was replaced by two boxes, one for Cold Receptors and one for Warm Receptors. Unlike man-made 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. This topic is discussed further in Chapter 11. The single Effectors box was also replaced by two boxes, one for Heat Loss mechanisms and one 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. Note 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."[37] The controlled variable in this case is body temperature. 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. In the early 1960s it became obvious that the constancy of the set point did not hold true in animals. The set point is *adjustable*, going up when skin temperature goes down, and going down when skin temperature goes up.[38] This phenomenon is the reason 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.

Set point is an important concept in thermal physiology. It conveniently explains the complex physiological processes involved in the phenomenon of *fever*. During the rising phase of fever, various thermoregulatory

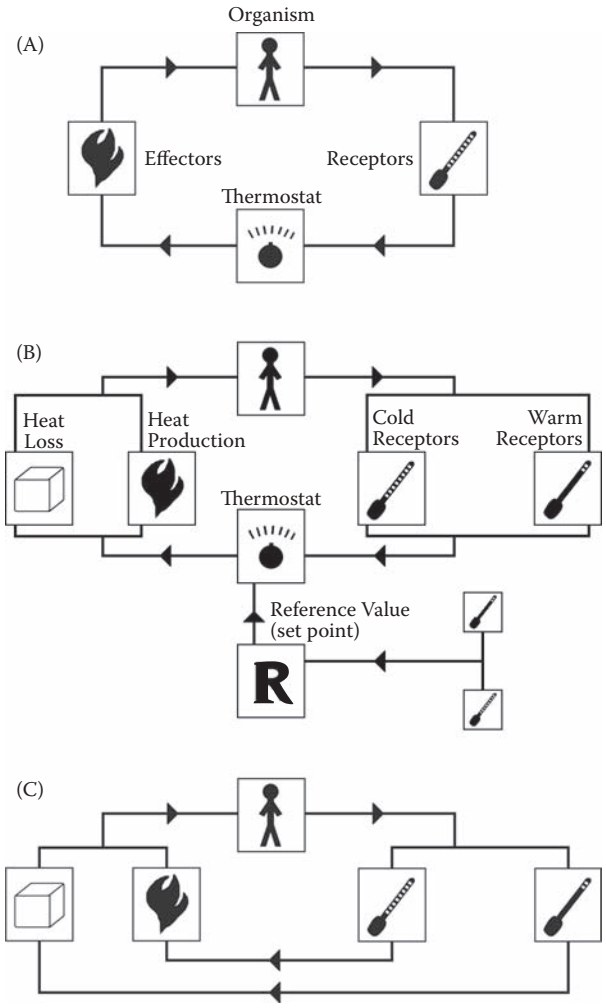


FIGURE 10.6 Homeostatic control of body temperature. The generic model of homeostasis (A) has been applied to body temperature regulation in a version that includes a central set point (B) and a version that does not (C). (Source: Adapted from Refinetti, R. (1988). The concept of set point (goal value) in thermal physiology. *Manuscrito* 11: 47–56.)

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.[39–44] 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), while in the later phase they prefer lower

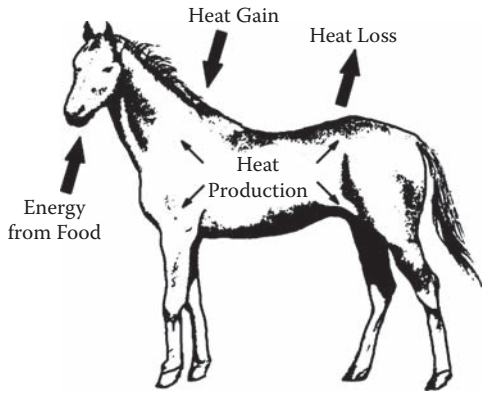


FIGURE 10.7 Heat exchange. Living beings exchange heat with the environment through radiation, conduction, and convection. In addition, they produce heat endogenously by breaking down the food that they eat.

temperatures than usual (which facilitates heat loss).[45–49] Even in ectothermic animals, which cannot produce a fever by autonomic means, higher temperatures are selected when the animals are infected with pyrogenic substances.[50–54]

The theoretical necessity of a central controller has been questioned by some thermal physiologists, who proposed an alternative view that could be called an *accelerator model*.[55] As diagrammed in Panel C of Figure 10.6, these physiologists proposed that heat loss mechanisms respond directly to signals from warm receptors, while heat production mechanisms respond directly to signals from cold receptors.[56–58] When temperature is elevated above thermoneutrality, heat loss mechanisms are activated; when temperature falls below thermoneutrality, heat production mechanisms are activated. The degree of activation is directly related to the temperature; as the temperature rises, more mechanisms of heat loss are activated; as the temperature falls, more mechanisms of heat production and conservation are activated— hence the term *accelerator*. The activation threshold and the response characteristics of the system are determined by the properties of the receptors. A master reference-signal generator (set point) is not needed.

The thermoregulatory system must manage the flow of energy through the body, regardless of the existence or absence of an explicit set point for body temperature. 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 body temperature. These responses may be employed on three basic time scales: as short-term responses, as seasonal acclimatization, or as part of the evolutionary process.[59,60] Table 10.1 lists the major thermoregulatory responses. All of these responses can be used on a short-term time scale, most of them can be modified by acclimatization, and some of them have acquired a more prominent role in different species along the evolutionary process.

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. & Bower, K. (1987). *Temperature Biology of Animals*. London: Chapman and Hall; Cabanac, M. (1996). The place of behavior in physiology. In: Fregley, M. J. & Blatteis, C. M. *Handbook of Physiology — Section 4: Environmental Physiology*, *Volume 2*. New York: Oxford University Press, pp. 1523–1536.

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 body temperature in a cold environment. It consists of small-amplitude, high-frequency contractions of skeletal muscles. It is employed by birds[61–69] and by mammals,[70–85] including humans.[86–88] Figure 10.8 shows an example. King penguin chicks (*Aptenodytes patagonicus*) were studied in a research station in Antarctica. The top graph (A) shows that the penguins elevate metabolic rate to increase heat production as ambient temperature is lowered to –20°C. The middle graph (B) shows that the elevation of metabolic rate is successful in preventing a fall in body temperature. The bottom panel (C) provides the integrated EMG activity data. EMG stands for *electromyogram* — the measurement of electrical activity of muscle cells. Note how closely the EMG activity follows the elevation of metabolic rate, which indicates that shivering is responsible for the elevated heat production.[61] Inactivation of shivering by blockade of neuromuscular synapses prevents the elevation of metabolic rate.[78]

All biological thermogenesis that does not involve shivering is called *nonshivering thermogenesis* (NST). NST involves obligatory as well as adaptive mechanisms.[4] Birds seem to rely primarily on shivering; if they exhibit thermoregulatory NST, the muscles are the probable source.[61,65,66,68,89–92] Mammals, however, use NST extensively in response to cold stress, and the capacity to use NST is strongly affected by acclimation (or acclimatization).[72,78–81,84,93–116] Mammalian thermoregulatory NST often relies on the activation of a specialized tissue, brown adipose tissue (BAT).[117–128] BAT is found in several depots

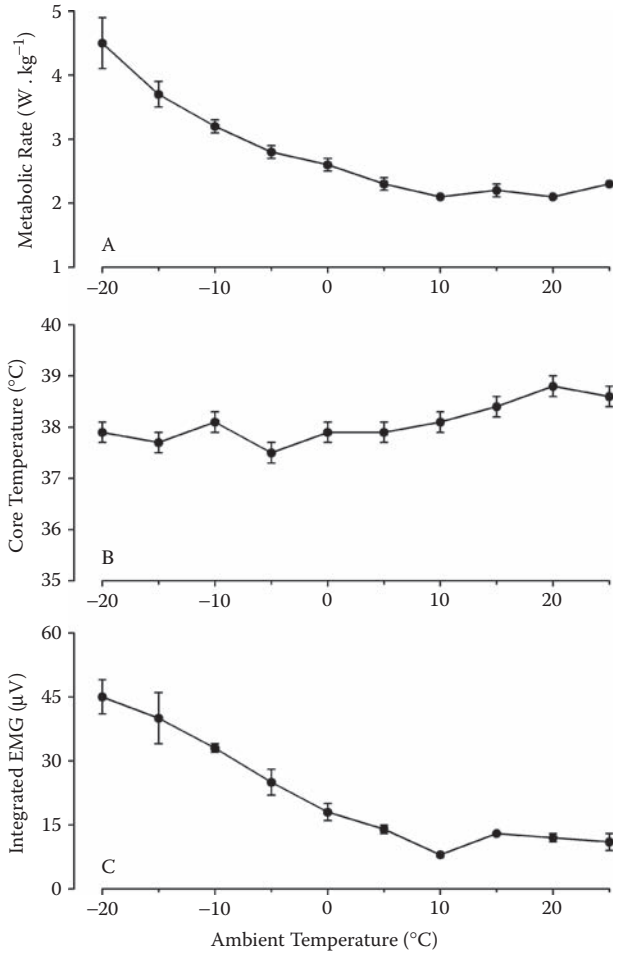


FIGURE 10.8 Autonomic temperature regulation: shivering. Birds and mammals have well-developed autonomic mechanisms of temperature regulation. These graphs show mean data (± SE) for eight young King penguins (*Aptenodytes patagonicus*) studied at a research station in Antarctica. Thermoregulatory thermogenesis is evident in the elevation of metabolic rate at lower ambient temperatures (top panel), which allows body temperature to remain constant despite the large variation in the temperature of the environment (middle panel). 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 (lower panel). EMG: electromyogram. (Source: Duchamp, C., Barré, H., Delage, D., Rouanet, J. L., Cohen-Adad, F. & Minaire, Y. (1989). Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. *American Journal of Physiology* 257: R744–R751.)

in the mammalian body.[129] 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.[106,130–146] 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, counter-current heat exchangers are used to cool the brains of running antelopes[147] and heat-stressed camels,[148] to reduce heat loss from the fins of dolphins[149] and the tongue of gray whales swimming in cold waters,[150] and to enable homeothermy in bluefin tuna.[151]

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 it in a hot environment. When air temperature exceeds body temperature, heat loss by vasodilation is not feasible, and evaporation becomes the primary mechanism of heat loss. Some animals sweat,[152–159] others pant,[160–168] and some spread saliva over their fur.[169–175] While 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 it. Therefore, it can serve as a mechanism of heat conservation in a cold environment.[165,176–178] That fur and feather coats are indeed effective is demonstrated by increased heat loss from animals that are experimentally shorn or plucked.[73,179–183] Some animals undergo molting twice a year, which provides large changes in insulation according to seasonal needs.[184–196] Animals living in cold environments have evolved thick fur coats, vascular heat exchangers, subcutaneous fat deposits, and other strategies to conserve body heat.[197,198]

As mentioned in Chapter 9, large body size alone can serve as an effective means of reducing heat loss[199] (Figure 10.10). According to Bergmann's rule, enunciated in 1847, mammals and birds increase in size toward the colder portion of the temperature range that they occupy in their natural habitats.[200] Allen's rule states that animals adapted to colder environments have smaller limbs and shorter protruding body parts.[201] The rationale is that larger body size and smaller extremities provide less relative surface area for heat loss and, therefore, constitute an adaptive strategy for life in a cold environment. Per Fredrik Scholander, a prominent 20th-century physiologist,

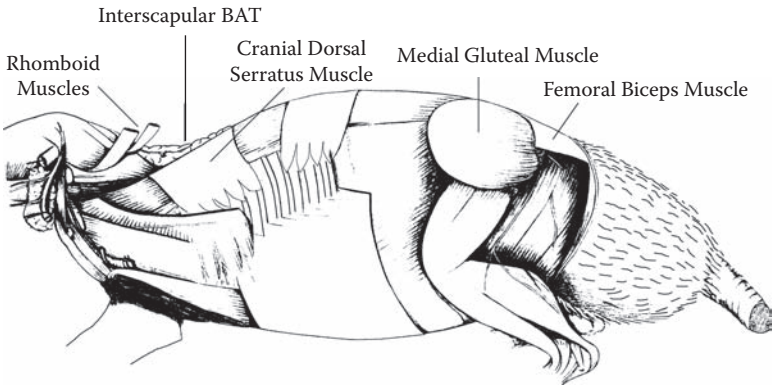


FIGURE 10.9 A different type of fat, a different type of bat. Brown adipose tissue (BAT), also known as brown fat, is a form of fat different from the ordinary white fat. It is a highly thermogenic tissue believed to play an important role in cold-induced thermogenesis in mammals. The diagram indicates the location of interscapular BAT in the rat. (Source: Adapted from Popesko, P. et al. (1992). *A Colour Atlas of Small Laboratory Animals, Volume 2*. London: Wolfe Publishing.)

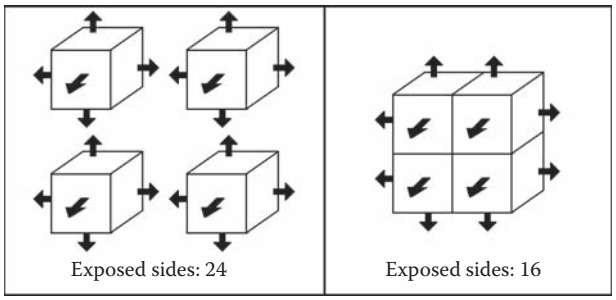


FIGURE 10.10 The deal about body size. Body size has consequences for insulation because it affects the surface-volume ratio of organisms. The two diagrams in this figure have the same volume (4 cubes), but different surface areas are exposed to the environment. If all cubes generate the same amount of heat, then small organisms made up of one cube (left panel) will lose more heat than the large organism made up of four cubes (right panel).

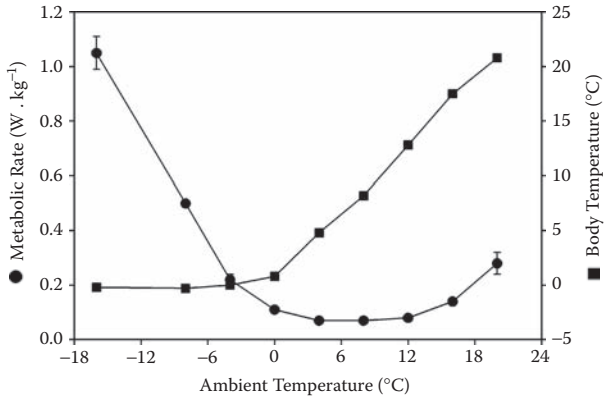


FIGURE 10.11 Hibernation is a regulated process. The graph shows the mean metabolic rate (± SE) and mean body core temperature (± SE) of eight Arctic ground squirrels (*Spermophilus parryii*) hibernating in the laboratory at different ambient temperatures. Note that body temperature is allowed to fall with ambient temperature only down to 0°C. At ambient temperatures below 0°C, body temperature is maintained constant as a result of an increase in metabolic heat production. (Source: Buck, C. L. & 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.)

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.[202] Bergmann's rule and Allen's rule may still be meaningful within individual species.[203] Pigs raised in the cold had shorter tails and smaller ears than their littermates raised at thermoneutrality,[204] and mice raised in the cold had shorter tails and smaller feet than mice raised at thermoneutrality.[205,206] However, reduced blood flow due to vasoconstriction may have prevented the extremities from developing properly.[204] If this suggestion is correct, 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 body temperature to deviate from the normothermic level. However, it is a potent mechanism of energy conservation, and its status is monitored by the thermoregulatory system. Chapter 4 discussed hibernation; daily torpor is discussed in detail later in this chapter. The data shown in Figure 10.11 demonstrate that hibernation is a regulated process, not just dormancy imposed by low ambient temperature. Eight arctic ground squirrels (*Spermophilus parryii*) were studied in the laboratory during the hibernating season. The graph shows the mean metabolic rate and the mean body temperature of the animals at different ambient temperatures. Note that body temperature falls along with ambient temperature only down to 0°C. At temperatures below 0°C, metabolic rate is increased and body temperature is prevented from falling further.[207] Thus, although the animals allow their body temperature to fall drastically during

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. (Source: Livecam of Jigokudani Monkey Park at www.jigokudani-yaenkoen.co.jp.)

hibernation, they do not give up temperature regulation altogether.

Both endotherms and ectotherms use behavior to regulate temperature. As a result, in the animal kingdom, behavioral responses are used more widely than any of the autonomic responses. The behavioral response of *relocation* is the most widely used thermoregulatory response. It refers to the movement of an organism from a location in which temperature is too low or too high to a location in which temperature is closer to thermoneutrality (Figure 10.12). Using a scale of seconds or hours, researchers have documented thermoregulatory behavior in a variety of animals, including insects,[208–211] arachnids,[53,212] crustaceans,[213–222] fishes,[50,223–236] amphibians,[52,237–239] reptiles,[240–265] birds,[266,267] and mammals.[47,268–294] Relocation along a temperature gradient has been documented even in unicellular organisms[295,296] and human spermatozoa.[297] 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.[21,298–301] 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. Thus, it 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).[164,165,172,174,210,302–315]

Huddling is a potent mechanism of heat conservation.[283,291,310,311,316–331] 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 huddle. Figure 10.13 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),[332] but by clustering together in groups of thousands of individuals they essentially create a "big bee" that functions as an endothermic unit.[333–335] A large cluster of bees was reported to maintain a core temperature of 34°C as ambient temperature was lowered to –80°C![335]

Modulation of food intake is an auxiliary thermoregulatory response, in that increased food intake is required to maintain elevated thermogenesis in a cold environment. Within limits, animals eat more at lower ambient temperatures,[279,336–352] and fasting animals allow their body temperature to fall even in a thermoneutral environment.[61,284,353–359] Low temperature during a meal (without long-term cold exposure) is sufficient to elicit greater food consumption.[360–363] This response may be an anticipatory reaction 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. However, the increase in heat loss caused by the greater exposure to the environment might offset the increased thermogenesis. Some studies suggest that heat from forced exercise can replace cold-induced thermogenesis in the maintenance of body temperature,[364–368] but others studies disagree with this finding.[369–371] Also, although some studies have indicated that animals will voluntarily increase activity in the cold,[372–374] others found no evidence that increased activity occurs.[338,375]

Building of microenvironments is an effective thermoregulatory response, although the thermoregulatory benefits cannot always be distinguished from the safety benefits (i.e., defense from predators). Humans are the animals most proficient in building microenvironments. Construction of human buildings considers indoor air quality (ventilation), thermal conditions (heating and air-conditioning), visual and acoustic conditions, electromagnetic fields, static electricity, and vibration.[376] The design of thermal conditions considers the location and orientation of the building, the materials, the external color, and the floor plan.[377] Many other animals build nests, often in burrows deep in the ground, to shelter themselves from environmental cold or heat (Figure 10.14). The use or construction of microenvironments has been

FIGURE 10.13 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. (Source: Photograph by Frank Todd. © Alexander Photography. Reproduced with permission.)

FIGURE 10.14 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. (Source: Northern Prairie Wildlife Research Center, U.S. Department of the Interior.)

studied in numerous species, including mollusks and crustaceans,[214,378] insects,[210,379–382] reptiles and birds,[69,245,251,383,384] rodents,[279,320,324,329,350,385–403] and other mammals.[304,404–410]

The thermoregulatory responses discussed so far are reflexive or instinctual, but many responses can be acquired by *learning*. Humans learn how to knit sweaters, weave blankets, 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,[411] goldfish,[412] several species of birds,[413–419] mice,[420–422] rats,[108,358,423–441] dogs,[45] pigs,[442–444] and squirrel monkeys.[445–447]

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 are integrated for the benefit of temperature homeostasis, as exemplified in Figure 10.15. 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 (the autonomic response) could be determined. The amount of heat obtained through the behavioral response ("heat intake") was calculated[448] and compared with the amount of heat obtained autonomically ("heat production"). Note that when the force required to

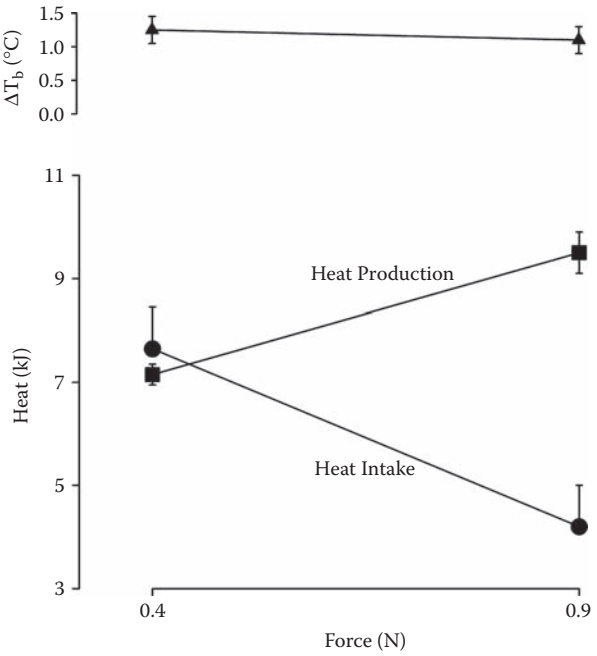


FIGURE 10.15 Trade-off of autonomic and behavioral responses. Rats were kept in the cold (3°C) for 30-minute sessions and could obtain 3-second heat rewards each time they pressed a lever. Metabolic heat production and number of lever presses were recorded. The graphs show heat production, heat intake, and the change in body temperature 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 body temperature. (Source: Refinetti, R. & 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.)

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 body temperature 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.[449] Thus, the trade-off between heat intake and heat production shown in Figure 10.15 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 body temperature.[450] Similar trade-offs between autonomic and behavioral responses were observed in other studies on birds and mammals exposed to cold[447,451,452] or heat stress.[271,293,453]

10.1.2 Circadian Control of Body Temperature

Previous chapters provided a considerable amount of information about the circadian control of body temperature. The phenomenology of the body temperature rhythm was covered in Chapter 5, and its relationship with other rhythms was discussed in Chapter 9. This section provides additional information about circadian control of body temperature.

Consider the relationship between the body size of a species and the parameters of its circadian rhythm of body temperature. In one of his many excellent review articles, Aschoff pointed out that, within the range of 10 g to 1 kg, the amplitude of the temperature rhythm is three to six times smaller in large animals than in small animals.[454] Based on data from 141 independent studies in various laboratories, I can confirm that the amplitude is about three times smaller in large animals with body weight ranging from 10 g to 1000 kg, and up to six times smaller in the restricted body weight range of 10 g to 1 kg (Figure 10.16, lower panel). Presumably, large bodies buffer the effects of the oscillations in heat production and heat loss responsible for the body temperature rhythm. Body size also has an effect on the mean level of the temperature rhythm (Figure 10.16, upper panel). Animals in the 1000-kg range have, on average, body temperatures 0.7°C higher than the body temperatures of animals in the 10-g range. Again, this is presumably due to the greater thermal inertia of large animals.

A recent literature survey based on 125 independent studies in mammals also confirmed Aschoff's prediction.[455] The amplitude of the body temperature rhythm was found to be smaller, and the mean level to be higher, in large animals than in small animals. However, 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 body temperature.[456] I attribute the failure to the smaller sample size and to the fact that pre-1950 studies did not measure body temperature telemetrically over full circadian cycles (instead, measurements were occasionally conducted with rectal probes, which could easily overestimate the body temperatures of small animals).[457–461] A recent compilation of 267 studies in animals weighing under 1 kg also did not identify a correlation between body size and body temperature.[462] This lack of correlation does not present a conflict, however, because the data in Figure 10.16 (upper panel) do not evince a correlation for the subgroup of animals under 1 kg. The effect obviously is dependent on large differences in body size.

Very little data are available about the robustness of the temperature rhythm. Figure 10.17 shows data from 16 mammalian species. 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.

The effect of ambient temperature on the body temperature rhythm also should be considered. Most studies in circadian physiology are conducted under thermoneutral conditions in the laboratory or under uncontrolled

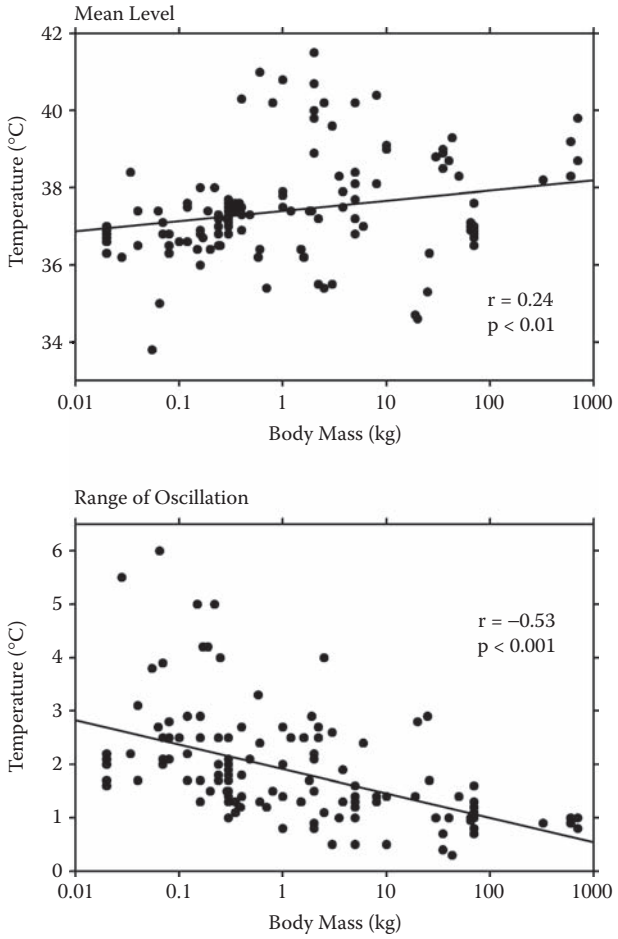


FIGURE 10.16 Mean level and amplitude of body temperature rhythm vary with body size across species. The top graph shows the relationship between the mean level of the daily rhythm of body temperature of a species and the body size of the species, as reported for various species of birds and mammals. The bottom graph 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. Note that both the mean level and the range of oscillation of the temperature rhythm are significantly correlated with body size, although in opposite directions. (Sources: See references 33, 36, 285, 287–289, 304, 308, 310, 348, 356, 358, 373, 410, 458, 459, 464, 469, 481, 487, 497–499, 509–511, 533, 547, 550, 570–573, 596, 597, 615, 617, 639, 649–653, 735–815 in the *Literature Cited* section.)

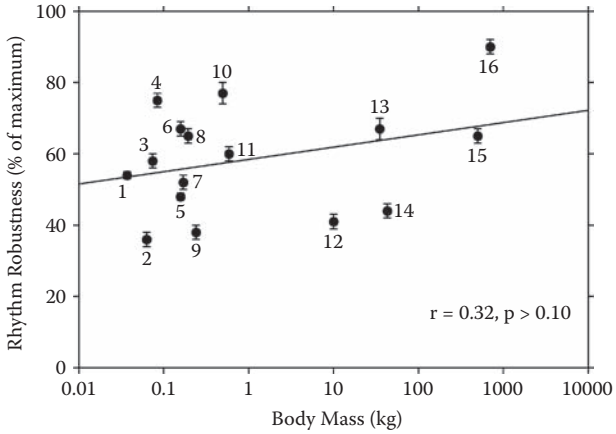


FIGURE 10.17 Does rhythm robustness depend on body size? The graph shows the relationship between the robustness of the daily rhythms of body temperature of 16 mammalian species and their average body sizes. The abscissa is scaled logarithmically. Although some correlation exists between the two variables (r = 0.32), the correlation is not statistically significant (because of the small sample size as compared with that in Figure 10.16). 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. (Sources: Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology A* 119: 295–300; 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; Refinetti, R. & Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937; Piccione, G., Caola, G. & Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541; Piccione, G., Caola, G. & Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119; Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.)

conditions in the field. However, several laboratory studies have addressed the issue with controlled changes in ambient temperature. Figure 10.18 provides an example. A tree shrew (*Tupaia belangeri*) was maintained under several ambient temperatures for several days, and its body temperature was monitored by telemetry. Note 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 to 32°C range) was found on the body temperature rhythms of rats, golden hamsters, or fat-tailed gerbils.[463] Greater rhythm amplitude in the cold was observed in studies on squirrel monkeys,[464] thirteen-lined ground squirrels,[33] pigeons,[67] mousebirds,[465] and sunbirds.[466] No effect of ambient temperature on the amplitude of the body temperature rhythm was found in rats[374,467] or mouse lemurs.[373] 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. What is normal in the cold need not be normal in the heat. If the notion of "normal" is forced, one could call

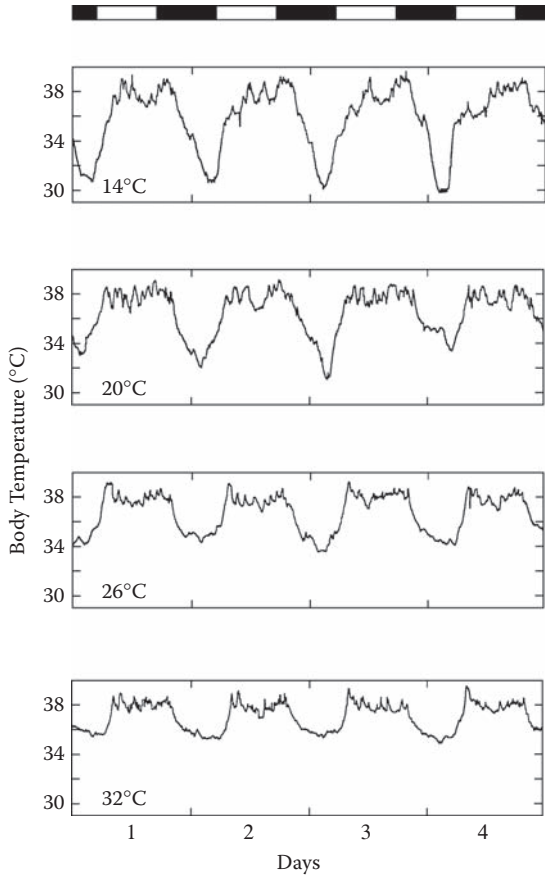


FIGURE 10.18 How ambient temperature affects the rhythm of body core temperature. The graphs show 4-day segments of the body temperature records of a tree shrew (*Tupaia belangeri*) recorded by telemetry in 6-minute intervals while the animal was housed under four different ambient temperatures. A 24-hour 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. (Source: Adapted from 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.)

"normal" the amplitude observed when the organism is kept 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)."[4] For sedentary laboratory rodents, the thermoneutral zone usually ranges from about 25°C to about 32°C.[468]

An important consideration about the circadian oscillation of body temperature is whether the oscillation is symmetrical about a neutral level or is caused by an elevation or a lowering of temperature. To answer this question, circadian physiologists can study organisms that do not exhibit circadian rhythmicity of body temperature. One way is to examine the temporal pattern of body temperature of newborn animals as they develop the temperature rhythm. Figure 10.19 shows data from young calves (*Bos taurus*) maintained under a natural photoperiod. As previously discussed in Chapter 9, a daily rhythm of body temperature is first observed on the ninth day after birth in this species. Note 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.[469] A similar pattern was observed in rats[470] but not in lambs and foals.[471] 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 body temperature is to surgically destroy their master circadian pacemakers. Figure 10.20 shows that regardless of the ambient temperature at which the animals are housed, the body temperature rhythm of golden hamsters with ablated pacemakers (SCNX) has the same mean level as the rhythm of normal hamsters (Control).[472] This finding 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[473] and ground squirrels.[474] In rats, one study found equivalent mean temperatures in rhythmic and arrhythmic animals,[475] another study found the mean temperature to be higher in arrhythmic animals,[476] and a third study found the mean temperature to be slightly lower in arrhythmic animals.[477] Until further studies are conducted, one can provisionally conclude that rhythmicity is established by development of oscillations both above and below a "neutral" level.

10.1.3 Integration of Homeostasis and Circadian Rhythmicity

Clearly, the homeostatic control of body temperature has the goal of ensuring stability — that is, of preventing deviations from an ideal set point. The circadian control of body temperature, however, imposes a persistent oscillation in body temperature. 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 body temperature is primarily under homeostatic control and is secondarily modulated by the circadian system through a daily oscillation in the thermoregulatory set point.[478–481] This assumption is diagrammed in the upper panel of Figure 10.21. According to this view, the circadian pacemaker acts on the thermoregulatory thermostat so that the set point is elevated during subjective day

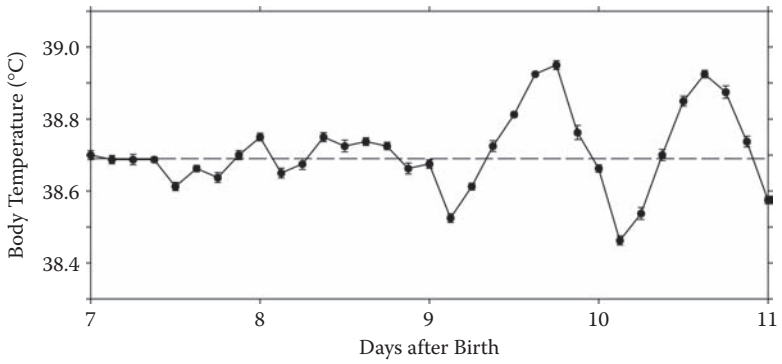


FIGURE 10.19 Developing the rhythm of body temperature. The graph shows the mean rectal temperature (± SE) of eight newborn calves (*Bos taurus*) recorded at 3-hour intervals during the second week of life, when the daily rhythm of body temperature develops in this species. The rhythm is established by oscillations both below and above the daily mean. (Source: Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.)

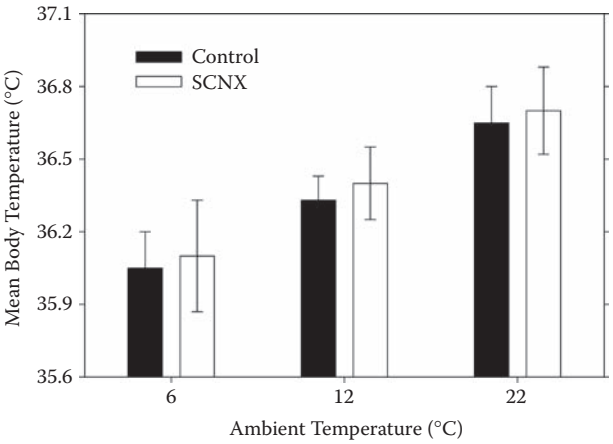


FIGURE 10.20 Disrupting the rhythm of body temperature. When the body temperature rhythm is eliminated as a consequence of surgical ablation of the circadian clock in the brain, the mean level of body temperature is not affected — which indicates that the rhythm was maintained by oscillations both below 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 twelve control hamsters were tested. (Source: Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.)

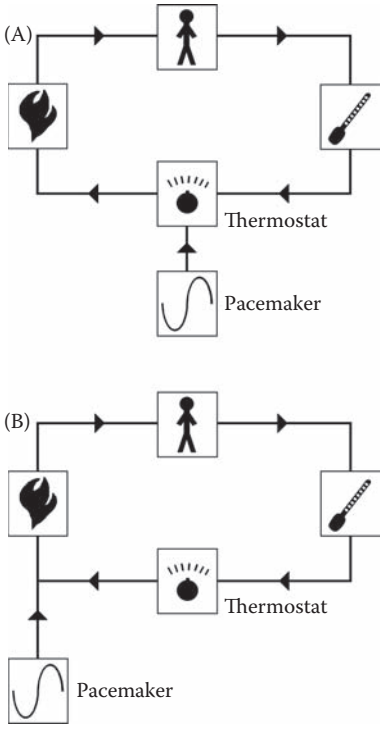


FIGURE 10.21 Change in set point or independent control? Integration of the homeostatic mechanism and the circadian mechanism that control body temperature can, in principle, be achieved either by circadian modulation of the thermostat (A) or by independent action of the two mechanisms on effector organs (B).

and lowered during subjective night in diurnal animals (or vice versa in nocturnal animals). However, from the viewpoint of the circadian physiologist, it is more sensible to assume that the circadian oscillation in body temperature is primarily under circadian control, bypassing the thermoregulatory set point, and is secondarily modulated by the thermoregulatory system (lower panel in Figure 10.21). Which of these two hypotheses is correct?

One problem with the traditional hypothesis (that the set point oscillates through the circadian cycle) is the concept of the set point. As discussed previously, thermal physiologists disagree about the existence of a central reference-signal generator (Figure 10.6). Even if a central reference-signal generator exists, extensive evidence indicates that thermoregulatory responses can be integrated at multiple levels of the neural axis.[482] The thermoregulatory system can be thought of as an assemblage of hierarchically

arranged integrating units.[483,484] Therefore, circadian modulation of the set point would have to involve modulation of multiple set points, some of which probably do not exist.

If a master thermoregulatory set point does exist, the set-point-change hypothesis still must explain why, as seen in the preceding section, the amplitude of the temperature rhythm is smaller in larger animals (Figure 10.16, lower panel). 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 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 body temperature that accompanies physical exercise is due not to an elevation of the set point but to a limitation of heat loss mechanisms.[485,486]

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 10.18). 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, the set point must be measured. One way to obtain this value is to measure thermoregulatory responses to environmental stress at different circadian times. If, for example, a greater thermogenic response is evoked by the same thermal stimulus during the circadian phase when body temperature is high than during the circadian phase when body temperature is low, then it is reasonable to assume that the elevated body temperature 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 the example, by generating more metabolic heat than when the set point is lower. Figure 10.22 shows the results from one study that used this approach. 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, metabolic rate 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, metabolic rate was not higher in the dark phase — it was actually lower in the dark phase than in the light phase.[487] Thus, cold-induced thermogenesis (the difference between the metabolic rates in the cold environment and in the neutral environment) was much greater during the light phase. In nocturnal animals such as the hamster, body temperature is lower during the light phase, which means that cold-induced thermogenesis was higher when body temperature was lower — the opposite of what the set-point-change hypothesis predicted.

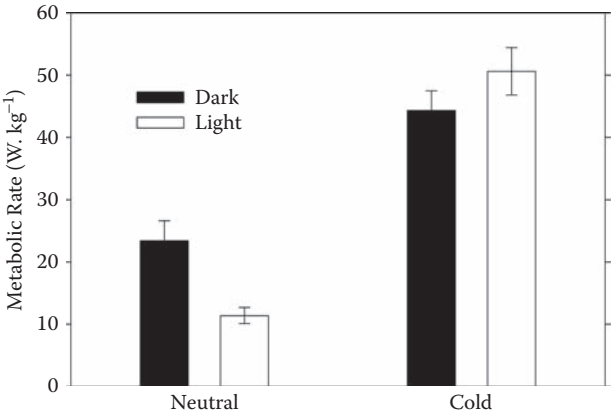


FIGURE 10.22 Autonomic thermoregulatory response opposes body temperature rhythm. The graph shows the mean metabolic rates (± SE) of ten golden hamsters (*Mesocricetus auratus*) measured in a neutral (24°C) and a cold (-10°C) environment during the dark and the light phases of the prevailing light–dark cycle. Note that cold-induced thermogenesis (that is, the difference between measurements in the cold and measurements in the neutral environment) is greater during the light phase (when body temperature is lower in this nocturnal species). (Source: Watts Jr., R. H. & Refinetti, R. (1996). Circadian modulation of cold-induced thermogenesis in the golden hamster. *Biological Rhythm Research* 27: 87–94.)

Similar results to those seen in golden hamsters were obtained in fat-tailed gerbils.[285] However, several studies involving autonomic thermoregulatory responses of heat gain and heat loss yielded results supporting the set-point-change hypothesis in rats,[137] pigeons,[488,489] and humans.[159,490–492] The discrepancy of results makes one wonder if the experimental approach used in all these studies is reliable. No researcher questions the existence of a circadian rhythm of body temperature. Also, it is a physical necessity that changes in body temperature 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 occur 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 demonstrate anything about the state of the set point. It indicates only that heat production and heat loss are changing. To measure the state of the set point, one needs a variable that normally is not required for the production of the body temperature rhythm but that, at the same time, reflects the operation of the set point. Section 10.1.1 showed that autonomic and behavioral

thermoregulatory responses can complement each other in the homeostatic control of body temperature, and that the body temperature rhythm of endotherms does not require behavioral responses. Therefore, the use of behavioral responses can provide a reliable measure of the state of the set point.

In 1978, Herbert Hensel — possibly the most influential thermal physiologist of the 20th century — studied the thermal sensation evoked by warming the hand of human subjects at different times of the day. He noticed that warm stimuli were perceived as more pleasant during the circadian phase of low body temperature than during the phase of high temperature.[493] 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 body temperature than during the phase of high body temperature.[494] Research in many other laboratories over the years has documented that higher ambient temperatures are preferred during the phase of low body temperature and lower ambient temperatures are preferred during the phase of high body temperature, in rats,[273,286,287,359,495,496] mice,[283] golden hamsters,[286,497] fat-tailed gerbils,[285] degus,[288] stripe-faced dunnarts,[498] tree shrews,[289] flying squirrels,[289] and humans.[499–502] An example is given in Figure 10.23. Fat-tailed gerbils were housed in a temperature gradient such as that described in Figure 2.27 (Chapter 2). Their preferred ambient temperature and body temperature were recorded in 6-minute intervals for 10 or more days. Note that, as expected for a nocturnal animal, body temperature is high during the night and low during the day. Note also that the rhythm of behavioral temperature selection is 180° out of phase with the rhythm of body temperature. Clearly, higher environmental temperatures are selected when body temperature is low, and vice versa. Thus, the oscillation of the set point cannot possibly be responsible for the temperature rhythm. In fact, there is no reason to assume that the set point oscillates at all.[503,504] As *body temperature* oscillates, the animals behaviorally counteract the oscillation to defend the unaltered set point. The thermoregulatory system actually opposes the oscillation of body temperature imposed by the circadian system (Figure 10.24).

Evidently, the opposition of the thermoregulatory system to the circadian oscillation of body temperature is not entirely successful, as shown by the existence of the rhythm. However, the amplitude of the temperature rhythm is effectively reduced by the action of the thermoregulatory system. This reduction has been shown in two ways. One way compared 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 body temperature rhythm was reduced

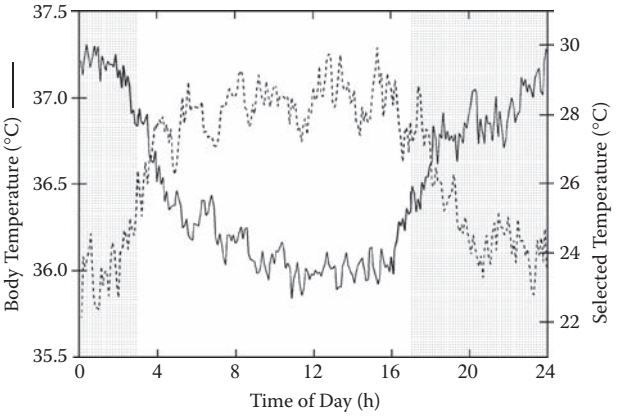


FIGURE 10.23 Rhythm of temperature selection is 180° out of phase with rhythm of body temperature. The graph shows the average rhythm of body temperature and the average rhythm of selected ambient temperature of fat-tailed gerbils (*Pachyuromys duprasi*) maintained in a temperature gradient under a 24-hour light–dark cycle (as indicated by the shading). The average rhythms are derived from five gerbils, each studied over 10 consecutive days. Higher ambient temperatures were selected when body temperature was low, and lower ambient temperatures were selected when body temperature was high. (Source: Adapted from Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology* A 119: 295–300.)


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 10.24 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.

in tree shrews and flying squirrels that were allowed to select their environmental temperature.[289] The other way impaired the thermoregulatory system through surgical

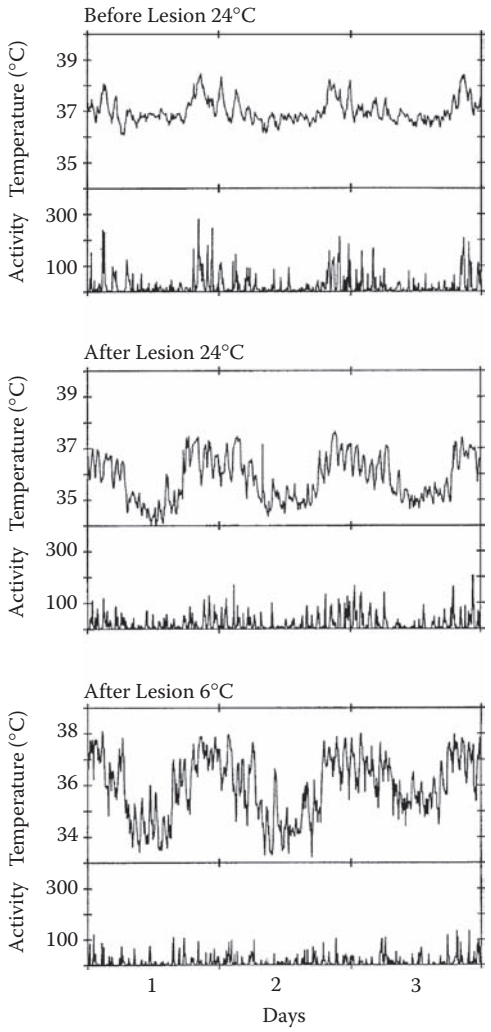


FIGURE 10.25 Disrupting the thermoregulatory system. When the thermoregulatory system is disrupted as a consequence of surgical ablation of the main thermostat in the brain, the amplitude of the body temperature rhythm is increased — which indicates that prior to the ablation, the amplitude of the rhythm was reduced by the thermoregulatory system. These graphs show 3-day segments of the body temperature 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. (Source: Adapted from Osborne, A. R. & Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.)

ablation of the main thermoregulatory center in the preoptic area of the brain. The amplitude of the body temperature rhythm was greatly enhanced in rats and golden hamsters with preoptic lesions.[473,505,506] Figure 10.25 shows that the amplitude of the body temperature 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

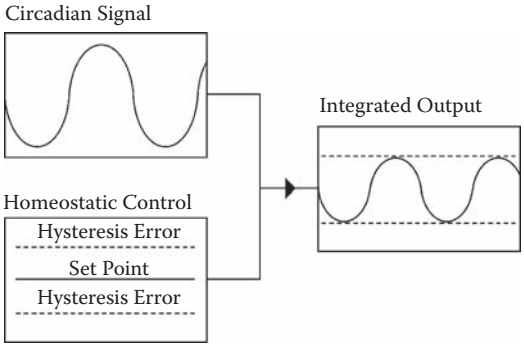


FIGURE 10.26 How the homeostatic and circadian mechanisms are integrated for the generation of the body temperature rhythm. The circadian system generates a signal for rhythmicity with relatively large amplitude. At the same time, but independently, the homeostatic system sets the limits of allowable variation around the set point. The integrated output is an oscillation with reduced amplitude.

of the preoptic area releases the circadian oscillation of body temperature from inhibitory control. Thus, the thermoregulatory center in the preoptic area of unlesioned animals restricts the oscillation of body temperature to an acceptable range. The logic of the regulatory process is diagrammed in Figure 10.26. 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 body temperature rhythm are not intuitive. Consider Figure 10.27, which shows 3-day segments of the records of body temperature, metabolic heat production, and dry heat loss of a laboratory rat kept in constant darkness at 24°C. Note that the oscillation of body temperature parallels the oscillation of heat production. Thus, the oscillation of heat production could potentially explain the oscillation of body temperature. However, heat loss also parallels heat production. That is, although heat production is high when body temperature is high, heat loss is also high. All three variables oscillate together, which is counter-intuitive. Because a change in body temperature must result from an imbalance between heat production and heat loss, one is tempted to conclude that animal physiology violates the laws of thermodynamics!

One cannot violate physical laws. The appearance of law violation comes from the presumption that a great amount of heat is needed to generate the body temperature rhythm. In actuality, very little heat is needed for it. Figure 10.28 shows the fractions of the total daily energetic budget of a rat that are associated with essential life processes,

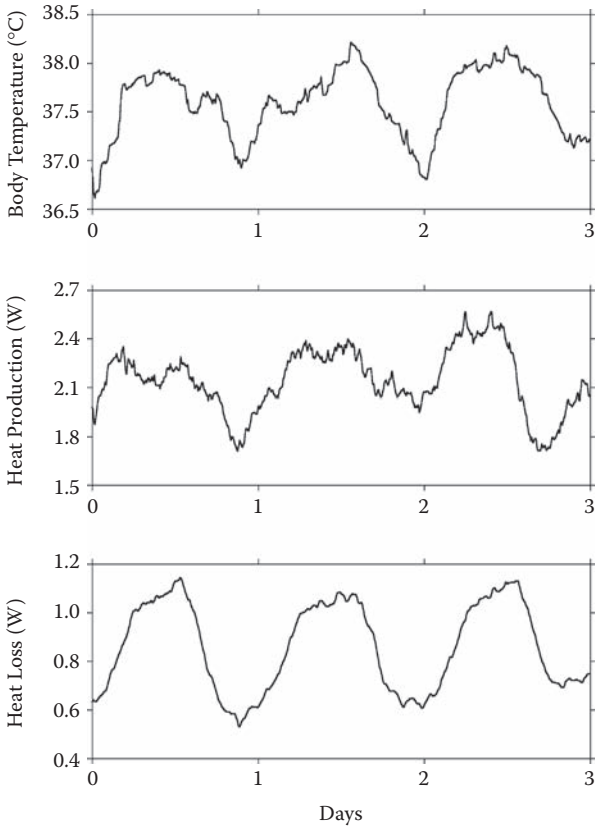


FIGURE 10.27 Matching gains and losses. The graphs show 3-day segments of the records of body temperature (measured by telemetry), heat production (computed from measurements of oxygen consumption), and heat loss (measured by direct calorimetry) of a laboratory rat (*Rattus norvegicus*) maintained in constant darkness. The absolute values of heat loss are relatively low because they do not include convective and evaporative losses. Note that the rhythm of body temperature parallels the rhythm of heat production but that the rhythm of heat loss also parallels the rhythm of heat production. (Source: Adapted from Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429.)

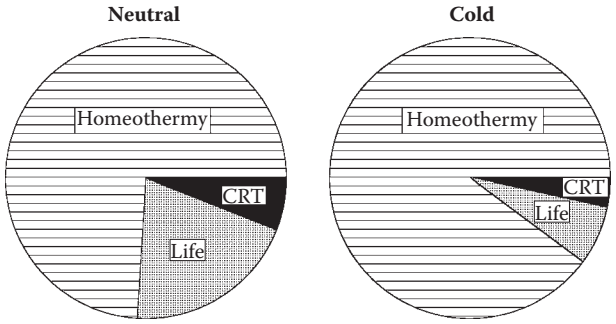


FIGURE 10.28 What is the cost of the body temperature rhythm? These pie charts indicate the fractions of the total daily energetic budget of a rat associated with essential life processes, the maintenance of homeothermy, and the circadian rhythm of body temperature (CRT) in a thermoneutral (24°C) and 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. (Sources: Templeton, J. R. (1970). Reptiles. In: Whittow, G. C. *Comparative Physiology of Thermoregulation*, Volume 1. New York: Academic Press, pp. 167–221; Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429; Refinetti, R. & 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; Refinetti, R. (1990). Peripheral nervous control of cold-induced reduction in the respiratory quotient of the rat. *International Journal of Biometeorology* 34: 24–27.)

the maintenance of homeothermy, and the circadian rhythm of body temperature (CRT) in a thermoneutral and a cold environment. Most of the energy used (which is directly proportional to heat production) is associated with the maintenance of homeothermy. The body temperature rhythm accounts for only 6% of the energy expended at thermoneutrality and only 3% of the energy expended in the cold. Thus, most of the daily oscillation in heat production and heat loss has nothing to do with the body temperature rhythm.

When considering the temporal coordination of the mechanisms of heat production and heat loss necessary for the generation of the body temperature rhythm, Aschoff reasoned that both heat production and heat loss need to oscillate, and that the oscillation of heat loss must lag behind the oscillation of heat production.[507] This prediction has been confirmed in rats,[508,509] squirrel monkeys,[481,510] and humans.[511] Figure 10.29 shows an example. Cosine waves were fitted to the raw data of body temperature, heat production, and heat loss of a rat (top three graphs). The vertical dashed lines indicate the acrophases of the rhythms. Note that heat production leads body temperature by 1.3 hours, while heat loss trails body temperature by 0.9 hour. If *heat balance* is calculated (bottom panel), a phase difference of 6 hours is found. This phase difference presumably is due to thermal inertia of the body, and it should be different in animals of different body sizes.[454]

One may wonder why a circadian rhythm of body temperature exists. It exists for the same reason that circadian rhythmicity exists. Chapter 9 showed that circadian rhythmicity is an old evolutionary trait that most likely existed before the appearance of the first animals. Seeking warm temperatures during (or in anticipation of) the active

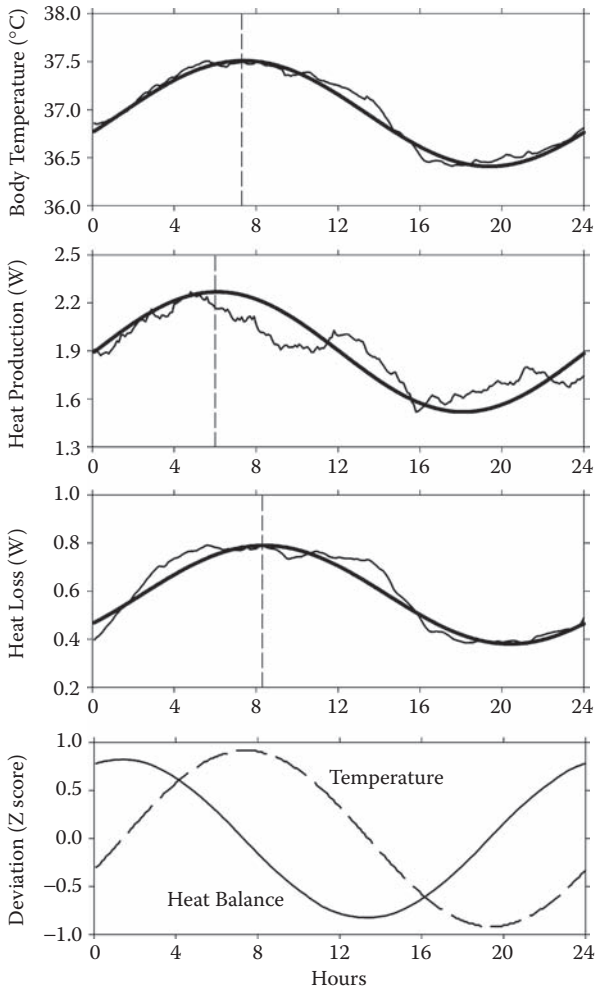


FIGURE 10.29 Thermal inertia and the body temperature rhythm. The graphs provide a close look at the records of body temperature, heat production, and heat loss of a laboratory rat maintained in constant darkness (compare with Figure 10.27). Thin lines correspond to actual data. Thick lines are cosine waves fit to the data. Dashed vertical lines indicate the acrophases of the rhythms. The bottom graph compares the body temperature rhythm with the heat-balance rhythm (i.e., the difference between the normalized values of heat production and heat loss). Note that the heat-balance rhythm leads the temperature rhythm by 6 hours. (Source: Adapted from Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429.)

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.[512] Because homeothermy is found today only in mammals and birds (and a few fishes), it must have appeared much later than circadian rhythmicity. Thus, in homeotherms, the new "drive" to maintain homeostasis conflicts with the old "drive" to oscillate body temperature — and this conflict explains the opposition between the thermoregulatory system and the circadian system in the control of body temperature. One reason to retain rhythmicity of body temperature in homeotherms is the ability to use body temperature as an internal nonphotic zeitgeber for the entrainment of multiple slave pacemakers distributed all over the body.[513–515] It has also been suggested that increased heat loss through peripheral vasodilation during the inactive phase may have an immunological host-defense function, as increased blood flow to the skin provides greater transport of leukocytes to this first-defense area of the body.[484] Energy savings — in a smaller but similar fashion to that attained in hibernators — might be another advantage of the body temperature rhythm. However, as shown previously (Figure 10.28), the fraction of energy expenditure associated with the body temperature 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 body temperature at night (assuming a mean metabolic rate of 90 W and a Q_{10} of 2). Eating an extra apple a day would be sufficient to offset the costs of maintaining body temperature at 37.0°C all day long. "An apple a day keeps nocturnal hypothermia away."

10.2 SLEEP, FEEDING, AND ENERGY EXPENDITURE

Other variables, in addition to body temperature, are homeostatically controlled and exhibit circadian rhythmicity. As a result, conflicts between homeostasis and circadian rhythmicity can potentially exist in the control of these variables. This section examines 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 10.30). If you have ever stayed up all night studying for an exam, you know that sleep is homeostatically controlled. The longer you stay awake, the stronger the urge to go to sleep and the less capable you are of performing various tasks. The effects of sleep deprivation on human performance are illustrated in Figure 10.31. Performance was evaluated by a psychomotor vigilance test (PVT), 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), while others were allowed to sleep 4, 6, or 8 hours per

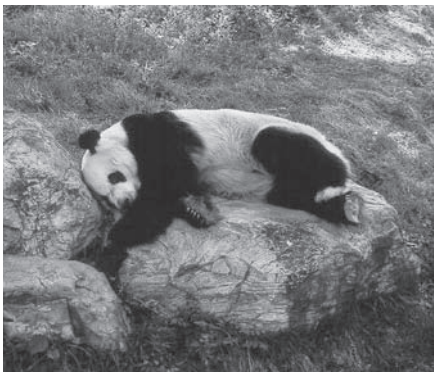


FIGURE 10.30 A sleeping panda. Most mammals and birds sleep at least a few hours each day. (Source: © ArtToday, Tucson, AZ.)

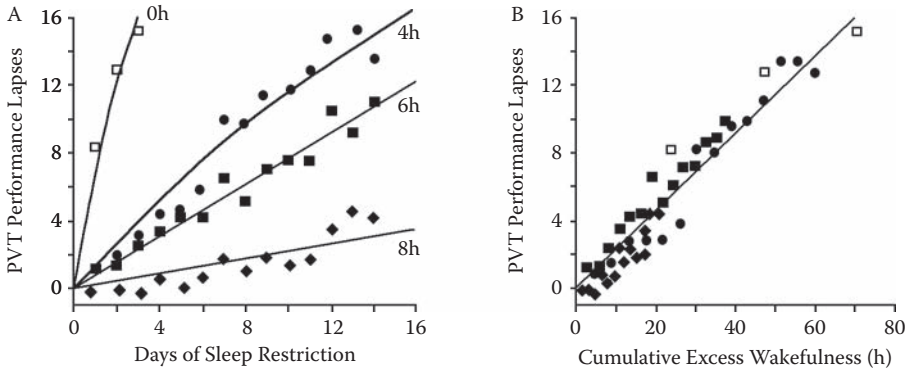


FIGURE 10.31 The effects of chronic sleep restriction. Chronic sleep restriction (that is, reduced number of hours of sleep on several consecutive nights) leads to an increase in performance lapses in a psychomotor vigilance test (PVT). Adult humans generally need slightly more than 8 hours of sleep 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 (that is, how long one has gone without getting enough sleep). Symbols refer to different conditions of sleep restriction as indicated by adjacent labels. (Source: Van Dongen, H. P. A., Maislin, G., Mullington, J. M. & 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.)

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 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.[516] The difference is subtle but mathematically meaningful.)

Sleep does not require a concept of set point because theoretically an absolute zero exists for sleepiness, so that sleepiness can only rise 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.[517–521] These findings also have been documented in animals devoid of circadian rhythmicity by surgical ablation of the master circadian pacemaker,[522–524] by deactivation of the pacemaker through genetic engineering,[525] and by the bizarre elimination of rhythmicity in Siberian hamsters submitted to a phase shift of the light–dark cycle.[526] Figure 10.32 shows an example of "sleep rebound" after sleep deprivation in a rat whose pacemaker had been previously ablated.

The exact cause of sleep is still not fully known, but one possibility is the accumulation of *adenosine* during wake hours.[527] Adenosine is the *A* in ATP (adenosine triphosphate), the primary energy currency of cells. The greater the breakdown of ATP, the greater 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,

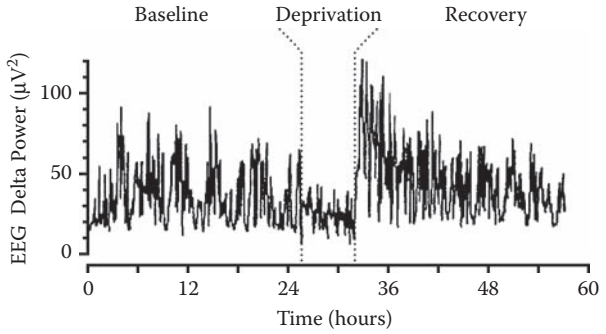


FIGURE 10.32 Sleep is a homeostatic process. The graph shows the variation in EEG delta power (a standard measure of sleepiness) of a laboratory rat (*Rattus norvegicus*) during a baseline interval of 24 hours, a sleep-deprivation interval of 6 hours, and a recovery interval of 24 hours. Daily rhythmicity of sleepiness had been eliminated as a consequence of surgical ablation of the circadian clock in the brain. Note a clear homeostatic rebound in EEG delta power immediately after the imposed interval of sleep deprivation. (Source: Adapted from Trachsel, L., Edgar, D. M., Seidel, W. F., Heller, H. C. & Dement, W. C. (1992). Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. *Brain Research* 589: 253–261.)

will accumulate more adenosine and 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.

If you have stayed up all night at least once, you probably noticed that sleep is more than just a 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. As shown in Chapter 5, daily or circadian rhythmicity of electroencephalographically monitored sleep has been documented in controlled laboratory conditions in a variety of animals, including rodents,[475,496,518,519,525,526,528–540] humans,[541–547] and other vertebrates.[357,517,548–551] 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[552] and has been supported by numerous empirical studies since then.[543,553–564]

Figure 10.33 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

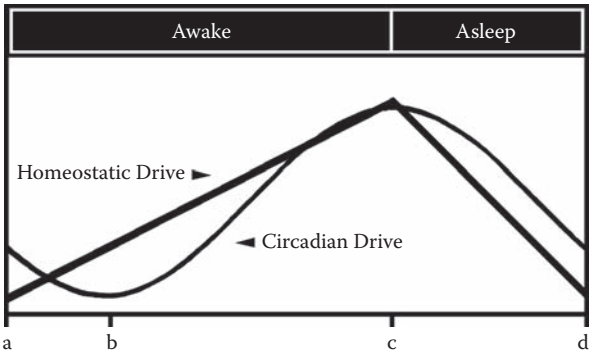


FIGURE 10.33 Sleep is under both homeostatic and circadian control. Under ideal conditions, the homeostatic drive to sleep and the circadian drive to sleep reinforce each other, as shown in this diagram. The homeostatic drive is depicted as a linear function for simplicity. See text for details.

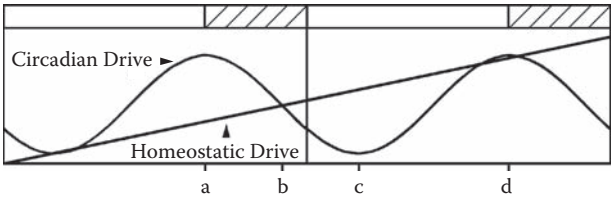


FIGURE 10.34 The homeostatic and circadian components of sleep may oppose each other. Attempts to stay up for 2 consecutive nights create the conditions for a conflict between the homeostatic and the circadian components of sleep regulation, as shown in this diagram. The hatched rectangles indicate sleep time. See text for details.

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 time. From time point *b* onwards, 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 time at point *d* (which is the same as *a*).

The diagram in Figure 10.34 can help you understand what happened that night when you did not sleep. Time point *a* was your usual bed time. 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 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*,

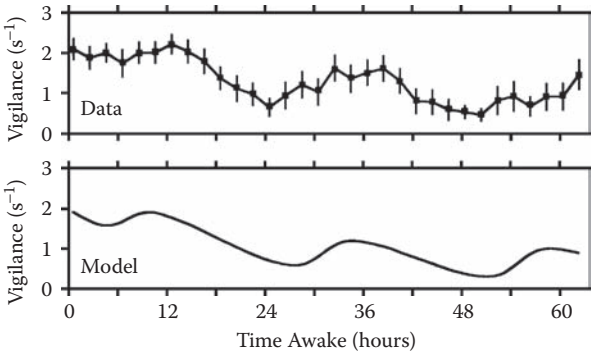


FIGURE 10.35 Measuring vigilance. The graphs show actual ("Data") and predicted ("Model") variations in vigilance of human subjects undergoing 64 consecutive hours of wakefulness. The data points correspond to the means (± SE) of 13 subjects. Vigilance is expressed as the reciprocal of reaction time 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. (Source: Adapted from Van Dongen, H. P. A. & 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.)

when the circadian drive reached its peak *and* you had accumulated 40 consecutive hours of wakefulness. You went home and crashed in bed.

If you compute the exact expected waveform of vigilance during a 64-hour interval of continuous wakefulness, you obtain the curve shown in the lower panel of Figure 10.35. For comparison, the curve in the upper panel describes the actual average performance of 13 subjects in a reaction time task. Note 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.[559]

Figure 10.36 shows that the homeostatic and circadian components of sleep can be disentangled by means of the *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 body temperature rhythm and the imposed 28-hour sleep-wake rhythm. Consequently, the subjects' waking hours occur at all different phases of the body temperature cycle, so that tests of alertness can be conducted during circadian times when the subjects would normally be asleep (and,

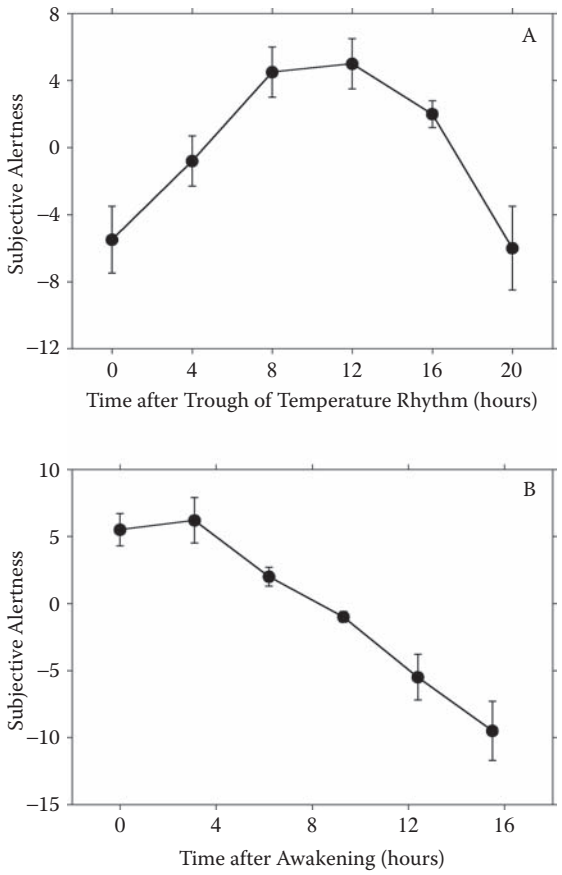


FIGURE 10.36 Isolating the effects of the homeostatic and circadian components. The experimental procedure of forced desynchrony allows the isolation of the effects of the homeostatic and circadian determinants of alertness. Human subjects were forced to live under 28-hour days, so that the endogenous circadian rhythm could not follow the imposed rhythm of sleep and rest. The data points shown correspond to the means (± SE) of nine 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. (Source: Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M. & 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.)

therefore, unable to perform any requested tasks).[554] The upper panel in Figure 10.36 shows the variation in subjective alertness as a function of time after the trough of the body temperature rhythm. A clear circadian pattern is present, which reveals the circadian component of alertness (or its opposite, sleepiness). The lower panel shows the variation in subjective alertness as a function of time after awakening. Alertness clearly decreases progressively during the hours of wakefulness, which reveals the homeostatic component of alertness (or sleepiness).

FIGURE 10.37 Lunch time! Eating is an essential part of the life of all animals. (Source: © ArtToday, Tucson, AZ.)

10.2.2 Homeostatic and Circadian Control of Feeding

The two preceding subsections showed that homeostatic and circadian mechanisms interact in the control of body temperature and sleep. One can expect that similar interactions will characterize the control of all other processes in the body. This subsection considers the process of *feeding* (Figure 10.37).

Animals, unlike plants, cannot photosynthesize. 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 10.38). 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 and cold-induced thermogenesis. The net energy is used for maintenance of tissues, performance of muscular work, growth, and production of various life-sustaining substances such as hormones, neurotransmitters, and reproductive cells.[565]

Although food intake is determined by a variety of signals that are discussed in Chapter 11, it is evident that the ingestion of nutrients must match the energetic needs of the organism. Ingestive behavior serves the homeostatic purpose of maintaining a relatively stable supply of nutrients in the body. Figure 10.39 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. Meal duration clearly increased during the period of diet dilution as a homeostatic adjustment for the reduction in the energy content of the diet.

You probably think of being hungry or being satiated as opposite states. If you do not eat for many hours, you get hungry; if you eat, you become satiated. However, if you think about it more carefully, you will realize that hunger and satiety must involve distinct processes (Figure 10.40). Hunger can be evoked as a response to the depletion of energy stores in the body, but, because the digestion

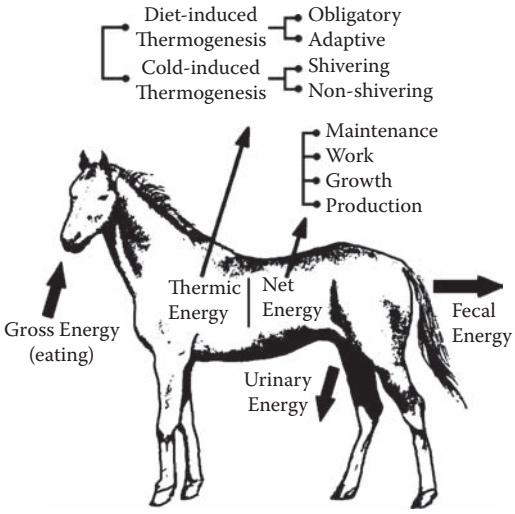


FIGURE 10.38 Where does the food go? In animals, ingested food is the sole source of energy that can be converted for multiple uses. Some of the ingested energy cannot be used and eventually is lost through the feces and urine. Some of the energy is "wasted" in thermal processes such as cold-induced thermogenesis and diet-induced thermogenesis. 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. (Adapted from Girardier, L. & Stock, M. J. (1983). *Mammalian Thermogenesis*. London: Chapman and Hall.)

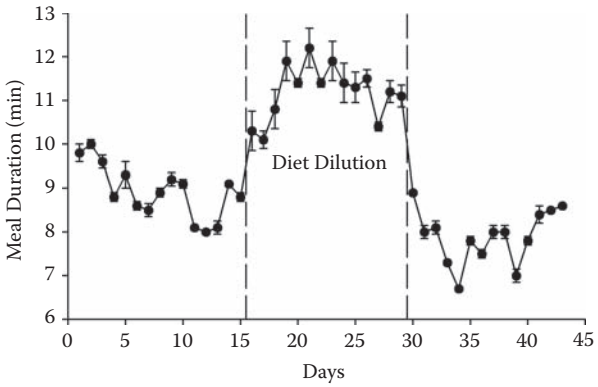


FIGURE 10.39 Ingestive behavior is homeostatically controlled. Rats (*Rattus norvegicus*) eat about ten meals a day, spending about 9 minutes on each meal. The graph shows the mean (± SE) meal duration of rats whose diet was diluted temporarily with cellulose (thus yielding 75% of the energy of the unadulterated diet). Meal duration increased during the period of diet dilution as a homeostatic adjustment for the reduction in the energy content of the diet. (Source: Adapted from Strubbe, J. H. & 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.)

of a meal and the assimilation of its nutrients into body organs takes many hours, you would continue eating if you did not have a short-term satiety mechanism to terminate the meal. Thus, there must be separate hunger

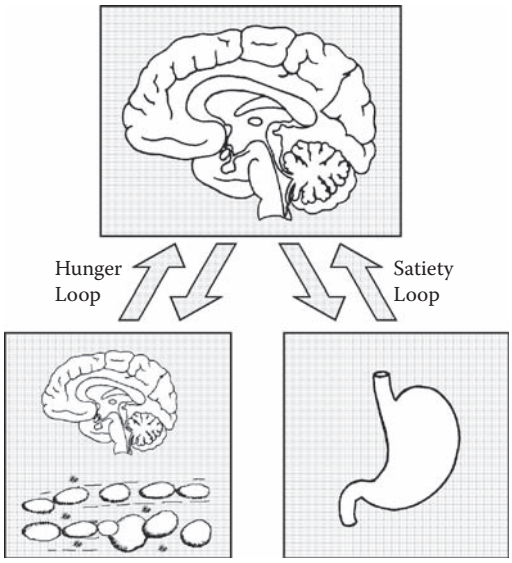


FIGURE 10.40 Two homeostatic loops. Although meals are normally initiated in response to hunger (and feeding eventually leads to a reduction in hunger), meals are terminated much before the digestive system has fully absorbed the ingested nutrients. Meal termination requires a satiety loop to temporarily inhibit the hunger loop.

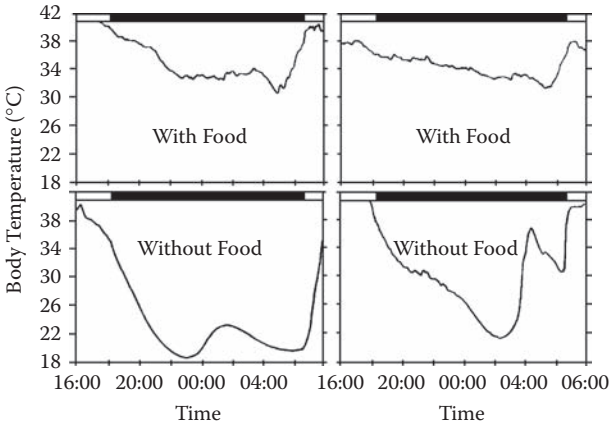


FIGURE 10.41 Starvation-induced hypothermia in a small bird. Food deprivation for even a single day greatly enhances the nocturnal fall in the body temperature of the speckled mousebird (*Colius striatus*), a 50-gram passerine bird. The graphs show segments of the body temperature records of two mousebirds maintained under a 24-hour light–dark cycle (LD 12:12) at 15°C with and without food. (Source: Adapted from McKechnie, A. E. & Lovegrove, B. G. (2001). Heterothermic responses in the speckled mousebird (*Colius striatus*). *Journal of Comparative Physiology B* 171: 507–518.)

and satiety loops. The hunger loop is based on the monitoring of energy stored in the form of adipose tissue, while the satiety loop is based on the monitoring of meal-generated signals such as gastric distention and various peptides secreted by the gastrointestinal system.[566,567]

Chapter 5 showed that daily and/or circadian rhythmicity of feeding has been extensively documented in laboratory rats[508,568–589] as well as in other rodents,[530,532,590–594] birds,[356,595–606] and other animals,[607–615] including nonhuman primates[481,616–622] and humans.[623–625] Consequently, the circadian system places temporal constraints on the homeostatic regulation of feeding.[626] 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, while meal frequency is associated mostly with the satiety loop.[566,626]

The control of feeding involves an interaction of homeostatic and circadian processes similar to that previously discussed for the control of body temperature and sleep. A peculiar feature of the control of feeding is its interaction with the control of overall metabolism and body temperature. This chapter earlier showed that fasted animals experience a reduction in metabolic rate and a fall in body temperature.[61,284,353–359] To better describe this phenomenon, Figure 10.41 shows body temperature records of two speckled mousebirds (*Colius striatus*) with food freely available (top panel) and after food deprivation for a day (bottom panel). Note the exaggerated fall in body temperature 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,[33,320,498,627–637] but various true homeotherms exhibit circadian-modulated starvation-induced hypothermia. This finding has been documented in doves,[638] pigeons,[356,357,639–641] quail,[642] mousebirds,[465,643] finches,[644] pygmy mice,[645] deer mice,[646] domestic mice,[647] rats,[358,359,476,648,649] lemurs,[650,651] sheep,[652] and goats.[653] Figure 10.42 exemplifies the phenomenon in sheep. The body temperatures of five sheep were recorded every 2 hours as the animals spent 5 days without food. Although mean body temperature initially fell gradually during the day and the night, a partial circadian modulation of starvation-induced hypothermia was evident. At sunrise on Day 4, mean body temperature 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 body temperature rose continuously for the next 24 hours, resuming the normal daily rhythm on the following day.

Starvation-induced hypothermia clearly is an energy-saving process similar to torpor and hibernation. Siberian hamsters (*Phodopus sungorus*) exhibit spontaneous torpor (that is, torpor when food is freely available) only under winter-like short photoperiods,[628,629] but they also exhibit

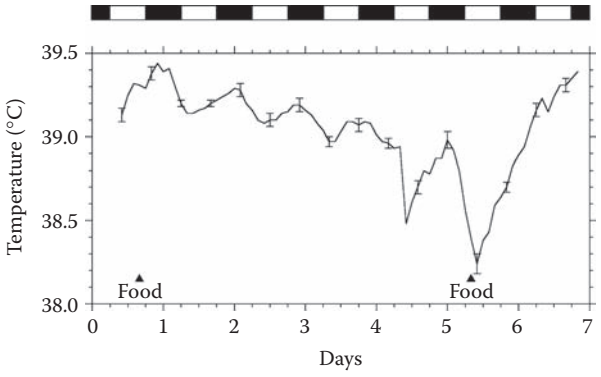


FIGURE 10.42 Starvation-induced hypothermia in a much larger mammal. Food deprivation for several days enhances the amplitude of the daily rhythm of body temperature in the sheep (*Ovis aries*), a 40-kg mammal. The graph shows the mean (± SE) rectal temperature of five sheep measured at 2-hour intervals 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. Note that body temperature is gradually lowered (without a change in the amplitude of the rhythm) for the first 3 days of food deprivation. On the fourth and fifth days, the daily fall is greatly enhanced. (Source: Piccione, G., Caola, G. & Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.)

starvation-induced hypothermia when deprived of food under long photoperiods.[654,655] The lowering of metabolic rate and body temperature 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. The relationship between the lowering of metabolic rate and the lowering of body temperature has been the topic of some controversy. Two theories exist: either *metabolic rate* is actively down-regulated (and body temperature follows along because of the reduced heat production) or *body temperature* is down-regulated (and metabolic rate follows along according to a natural Q_{10} effect). The truth seems to be that the two processes are used at different stages of the torpid state. Figure 10.43 shows records of body temperature (BT) and metabolic rate (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, but more clearly so in the speckled mousebird (A), metabolic rate starts to fall before body temperature. Therefore, the fall in body temperature cannot be causing the lowering in metabolic rate.[656] Also, as shown earlier (Figure 10.11), during deep hibernation, metabolic rate is raised when body temperature approaches dangerously low levels.[207,657] In contrast, during most of the stable stage of torpor and hibernation, metabolic rate falls along with body temperature, according to a Q_{10} between 2 and 3.[658–661]

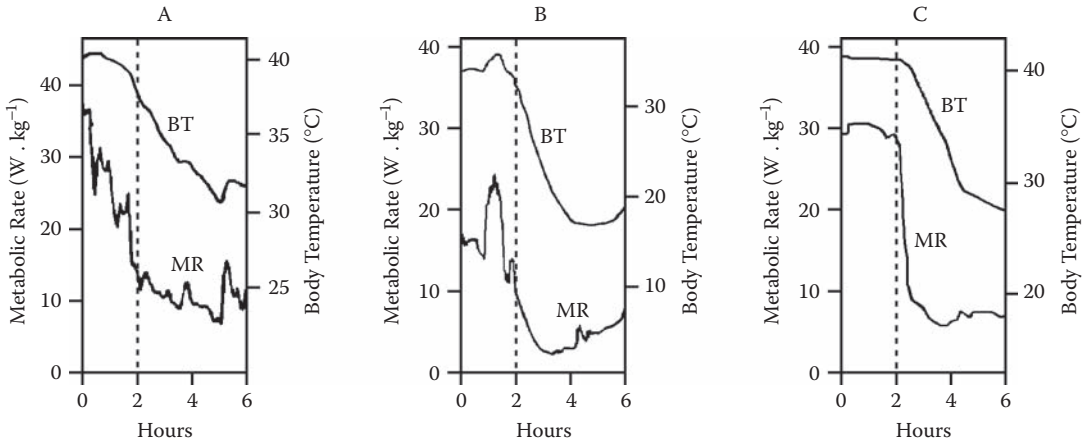


FIGURE 10.43 Fall in metabolic rate precedes fall in body temperature. In animals entering daily torpor, the fall in metabolic rate (MR) generally precedes the fall in body temperature (BT), as indicated by these records from representative individuals of three species. A: speckled mousebird (*Colius 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. (Sources: McKechnie, A. E. & Lovegrove, B. G. (2001). Heterothermic responses in the speckled mousebird (*Colius striatus*). *Journal of Comparative Physiology B* 171: 507–518; Heldmaier, G. & Ruf, T. (1992). Body temperature and metabolic rate during natural hypothermia in endotherms. *Journal of Comparative Physiology B* 162: 696–706; Prinzinger, R., Schleucher, E. & 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.)

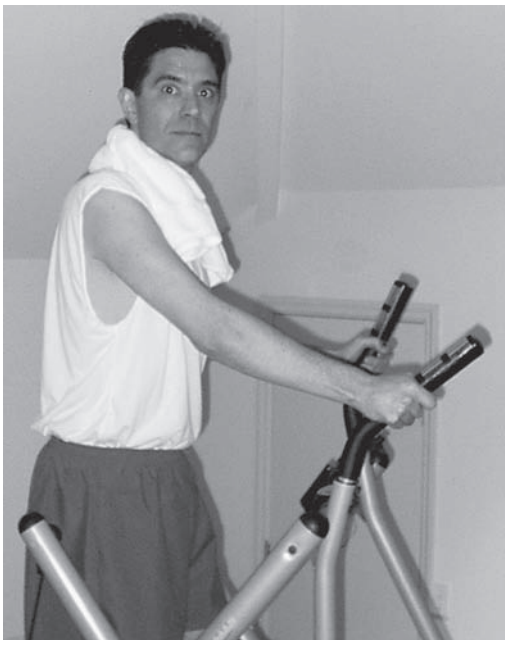


FIGURE 10.44 Working up a sweat. Physical exercise requires an elevation in energy expenditure. (Source: Photograph by Theresa Tolleson.)

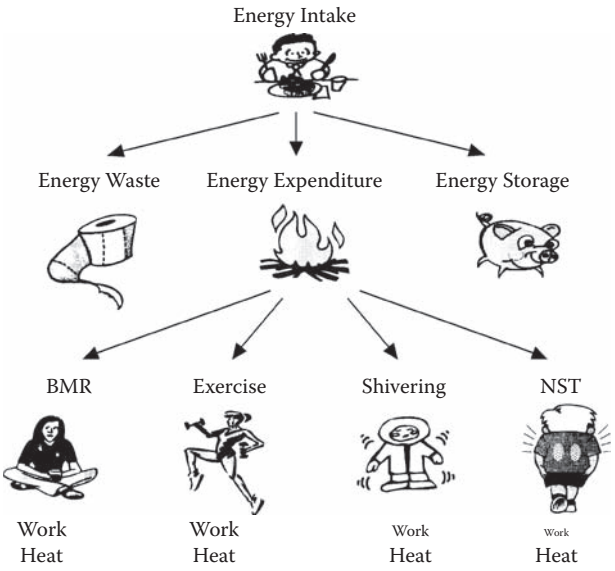


FIGURE 10.45 Energy expenditure. Energy that is ingested and not wasted or stored is eventually expended. Energy expenditure generally falls into one of four categories: basal metabolic rate (BMR), physical exercise, shivering, and nonshivering thermogenesis (NST).

10.2.3 Homeostatic and Circadian Control of Energy Expenditure

This last subsection of the chapter examines an interesting aspect of the interaction of homeostasis and circadian rhythmicity in the control of *energy expenditure* (Figure 10.44). Energy expenditure was addressed briefly in the discussion of feeding in the preceding subsection. As diagrammed in Figure 10.45, the energy acquired through feeding may be wasted (if it is not metabolizable), expended, or stored for later use. Energy is expended for the performance of essential body functions (basal metabolic rate, or BMR), for exercise, for thermoregulatory shivering, or for nonshivering thermogenesis (which includes both diet- and cold-induced thermogenesis).[565]

Basal metabolic rate 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 (that is, after food has been digested and absorbed). Larger animals have higher basal metabolic rates, as exemplified for rats in Figure 10.46. This phenomenon is quite intuitive, as larger animals are made up of a larger number of cells. However, note that the relationship between body mass and metabolic rate is not linear. The function is decelerated, which means that larger animals have lower metabolic rates per unit body mass.[662] This phenomenon is quite evident when a mouse and an elephant are compared. As indicated in Figure 10.47, a mouse weighs about 0.03 kg and has a metabolic rate of 0.2 W. An elephant

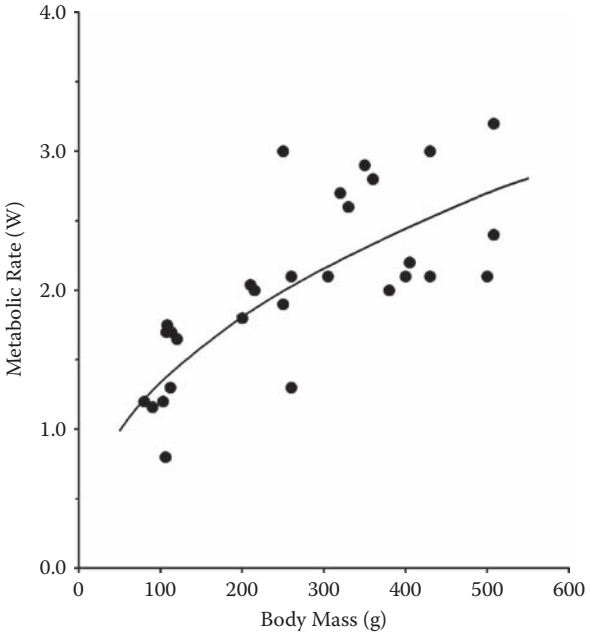


FIGURE 10.46 Energy expenditure and body size. The graph shows the relationship between basal metabolic rate and body size for 30 laboratory rats (*Rattus norvegicus*). Note the deceleration of the function as body size increases. (Source: Refinetti, R. (1989). Body size and metabolic rate in the laboratory rat. *Experimental Biology* 48: 291–294.)

weighs as much as 100,000 mice (3000 kg) and, therefore, should have a metabolic rate of 22,000 W. Yet, the elephant's actual metabolic rate is only 1500 W.

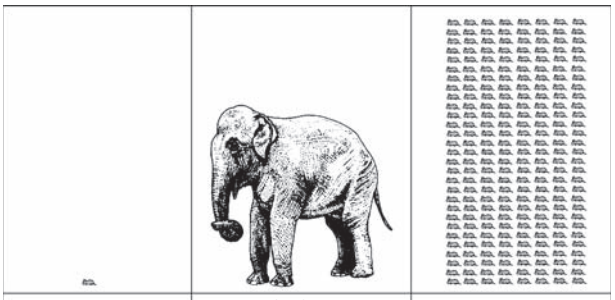


FIGURE 10.47 Big but slow. The elephant is bigger that the mouse and has a greater metabolic rate. However, its metabolic rate is much lower than that of a group of mice large enough to match the elephant's body size. (The mouse is drawn out of proportion so that it can be visible in the figure.)

Why do larger animals have proportionally lower metabolic rates? Researchers have argued about the answer to this question for generations. As early as 1838, before data had been collected, Pierre Sarrus and J. F. Rameaux proposed that metabolic rate should be proportional to the surface area of an animal — because heat can leave the body only through its surface.[663] 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, to prevent overheating, metabolic rate in large animals would have to be proportional not to M^1 but to $M^{2/3}$. This theory could account for the decelerated function relating metabolic rate to body mass. Experimental data indicating a decelerated function were collected by other investigators in the 19th century.[664,665] However, in 1947, when Max Kleiber compiled data from a large number of studies conducted during the previous half-century, he found that metabolic rate was proportional to $M^{3/4}$, not to $M^{2/3}$.[666] This finding meant that the surface-area explanation was incorrect, but the correct explanation was still unknown. The issue has been debated since then,[667–679] with no clear answer.

Exercise is a second major avenue of energy expenditure. As physical activity increases, metabolic rate increases.[318,366,369,370,680–683] Figure 10.48 provides an example. Antelope ground squirrels (*Ammospermophilus leucurus*) were forced to run in a motor-driven treadmill at different speeds, and their metabolic rate was recorded. The function is decelerated, suggesting lower energy expenditure at high speeds. Note, 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 metabolic rate is generally linear.[6]

Shivering was discussed earlier in this chapter. One form of *nonshivering thermogenesis*, cold-induced thermogenesis (CIT), was also discussed. This section will address the other form of nonshivering thermogenesis, diet-induced thermogenesis (DIT), which is the fraction of energy expenditure induced by the ingestion of food. Some authors include basal metabolic rate in the definition of diet-induced thermogenesis.[4] Two types of diet-induced thermogenesis exist. One is called *obligatory* because it cannot be avoided. After a meal is ingested, metabolic rate is temporarily elevated.[511,684–693] 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").[694–700] The other type of diet-induced thermogenesis is called *adaptive* because its magnitude can be adapted to conditions of shortage or excess of food supply. That is, diet-induced thermogenesis can be increased after overeating and reduced during starvation or food restriction.[685,701-710] As with cold-induced thermogenesis, diet-induced thermogenesis in mammals seems to depend strongly on the activation of brown adipose tissue.[711–717] The drug *ephedrine*, used in several weight-loss products, is a potent stimulator of diet-induced thermogenesis in brown adipose tissue.[122,718–722]

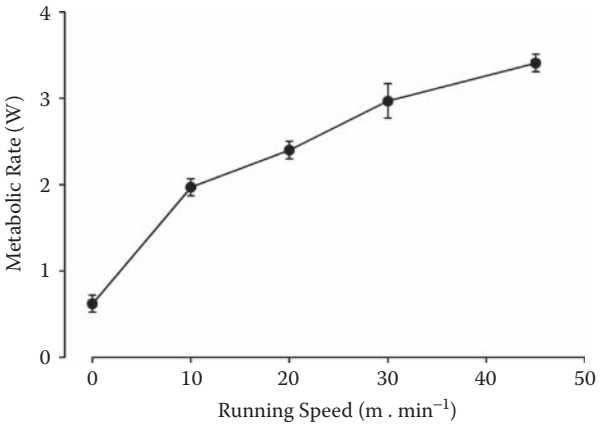


FIGURE 10.48 Rodent gym. The graph shows the average metabolic rates of antelope ground squirrels (*Ammospermophilus leucurus*) running on a motor-driven treadmill at different speeds. The data points correspond to the means (± SE) of ten squirrels. Note that more energy is expended at higher speeds. (Source: Yousef, M. K., Robertson, W. D., Dill, D. B. & Johnson, H. D. (1973). Energetic cost of running in the antelope ground squirrel *Ammospermophilus leucurus*. *Physiological Zoology* 46: 139–147.)

According to the traditional homeostatic view, animals and humans expend metabolic energy at a constant rate, in the same way a light bulb expends electrical energy, so that energy expenditure can be expressed in units of joules per second (i.e., in watts). Forty years ago, Aschoff raised the hypothesis that energy expenditure in animals might be primarily under circadian control rather than homeostatic control.[723] Because the natural alternation of day and night imposes on most animals a daily cycle of food

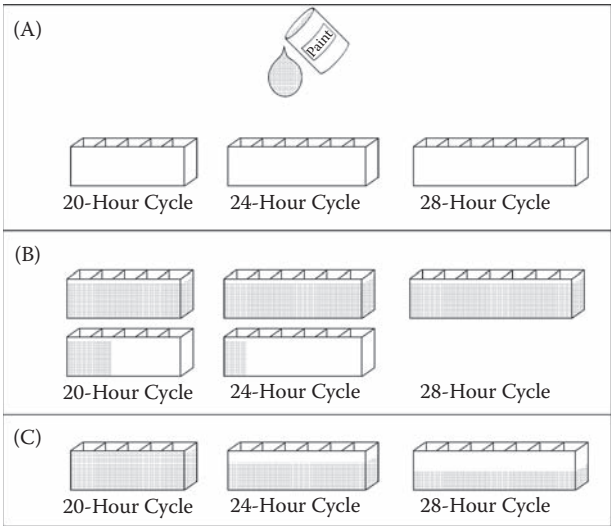


FIGURE 10.49 Circadian conservation of energy expenditure? These diagrams provide an analogy for the issue of the temporal organization of energy expenditure. Assume that some paint must be poured into containers of different capacities (A). Pouring at a constant rate requires more small containers than large containers (B), but an equal number of containers of different capacities can be used if the rate of pouring is adjusted proportionally (C).

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, metabolic rate would be properly expressed not in units of joules per second but in units of joules per circadian cycle. Consider Figure 10.49. Instead of metabolic energy, think of paint. If you pour paint into three different containers of different sizes (Panel A), two outcomes are possible depending on how you proceed. If you 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 procedure corresponds to the *homeostatic* control of energy expenditure. However, if you 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 procedure 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).[724] Assuming that body mass was not affected, these results suggest that energy expenditure is regulated on a circadian basis (i.e., not on a constant-rate basis) and that metabolic rate should be expressed not in watts but in "aschoffs" (i.e., joules per circadian cycle). This new conception of metabolic rate would instate a true revolution in the study of homeostasis. It might not affect normal humans living under typical conditions in which the duration of a day is the same as the duration of the entrained circadian cycle (i.e., 24 hours). However, it would have great practical importance for humans living under artificial working conditions. The new conception of metabolic rate would affect the planning of food supply for special worker groups (such as sailors in submarines and workers in underground facilities in which the duration of a day is not 24 hours). In addition, seasonal changes in the photoperiod have physiological effects similar to changes in the circadian period, as in both cases the daily segment of activity is reduced or extended. 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 segment 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.

In later studies, Aschoff determined that the amount of locomotion and the pattern of defecation are also conserved on a circadian basis rather than on a homeostatic basis.[725,726] However, a separate group of investigators found that human subjects free-running with a period of 25 hours consumed less food per cycle than when entrained to a 24-hour routine.[623] 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*.[588]

Two studies published in 1997 took advantage of the *tau* mutation in the golden hamster. Chapter 6 showed 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 metabolic rates 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).[727] 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 10.50. Note 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.[728] Similar results were obtained in a third laboratory.[729] Also, running-wheel activity of mice maintained under 24-hour light–dark cycles with

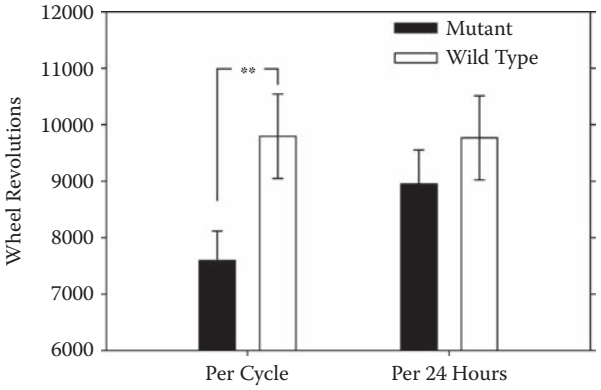


FIGURE 10.50 Running-wheel activity: homeostasis by circadian period. The graph shows the mean number of wheel revolutions of *tau*-mutant and wild-type golden hamsters (*Mesocricetus auratus*) maintained in constant darkness. The bars correspond to the means (± SE) of 32 mutants (τ = 20.4 hours) and 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. (Source: Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.)

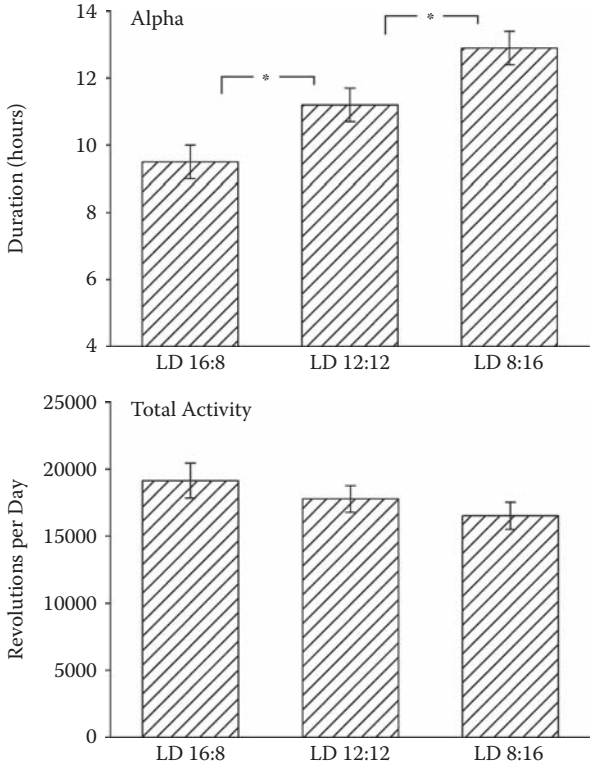


FIGURE 10.51 Running-wheel activity: homeostasis by photoperiod. The graphs show the mean duration of the activity phase (Alpha) and the number of wheel revolutions per day of domestic mice (*Mus musculus*) maintained under light–dark cycles with different proportions of light and darkness. Each bar corresponds to the mean (± SE) of 30 mice. Note that Alpha is longer when the dark phase of the light–dark cycle is longer, but total daily activity is not significantly different among the groups. (Source: Refinetti, R. (2001). Compression and expansion of circadian rhythm in mice under long and short photoperiods. *Integrative Physiological and Behavioral Science* 37: 114–127.)

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 10.51).

Figure 10.52 shows the metabolic rate data from the study on *tau*-mutant hamsters. Note 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 finding indicates that energy expenditure is conserved on the traditional unit-time basis, not on a circadian basis. Why the results of this study[728] contradict the results of the other study[727] is not clear. In the other study, the wild-type hamsters were significantly heavier (132 g) than the mutants (107 g). Difficulties in comparing metabolic rate between animals of different body sizes are well recognized in the physiological literature.[670,730–734] If the difference in weight caused the difference in the results of the two studies, then Aschoff's hypothesis of circadian conservation of energy expenditure cannot be supported. My laboratory is currently engaged in research aimed at elucidating this issue.

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 body temperature. The circadian system generates the circadian rhythmicity of body temperature. 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 it is unclear whether it is conserved on a circadian basis or on a homeostatic basis.

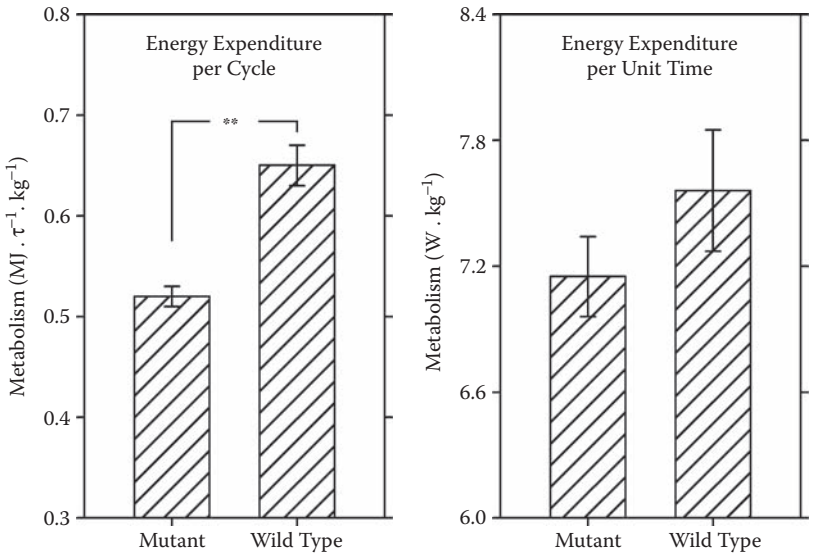


FIGURE 10.52 Metabolism: homeostasis by circadian period. The graph shows the mean energy expenditure of *tau*-mutant and wild-type golden hamsters (*Mesocricetus auratus*) maintained in constant darkness. The bars correspond to the means (± SE) of eight mutants (τ = 20.4 hours) and 8 wild types (τ = 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. (Source: Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.)

EXERCISES

Exercise 10.1 Circadian modulation of thermoregulatory behavior

Section 10.1 discussed the interaction between homeostasis and circadian rhythmicity in the control of body temperature. Behavioral adjustment is one of the mechanisms used by the homeostatic (thermoregulatory) system to control body temperature. This exercise examines how a behavioral response (thermal sensation) is affected by the circadian system.

1. You can serve as your own subject, but it will be easier to work in groups of two.
2. In previous exercises, you measured your body temperature rhythm. You should have created a graph that looks like the one in the left panel (A) in Figure 10.53. Use your graph to determine the approximate time of day when your temperature rhythm reaches its lowest value and its highest value. For most people, the lowest temperature occurs around wake time, and the highest temperature occurs 10 to 12 hours later.
3. For this exercise you will need a set of five constant-temperature water containers. Thermostatically controlled water baths are ideal, but a set of five small Styrofoam boxes filled with appropriate mixtures of cold and hot water are adequate. If you use Styrofoam boxes, you will need an accurate thermometer to measure the water temperature. Also, each container should be large enough for you to place your hand inside it.

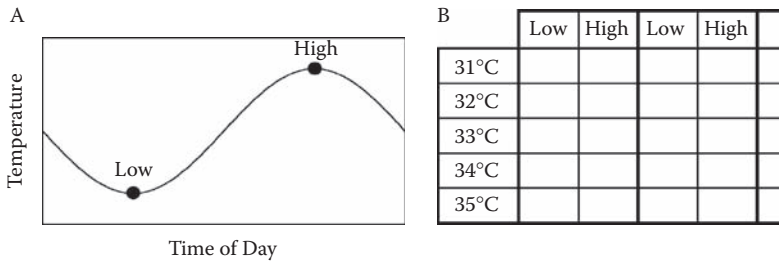


FIGURE 10.53 Testing the daily variation in thermal sensation. This figure will help you conduct Exercise 10.1. Panel A indicates the two daily time points for data collection (in reference to your body temperature rhythm). Panel B illustrates a form for registering the data.

4. Set the temperature of the water baths 1°C (2°F) apart, starting at 31°C (88°F). Usually, 33°C feels neither cold nor warm, so the two boxes set above 33°C will feel warmer and the two boxes set below 33°C will feel cooler. (If you have trouble distinguishing the temperature differences between boxes, you can adjust the range up or down.)
5. The person serving as the test subject will subjectively assess the temperature of the water baths with his or her hand. (If you serve as your own subject, your knowledge of the water temperature in each box may interfere with your true sensations. In this case, you should put your hand in each box 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.) The test subject should dry his or her hand after dipping it in each box and also wait a few minutes between boxes.
6. Refer to the right panel (B) in Figure 10.53 as a guide for collecting your data. 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. Concentrating on the "pleasantness" of the sensation, rather than on an objective measurement of temperature, is likely to yield more robust results. You may feel uncertain when you test the neutral temperature, but you should decide whether the water is warm or cold.
7. When you finish collecting the data, start to analyze them. You should have identified all 35°C stimuli as warm and all 31°C stimuli as cold. The interesting values are those between these two extremes. Can you see a consistent difference between the early morning values (low) and the late afternoon values (high)? Did you judge more stimuli as warm in the late afternoon? If you did, it probably was 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 stimuli as warmer than they are). That is, the homeostatic system tried to reduce the elevation in body temperature caused by the circadian system.

Exercise 10.2 Homeostatic and circadian control of sleep

Section 10.2 discussed the interaction between homeostasis and circadian rhythmicity in the control of sleep. In this exercise, you will experience the interaction yourself.

1. You will be your own subject. The central variable of study will be general sleepiness. To measure sleepiness, use the same subjective scale (0: very awake, 5: very sleepy) used in Exercise 9.1. You should also monitor the state of your circadian system by measuring your own body temperature. (Refer to Exercise 1.3 if you need instructions on monitoring your body temperature rhythm.)
2. The goal of this exercise is to uncouple the two systems that control sleep. You will need to maintain the circadian system in an undisturbed state while manipulating the homeostatic system. This uncoupling will be achieved by sleep deprivation.
3. Start by recording sleepiness and body temperature every waking hour for at least 1 day (2 or 3 days would be better). Then, stay awake all night so that you can record data throughout the night.
4. After you stay up all night, you may not be able to sleep in the morning. However, it is important that you try to sleep in the afternoon so that you will be refreshed for your second consecutive "all-nighter." Yes, you will stay awake for a second night. During the first "all-nighter," you simply skipped a night of sleep. For the second night, you will try to sleep earlier in the day so that you can be awake during your usual bed time later in the evening. You will still stay awake all night, but you will not be sleep deprived.
5. To sleep in the afternoon, 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. A good meal at lunch time often helps initiate sleep in the afternoon.
6. When you finish collecting data, plot the two variables on Cartesian coordinates (time in the X axis, variable in the Y axis).
7. Your body temperature records should show that your circadian system was not disturbed by the experimental procedure (that is, the daily oscillations will be similar during the baseline days and the 2 experimental days).
8. 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 bed time 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 between the data from the first sleepless night and the data from the second sleepless night. Although the influence of the circadian system was the same on both nights, the influence of the homeostatic system should have been much greater on the first night than on 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

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Van Dongen, H. P. A., Maislin, G., Mullington, J. M. & 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. This research article 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-to-read book on the homeostatic aspect of body temperature regulation, written for general audiences.

Mrosovsky, N. (1990). *Rheostasis: The Physiology of Change*. New York: Oxford University Press. A 15-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. & Glotzbach, S. F. (1996). Sleep, thermoregulation, and circadian rhythms. In: Fregly, M. J. & Blatteis, C. M. (Eds.). *Handbook of Physiology. Section 4: Environmental Physiology. Volume 2*. New York: Oxford University Press, pp. 1361–1374. A review article, with emphasis on animal studies, that discusses the interactions between and among sleep, circadian rhythmicity, and hibernation.

Dement, W. C. & 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. & Blatteis, C. M. (Eds.). *Handbook of Physiology. Section 4: Environmental Physiology. Volume 2*. New York: Oxford University Press, pp. 1375–1387. A review article on the interactions between the homeostatic mechanisms controlling food intake and sleep.

WEB SITES TO EXPLORE

American Physiological Society:
http://www.The-APS.org

Center for Human Sleep Research at Stanford University:
http://www.med.stanford.edu/school/psychiatry/humansleep/main.html

Center for Sleep & Circadian Biology, Northwestern Univ.:
http://www.northwestern.edu.cscb/

Lecture on Homeostasis:
http://pespmc1.vub.ac.be/HOMEOSTA.html

Thermophysiology Home Page:
http://physio1.utmem.edu/THERMOPHYSIOLOGY

LITERATURE CITED

1. Bernard, C. (1872). *De la Physiologie Générale*. Paris: Librairie Hachette.
2. Mrosovsky, N. (1990). *Rheostasis: The Physiology of Change*. New York: Oxford University Press.
3. Hirsch, E. D. (1987). *Cultural Literacy: What Every American Needs to Know*. Boston: Houghton Mifflin.
4. IUPS Thermal Commission (2001). Glossary of terms for thermal physiology. *Japanese Journal of Physiology* 51: 245–280.
5. Cossins, A. R. & Bowler, K. (1987). *Temperature Biology of Animals*. London: Chapman and Hall.
6. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th Edition. New York: Cambridge University Press.
7. Montgomery, J. C. & Macdonald, J. A. (1990). Effects of temperature on nervous system: implications for behavioral performance. *American Journal of Physiology* 259: R191–R196.
8. Kashefi, K. & Lovley, D. R. (2003). Extending the upper temperature limit for life. *Science* 301: 934.
9. Costanzo, J. P. & Lee, R. E. (1994). Biophysical and physiological responses promoting freeze tolerance in vertebrates. *News in Physiological Sciences* 9: 252–256.
10. Fletcher, G. L., Hew, C. L. & Davies, P. L. (2001). Antifreeze proteins of teleost fishes. *Annual Review of Physiology* 63: 359–390.
11. Stevens, J. C. & Stevens, S. S. (1960). Warmth and cold: dynamics of sensory intensity. *Journal of Experimental Psychology* 60: 183–192.
12. 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.
13. Stevens, J. C. (1979). Variation of cold sensitivity over the body surface. *Sensory Processes* 3: 317–326.

14. Refinetti, R. (1988). Magnitude estimation of warmth in children. *Journal of Thermal Biology* 13: 85–88.
15. Refinetti, R. (1990). Is there modal opponency in the thermal senses? *Journal of Thermal Biology* 15: 255–258.
16. McArthur, A. J. & Clark, J. A. (1988). Body temperature of homeotherms and the conservation of energy and water. *Journal of Thermal Biology* 13: 9–13.
17. Else, P. L. & Hulbert, A. J. (1981). Comparison of the "mammal machine" and the "reptile machine": energy production. *American Journal of Physiology* 240: R3–R9.
18. Hulbert, A. J. & Else, P. L. (1989). Evolution of mammalian endothermic metabolism: mitochondrial activity and cell composition. *American Journal of Physiology* 256: R63–R69.
19. Graham, J. B. & Dickson, K. A. (1981). Physiological thermoregulation in the albacore *Thunnus alalunga*. *Physiological Zoology* 54: 470–486.
20. Holland, K. N., Brill, R. W., Chang, R. K. C., Sibert, J. R. & Fournier, D. A. (1992). Physiological and behavioral thermoregulation in bigeye tuna (*Thunnus obesus*). *Nature* 358: 410–412.
21. Block, B. A., Dewar, H., Blackwell, S. B., Williams, T. D., Prince, E. D., Farwell, C. J., Boustany, A., Teo, S. L. H., Seitz, A., Walli, A. & Fudge, D. (2001). Migratory movements, depth preferences, and thermal biology of Atlantic bluefin tuna. *Science* 293: 1310–1314.
22. Chown, S. L. & Scholtz, C. H. (1993). Temperature regulation in the nocturnal melolonthine *Sparrmannia flava*. *Journal of Thermal Biology* 18: 25–33.
23. Knutson, R. M. (1974). Heat production and temperature regulation in eastern skunk cabbage. *Science* 186: 746–747.
24. Nagy, K. A., Odell, D. K. & Seymour, R. S. (1972). Temperature regulation by the inflorescence of philodendron. *Science* 178: 1195–1197.
25. Brattstrom, B. H. (1972). Temperature changes in heat producing plants. *Bulletin of the Southern California Academy of Sciences* 71: 54–55.
26. Seymour, R. S. & Schultze-Motel, P. (1996). Thermoregulating lotus flowers. *Nature* 383: 305.
27. Lamprecht, I., Seymour, R. S. & Schultze-Motel, P. (1998). Direct and indirect calorimetry of thermogenic flowers of the sacred lotus, *Nelumbo nucifera*. *Thermochimica Acta* 309: 5–16.
28. Buggeln, R. G. & Meeuse, B. J. D. (1971). Hormonal control of the respiratory climateric in *Sauromatum guttatum* (Araceae). *Canadian Journal of Botany* 49: 1373–1377.
29. Raskin, I., Ehmann, A., Melander, W. R. & Meeuse, B. J. D. (1987). Salicylic acid: a natural inducer of heat production in *Arum* lilies. *Science* 237: 1601–1602.
30. Vane, J. R. & Botting, R. M. (2003). The mechanism of action of aspirin. *Thrombosis Research* 110: 255–258.
31. Wooden, K. M. & 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.
32. Wooden, K. M. & Walsberg, G. E. (2004). Body temperature and locomotor capacity in a heterothermic rodent. *Journal of Experimental Biology* 207: 41–46.
33. Refinetti, R. (1996). The body temperature rhythm of the thirteen-lined ground squirrel, *Spermophilus tridecemlineatus*. *Physiological Zoology* 69: 270–275.
34. Bennett, N. C., Jarvis, J. U. M. & 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.
35. Buffenstein, R. & Yahav, S. (1991). Is the naked mole-rat *Heterocephalus glaber* an endothermic yet poikilothermic mammal? *Journal of Thermal Biology* 16: 227–232.
36. Bligh, J., Ingram, D. L., Keynes, R. D. & 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.
37. Hardy, J. D. (1965). The "set-point" concept in physiological temperature regulation. In: Yamamoto, W. S. & Brobeck, J. R. (Eds.). *Physiological Controls and Regulations*. Philadelphia: Saunders, pp. 98–116.
38. Hammel, H. T., Jackson, D. C., Stolwijk, J. A. J., Hardy, J. D. & Strømme, S. B. (1963). Temperature regulation by hypothalamic proportional control with an adjustable set point. *Journal of Applied Physiology* 18: 1146–1154.
39. Lipton, J. M. & 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.
40. Morimoto, A., Watanabe, T., Ono, T., Sakata, Y. & Murakami, N. (1986). Rat endogenous pyrogen and fever. *American Journal of Physiology* 250: R776–R782.
41. Dascombe, M. J., Rothwell, N. J., Sagay, B. O. & Stock, M. J. (1989). Pyrogenic and thermogenic effects of interleukin 1-β in the rat. *American Journal of Physiology* 256: E7–E11.
42. Horan, M. A., Little, R. A., Rothwell, N. J. & 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.
43. Tanaka, H., Kanosue, K., Yanase, M. & Nakayama, T. (1990). Effects of pyrogen administration on temperature regulation in exercising rats. *American Journal of Physiology* 258: R842–R847.
44. Bruguerolle, B. & Roucoules, X. (1994). Time-dependent changes in body temperature rhythm induced in rats by brewer's yeast injection. *Chronobiology International* 11: 180–186.
45. Cabanac, M., Duclaux, R. & Gillet, A. (1970). Thermorégulation comportementale chez le chien: effets de la fièvre et de la thyroxine. *Physiology and Behavior* 5: 697–704.
46. Blatteis, C. M. & Smith, K. A. (1980). Behavioral fever induced in guinea-pigs by intrapreoptic pyrogens. *Experientia* 36: 1086–1088.

47. Marques, P. R., Spencer, R. L., Burks, T. F. & 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.
48. Sugimoto, N., Shido, O., Sakurada, S. & Nagasaka, T. (1996). Day-night variations of behavioral and autonomic thermoregulatory responses to lipopolysaccharide in rats. *Japanese Journal of Physiology* 46: 451–456.
49. Briese, E. (1997). Selected temperature correlates with intensity of fever in rats. *Physiology and Behavior* 61: 659–660.
50. Reynolds, W. W., Casterlin, M. E. & Covert, J. B. (1976). Behavioural fever in teleost fishes. *Nature* 259: 41–42.
51. Casterlin, M. E. & Reynolds, W. W. (1977). Behavioral fever in crayfish. *Hydrobiologia* 56: 99–101.
52. Myhre, K., Cabanac, M. & Myhre, G. (1977). Fever and behavioural temperature regulation in the frog *Rana esculenta*. *Acta Physiologica Scandinavica* 101: 219–229.
53. Cabanac, M. & Le Guelte, L. (1980). Temperature regulation and prostaglandin E_1 fever in scorpions. *Journal of Physiology* 303: 365–370.
54. Cabanac, M. (1989). Fever in the leech, *Nephelopsis obscura* (Annelida). *Journal of Comparative Physiology B* 159: 281–285.
55. Refinetti, R. (1988). The concept of set point (goal value) in thermal physiology. *Manuscrito* 11: 47–56.
56. Bligh, J. (1979). The central neurology of mammalian thermoregulation. *Neuroscience* 4: 1213–1236.
57. Werner, J. (1980). The concept of regulation for human body temperature. *Journal of Thermal Biology* 5: 75–82.
58. Bligh, J. (1984). Temperature regulation: a theoretical consideration incorporating Sherringtonian principles of central neurology. *Journal of Thermal Biology* 9: 3–6.
59. Hochachka, P. W. & Somero, G. N. (1984). *Biochemical Adaptation*. Princeton, N.J.: Princeton University Press.
60. Blatteis, C. M. & Fregly, M. J. (1996). Introduction. In: Fregly, M. J. & Blatteis, C. M. (Eds.). *Handbook of Physiology, Section 4: Environmental Physiology. Volume 1*. New York: Oxford University Press, pp. xix–xxii.
61. Duchamp, C., Barré, H., Delage, D., Rouanet, J. L., Cohen-Adad, F. & Minaire, Y. (1989). Nonshivering thermogenesis and adaptation to fasting in king penguin chicks. *American Journal of Physiology* 257: R744–R751.
62. West, G. C. (1965). Shivering and heat production in wild birds. *Physiological Zoology* 38: 111–120.
63. Hohtola, E. & 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.
64. Steen, J. & Enger, P. S. (1957). Muscular heat production in pigeons during exposure to cold. *American Journal of Physiology* 191: 157–158.
65. Saarela, S. & 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.
66. Barré, H., Cohen-Adad, F., Duchamp, C. & Rouanet, J. L. (1986). Multilocular adipocytes from muscovy ducklings differentiated in response to cold acclimation. *Journal of Physiology* 375: 27–38.
67. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. I. Effector mechanisms. *Pflügers Archiv* 386: 173–179.
68. Marjoniemi, K. & 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.
69. Korhonen, K. (1989). Heat loss of willow grouse (*Lagopus lagopus*) in a snowy environment. *Journal of Thermal Biology* 14: 27–31.
70. Lim, T. P. K. (1960). Central and peripheral control mechanisms of shivering and its effects on respiration. *Journal of Applied Physiology* 15: 567–574.
71. Pohl, H. (1965). Temperature regulation and cold acclimation in the golden hamster. *Journal of Applied Physiology* 20: 405–410.
72. Oufara, S., Barré, H., Rouanet, J. L. & Chatonnet, J. (1987). Adaptation to extreme ambient temperatures in cold-acclimated gerbils and mice. *American Journal of Physiology* 253: R39–R45.
73. Davis, T. R. A. & Mayer, J. (1955). Nature of the physiological stimulus for shivering. *American Journal of Physiology* 181: 669–674.
74. Spaan, G. & Klussmann, F. W. (1970). Die Frequenz des Kältezitterns bei Tierarten verschiedener Größe. *Pflügers Archiv* 320: 318–333.
75. Slee, J. (1972). Habituation and acclimatization of sheep to cold following exposures of varying length and severity. *Journal of Physiology* 227: 51–70.
76. Fuller, C. A., Horwitz, B. A. & Horowitz, J. M. (1975). Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming. *American Journal of Physiology* 228: 1519–1524.
77. Sellers, E. A., Scott, J. W. & Thomas, N. (1954). Electrical activity of skeletal muscle of normal and acclimated rats on exposure to cold. *American Journal of Physiology* 177: 372–376.
78. 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.
79. Hart, J. S., Heroux, O. & Depocas, F. (1956). Cold acclimation and the electromyogram of unanesthetized rats. *Journal of Applied Physiology* 9: 404–408.
80. Heroux, O., Hart, J. S. & 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.

81. Moriya, K., Arnold, F. & LeBlanc, J. (1988). Shivering and nonshivering thermogenesis in exercised cold-deacclimated rats. *European Journal of Applied Physiology* 57: 467–473.
82. Harri, M., Dannenberg, T., Oksanen-Rossi, R., Hohtola, E. & Sundin, U. (1984). Related and unrelated changes in response to exercise and cold in rats: a reevaluation. *Journal of Applied Physiology* 57: 1489–1497.
83. Pohl, H. & Hart, J. S. (1965). Thermoregulation and cold acclimation in a hibernator, *Citellus tridecemlineatus*. *Journal of Applied Physiology* 20: 398–404.
84. Heath, M. & Ingram, D. L. (1983). Thermoregulatory heat production in cold-reared and warm-reared pigs. *American Journal of Physiology* 244: R273–R278.
85. Berthon, D., Herpin, P. & Le Dividich, J. (1994). Shivering thermogenesis in the neonatal pig. *Journal of Thermal Biology* 19: 413–418.
86. Israel, D. J. & Pozos, R. S. (1989). Synchronized slow-amplitude modulations in the electromyograms of shivering muscles. *Journal of Applied Physiology* 66: 2358–2363.
87. Brück, K., Baum, E. & 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.
88. Bawa, P., Matthews, P. B. C., & Mekjavic, I. B. (1987). Electromyographic activity during shivering of muscles acting at the human elbow. *Journal of Thermal Biology* 12: 1–4.
89. Barré, H., Bailly, L. & 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.
90. Duchamp, C., Barré, H., Rouanet, J. L., Lanni, A., Cohen-Adad, F., Berne, G. & Brebion, P. (1991). Nonshivering thermogenesis in king penguin chicks. I. Role of skeletal muscle. *American Journal of Physiology* 261: R1438–R1445.
91. Duchamp, C., Cohen-Ada, F., Rouanet, J. L. & Barré, H. (1992). Histochemical arguments for muscular nonshivering thermogenesis in muscovy ducklings. *Journal of Physiology* 457: 27–45.
92. Chaffee, R. R. J., Mayhew, W. W., Drebin, M. & Cassuto, Y. (1963). Studies on thermogenesis in cold-acclimated birds. *Canadian Journal of Biochemistry and Physiology* 41: 2215–2220.
93. Kronfeld-Schor, N., Haim, A., Dayan, T., Zisapel, N., Klingenspor, M. & Heldmaier, G. (2000). Seasonal thermogenic acclimation of diurnally and nocturnally active desert spiny mice. *Physiological and Biochemical Zoology* 73: 37–44.
94. Haim, A. & Martinez, J. J. (1992). Seasonal acclimatization in the migratory hamster *Cricetulus migratorius*: the role of photoperiod. *Journal of Thermal Biology* 17: 347–351.
95. 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.
96. 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.
97. Haim, A. & 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.
98. Wünnenberg, W. (1983). Thermosensitivity of the preoptic region and the spinal cord in the golden hamster. *Journal of Thermal Biology* 8: 381–384.
99. Banin, D., Haim, A. & Arad, Z. (1994). Metabolism and thermoregulation in the Levant vole *Microtus guentheri*: the role of photoperiodicity. *Journal of Thermal Biology* 19: 55–62.
100. Saarela, S. & 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.
101. Cottle, W. H. (1963). Calorigenic response of cold-adapted rabbits to adrenaline and to noradrenaline. *Canadian Journal of Biochemistry and Physiology* 41: 1334–1337.
102. Heldmaier, G., Steinlechner, S. & Rafael, J. (1982). Nonshivering thermogenesis and cold resistance during seasonal acclimatization in the Djungarian hamster. *Journal of Comparative Physiology* 149: 1–9.
103. Heldmaier, G. & Jablonka, B. (1985). Seasonal differences in thermogenic adaptation evoked by daily injections of noradrenaline. *Journal of Thermal Biology* 10: 97–99.
104. Davis, T. R. A., Johnston, D. R., Bell, F. C. & Cremer, B. J. (1960). Regulation of shivering and non-shivering heat production during acclimation of rats. *American Journal of Physiology* 198: 471–475.
105. Hsieh, A. C. L., Carlson, L. D. & 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.
106. Cottle, W. H. & Carlson, L. D. (1956). Regulation of heat production in cold-adapted rats. *Proceedings of the Society for Experimental Biology and Medicine* 92: 845–849.
107. Davis, T. R. A. & 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.
108. Morimoto, A., Murakami, N., Nakamori, T. & Watanabe, T. (1986). Suppression of non-shivering thermogenesis in the rat by heat-seeking behaviour during cold exposure. *Journal of Physiology* 380: 541–549.
109. Arnold, J., LeBlanc, J., Cote, J., Lalonde, J. & Richard, D. (1986). Exercise suppression of thermoregulatory thermogenesis in warm- and cold-acclimated rats. *Canadian Journal of Physiology and Pharmacology* 64: 922–926.
110. Banet. M. & Hensel, H. (1977). The control of shivering and non-shivering thermogenesis in the rat. *Journal of Physiology* 269: 669–676.

111. Vallerand, A. L., Pérusse, F. & 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.
112. McDonald, R. B., Horwitz, B. A., Hamilton, J. S. & Stern, J. S. (1988). Cold- and norepinephrine-induced thermogenesis in younger and older Fischer 344 rats. *American Journal of Physiology* 254: R457–R462.
113. Nagashima, T., Kuroshima, A. & 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.
114. Haim, A., Racey, P. A., Speakman, J. R., Ellison, G. T. H. & Skinner, J. D. (1991). Seasonal acclimatization and thermoregulation in the pouched mouse *Saccostomus campestris*. *Journal of Thermal Biology* 16: 13–17.
115. 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.
116. Tomasi, T. E., Hamilton, J. S., & Horwitz, B. A. (1987). Thermogenic capacity in shrews. *Journal of Thermal Biology* 12: 143–147.
117. Smith, R. E. & Roberts, J. C. (1964). Thermogenesis of brown adipose tissue in cold-acclimated rats. *American Journal of Physiology* 206: 143–148.
118. Holloway, B. R., Stribling, D., Freeman, S. & Jamieson, L. (1985). The thermogenic role of adipose tissue in the dog. *International Journal of Obesity* 9: 423–432.
119. Huttunen, P., Hirvonen, J. & Kinnula, V. (1981). The occurrence of brown adipose tissue in outdoor workers. *European Journal of Applied Physiology* 46: 339–345.
120. Smith, R. E. & Hock, R. J. (1963). Brown fat: thermogenic effector of arousal in hibernators. *Science* 140: 199–200.
121. Heim, T. & Hull, D. (1966). The blood flow and oxygen consumption of brown adipose tissue in the new-born rabbit. *Journal of Physiology* 186: 42–55.
122. Astrup, A., Bülow, J., Madsen, J. & Christensen, N. J. (1985). Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *American Journal of Physiology* 248: E507–E515.
123. Foster, D. O. & 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.
124. Bukowiecki, L., Collet, A. J., Follea, N., Guay, G. & Jahjah, L. (1982). Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia. *American Journal of Physiology* 242: E353–E359.
125. Schönbaum, E., Johnson, G. E., Sellers, E. A. & Gill, M. J. (1966). Adrenergic β-receptors and non-shivering thermogenesis. *Nature* 210: 426.
126. Rafael, J. & Vsiansky, P. (1985). Photoperiodic control of the thermogenic capacity in brown adipose tissue of the Djungarian hamster. *Journal of Thermal Biology* 10: 167–170.
127. Bourhim, M., Barré, H., Oufara, S., Minaire, Y., Chatonnet, J., Cohen-Adad, F. & 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.
128. 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.
129. Rothwell, N. J. & Stock, M. J. (1985). Biological distribution and significance of brown adipose tissue. *Comparative Biochemistry and Physiology A* 82: 745–751.
130. 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.
131. Brown, R. T. & Baust, J. G. (1980). Time course of peripheral heterothermy in a homeotherm. *American Journal of Physiology* 239: R126–R129.
132. Rand, R. P., Burton, A. C. & Ing, T. (1965). The tail of the rat, in temperature regulation and acclimatization. *Canadian Journal of Physiology and Pharmacology* 43: 257–267.
133. Raman, E. R., Roberts, M. F. & Vanhuyse, V. J. (1983). Body temperature control of rat tail blood flow. *American Journal of Physiology* 245: R426–R432.
134. Young, A. A. & 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.
135. Romanovsky, A. A., Ivanov, A. I. & 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.
136. Gwosdow, A. R. & Besch, E. L. (1985). Effect of thermal history on the rat's response to varying environmental temperature. *Journal of Applied Physiology* 59: 413–419.
137. Shido, O. (1987). Day-night variation of thermoregulatory responses to intraperitoneal electric heating in rats. *Journal of Thermal Biology* 12: 273–279.
138. Caputa, M., Kamari, A. & Wachulec, M. (1991). Selective brain cooling in rats resting in heat and during exercise. *Journal of Thermal Biology* 16: 19–24.
139. Caputa, M., Demicka, A., Dokladny, K. & Kurowicka, B. (1996). Anatomical and physiological evidence for efficacious selective brain cooling in rats. *Journal of Thermal Biology* 21: 21–28.
140. Folkow, B., Fox, R. H., Krog, J., Odelram, H. & Thorén, O. (1963). Studies on the reactions of the cutaneous vessels to cold exposure. *Acta Physiologica Scandinavica* 58: 342–354.
141. Grant, R. T. (1963). Vasodilatation and body warming in the rat. *Journal of Physiology* 167: 311–317.
142. Webb-Peploe, M. M. & Shepherd, J. T. (1968). Response of dogs' cutaneous veins to local and central temperature changes. *Circulation Research* 23: 693–699.

143. Webb-Peploe, M. M. & Shepherd, J. T. (1968). Peripheral mechanism involved in response of dogs' cutaneous veins to local temperature change. *Circulation Research* 23: 701–708.
144. Mohler, F. S. & Heath, J. E. (1988). Comparison of IR thermography and thermocouple measurement of heat loss from rabbit pinna. *American Journal of Physiology* 254: R389–R395.
145. Grayson, J. (1949). Reactions of the peripheral circulation to external heat. *Journal of Physiology* 109: 53–63.
146. Werner, J. (1977). Influences of local and global temperature stimuli on the Lewis reaction. *Pflügers Archiv* 367: 291–294.
147. Taylor, C. R. & Lyman, C. P. (1972). Heat storage in running antelopes: independence of brain and body temperatures. *American Journal of Physiology* 222: 114–117.
148. Schroter, R. C., Robertshaw, D. & Filali, R. Z. (1989). Brain cooling and respiratory heat exchange in camels during rest and exercise. *Respiration Physiology* 78: 95–105.
149. Scholander, P. F. & Schevill, W. E. (1955). Countercurrent vascular heat exchange in the fins of whales. *Journal of Applied Physiology* 8: 279–282.
150. Heyning, J. E. & Mead, J. G. (1997). Thermoregulation in the mouths of feeding gray whales. *Science* 278: 1138–1139.
151. Carey, F. G., Teal, J. M., Kanwisher, J. W., Lawson, K. D. & Beckett, J. S. (1971). Warm-bodied fish. *American Zoologist* 11: 137–145.
152. Randall, W. C. (1947). Local sweat gland activity due to direct effects of radiant heat. *American Journal of Physiology* 150: 365–371.
153. Fox, R. H., Goldsmith, R., Hampton, I. F. G. & Lewis, H. E. (1964). The nature of the increase in sweating capacity produced by heat acclimatization. *Journal of Physiology* 171: 368–376.
154. Elizondo, R. S. & Bullard, R. W. (1971). Local determinants of sweating and the assessment of the "set point." *International Journal of Biometeorology* 15: 273–280.
155. Nadel, E. R., Bullard, R. W. & Stolwijk, J. A. J. (1971). Importance of skin temperature in the regulation of sweating. *Journal of Applied Physiology* 31: 80–87.
156. Nadel, E. R., Mitchell, J. W. & Stolwijk, J. A. J. (1973). Differential thermal sensitivity in the human skin. *Pflügers Archiv* 340: 71–76.
157. Sato, K. & Sato, F. (1983). Individual variations in structure and function of human eccrine sweat gland. *American Journal of Physiology* 245: R203–R208.
158. Amoros, C., Sagot, J. C., Libert, J. P. & Candas, V. (1986). Sweat gland response to local heating during sleep in man. *Journal de Physiology* 81: 209–215.
159. Aoki, K., Kondo, N., Shibasaki, M., Takano, S., Tominaga, H. & Katsuura, T. (1997). Circadian variation of sweating responses to passive heat stress. *Acta Physiologica Scandinavica* 161: 397–402.
160. Adolph, E. F. (1947). Tolerance to heat and dehydration in several species of mammals. *American Journal of Physiology* 151: 564–575.
161. Bartholomew, G. A. & Wilke, F. (1956). Body temperature in the northern fur seal, *Callorhinus ursinus*. *Journal of Mammalogy* 37: 327–337.
162. 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.
163. Blatt, C. M., Taylor, C. R. & Habal, M. B. (1972). Thermal panting in dogs: the lateral nasal gland, a source of water for evaporative cooling. *Science* 177: 804–805.
164. Le Maho, Y., Goffart, M., Rochas, A., Felbabel, H. & Chatonnet, J. (1981). Thermoregulation in the only nocturnal simian: the night monkey *Aotus trivirgatus*. *American Journal of Physiology* 240: R156–R165.
165. Whittow, G. C., Pettit, T. N., Ackerman, R. A. & 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.
166. 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.
167. Kasa, I. W. & Thwaites, C. J. (1992). The effect of infrared radiation on rectal temperature and respiration rate of unacclimated female New Zealand white rabbits. *Journal of Thermal Biology* 17: 293–296.
168. Ellis, H. I., Maskrey, M., Pettit, T. N. & Whittow, G. C. (1995). Thermoregulation in the brown noddy (*Anous stolidus*). *Journal of Thermal Biology* 20: 307–313.
169. Hainsworth, F. R. (1967). Saliva spreading, activity, and body temperature regulation in the rat. *American Journal of Physiology* 212: 1288–1292.
170. Malan, A. & 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.
171. Hainsworth, F. R. (1968). Evaporative water loss from rats in the heat. *American Journal of Physiology* 214: 979–982.
172. Roberts, W. W., Mooney, R. D. & Martin, J. R. (1974). Thermoregulatory behaviors of laboratory rodents. *Journal of Comparative and Physiological Psychology* 86: 693–699.
173. Refinetti, R. (1983). Conflito entre ingestão de água e termorregulação no rato. *Ciência e Cultura* 35(S): 821.
174. Buffenstein, R. (1984). The importance of microhabitat in thermoregulation and thermal conductance in two Namib rodents. *Journal of Thermal Biology* 9: 235–241.
175. Yanase, M., Kanosue, K., Yasuda, H. & Tanaka, H. (1991). Salivary secretion and grooming behaviour during heat exposure in freely moving rats. *Journal of Physiology* 432: 585–592.
176. Hahn, P. (1956). The development of thermoregulation. III. The significance of fur in the development of thermoregulation in rats. *Physiologia Bohemoslovenica* 5: 428–431.
177. Johansen, K. (1962). Buoyancy and insulation in the muskrat. *Journal of Mammalogy* 43: 64–68.

178. Walsberg, G. E. (1988). Heat flow through avian plumages: the relative importance of conduction, convection, and radiation. *Journal of Thermal Biology* 13: 89–92.
179. Sellers, E. A., You, S. S. & 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.
180. Bartlett, R. G., Mantel, N., Foster, G. L. & Bernstein, P. (1958). Core to surface thermal gradients in the rat at several environmental temperatures. *American Journal of Physiology* 193: 541–546.
181. Herreid, C. F. & Kessel, B. (1967). Thermal conductance in birds and mammals. *Comparative Biochemistry and Physiology* 21: 405–414.
182. Piccione, G., Caola, G. & Refinetti, R. (2002). Effect of shearing on the core body temperature of three breeds of Mediterranean sheep. *Small Ruminant Research* 46: 211–215.
183. Kauffman, A. S., Paul, M. J. & Zucker, I. (2004). Increased heat loss affects hibernation in golden-mantled ground squirrels. *American Journal of Physiology* 287: R167–R173.
184. Rust, C. C., Shackelford, R. M. & Meyer, R. K. (1965). Hormonal control of pelage cycles in the mink. *Journal of Mammalogy* 46: 549–565.
185. Rust, C. C. (1962). Temperature as a modifying factor in the spring pelage change of sort-tailed weasels. *Journal of Mammalogy* 43: 323–328.
186. Cooper, S. J. (2002). Seasonal metabolic acclimatization in mountain chickadees and juniper titmice. *Physiological and Biochemical Zoology* 75: 386-395.
187. Wingfield, J. C., Hahn, T. P., Maney, D. L., Schoech, S. J., Wada, M. & 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.
188. Harris, G. D., Huppi, H. D., & Gessaman, J. A. (1985). The thermal conductance of winter and summer pelage of *Lepus californicus*. *Journal of Thermal Biology* 10: 79–81.
189. Walsberg, G. E. (1991). Thermal effects of seasonal coat change in three subarctic mammals. *Journal of Thermal Biology* 16: 291–296.
190. Al-Hilli, F. & Wright, E. A. (1988). The effects of environmental temperature on the hair coat of the mouse. *Journal of Thermal Biology* 13: 21–24.
191. Duncan, M. J. & 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.
192. Goldman, S. L., Dhandapani, K. & Goldman, B. D. (2000). Genetic and environmental influences on short-day responsiveness in Siberian hamsters (*Phodopus sungorus*). *Journal of Biological Rhythms* 15: 417–428.
193. Kuhlmann, M. T., Clemen, G. & Schlatt, S. (2003). Molting in the Djungarian hamster (*Phodopus sungorus* Pallas): seasonal or continuous process? *Journal of Experimental Zoology* 295A: 160–171.
194. Palchykova, S., Deboer, T. & Tobler, I. (2003). Seasonal aspects of sleep in the Djungarian hamster. *BMC Neuroscience* 4: art. 9.
195. Burkhardt, J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *Journal of Agricultural Science* 37: 64–68.
196. Lincoln, G. A., Andersson, H. & 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.
197. 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.
198. Jansky, L. (1967). Evolutionary adaptations of temperature regulation in mammals. *Zeitschrift für Säugetierkunde* 32: 167–172.
199. Paladino, F. V., O'Connor, M. P. & Spotila, J. R. (1990). Metabolism of leatherback turtles, gigantothermy, and thermoregulation of dinosaurs. *Nature* 344: 858–860.
200. Bergmann, C. (1847). Über die Verhältnisse der wärmeökonomie der Thiere zu ihrer Größe. *Göttinger Studien (Abtheilung 1)* 3: 595–708.
201. Allen, J. A. (1877). The influence of physical conditions in the genesis of species. *Radical Review* 1: 108–140.
202. Scholander, P. F. (1955). Evolution of climatic adaptation in homeotherms. *Evolution* 9: 15–26.
203. Mayr, E. (1956). Geographical character gradients and climatic adaptation. *Evolution* 10: 105–108.
204. Weaver, M. E. & Ingram, D. L. (1969). Morphological changes in swine associated with environmental temperature. *Ecology* 50: 710–713.
205. Sumner, F. B. (1915). Some studies of environmental influence, heredity, correlation and growth, in the white mouse. *Journal of Experimental Zoology* 18: 325–432.
206. Ogle, C. (1934). Climatic influence on the growth of the male albino mouse. *American Journal of Physiology* 107: 635–640.
207. Buck, C. L. & 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.
208. Herter, K. (1924). Untersuchungen über den Temperatursinn einiger Insekten. *Zeitschrift für Vergleichende Physiologie* 1: 221–288.
209. Refinetti, R. (1982). Termopreferendum da formiga saúva fora do ninho. *Ciência e Cultura* 34(S): 897.
210. Dreisig, H. (1980). Daily activity, thermoregulation and water loss in the tiger beetle *Cicindela hybrida*. *Oecologia* 44: 376–389.
211. 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.

212. Krafft, B. (1967). Thermopreferendum de l'araignée sociale *Agelena consociata* Denis. *Insectes Sociaux* 14: 161–182.
213. Barlow, C. A. & Kuenen, D. J. (1957). A new thermopreferendum apparatus for terrestrial isopods. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen, Ser. C* 60: 240–250.
214. Refinetti, R. (1984). Behavioral temperature regulation in the pill bug, *Armadillidium vulgare* (Isopoda). *Crustaceana* 47: 29–43.
215. Taylor, R. C. (1984). Thermal preference and temporal distribution in three crayfish species. *Comparative Biochemistry and Physiology A* 77: 513–517.
216. Crawshaw, L. I. (1971). Temperature selection and activity in the crayfish, *Orconectes immunis*. *Journal of Comparative Physiology* 95: 315–322.
217. Díaz-Iglesias, E., Díaz-Herrera, F., Re-Araujo, A. D., Báez-Hidalgo, M., López-Zenteno, M., Valdés-Sáanchez, G. & 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.
218. Bernardi, N. (1990). Temperature influence upon food ingestion and spontaneous locomotion of the freshwater prawn *Macrobrachium acanthurus*. *Journal of Thermal Biology* 15: 33–36.
219. Kivivuori, L. & Lagerspetz, K. Y. H. (1990). Temperature selection behaviour of the Isopod *Saduria entomon*. *Journal of Thermal Biology* 15: 83–86.
220. Espina, S., Herrera, F. D. & Bückle, L. F. (1993). Preferred and avoided temperatures in the crawfish *Procambarus clarkii* (Decapoda: Cambaridae). *Journal of Thermal Biology* 18: 35–39.
221. Kivivuori, L. A. (1994). Temperature selection behaviour of cold- and warm-acclimated crayfish (*Astacus astacus*). *Journal of Thermal Biology* 19: 291–297.
222. Bückle-Ramírez, L. F., Díaz-Herrera, F., Correa-Sandoval, F., Barón-Sevilla, B. & Hernández-Rodríguez, M. (1994). Diel thermoregulation of the crawfish *Procambarus clarkii* (Crustacea: Cambaridae). *Journal of Thermal Biology* 19: 419–422.
223. Neill, W, H., Magnuson, J. J. & Chipman, G. G. (1972). Behavioral thermoregulation by fishes: a new experimental approach. *Science* 176: 1443–1445.
224. Roy, A. W. & Johansen, P. H. (1970). The temperature selection of small hypophysectomized goldfish (*Carassius auratus* L.). *Canadian Journal of Zoology* 48: 323–326.
225. Ingersoll, C. G. & 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.
226. McCauley, R. W. & 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.
227. Reynolds, W. W., Thomson, D. A. & Casterlin, M. E. (1977). Responses of young California grunion, *Leuresthes tenuis*, to gradients of temperature and light. *Copeia* 1977: 144–149.
228. Reynolds, W. W., McCauley, R. W., Casterlin, M. E. & Crawshaw, L. I. (1976). Body temperatures of behaviorally thermoregulating largemouth blackbass (*Micropterus salmoides*). *Comparative Biochemistry and Physiology A* 54: 461–463.
229. Sullivan, C. M. & Fisher, K. C. (1954). The effects of light on temperature selection in speckled trout *Salvelinus fontinalis* (Mitchill). *Biological Bulletin* 107: 278–288.
230. Beitinger, T. L. & 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.
231. Carpenter, S. J., Erickson, J. M. & Holland, F. D. (2003). Migration of a Late Cretaceous fish. *Nature* 423: 70–74.
232. Reynolds, W. W., Casterlin, M. E., Matthey, J. K., Millington, S. T. & 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.
233. Kavaliers, M. & 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.
234. 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.
235. Nelson, D. O. & 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.
236. Schurmann, H. & 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.
237. Feder, M. E. & Pough, F. H. (1975). Temperature selection by red-backed salamander, *Plethodon c. cinereus* (Green) (Caudata: Plethodontidae). *Comparative Biochemistry and Physiology A* 50: 91–98.
238. Duclaux, R., Fantino, M. & Cabanac, M. (1973). Comportement thermoregulateur chez *Rana esculenta*. *Pflügers Archiv* 342: 347–358.
239. Sievert, L. M. (1991). Thermoregulatory behaviour in the toads *Bufo marinus* and *Bufo cognatus*. *Journal of Thermal Biology* 16: 309–312.
240. Smith, E. N. (1975). Thermoregulation of the American alligator, *Alligator mississippiensis*. *Physiological Zoology* 48: 177–194.
241. Crawshaw, L. I., Johnston, M. H. & Lemons, D. E. (1980). Acclimation, temperature selection, and heat exchange in the turtle, *Chrysemys scripta*. *American Journal of Physiology* 238: R443–R446.

242. Myhre, K. & Hammel, H. T. (1969). Behavioral regulation of internal temperature in the lizard *Tiliqua scincoides*. *American Journal of Physiology* 217: 1490–1495.
243. Gleeson, T. T. (1981). Preferred body temperature, aerobic scope, and activity capacity in the monitor lizard, *Varanus salvator*. *Physiological Zoology* 54: 423–429.
244. Schuett, G. W. & Gatten, R. E. (1980). Thermal preference in snapping turtles *(Chelydra serpentina*). *Copeia* 1980: 149–152.
245. King, D., Green, B. & Herrera, E. (1994). Thermoregulation in a large teiid lizard, *Tupinambis teguixin*, in Venezuela. *Copeia* 1994: 806–808.
246. Innicenti, A., Minutini, L. & Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
247. Tosini, G. & 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.
248. Sievert, L. M. & Hutchison, V. H. (1988). Light versus heat: thermoregulatory behavior in a nocturnal lizard (*Gekko gecko*). *Herpetologica* 44: 266–273.
249. Autumn, K. & De Nardo, D. F. (1995). Behavioral thermoregulation increases growth rate in a nocturnal lizard. *Journal of Herpetology* 29: 157–162.
250. Brown, R. P. (1996). Thermal biology of the gecko *Tarentola boettgeri*: comparisons among populations from different elevations within Gran Canaria. *Herpetologica* 52: 396–405.
251. Keraney, M. & Predavec, M. (2000). Do nocturnal ectotherms thermoregulate? A study of the temperate gecko *Christinus marmoratus*. *Ecology* 81: 2984–2996.
252. Regal, P. J. (1967). Voluntary hypothermia in reptiles. *Science* 155: 1551–1553.
253. Cowgell, J. & Underwood, H. (1979). Behavioral thermoregulation in lizards: a circadian rhythm. *Journal of Experimental Zoology* 210: 189–194.
254. Rismiller, P. D. & Heldmaier, G. (1991). Seasonal changes in daily metabolic patterns of *Lacerta viridis*. *Journal of Comparative Physiology B* 161: 482–488.
255. Refinetti, R. & Susalka, S. J. (1997). Circadian rhythm of temperature selection in a nocturnal lizard. *Physiology and Behavior* 62: 331–336.
256. Wilhoft, D. C. & Anderson, J. D. (1960). Effect of acclimation on the preferred body temperature of the lizard, *Sceloporus occidentalis*. *Science* 131: 610–611.
257. Cannon, J. G. & Kluger, M. J. (1985). Altered thermoregulation in the iguana *Dipsosaurus dorsalis* following exercise. *Journal of Thermal Biology* 10: 41–45.
258. Christian, K. A., Tracy, C. R., & 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.
259. van Damme, R., Bauwens, D., & Verheyen, R. F. (1986). Selected body temperature in the lizard *Lacerta vivipara*: variation within and between populations. *Journal of Thermal Biology* 11: 219–222.
260. Sievert, L. M. & 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.
261. Jarling, C., Scarperi, M., & 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.
262. Shine, R. & Lambeck, R. (1990). Seasonal shifts in the thermoregulatory behaviour of Australian blacksnakes, *Pseudechis porphyriacus* (Serpentes: Elapidae). *Journal of Thermal Biology* 15: 301–305.
263. 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.
264. Sievert, L. M. & 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.
265. Christian, K. & Bedford, G. (1996). Thermoregulation by the spotted tree monitor, *Varanus scalaris*, in the seasonal tropics of Australia. *Journal of Thermal Biology* 21: 67–73.
266. Cotton, P. A. (2003). Avian migration phenology and global climate change. *Proceedings of the National Academy of Sciences U.S.A.* 100: 12219–12222.
267. Modrey, P. & Nichelmann, M. (1992). Development of autonomic and behavioural thermoregulation in turkeys (*Meleagris gallopavo*). *Journal of Thermal Biology* 17: 287–292.
268. Hull, J. & Hull, D. (1982). Behavioral thermoregulation in newborn rabbits. *Journal of Comparative and Physiological Psychology* 96: 143–147.
269. Gumma, M. R. & South, F. E. (1970). Hypothermia and behavioural thermoregulation by the hamster (*Mesocricetus auratus*). *Animal Behaviour* 18: 504–511.
270. Herter, K. (1936). Das thermotaktische Optimum bei Nagetieren, ein mendelndes art- und Rassenmerkmal. *Zeitschrift für Vergleichende Physiologie* 23: 605–650.
271. Gordon, C. J. (1983). Behavioral and autonomic thermoregulation in mice exposed to microwave radiation. *Journal of Applied Physiology* 55: 1242–1248.
272. Carlisle, H. J. & Dubuc, P. U. (1984). Temperature preference of genetically obese (ob/ob) mice. *Physiology and Behavior* 33: 899–902.
273. Briese, E. (1985). Rats prefer ambient temperatures out of phase with their body temperature circadian rhythm. *Brain Research* 345: 389–393.
274. Gordon, C. J. (1986). Relationship between behavioral and autonomic thermoregulation in the guinea pig. *Physiology and Behavior* 38: 827–831.
275. Gordon, C. J. (1987). Relationship between preferred ambient temperature and autonomic thermoregulatory function in rat. *American Journal of Physiology* 252: R1130–R1137.
276. Gordon, C. J. (1985). Relationship between autonomic and behavioral thermoregulation in the mouse. *Physiology and Behavior* 34: 687–690.

277. Gordon, C. J., Fehlner, K. S. & Long, M. D. (1986). Relationship between autonomic and behavioral thermoregulation in the golden hamster. *American Journal of Physiology* 251: R320–R324.
278. Ogilvie, D. M. & Stinson, R. H. (1966). Temperature selection in *Peromyscus* and laboratory mice, *Mus musculus*. *Journal of Mammalogy* 47: 655–660.
279. Lynch, G. R., Lynch, C. B., Dube, M. & Allen, C. (1976). Early cold exposure: effects on behavioral and physiological thermoregulation in the house mouse, *Mus musculus*. *Physiological Zoology* 49: 191–199.
280. Refinetti, R. & Horvath, S. M. (1989). Thermopreferendum of the rat: inter- and intra-subject variability. *Behavioral and Neural Biology* 52: 87–94.
281. Laughter, J. S. & Blatteis, C. M. (1985). A system for the study of behavioral thermoregulation of small animals. *Physiology and Behavior* 35: 993–997.
282. Gordon, C. J., Lee, K. A., Chen, T. A., Killough, P. & Ali, J. S. (1991). Dynamics of behavioral thermoregulation in the rat. *American Journal of Physiology* 261: R705–R711.
283. Gordon, C. J., Becker, P. & Ali, J. S. (1998). Behavioral thermoregulatory responses of single- and group-housed mice. *Physiology and Behavior* 65: 255–262.
284. Klein, M. S., Conn, C. A. & Kluger, M. J. (1992). Behavioral thermoregulation in mice inoculated with influenza virus. *Physiology and Behavior* 52: 1133–1139.
285. Refinetti, R. (1998). Homeostatic and circadian control of body temperature in the fat-tailed gerbil. *Comparative Biochemistry and Physiology A* 119: 295–300.
286. Gordon, C. J. (1993). Twenty-four hour rhythms of selected ambient temperature in rat and hamster. *Physiology and Behavior* 53: 257–263.
287. 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.
288. 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.
289. Refinetti, R. (1998). Body temperature and behavior of tree shrews and flying squirrels in a thermal gradient. *Physiology and Behavior* 63: 517–520.
290. Gelineo, S. & 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.
291. Leonard, C. M. (1982). Shifting strategies for behavioral thermoregulation in developing golden hamsters. *Journal of Comparative and Physiological Psychology* 96: 234–243.
292. Fewell, J. E., Kang, M. & Eliason, H. L. (1997). Autonomic and behavioral thermoregulation in guinea pigs during postnatal maturation. *Journal of Applied Physiology* 83: 830–836.
293. Gordon, C. J., Long, M. D., & Fehlner, K. S. (1984). Behavioural and autonomic thermoregulation in hamsters during microwave-induced heat exposure. *Journal of Thermal Biology* 9: 271–277.
294. Downs, C. T. & Perrin, M. R. (1990). Thermal parameters of four species of *Gerbillurus*. *Journal of Thermal Biology* 15: 291–300.
295. Malvin, G. M. & Wood, S. C. (1992). Behavioral hyothermia and survival of hypoxic protozoans *Paramecium caudatum*. *Science* 255: 1423–1425.
296. 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.
297. Jeddi, E., Guerin, J. F. & Czyba, J. C. (1977). Have human spermatozoa a thermopreferendum? *Biology of Reproduction* 16: 561–563.
298. Hernandez, J., Bonnedahl, J., Waldenström, J., Palmgren, H. & Olsen, B. (2003). *Salmonella* in birds migrating through Sweden. *Emerging Infectious Diseases* 9: 753–755.
299. Froy, O., Gotter, A. L., Casselman, A. L. & Reppert, S. M. (2003). Illuminating the circadian clock in monarch butterfly migration. *Science* 300: 1303–1305.
300. Hunter, E., Metcalfe, J. D. & 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.
301. Rattenborg, N. C., Mandt, B. H., Obermeyer, W. H., Winsauer, P. J., Huber, R., Wikelski, M. & Benca, R. M. (2004). Migratory sleeplessness in the white-crowned sparrow (*Zonotrichia leucophrys gambelii*). *PLOS Biology* 2: 924–936.
302. Le Maho, Y., Delclitte, P. & Chatonnet, J. (1976). Thermoregulation in fasting emperor penguins under natural conditions. *American Journal of Physiology* 231: 913–922.
303. Lustick, S., Battersby, B. & Kelty, M. (1978). Behavioral thermoregulation: orientation toward the sun in herring gulls. *Science* 200: 81–83.
304. Wells, R. T. (1978). Thermoregulation and activity rhythms in the hairy-nosed wombat, Lasiorhinus latifrons (Owen), (Vombatidae). *Australian Journal of Zoology* 26: 639–651.
305. Swift, R. W. & Forbes, R. M. (1939). The heat production of the fasting rat in relation to the environmental temperature. *Journal of Nutrition* 18: 307–318.
306. Schmidek, W. R., Hoshino, K., Schmidek, M. & Timo-Iaria, C. (1972). Influence of environmental temperature on the sleep-wakefulness cycle in the rat. *Physiology and Behavior* 8: 363–371.
307. McEwen, G. N. & Heath, J. E. (1973). Resting metabolism and thermoregulation in the unrestrained rabbit. *Journal of Applied Physiology* 35: 884–886.
308. Chevillard-Hugot, M. C., Müller, E. F. & Kulzer, E. (1980). Oxygen consumption, body temperature and heart rate in the coati (*Nasua nasua*). *Comparative Biochemistry and Physiology A* 65: 305–309.
309. Øritsland, N. A. (1970). Temperature regulation of the polar bear (*Thalarctos maritimus*). *Comparative Biochemistry and Physiology* 37: 225–233.
310. Macari, M. (1983). Efeito do cruzamento de suínos sobre o comportamento termoregulador. *Ciência e Cultura* 35: 1145–1150.

311. Moinard, C., Doncaster, C. P. & Barré, H. (1992). Indirect calorimetry measurements of behavioral thermoregulation in a semiaquatic social rodent, *Myocastor coypus*. *Canadian Journal of Zoology* 70: 907–911.
312. Pivnick, K. A. & McNeil, J. N. (1986). Sexual differences in the thermoregulation of *Thymelicus lineola* adults (Lepidoptera: Hesperiidae). *Ecology* 67: 1024–1035.
313. Rutowski, R. L., Demlong, M. J. & Leffingwell, T. (1994). Behavioural thermoregulation at mate encounter sites by male butterflies (*Asterocampa leilia*, Nymphalidae). *Animal Behaviour* 48: 833–841.
314. Lillywhite, H. B. (1970). Behavioral temperature regulation in the bullfrog, *Rana catesbeiana*. *Copeia* 1970: 158–168.
315. Bogert, C. M. (1959). How reptiles regulate their body temperature. *Scientific American* 200(4): 105–120.
316. Kleiber, M. & Winchester, C. (1933). Temperature regulation in baby chicks. *Proceedings of the Society for Experimental Biology and Medicine* 31: 158–159.
317. Webb, D. R. (1993). Maternal-nestling contact geometry and heat transfer in an altricial bird. *Journal of Thermal Biology* 18: 117–124.
318. Mount, L. E. & 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.
319. Sealander, J. A. (1952). The relationship of nest protection and huddling to survival of *Peromyscus* at low temperature. *Ecology* 33: 63–71.
320. Vogt, F. D. & Lynch, G. R. (1982). Influence of ambient temperature, nest availability, huddling, and daily torpor on energy expenditure in the white-footed mouse *Peromyscus leucopus*. *Physilogical Zoology* 55: 56–63.
321. Alberts, J. R. (1978). Huddling by rat pups: group behavioral mechanisms of temperature regulation and energy conservation. *Journal of Comparative and Physiological Psychology92* 92: 231–245.
322. 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.
323. Batchelder, P., Kinney, R. O., Demlow, L. & Lynch, C. B. (1983). Effects of temperature and social interactions on huddling behavior in *Mus musculus*. *Physiology and Behavior* 31: 97–102.
324. Withers, P. C. & 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.
325. Contreras, L. C. (1984). Bioenergetics of huddling: test of a psycho-physiological hypothesis. *Journal of Mammalogy* 65: 256–262.
326. Andrews, R. V. & Belknap, R. W. (1986). Bioenergetic benefits of huddling by deer mice (*Peromyscus maniculatus*). *Comparative Biochemistry and Physiology A* 85: 775–778.
327. Newkirk, K. D., Silverman, D. A. & Wynne-Edwards, K. E. (1995). Ontogeny of thermoregulation in the Djungarian hamster (*Phodopus campbelli*). *Physiology and Behavior* 57: 117–124.
328. Sokoloff, G., Blumberg, M. S. & 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.
329. Arnold, W., Heldmaier, G., Ortmann, S., Pohl, H., Ruf, T. & Steinlechner, S. (1991). Ambient temperatures in hibernacula and their energetic consequences for Alpine marmots (*Marmota marmota*). *Journal of Thermal Biology* 16: 223–226.
330. 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.
331. Schino, G. & Troisi, A. (1990). Behavioral thermoregulation in long-tailed macaques: effect on social preference. *Physiology and Behavior* 47: 1125–1128.
332. Harrison, J. F., Fewell, J. H., Roberts, S. P. & Hall, H. G. (1996). Achievement of thermal stability by varying metabolic heat production in flying honeybees. *Science* 274: 88–90.
333. Heinrich, B. (1981). The regulation of temperature in the honeybee swarm. *Scientific American* 244(6): 120–129.
334. Kronenberg, F. & Heller, H. C. (1982). Colonial thermoregulation in honey bees (*Apis mellifera*). *Journal of Comparative Physiology* 148: 65–76.
335. Southwick, E. E. (1987). Cooperative metabolism in honey bees: an alternative to antifreeze and hibernation. *Journal of Thermal Biology* 12: 155–158.
336. Gasnier, A. & 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.
337. Brobeck, J. R. (1948). Food intake as a mechanism of temperature regulation. *Yale Journal of Biology and Medicine* 20: 545–549.
338. Fregly, M. J., Marshall, N. B. & 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.
339. Iampietro, P. F., Bass, D. E. & Buskirk, E. R. (1958). Caloric intake during prolonged cold exposure. *Metabolism* 7: 149–153.
340. Witty, R. T. & Long, J. F. (1970). Effect of ambient temperature on gastric secretion and food intake in the rat. *American Journal of Physiology* 219: 1359–1363.
341. Jakubczak, L. F. (1976). Food an water intakes of rats as a function of strain, age, temperature, and body weight. *Physiology and Behavior* 17: 251–258.
342. 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.

343. Yamauchi, C., Fujita, S., Obara, T. & 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.
344. Nakatsuka, H., Shoji, Y. & Tsuda, T. (1983). Effects of cold exposure on gaseous metabolism and body composition in the rat. *Comparative Biochemistry and Physiology A* 75: 21–25.
345. Armitage, G., Harris, R. B. S., Hervey, G. R. & 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.
346. Savory, C. J. (1986). Influence of ambient temperature on feeding activity parameters and digestive function in domestic fowls. *Physiology and Behavior* 38: 353–357.
347. Manning, J. M. & Bronson, F. H. (1990). The effects of low temperature and food intake on ovulation in domestic mice. *Physiological Zoology* 63: 938–948.
348. Hahn, G. L., Chen, Y. R., Nienaber, J. A., Eigenberg, R. A. & Parkhurst, A. M. (1992). Characterizing animal stress through fractal analysis of thermoregulatory responses. *Journal of Thermal Biology* 17: 115–120.
349. Henderson, D., Fort, M. M., Rashotte, M. E. & Henderson, R. P. (1992). Ingestive behavior and body temperature of pigeons during long-term cold exposure. *Physiology and Behavior* 52: 455–469.
350. 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.
351. Agetsuma, N. (2000). Influence of temperature on energy intake and food selection by macaques. *International Journal of Primatology* 21: 103–111.
352. Kauffman, A. S., Cabrera, A. & Zucker, I. (2001). Energy intake and fur in summer- and winter-acclimated Siberian hamsters (*Phodopus sungorus*). *American Journal of Physiology* 281: R519–R527.
353. Horst, K., Mendel, L. B. & Benedict, F. G. (1930). The metabolism of the albino rat during prolonged fasting at two different environmental temperatures. *Journal of Nutrition* 3: 177–200.
354. Campbell, B. A. & 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.
355. Bolles, R. C. & Duncan, P. M. (1969). Daily course of activity and subcutaneous body temperature in hungry and thirsty rats. *Physiology and Behavior* 4: 87–89.
356. Rashotte, M. E., Basco, P. S. & 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.
357. Rashotte, M. E., Pastukhov, I. F., Poliakov, E. L. & 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.
358. Yoda, T., Crawshaw, L. I., Yoshida, K., Su, L., Hosono, T., Shido, O., Sakurada, S., Fukuda, Y. & 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.
359. Sakurada, S., Shido, O., Sugimoto, N., Hiratsuka, Y., Yoda, T. & Kanosue, K. (2000). Autonomic and behavioural thermoregulation in starved rats. *Journal of Physiology* 526: 417–424.
360. Ingram, D. L. & Legge, K. F. (1974). Effects of environmental temperature on food intake in growing pigs. *Comparative Biochemistry and Physiology A* 48: 573–581.
361. Kraly, F. S. & Blas, E. M. (1976). Mechanisms for enhanced feeding in the cold in rats. *Journal of Comparative and Physiological Psychology* 90: 714–726.
362. Macari, M., Dauncey, M. J. & 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.
363. 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.
364. Caputa, M. & Cabanac, M. (1980). Muscular work as thermal behavior. *Journal of Applied Physiology* 48: 1020–1023.
365. Cabanac, M. & Leblanc, J. (1983). Physiological conflict in humans: fatigue vs. cold discomfort. *American Journal of Physiology* 244: R621–R628.
366. Paladino, F. V. & King, J. R. (1984). Thermoregulation and oxygen consumption during terrestrial locomotion by white-crowned sparrows *Zonotrichia leucophrys gambelii*. *Physiological Zoology* 57: 226–236.
367. Webster, M. D. & 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.
368. Zerba, E. & Walsberg, G. E. (1992). Exercise-generated heat contributes to thermoregulation by Gambel's quail in the cold. *Journal of Experimental Biology* 171: 409–422.
369. Wunder, B. A. (1970). Energetics of running activity in Merriam's chipmunk, *Eutamias merriami*. *Comparative Biochemistry and Physiology* 33: 821–836.
370. Yousef, M. K., Robertson, W. D., Dill, D. B. & Johnson, H. D. (1973). Energetic cost of running in the antelope ground squirrel *Ammospermophilus leucurus*. *Physiological Zoology* 46: 139–147.
371. Girardier, L., Clark, M. G. & Seydoux, J. (1995). Thermogenesis associated with spontaneous activity: an important component of thermoregulatory needs in rats. *Journal of Physiology* 488: 779–787.
372. Doss, D. & Ohnesorge, F. K. (1966). Motilität und Körpertemperatur von Mäusen in verschiedenen Umgebungstemperaturen. *Pflügers Archiv* 289: 91–97.
373. Aujard, F. & 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.

374. Yang, Y. & 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.
375. Lyman, C. P. (1954). Activity, food consumption and hoarding in hibernators. *Journal of Mammalogy* 35: 545–552.
376. Olesen, B. W. & Seelen, J. (1993). Criteria for a comfortable indoor environment in buildings. *Journal of Thermal Biology* 18: 545–549.
377. Givoni, B. (1974). Architectural and urban planning in relation to weather and climate. *Progress in Biometeorology* 1: 183–193.
378. Leff, L. G. & 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.
379. Grassé, P. P. & Noirot, C. (1961). Nouvelles recherches sur la systématique et l'éthologie des termites champignonnistes du genre *Bellicositermes* Emerson. *Insectes Sociaux* 8: 311–357.
380. Scherba, G. (1962). Mound temperatures of the ant *Formica ulkei* Emery. *American Midland Naturalist* 67: 373–385.
381. Lensky, Y. (1964). Comportement d'une colonie d'abeilles a des temperatures extremes. *Journal of Insect Physiology* 10: 1–12.
382. Degrandi-Hoffman, G., Spivak, M. & 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.
383. McGinnis, S. M. & Voigt, W. G. (1971). Thermoregulation in the desert tortoise, *Gopherus agassizii*. *Comparative Biochemistry and Physiology A* 40: 119–126.
384. Autumn, K., Weinstein, R. B. & Full, R. J. (1994). Low cost of locomotion increases performance at low temperature in a nocturnal lizard. *Physiological Zoology* 67: 238–262.
385. Coulianos, C. C. & Johnels, A. G. (1963). Note on the subnivean environment of small mammals. *Arkiv för Zoologi* 15: 363–370.
386. Elvert, R., Kronfeld, N., Dayan, T., Haim, A., Zisapel, N. & 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.
387. Lovegrove, B. G., Heldmaier, G. & 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.
388. 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.
389. Rode, P. (1929). Contribuition a l'étude du fouissement chez les petits rongeurs. *Bulletin de la Société Zoologique de France* 54: 573–588.
390. 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.
391. Stark, H. E. (1963). Nesting habits of the California vole, *Microtus californicus*, and microclimatic factors affecting its nests. *Ecology* 44: 663–669.
392. Lee, C. T. & Wong, P. T. P. (1970). Temperature effect and strain differences in the nest-building behavior of inbred mice. *Psychonomic Science* 20: 9–10.
393. Kenagy, G. J., Vásquez, R. A., Barnes, B. M. & Bozinovic, F. (2004). Microstructure of summer activity bouts of degus in a thermally heterogeneous habitat. *Journal of Mammalogy* 85: 260–267.
394. Lynch, C. B. (1973). Environmental modification of nest-building in the white-footed mouse, *Peromyscus leucopus*. *Animal Behaviour* 22: 405–409.
395. Ivey, R. D. (1949). Life history notes on three mice from the Florida east coast. *Journal of Mammalogy* 30: 157–162.
396. Puchalski, W., Bulova, S. J., Lynch, C. B. & Lynch, G. R. (1988). Photoperiod, temperature and melatonin effects on thermoregulatory behavior in Djungarian hamsters. *Physiology and Behavior* 42: 173–177.
397. Kinder, E. F. (1927). A study of the nest-building activity of the albino rat. *Journal of Experimental Zoology* 47: 117–161.
398. 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.
399. Pisano, R. G. & Storer, T. I. (1948). Burrows and feeding of the Norway rat. *Journal of Mammalogy* 29: 374–383.
400. Refinetti, R. (1983). Construção de ninho no rato: efeitos da temperatura na fase de coleta. *Ciência e Cultura* 35(S): 820–821.
401. McDevitt, R. M. & 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.
402. Hut, R. A., Barnes, B. M. & 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.
403. Mayer, W. V. (1955). The protective value of the burrow system to the hibernating arctic squirrel, *Spermophilus undulatus*. *Anatomical Record* 122: 437–438.
404. Fadem, B. H., Kraus, D. B. & Sheffet, R. H. (1986). Nest-building in gray short-tailed opossums: temperature effects and sex differences. *Physiology and Behavior* 36: 667–670.
405. Deutsch, J. A. (1957). Nest building behaviour of domestic rabbits under semi-natural conditions. *British Journal of Animal Behaviour* 5: 53–54.
406. Buech, R. R. & Rugg, D. J. (1989). Temperature in beaver lodges and bank dens in a near-boreal environment. *Canadian Journal of Zoology* 67: 1061–1066.

407. Dyck, A. P. & MacArthur, R. A. (1992). Seasonal patterns of body temperature in free-ranging beaver (*Castor canadensis*). *Canadian Journal of Zoology* 70: 1668–1672.
408. Lancia, R. A., Dodge, W. E. & Larson, J. S. (1982). Winter activity patterns of two radio-marked beaver colonies. *Journal of Mammalogy* 63: 598–606.
409. Linduska, J. P. (1947). Winter den studies of the cottontail in southern Michigan. *Ecology* 28: 448–454.
410. Bakko, E. B., Porter, W. P. & Wunder, B. A. (1988). Body temperature patterns in black-tailed prairie dogs in the field. *Canadian Journal of Zoology* 66: 1783–1789.
411. Downey, P. & Jahan-Parwar, B. (1972). Cooling as reinforcing stimulus in *Aplysia*. *American Zoologist* 12: 507–512.
412. Rozin, P. N. & Mayer, J. (1961). Thermal reinforcement and thermoregulatory behavior in the goldfish, *Carassius auratus*. *Science* 134: 942–943.
413. Laudenslager, M. L. & Hammel, H. T. (1977). Environmental temperature selection by the Chukar partridge, *Alectoris chukar*. *Physiology and Behavior* 19: 543–548.
414. Schmidt, I. & Rautenberg, W. (1975). Instrumental thermoregulatory behavior in pigeons. *Journal of Comparative Physiology* 101: 225–235.
415. Schmidt, I. (1976). Effect of central thermal stimulation on the thermoregulatory behavior of the pigeon. *Pflügers Archiv* 363: 271–272.
416. Horowitz, K. A., Scott, N. R., Hillman, P. E. & Van Tienhoven, A. (1978). Effects of feathers on instrumental thermoregulatory behavior in chickens. *Physiology and Behavior* 21: 233–238.
417. Budgell, P. (1971). Behavioural thermoregulation in the Barbary dove (*Streptopelia risoria*). *Animal Behaviour* 19: 524–531.
418. Richards, S. A. (1976). Behavioural temperature regulation in the fowl. *Journal of Physiology* 258: 122P–123P.
419. Refinetti, R. (1983). Thermoregulatory behaviour of Budgerigars in a cold environment. *Bird Behaviour* 4: 90–92.
420. Revusky, S. H. (1966). Cold acclimatization in hairless mice measured by behavioral thermoregulation. *Psychonomic Science* 6: 209–210.
421. Baldwin, B. A. (1968). Behavioural thermoregulation in mice. *Physiology and Behavior* 3: 401–407.
422. Dauncey, M. J. (1984). Behavioural and autonomic thermoregulation in lean and genetically obese (*ob*/*ob*) mice. *Journal of Thermal Biology* 9: 247–253.
423. Chen, X. M., Hosono, T., Mizuno, A., Yoda, T., Yoshida. K., Aoyagi, Y. & Kanosue, K. (1998). New apparatus for studying behavioral thermoregulation in rats. *Physiology and Behavior* 64: 419–424.
424. Epstein, A. N. & Milestone, R. (1968). Showering as a coolant for rats exposed to heat. *Science* 160: 895–896.
425. Carlton, P. L. & Marks, R. A. (1958). Cold exposure and heat reinforced operant behavior. *Science* 128: 1344.
426. Weiss, B. (1957). Thermal behavior of the subnourished and pantothenic-acid-deprived rat. *Journal of Comparative and Physiological Psychology* 50: 481–485.
427. Sabourin, M., Deschambault, A. & Ducharme, R. (1973). Effect of light on thermal reinforcement in behavioral thermoregulation. *Perceptual and Motor Skills* 37: 611–614.
428. Wright, J. W. & Meyer, M. E. (1969). A new technique for measuring thermoregulatory behavior in the rat. *Journal of the Experimental Analysis of Behavior* 12: 999–1002.
429. Carlisle, H. J. (1968). Initiation of behavioral responding for heat in a cold environment. *Physiology and Behavior* 3: 827–830.
430. Carlisle, H. J. (1969). Effect of fixed-ratio thermal reinforcement on thermoregulatory behavior. *Physiology and Behavior* 4: 23–28.
431. Matthews, T. J. (1971). Thermal motivation in the rat. *Journal of Comparative and Physiological Psychology* 74: 240–247.
432. Leeming, F. C. (1968). Response rate as a function of magnitude and schedule of heat reinforcement. *Journal of Experimental Psychology* 76: 74–77.
433. Scott, D. M. & Blackwood, V. (1973). Response latencies of rats during behavioral thermoregulation. *Perception and Psychophysics* 14: 531–541.
434. Weiss, B. & Laties, V. G. (1960). Magnitude of reinforcement as a variable in thermoregulatory behavior. *Journal of Comparative and Physiological Psychology* 53: 603–608.
435. Kristt, D. A. & 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.
436. Refinetti, R. & Carlisle, H. J. (1987). Behavioral heat intake as a function of reward duration in rats. *Animal Learning and Behavior* 15: 228–237.
437. Szymusiak, R., DeMory, A., Kittrell, E. M. W. & Satinoff, E. (1985). Diurnal changes in thermoregulatory behavior in rats with medial preoptic lesions. *American Journal of Physiology* 249: R219–R227.
438. Cabanac, M. & Dib, B. (1983). Behavioural responses to hypothalamic cooling and heating in the rat. *Brain Research* 264: 79–87.
439. Jakubczak, L. F. (1966). Behavioral thermoregulation in young and old rats. *Journal of Applied Physiology* 21: 19–21.
440. Schulze, G. & Bürgel, P. (1977). The influence of age and drugs on the thermoregulatory behaviour of rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 298: 143–147.
441. Jänicke, B. & Schulze, G. (1986). Capacity of thermoregulation in different aged rats: age as a factor modifying thermoregulation. *Pharmacopsychiatry* 19: 312–313.
442. Baldwin, B. A. & Ingram, D. L. (1968). Factor influencing behavioral thermoregulation in the pig. *Physiology and Behavior* 3: 409–415.
443. Baldwin, B. A. & 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.

444. Carlisle, H. J. & 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.
445. Carlisle, H. J. (1966). Heat intake and hypothalamic temperature during behavioral temperature regulation. *Journal of Comparative and Physiological Psychology* 61: 388–397.
446. Adair, E. R., Casby, J. U. & 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.
447. Adair, E. R. & Wright, B. A. (1976). Behavioral thermoregulation in the squirrel monkey when response effort is varied. *Journal of Comparative and Physiological Psychology* 90: 179–184.
448. Refinetti, R. & Carlisle, H. J. (1987). A computational formula for heat influx in animal experiments. *Journal of Thermal Biology* 12: 263–266.
449. Refinetti, R. (1982). Custo da resposta termoregulatória de pressão à barra. *Ciência e Cultura* 34(S): 896–897.
450. Refinetti, R. & 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.
451. 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.
452. Schmidt, I. & Simon, E. (1979). Interaction of behavioral and autonomic thermoregulation in cold exposed pigeons. *Journal of Comparative Physiology* 133: 151–157.
453. Schmidt, I. (1978). Interactions of behavioral and autonomic thermoregulation in heat stressed pigeons. *Pflügers Archiv* 374: 47–55.
454. Aschoff, J. (1982). The circadian rhythm of body temperature as a function of body size. In: Taylor, C. R., Johansen, K. & Bolis, L. (Eds.). *A Companion to Animal Physiology.* Cambridge, UK: Cambridge University Press, pp. 173–188.
455. Mortola, J. P. & 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.
456. Morrison, P. R. & Ryser, F. A. (1952). Weight and body temperature in mammals. *Science* 116: 231–232.
457. Poole, S. & Stephenson, J. D. (1977). Core temperature: some shortcomings of rectal temperature measurements. *Physiology and Behavior* 18: 203–205.
458. Georgiev, J. (1978). Influence of environmental conditions and handling on the temperature rhythm of the rat. *Biotelemetry and Patient Monitoring* 5: 229–234.
459. Berkey, D. L., Meeuwsen, K. W. & Barney, C. C. (1990). Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. *American Journal of Physiology* 258: R743–R749.
460. Cabanac, A. & Briese, E. (1991). Handling elevates the colonic temperature of mice. *Physiology and Behavior* 51: 95–98.
461. Harkin, A., Connor, T. J., O'Donnell, J. M. & Kelly, J. P. (2002). Physiological and behavioral responses to stress: what does a rat find stressful? *Lab Animal* 31: 42–50.
462. 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.
463. 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.
464. Fuller, C. A. (1984). Circadian brain and body temperature rhythms in the squirrel monkey. *American Journal of Physiology* 246: R242–R246.
465. McKechnie, A. E. & Lovegrove, B. G. (2001). Heterothermic responses in the speckled mousebird (*Colius striatus*). *Journal of Comparative Physiology B* 171: 507–518.
466. Downs, C. T. & Brown, M. (2002). Nocturnal heterothermy and torpor in the Malachite sunbird (*Nectarinia famosa*). *Auk* 119: 251–260.
467. Roussel, B., Chouvet, G. & Debilly, G. (1976). Rythmes circadiens des températures internes et ambiance thermique chez le rat. *Pflügers Archiv* 365: 183–189.
468. Gordon, C. J. (1993). *Temperature Regulation in Laboratory Rodents*. New York: Cambridge University Press.
469. Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
470. Kittrell, E. M. W. & Satinoff, E. (1986). Development of the circadian rhythm of body temperature in rats. *Physiology and Behavior* 38: 99–104.
471. Piccione, G., Caola, G. & Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.
472. Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.
473. Osborne, A. R. & Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.
474. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. & Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
475. Stephan, F. K. & 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.
476. Liu, S., Chen, X. M., Yoda, T. Nagashima, K., Fukuda, Y. & Kanosue, K. (2002). Involvement of the suprachiasmatic nucleus in body temperature modulation by food deprivation in rats. *Brain Research* 929: 26–36.

477. Wachulec, M., Li, H., Tanaka, H., Peloso, E. & Satinoff, E. (1997). Suprachiasmatic nuclei lesions do not eliminate homeostatic thermoregulatory responses in rats. *Journal of Biological Rhythms* 12: 226–234.
478. Aschoff, J. (1970). Circadian rhythm of activity and of body temperature. In: Hardy, J. D., Gagge, A. P. & Stolwijk, J. A. J. (Eds.). *Physiological and Behavioral Temperature Regulation*. Springfield, IL: Charles C Thomas, pp. 905–919.
479. Hensel, H. (1981). *Thermoreception and Temperature Regulation*. New York: Academic Press.
480. Kluger, M. J. (1991). Fever: role of pyrogens and cryogens. *Physiological Reviews* 71: 93–128.
481. Robinson, E. L. & Fuller, C. A. (1999). Endogenous thermoregulatory rhythms of squirrel monkeys in thermoneutrality and cold. *American Journal of Physiology* 276: R1397–R1407.
482. Simon, E., Pierau, F. K. & Taylor, D. C. M. (1986). Central and peripheral thermal control of effectors in homeothermic temperature regulation. *Physiological Reviews* 66: 235–300.
483. Satinoff, E. (1978). Neural organization and evolution of thermal regulation in mammals. *Science* 201: 16–22.
484. Van Someren, E. J. W., Raymann, R. J. E. M., Scherder, E. J. A., Daanen, H. A. M. & Swaab, D. F. (2002). Circadian and age-related modulation of thermoreception and temperature regulation: mechanisms and functional implications. *Ageing Research Reviews* 1: 721–778.
485. Cabanac, M., Cunningham, D. J. & Stolwijk, J. A. J. (1971). Thermoregulatory set point during exercise: a behavioral approach. *Journal of Comparative and Physiological Psychology* 76: 94–102.
486. Nadel, E. R. (1983). Factors affecting the regulation of body temperature during exercise. *Journal of Thermal Biology* 8: 165–169.
487. Watts Jr., R. H. & Refinetti, R. (1996). Circadian modulation of cold-induced thermogenesis in the golden hamster. *Biological Rhythm Research* 27: 87–94.
488. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. II. Spinal thermosensitivity. *Pflügers Archiv* 386: 181–185.
489. Heller, H. C., Graf, R. & Rautenberg, W. (1983). Circadian and arousal state influences on thermoregulation in the pigeon. *American Journal of Physiology* 245: R321–R328.
490. Wenger, C. B., Roberts, M. F., Stolwijk, J. A. J. & Nadel, E. R. (1976). Nocturnal lowering of thresholds for sweating and vasodilation. *Journal of Applied Physiology* 41: 15–19.
491. Stephenson, L. A., Wenger, C. B., O'Donovan, B. H. & Nadel, E. R. (1984). Circadian rhythm in sweating and cutaneous blood flow. *American Journal of Physiology* 246: R321–R324.
492. Aoki, K., Stephens, D. P., Saad, A. R. & 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.
493. Beste, R., Kaiser, K., Issing, K. & Hensel, H. (1978). Perception of local thermal stimuli in the course of day. *Pflügers Archiv* 377S: R56.
494. Carlisle, H. J., Wilkinson, C. W., Laudenslager, M. L. & Keith, L. D. (1979). Diurnal variation of heat intake in ovariectomized, steroid-treated rats. *Hormones and Behavior* 12: 232–242.
495. Briese, E. (1986). Circadian body temperature rhythm and behavior of rats in thermoclines. *Physiology and Behavior* 37: 839–847.
496. Ray, B., Mallick, H. N. & 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.
497. Refinetti, R. (1995). Rhythms of temperature selection and body temperature are out of phase in the golden hamster. *Behavioral Neuroscience* 109: 523–527.
498. Song, X., Körtner, G. & Geiser, F. (1998). Temperature selection and use of torpor by the marsupial *Sminthopsis macroura*. *Physiology and Behavior* 64: 675–682.
499. Terai, Y., Asayama, M., Ogawa, T., Sugenoya, J., & Miyagawa, T. (1985). Circadian variation of preferred environmental temperature and body temperature. *Journal of Thermal Biology* 10: 151–156.
500. Pöllmann, L. (1994). Circadian and circannual variations in the evaluation of thermal comfort in a constant climate. *Indoor Environment* 3: 145–148.
501. Kim, H. E. & 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.
502. Shoemaker, J. A. & Refinetti, R. (1996). Day-night difference in the preferred ambient temperature of human subjects. *Physiology and Behavior* 59: 1001–1003.
503. Refinetti, R. (1997). Homeostasis and circadian rhythmicity in the control of body temperature. *Annals of the New York Academy of Sciences* 813: 63–70.
504. Briese, E. (1998). Normal body temperature of rats: the setpoint controversy. *Neuroscience and Biobehavioral Reviews* 22: 427–436.
505. Satinoff, E., Liran, J. & Clapman, R. (1982). Aberrations of circadian body temperature rhythms in rats with medial preoptic lesions. *American Journal of Physiology* 242: R352–R357.
506. Briese, E. (1989). Do medial preoptic lesions interfere with the set-point of temperature regulation? *Brain Research Bulletin* 23: 137–144.
507. Aschoff, J. (1983). Circadian control of body temperature. *Journal of Thermal Biology* 8: 143–147.
508. Shido, O., Sugano, Y., & Nagasaka, T. (1986). Circadian change of heat loss in response to change in core temperature in rats. *Journal of Thermal Biology* 11: 199–202.
509. Refinetti, R. (2003). Metabolic heat production, heat loss and the circadian rhythm of body temperature in the rat. *Experimental Physiology* 88: 423–429.

510. Fuller, C. A., Sulzman, F. M. & 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.
511. Kräuchi, K. & 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.
512. Refinetti, R. & Menaker, M. (1992). The circadian rhythm of body temperature. *Physiology and Behavior* 51: 613–637.
513. Liu, Y., Merrow, M., Loros, J. J. & Dunlap, J. C. (1998). How temperature changes reset a circadian oscillator. *Science* 281: 825–829.
514. Brown, S. A., Zumbrunn, G., Fleury-Olela, F, Preitner, N. & Schibler, U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Current Biology* 12: 1574–1583.
515. Ruoff, P. & Rensing, L. (2004). Temperature effects on circadian clocks. *Journal of Thermal Biology* 29: 445–456.
516. Van Dongen, H. P. A., Maislin, G., Mullington, J. M. & 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.
517. Lancel, M., van Riezen, H. & 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.
518. Franken, P., Dijk, D. J., Tobler, I. & 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.
519. Yoshida, Y., Fujiki, N., Nakajima, T., Ripley, B., Matsumura, H., Yoneda, H., Mignot, E. & Nishino, S. (2001). Fluctuation of extracellular hypocretin-1 (orexin A) levels in the rat in relation to the light-dark cycle and sleep-wake activitties. *European Journal of Neuroscience* 14: 1075–1081.
520. Dijk, D. J. & 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.
521. Almirall, H., Aguirre, A., Rial, R. V., Daurat, A., Foret, J. & Benoit, O. (1993). Temperature drop and sleep: testing the contribution of SWS in keeping cool. *NeuroReport* 5: 177–180.
522. Mistlberger, R. E., Bergmann, B. M., Waldenar, W. & Rechtschaffen, A. (1983). Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep* 6: 217–233.
523. Trachsel, L., Edgar, D. M., Seidel, W. F., Heller, H. C. & Dement, W. C. (1992). Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. *Brain Research* 589: 253–261.
524. Mendelson, W. B., Bergmann, B. M. & Tung, A. (2003). Baseline and post-deprivation recovery sleep in SCN-lesioned rats. *Brain Research* 980: 185–190.
525. Shiromani, P. J., Xu, M., Winston, E. M., Shiromani, S. N., Gerashchenko, D. & Weaver, D. R. (2004). Sleep rhythmicity and homeostasis in mice with targeted disruption of *mPeriod* genes. *American Journal of Physiology* 287: R47–R57.
526. Larkin, J. E., Yokogawa, T., Heller, H. C., Franken, P. & Ruby, N. F. (2004). Homeostatic regulation of sleep in arrhythmic Siberian hamsters. *American Journal of Physiology* 287: R104–R111.
527. Porkka-Heiskanen, T., Strecker, R. E., Thakkar, M., Bjørkum, A. A., Greene, R. W. & McCarley, R. W. (1997). Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science* 276: 1265–1268.
528. Naylor, E., Bergmann, B. M., Krauski, K., Zee, P. C., Takahashi, J. S., Vitaterna, M. H. & Turek, F. W. (2000). The circadian clock mutation alters sleep homeostasis in the mouse. *Journal of Neuroscience* 20: 8138–8143.
529. Wisor, J. P., O'Hara, B. F., Terao, A., Selby, C. P., Kilduff, T. S., Sancar, A., Edgar, D. M. & Franken, P. (2002). A role for *cryptochromes* in sleep regulation. *BMC Neuroscience* 3: art. 20.
530. Edgar, D. M., Martin, C. E. & 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.
531. Sei, H., Oishi, K., Morita, Y. & Ishida, N. (2001). Mouse model for morningness/eveningness. *NeuroReport* 12: 1461–1464.
532. Edgar, D. M. & Dement, W. C. (1991). Regularly scheduled voluntary exercise synchronizes the mouse circadian clock. *American Journal of Physiology* 261: R928–R933.
533. Kas, M. J. H. & Edgar, D. M. (1998). Crepuscular rhythms of EEG sleep-wake in a hystricomorph rodent, *Octodon degus*. *Journal of Biological Rhythms* 13: 9–17.
534. Deboer, T., Vyazovskiy, V. V. & 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.
535. Borbély, A. A. & 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.
536. Eastman, C. & Rechtschaffen, A. (1983). Circadian temperature and wake rhythms of rats exposed to prolonged continuous illumination. *Physiology and Behavior* 31: 417–427.
537. Ikeda, M., Sagara, M. & 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.
538. Adrien, J., Dugovic, C. & Martin, P. (1991). Sleep-wakefulness patterns in the helpless rat. *Physiology and Behavior* 49: 257–262.

539. Deboer, T., Vansteensel, M. J., Détári, L. & Meijer, J. H. (2003). Sleep states alter activity of suprachiasmatic nucleus neurons. *Nature Neuroscience* 6: 1086–1090.

540. Li, H. & Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.

541. Tzischinsky, O. & Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.

542. Hayashi, M., Morikawa, T. & Hori, T. (2002). Circasemidian 12 h cycle of slow wave sleep under constant darkness. *Clinical Neurophysiology* 113: 1505–1516.

543. Tan, X., Uchiyama, M., Shibui, K., Tagaya, H., Suzuki, H., Kamei, Y., Kim, K., Aritaka, S., Ozaki, A. & Takahashi, K. (2003). Circadian rhythms in human's delta sleep electroencephalogram. *Neuroscience Letters* 344: 205–208.

544. Gradisar, M. & 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.

545. Czeisler, C. A., Weitzman, E. D., Moore-Ede, M. C., Zimmerman, J. C. & Knauer, R. S. (1980). Human sleep: its duration and organization depend on its circadian phase. *Science* 210: 1264–1267.

546. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J. & 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.

547. Weitzman, E. D., Moline, M. L., Czeisler, C. A. & Zimmerman, J. C. (1982). Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.

548. Berger, R. J. & 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.

549. 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.

550. Kuwabara, N., Seki, K. & 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.

551. Marks, G. A. & 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.

552. Daan, S., Beersma, D. G. M. & Borbély, A. A. (1984). Timing of human sleep: recovery process gated by a circadian pacemaker. *American Journal of Physiology* 246: R161–R178.

553. Dijk, D. J., Beersma, G. M., Daan, S. & Lewy, A. J. (1989). Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *American Journal of Physiology* 256: R106–R111.

554. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M. & 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.

555. Dijk, D. J. & 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.

556. Wyatt, J. K., Ritz-de-Cecco, A., Czeisler, C. A. & 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.

557. Klerman, E. B., Boulos, Z., Edgar, D. M., Mistlberger, R. E. & Moore-Ede, M. C. (1999). Circadian and homeostatic influences on sleep in the squirrel monkey: sleep after sleep deprivation. *Sleep* 22: 45–59.

558. Zeitzer, J. M., Buckmaster, C. L., Parker, K. J., Hauck, C. M., Lyons, D. M. & 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.

559. Van Dongen, H. P. A. & 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.

560. Hull, J. T., Wright, K. P. & 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.

561. Varkevisser, M. & Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: validating a self-constructed test battery. *Chronobiology International* 20: 109–121.

562. Horowitz, T. S., Cade, B. E., Wolfe, J. M. & 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.

563. Miró, E., Cano, M. C., Espinosa-Fernández, L. & Buela-Casal, G. (2003). Time estimation during prolonged sleep deprivation and its relation to activation measures. *Human Factors* 45: 148–159.

564. Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A. & Cajochen, C. (2004). Circadian and wake-dependent modulation of fastest and slowest reaction times during psychomotor vigilance task. *Physiology and Behavior* 80: 695–701.

565. Girardier, L. & Stock, M. J. (1983). Mammalian thermogenesis: an introduction. In: Girardier, L. & Stock, M. J. (Eds.). *Mammalian Thermogenesis*. London: Chapman and Hall, pp. 1–7.

566. Woods, S. C., Seeley, R. J., Porte, D. & Schwartz, M. W. (1998). Signals that regulate food intake and energy homeostasis. *Science* 280: 1378–1383.

567. Funahashi, H., Takenoys, F., Guan, J. L., Kageyama, H., Yada, T. & Shioda, S. (2003). Hypothalamic neuronal networks and feeding-related peptides involved in the regulation of feeding. *Anatomical Science International* 78: 123–138.
568. Fukagawa, K., Sakata, T., Yoshimatsu, H., Fujimoto, K. & 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.
569. Drewnowski, A., Grinker, J. A., Gruen, R. & 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.
570. Mistlberger, R. E., Lukman, H. & Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: assessment and intervention. *Appetite* 30: 255–267.
571. Shido, O., Sakurada, S., Kohda, W. & Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
572. Kittrell, E. M. W. & Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.
573. Ikeda, M. & 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.
574. Francis, A. J. P. & 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.
575. Balagura, S. & Coscina, D. V. (1968). Periodicity of food intake in the rat as measured by an operant response. *Physiology and Behavior* 3: 641–643.
576. Prins, A. A., de Jong-Nagelsmit, A., Keijser, J. & Strubbe, J. H. (1986). Daily rhythms of feeding in the genetically obese and lean Zucker rats. *Physiology and Behavior* 38: 423–426.
577. Cunningham, J. T., Beltz, T., Johnson, R. F. & 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.
578. Velasco-Plaza, A., Granda, T. G. & Cachero, M. T. G. (1993). Circadian rhythms of food and water intake and urine excretion in diabetic rats. *Physiology and Behavior* 54: 665–670.
579. Zucker, I. (1971). Light-dark rhythms in rat eating and drinking behavior. *Physiology and Behavior* 6: 115–126.
580. Barney, C. C., Vergoth, C., Renkema, L. A. & Meeuwsen, K. W. (1995). Nycthemeral variation in thermal dehydration-induced thirst. *Physiology and Behavior* 58: 329–335.
581. Williams, T. D., Chambers, J. B., May, O. L., Henderson, R. P., Rashotte, M. E. & 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.
582. Strubbe, J. H., Spiteri, N. J. & Prins, A. J. A. (1986). Effect of skeleton photoperiod and food availability on the circadian pattern of feeding and drinking in rats. *Physiology and Behavior* 36: 647–651.
583. Witte, K. & Lemmer, B. (1999). Development of inverse circadian blood pressure pattern in transgenic hypertensive TGR(mREN2)27 rats. *Chronobiology International* 16: 293–303.
584. Galbicka, G., Smurthwaite, S., Riggs, R. & Tang, L. W. (1997). Daily rhythms in a complex operant: targeted percentile shaping of run lengths in rats. *Physiology and Behavior* 62: 1165–1169.
585. De Castro, J. M. (1978). Diurnal rhythms of behavioral effects on core temperature. *Physiology and Behavior* 21: 883–886.
586. Rosenwasser, A. M., Boulos, Z. & Terman, M. (1983). Circadian feeding and drinking rhythms in the rat under complete and skeleton photoperiods. *Physiology and Behavior* 30: 353–359.
587. Rosenwasser, A. M. (1993). Circadian drinking rhythms in SHR and WKY rats: effects of increasing light intensity. *Physiology and Behavior* 53: 1035–1041.
588. Madrid, J. A., Sánchez-Vázquez, F. J., Lax, P., Matas, P., Cuenca, E. M. & Zamora, S. (1998). Feeding behavior and entrainment limits in the circadian system of the rat. *American Journal of Physiology* 275: R372–R383.
589. Fischette, C. T., Edinger, H. M. & Siegel, A. (1981). Temporary desynchronization among circadian rhythms with lateral fornix ablation. *Brain Research* 229: 85–101.
590. 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.
591. 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.
592. Oklejewicz, M., Overkamp, G. J. F., Stirland, J. A. & Daan, S. (2001). Temporal organization of feeding in Syrian hamsters with a genetically altered circadian period. *Chronobiology International* 18: 657–664.
593. Gerkema, M. P., Groos, G. A. & 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.
594. Edgar, D. M., Kilduff, T. S., Martin, C. E. & 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.
595. Shapiro, C. J. & Weathers, W. W. (1981). Metabolic and behavioral responses of American kestrels to food deprivation. *Comparative Biochemistry and Physiology A* 68: 11–114.
596. Kadono, H., Besch, E. L. & Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.

597. Aschoff, J. & von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
598. Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.
599. Lumineau, S. & 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.
600. Guyomarc'h, C., Lumineau, S. & 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.
601. Lumineau, S. & Guyomarc'h, C. (2003). Effect of light intensity on circadian activity in developing Japanese quail. *Biological Rhythm Research* 34: 101–113.
602. Ebihara, S. & Gwinner, E. (1992). Different circadian pacemakers control feeding and locomotor activity rhythms in European starlings. *Journal of Comparative Physiology A* 171: 63–67.
603. Pohl, H. (1999). Spectral composition of light as a zeitgeber for birds living in the high arctic summer. *Physiology and Behavior* 67: 327–337.
604. Hau, M. & Gwinner, E. (1992). Circadian entrainment by feeding cycles in house sparrows, *Passer domesticus*. *Journal of Comparative Physiology A* 170: 403–409.
605. Heigl, S. & Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
606. Reierth, E. & Stokkan, K. A. (1998). Dual entrainment by light and food in the Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Journal of Biological Rhythms* 13: 393–402.
607. Casey, T. M., Joos, B., Fitzgerald, T. D., Yurlina, M. E. & Young, P. A. (1988). Synchronized group foraging, thermoregulation, and growth of Eastern tent caterpillars in relation to microclimate. *Physiological Zoology* 61: 372–377.
608. Ryer, C. H. & 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.
609. Chen, W. M., Naruse, M. & 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.
610. Sánchez-Vázquez, F. J., Iigo, M., Madrid, J. A. & 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.
611. Bovet, J. (1994). Missed opportunities in the study of activity and body temperature of beaver (*Castor canadensis*). *Canadian Journal of Zoology* 72: 567–569.
612. Jilge, B. (1993). The ontogeny of circadian rhythms in the rabbit. *Journal of Biological Rhythms* 8: 247–260.
613. Jilge, B. (1991). Restricted feeding: a nonphotic zeitgeber in the rabbit. *Physiology and Behavior* 51: 157–166.
614. Bobbert, A. C. & 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.
615. Araki, C. T., Nakamura, R. M. & Kam, L. W. G. (1987). Diurnal temperature sensitivity of dairy cattle in a naturally cycling environment. *Journal of Thermal Biology* 12: 23–26.
616. Rauth-Widmann, B., Fuchs, E. & 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.
617. Hoban, T. M., Levine, A. H., Shane, R. B. & Sulzman, F. M. (1985). Circadian rhythms of drinking and body temperature of the owl monkey (*Aotus trivirgatus*). *Physiology and Behavior* 34: 513–518.
618. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Environmental synchronizers of squirrel monkey circadian rhythms. *Journal of Applied Physiology* 43: 795–800.
619. Moore-Ede, M. C., Kass, D. A. & Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
620. Fuller, C. A. & 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.
621. Boulos, Z., Frim, D. M., Dewey, L. K. & Moore-Ede, M. C. (1989). Effects of restricted feeding schedules on circadian organization in squirrel monkeys. *Physiology and Behavior* 45: 507–515.
622. Sulzman, F. M., Fuller, C. A. & Moore-Ede, M. C. (1977). Feeding time synchronizes primate circadian rhythms. *Physiology and Behavior* 18: 775–779.
623. Green, J., Pollak, C. P. & Smith, G. P. (1987). Meal size and intermeal interval in human subjects in time isolation. *Physiology and Behavior* 41: 141–147.
624. De Castro, J. M. (2001). Heritability of diurnal changes in food intake in free-living humans. *Nutrition* 17: 713–720.
625. De Castro, J. M. (1987). Circadian rhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology and Behavior* 40: 437–446.
626. Strubbe, J. H. & 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.
627. Tannenbaum, M. G. & 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.

628. Heldmaier, G., Steinlechner, S., Ruf, T., Wiesinger, H. & Klingenpor, M. (1989). Photoperiod and thermoregulation in vertebrates: body temperature rhythms and thermogenic acclimation. *Journal of Biological Rhythms* 4: 251–265.
629. Ruby, N. F., Ibuka, N., Barnes, B. M. & Zucker, I. (1989). Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *American Journal of Physiology* 257: R210–R215.
630. Ellison, G. T. H. & 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.
631. Prinzinger, R., Schäfer, T. & 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.
632. Geiser, F. & Masters, P. (1994). Torpor in relation to reproduction in the mulgara, *Dasycercus cristicauda* (Dasyuridae: Marsupialia). *Journal of Thermal Biology* 19: 33–40.
633. Holloway, J. C. & Geiser, F. (1996). Reproductive status and torpor of the marsupial *Sminthopsis crassicaudata*: effect of photoperiod. *Journal of Thermal Biology* 21: 373–380.
634. Ortmann, S. & Heldmaier, G. (1997). Spontaneous daily torpor in Malagasy mouse lemurs. *Naturwissenschaften* 84: 28–32.
635. Turbill, C., Law, B. S. & Geiser, F. (2003). Summer torpor in a free-ranging bat from subtropical Australia. *Journal of Thermal Biology* 28: 223–226.
636. Turbill, C., Körtner, G. & Geiser, F. (2003). Natural use of heterothermy by a small, tree-roosting bat during summer. *Physiological and Biochemical Zoology* 76: 868–876.
637. Cooper, C. E. & 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.
638. MacMillen, R. E. & Trost, C. H. (1967). Nocturnal hypothermia in the Inca dove, *Scardafella inca*. *Comparative Biochemistry and Physiology* 23: 243–253.
639. Rashotte, M. E. & 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.
640. Phillips, N. H. & 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.
641. Ostheim, J. (1992). Coping with food-limited conditions: feeding behavior, temperature preference, and nocturnal hypothermia in pigeons. *Physiology and Behavior* 51: 353–361.
642. Hohtola, E., Hissa, R., Pyörnilä, A., Rintamäki, H. & Saarela, S. (1991). Nocturnal hypothermia in fasting Japanese quail: the effect of ambient temperature. *Physiology and Behavior* 49: 563–567.
643. Prinzinger, R., Schleucher, E. & 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.
644. McKechnie, A. E. & 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.
645. Hudson, J. W. (1965). Temperature regulation and torpidity in the pigmy mouse, *Baiomys taylori*. *Physiological Zoology* 38: 243–254.
646. 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.
647. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F. & 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.
648. Pecoraro, N., Gomez, F., Laugero, K. & Dallman, M. F. (2002). Brief access to sucrose engages food-entrainable rhythms in food-deprived rats. *Behavioral Neuroscience* 116: 757–776.
649. Nagashima, K., Nakai, S., Matsue, K., Konishi, M., Tanaka, M. & Kanosue, K. (2003). Effects of fasting on thermoregulatory processes and the daily oscillations in rats. *American Journal of Physiology* 284: R1486–R1493.
650. Perret, M. & 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.
651. Génin, F. & 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.
652. Piccione, G., Caola, G. & Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
653. Piccione, G., Caola, G. & 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.
654. Ruby, N. F. & Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *American Journal of Physiology* 263: R353–R362.
655. Paul, M. J., Kauffman, A. S. & Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
656. Heldmaier, G. & Ruf, T. (1992). Body temperature and metabolic rate during natural hypothermia in endotherms. *Journal of Comparative Physiology B* 162: 696–706.

657. 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.
658. 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.
659. Song, X., Körtner, G. & Geiser, F. (1995). Reduction of metabolic rate and thermoregulation during daily torpor. *Journal of Comparative Physiology B* 165: 291–297.
660. Zimmer, M. B. & 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.
661. Gillooly, J. F., Brown, J. H., West, G. B., Savage, V. M. & Charnov, E. L. (2001). Effects of size and temperature on metabolic rate. *Science* 293: 2248–2251.
662. Refinetti, R. (1989). Body size and metabolic rate in the laboratory rat. *Experimental Biology* 48: 291–294.
663. Sarrus, P. & 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.
664. Regnault, V. & Reiset, J. (1849). Recherches chimiques sur la respiration des animaux des diverses classes. *Annales de Chimie et de Physique, Series 3* 26: 299–519.
665. 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.
666. Kleiber, M. (1947). Body size and metabolic rate. *Physiological Reviews* 27: 511–541.
667. Kayser, C. & Heusner, A. (1964). Étude comparative de métabolisme énergétique dans la série animale. *Journal de Physiologie* 56: 489–524.
668. Lasiewski, R. C. & Dawson, W. R. (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. *Condor* 69: 13–23.
669. Stahl, W. R. (1967). Scaling of respiratory variables in mammals. *Journal of Applied Physiology* 22: 453–460.
670. Thonney, M. L., Touchberry, R. W., Goodrich, R. D. & Meiske, J. C. (1976). Intraspecies relationship between fasting heat production and body weight. *Journal of Animal Science* 43: 692–704.
671. Poczopko, P. (1979). Metabolic rate and body size relationships in adult and growing homeotherms. *Acta Theriologica* 24: 125–136.
672. Bartels, H. (1982). Metabolic rate of mammals equals the 0.75 power of their body weight. In: Gärtner, K., Hackbarth, H. & Stolte, H. (Eds.). *Research Animals and Concepts of Applicability to Clinical Medicine*. Basel: S. Karger, pp. 1–11.
673. Feldman, H. A. & McMahon, T. A. (1983). The 3/4 mass exponent for energy metabolism is not a statistical artifact. *Respiration Physiology* 52: 149–163.
674. 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.
675. Heusner, A. A. (1984). Biological similitude: statistical and functional relationships in comparative physiology. *American Journal of Physiology* 246: R839–R845.
676. Calder, W. A. (1987). Scaling energetics of homeothermic vertebrates: an operational allometry. *Annual Review of Physiology* 49: 107–120.
677. Spatz, H. C. (1991). Circulation, metabolic rate, and body size in mammals. *Journal of Comparative Physiology B* 161: 231–236.
678. West, G. B., Brown, J. H. & Enquist, B. J. (1997). A general model for the origin of allometric scaling laws in biology. *Science* 276: 122–126.
679. West, G. B., Brown, J. H. & Enquist, B. J. (1999). The fourth dimension of life: fractal geometry and allometric scaling of organisms. *Science* 284: 1677–1679.
680. Lefèvre, J. & 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.
681. Margaria, R., Cerretelli, P., Aghemo, P. & Sassi, G. (1963). Energy cost of running. *Journal of Applied Physiology* 18: 367–370.
682. Taylor, C. R., Schmidt-Nielsen, K. & Raab, J. L. (1970). Scaling of energetic cost of running to body size in mammals. *American Journal of Physiology* 219: 1104–1107.
683. Saltin, B., Gagge, A. P. & Stolwijk, J. A. J. (1970). Body temperature and sweat during thermal transients caused by exercise. *Journal of Applied Physiology* 28: 318–327.
684. Wang, L. C. H. (1980). Modulation of maximum thermogenesis by feeding in the white rat. *Journal of Applied Physiology* 49: 975–978.
685. Forsum, E., Hillman, P. E. & 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.
686. Welle, S., Lilavivat, U. & 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.
687. Rothwell, N. J. & 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.
688. Glick, Z., Wickler, S. J., Stern, J. S. & 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.
689. Maehlum, S., Grandmontage, M., Newsholme, E. A. & Sejersted, O. M. (1986). Magnitude and duration of excess postexercise oxygen consumption in healthy young subjects. *Metabolism* 35: 425–429.
690. Poehlman, E. T., Melby, C. L. & Badylak, S. F. (1988). Resting metabolic rate and postprandial thermogenesis in highly trained and untrained males. *American Journal of Clinical Nutrition* 47: 793–798.
691. Hill, J. O., Anderson, J. C., Lin, D. & Yakubu, F. (1988). Effects of meal frequency on energy utilization in rats. *American Journal of Physiology* 255: R616–R621.

692. Romom, M., Edme, J. J., Boulenguez, C., Lescroart, J. L. & Frimat, P. (1993). Circadian variation of diet-induced thermogenesis. *American Journal of Clinical Nutrition* 57: 476–480.

693. Maffeis, C., Schutz, Y., Grezzani, A., Provera, S., Piacentini, G. & 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.

694. Diamond, P., Brondel, L. & LeBlanc, J. (1985). Palatability and postprandial thermogenesis in dogs. *American Journal of Physiology* 248: E75–E79.

695. LeBlanc, J. & Diamond, P. (1986). Effect of meal size and frequency on postprandial thermogenesis in dogs. *American Journal of Physiology* 250: E144–E147.

696. Heymsfield, S. B., Hill, J. O., Evert, M., Casper, K. & DiGirolamo, M. (1987). Energy expenditure during continuous intragastric infusion of fuel. *American Journal of Clinical Nutrition* 45: 526–533.

697. Diamond, P. & LeBlanc, J. (1987). Hormonal control of postprandial thermogenesis in dogs. *American Journal of Physiology* 253: E521–E529.

698. Allard, M. & LeBlanc, J. (1988). Effects of cold acclimation, cold exposure, and palatability on postprandial thermogenesis in rats. *International Journal of Obesity* 12: 169–178.

699. Arnold, J., Shipley, K. A., Scott, N. A., Little, R. A. & Irving, M. H. (1989). Thermic effect of parenteral nutrition in septic and nonseptic individuals. *American Journal of Clinical Nutrition* 50: 853–860.

700. LeBlanc, J., Mercier, I. & Nadeau, A. (1993). Components of postprandial thermogenesis in relation to meal frequency in humans. *Canadian Journal of Physiology and Pharmacology* 71: 879–883.

701. Grande, F., Anderson, J. T. & Keys, A. (1958). Changes of basal metabolic rate in man in semistarvation and refeeding. *Journal of Applied Physiology* 12: 230–238.

702. Bray, G. A. (1969). Effect of caloric restriction on energy expenditure in obese patients. *Lancet* 7617: 397–398.

703. Boyle, P. C., Storlien, L. H. & Keesey, R. E. (1978). Increased efficiency of food utilization following weight loss. *Physiology and Behavior* 21: 261–264.

704. 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.

705. Boyle, P. C., Storlien, L. H., Harper, A. E. & Keesey, R. E. (1981). Oxygen consumption and locomotor activity during restricted feeding and realimentation. *American Journal of Physiology* 241: R392–R397.

706. Gleeson, M., Brown, J. F. & Waring, J. J. (1982). The effects of physical exercise on metabolic rate and dietary-induced thermogenesis. *British Journal of Nutrition* 47: 173–181.

707. Stock, M. J. & Rothwell, N. J. (1982). Evidence for diet-induced thermogenesis in hyperphagic cafeteria-fed rats. *Proceedings of the Nutrition Society* 41: 133–135.

708. 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. & 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.

709. Levine, J. A., Eberhardt, N. L. & Jensen, M. D. (1999). Role of nonexercise activity thermogenesis in resistance to fat gain in humans. *Science* 283: 212–214.

710. Bachman, E. S., Dhillon, H., Zhang, C. Y., Cinti, S., Bianco, A. C., Kobilka, B. K. & Lowell, B. B. (2002). Beta-AR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297: 843–845.

711. Rothwell, N. J. & Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281: 31–35.

712. Himms-Hagen, J., Triandafillou, J. & Gwilliam, C. (1981). Brown adipose tissue of cafeteria-fed rats. *American Journal of Physiology* 241: E116–E120.

713. Trayhurn, P., Jones, P. M., McGuckin, M. M. & Goodbody, A. E. (1982). Effects of overfeeding on energy balance and brown fat thermogenesis in obese (*ob*/*ob*) mice. *Nature* 295: 323–325.

714. Triandafillou, J. & 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.

715. Glick, Z., Wickler, S. J., Stern, J. S. & 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.

716. Glick, Z., Wu, S. Y., Lupien, J., Reggio, R., Bray, G. A. & Fisher, D. A. (1985). Meal-induced brown fat thermogenesis and thyroid hormone metabolism in rats. *American Journal of Physiology* 249: E519–E524.

717. Himms-Hagen, J. (1990). Brown adipose tissue thermogenesis: interdisciplinary studies. *FASEB Journal* 4: 2890–2898.

718. Tulp, O. L. & Buck, C. L. (1986). Caffeine and ephedrine stimulated thermogenesis in LA-corpulent rats. *Comparative Biochemistry and Physiology C* 85: 17–19.

719. Bailey, C. J., Thornburn, C. C. & 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.

720. Dulloo, A. G. & 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.

721. Dulloo, A. G. & Miller, D. S. (1987). Prevention of genetic *fa*/*fa* obesity with an ephedrine-methylxanthines thermogenic mixture. *American Journal of Physiology* 252: R507–R513.

722. Dulloo, A. G. & 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.

723. Aschoff, J. (1964). Survival value of diurnal rhythms. *Symposia of the Zoological Society of London* 13: 79–98.
724. Aschoff, J., von Goetz, C., Wildbruber, C. & Wever, R. (1986). Meal timing in humans during isolation without time cues. *Journal of Biological Rhythms* 1: 151–162.
725. 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.
726. Aschoff, J. (1994). The timing of defecation within sleep-wake cycle of humans during temporal isolation. *Journal of Biological Rhythms* 9: 43–50.
727. Oklejewicz, M., Hut, R. A., Daan, S, Loudon, A. S. I. & Stirland, A. J. (1997). Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamster. *Journal of Biological Rhythms* 12: 413–422.
728. Refinetti, R. & Menaker, M. (1997). Is energy expenditure in the hamster primarily under homeostatic or circadian control? *Journal of Physiology* 501: 449–453.
729. Osiel, S., Golombek, D. A. & 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.
730. Conrad, M. C. & Miller, A. T. (1956). Age changes in body size, body composition and basal metabolism. *American Journal of Physiology* 186: 207–210.
731. Chiu, C. C. & Hsieh, A. C. L. (1960). A comparative study of four means of expressing the metabolic rate of rats. *Journal of Physiology* 150: 694–706.
732. Heusner, A. & 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.
733. 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.
734. Even, P. C., Rolland, V., Roseau, S., Bouthegourd, J. C. & 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.
735. Rubal, A., Choshniak, I. & Haim, A. (1992). Daily rhythms of metabolic rate and body temperature of two murids from extremely different habitats. *Chronobiology International* 9: 341–349.
736. Lovegrove, B. G. & 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.
737. Körtner, G. & Geiser, F. (1995). Body temperature rhythms and activity in reproductive *Antechinus* (Marsupialia). *Physiology and Behavior* 58: 31–36.
738. Gebczynski, A. K. & 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.
739. McElhinny, T. L., Smale, L. & 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.
740. Blanchong, J. A., McElhinny, T. L., Mahoney, M. M. & Smale, L. (1999). Nocturnal and diurnal rhythms in the unstriped Nile rate, *Arvicanthis niloticus*. *Journal of Biological Rhythms* 14: 364–377.
741. Rose, R. W., Swain, R. & 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.
742. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A. & 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.
743. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L. & 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.
744. Refinetti, R. & Piccione, G. (2003). Daily rhythmicity of body temperature in the dog. *Journal of Veterinary Medical Science* 65: 935–937.
745. Jessen, C. & Kuhnen, G. (1996). Seasonal variations of body temperature in goats living in an outdoor environment. *Journal of Thermal Biology* 21: 197–204.
746. Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D. & Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflügers Archiv* 436: 659–666.
747. Winget, C. M., Card, D. H. & Hetherington, N. W. (1968). Circadian oscillations of deep-body temperature and heart rate in a primate (*Cebus albafrons*). *Aerospace Medicine* 39: 350–353.
748. Oshima, I. & 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.
749. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
750. Harlow, H. J., Phillips, J. A. & 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.
751. Gemmell, R. T., Turner, S. J. & Krause, W. J. (1997). The circadian rhythm of body temperature of four marsupials. *Journal of Thermal Biology* 22: 301–307.
752. Piccione, G., Caola, G. & Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
753. Fowler, P. A. & 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.

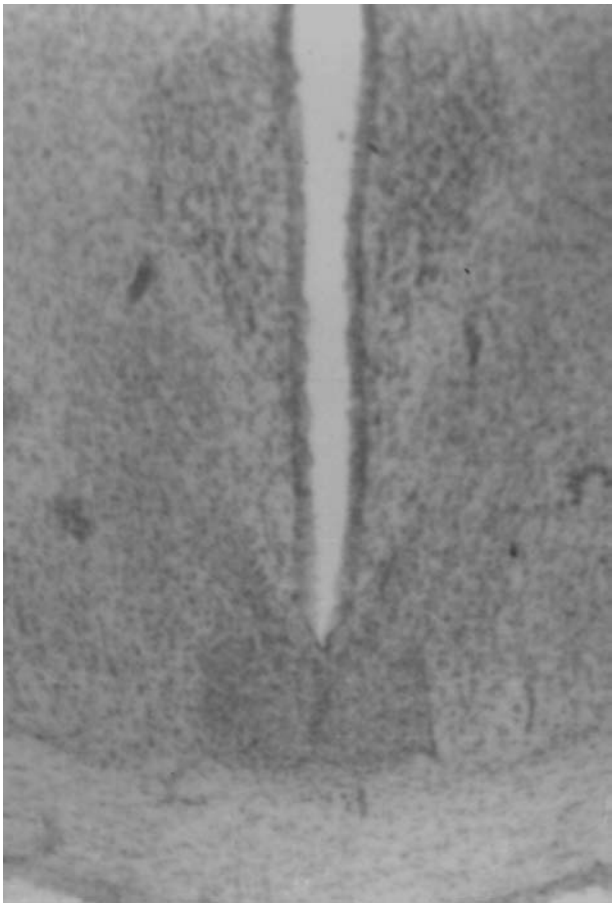
754. Johnson, R. F. & Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
755. Randall, W., Cunningham, J. T., Randall, S., Liittschwager, J., & Johnson, R. F. (1987). A two-peak circadian system in body temperature and activity in the domestic cat, *Felis catus*. *Journal of Thermal Biology* 12: 27–37.
756. Michels, H., Herremans, M., & 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.
757. Winget, C. M. & Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
758. Riccio, A. P. & Goldman, B. D. (2000). Circadian rhythms of body temperature and metabolic rate in naked mole-rats. *Physiology and Behavior* 71: 15–22.
759. Wever, R. & Zink, R. A. (1971). Fortlaufende Registrierung der Rectaltemperatur des Menschen unter extremen Bedingungen. *Pflügers Archiv* 327: 186–190.
760. Souetre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B. & 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.
761. Kleitman, N. & Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
762. Czeisler, C. A., Kronauer, R. E., Allan, J. S., Duffy, J. F., Jewett, M. E., Brown, E. N. & Ronda, J. M. (1989). Bright light induction of strong (Type 0) resetting of the human circadian pacemaker. *Science* 244: 1328–1333.
763. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J., Elliott, A. R., Prisk, G. K., West, J. B. & Czeisler, C. A. (2001). Sleep, performance, circadian rhythms, and light-dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
764. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
765. Barrett, J., Lack, L. & Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
766. Pollak, C. P. & Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
767. Cagnacci, A., Arangino, S., Tuveri, F., Paoletti, A. M. & 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.
768. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K. & Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
769. Kattapong, K. R., Fogg, L. F. & Eastman, C. I. (1995). Effect of sex, menstrual phase, and oral contraceptive use on circadian temperature rhythms. *Chronobiology International* 12: 257–266.
770. Aschoff, J., Gerecke, U. & Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
771. Scales, W. E., Vander, A. J., Brown, M. B. & Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
772. Lee, Y. H. & 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.
773. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T. & Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
774. Takasu, N., Nigi, H. & 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.
775. Tapp, W. N. & Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
776. Honnebier, M. B. O. M., Jenkins, S. L. & 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.
777. Simpson, S. & 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.
778. McCarron, H. C. K., Buffenstein, R., Fanning, F. D. & 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.
779. 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.
780. Yang, J., Morgan, J. L. M., Kirby, J. D., Long, D. W. & 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.
781. Folk, G. E. (1957). Twenty-four hour rhythms of mammals in a cold environment. *American Naturalist* 91: 153–166.

782. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
783. Conn, C. A., Borer, K. T. & 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.
784. Perret, M., Aujard, F., Séguy, M. & 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.
785. Kramer, K., Voss, H. P., Grimbergen, J. & 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.
786. Leon, L. R., Walker, L. D., DuBose, D. A. & Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
787. Irizarry, R. A., Tankersley, C., Frank, R. & Flanders, S. (2001). Assessing homeostasis through circadian patterns. *Biometrics* 57: 1228–1237.
788. Keeney, A. J., Hogg, S. & 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.
789. Weinert, D. & Waterhouse, J. (1998). Diurnally changing effects of locomotor activity on body temperature in laboratory mice. *Physiology and Behavior* 63: 837–843.
790. Conn, C. A., Franklin, B., Freter, R. & 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.
791. Kluger, M. J., Conn, C. A., Franklin, B., Freter, R. & Abrams, G. D. (1990). Effect of gastrointestinal flora on body temperature of rats and mice. *American Journal of Physiology* 258: R552–R557.
792. Goel, N. & Lee, T. M. (1995). Social cues accelerate reentrainment of circadian rhythms in diurnal female *Octodon degus* (Rodentia: Octodontidae). *Chronobiology International* 12: 311–323.
793. Labyak, S. E. & Lee, T. M. (1995). Estrus- and steroid-induced changes in circadian rhythms in a diurnal rodent, *Octodon degus*. *Physiology and Behavior* 58: 573–585.
794. Varosi, S. M., Brigmon, R. L. & Besch, E. L. (1990). A simplified telemetry system for monitoring body temperature in small animals. *Laboratory Animal Science* 40: 299–302.
795. Recabarren, S. E., Vergara, M., Llanos, A. J. & Serón-Ferré, M. (1987). Circadian variation of rectal temperature in newborn sheep. *Journal of Developmental Physiology* 9: 399–408.
796. Miles, G. H. (1962). Telemetering techniques for periodicity studies. *Annals of the New York Academy of Sciences* 98: 858–865.
797. Refinetti, R. (1990). Experimentally induced disruption of the diurnal rhythm of body temperature of the rat. *Biotemas* 3(2): 47–58.
798. Honma, K. & Hiroshige, T. (1978). Internal synchronization among several circadian rhythms in rats under constant light. *American Journal of Physiology* 235: R243–R249.
799. Satinoff, E., Kent, S. & Hurd, M. (1991). Elevated body temperature in female rats after exercise. *Medicine and Science in Sports and Exercise* 23: 1250–1253.
800. Refinetti, R., Ma, H. & Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
801. Meinrath, M. & D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
802. Deprés-Brummer, P., Metzger, G. & Lévi, F. (1996). Analyses des rythmes de la température corporelle du rat en libre cours. *Pathologie Biologie* 44: 150–156.
803. Benstaali, C., Bogdan, A. & 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.
804. Severinsen, T. & Øritsland, N. A. (1991). Endotoxin induced prolonged fever in rats. *Journal of Thermal Biology* 16: 167–171.
805. Peloso, E., Wachulec, M. & Satinoff, E. (2002). Stress-induced hyperthermia depends on both time of day and light condition. *Journal of Biological Rhythms* 17: 164–170.
806. 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.
807. Morley, R. M., Conn, C. A., Kluger, M. J. & Vander, A. J. (1990). Temperature regulation in biotelemetered spontaneously hypertensive rats. *American Journal of Physiology* 258: R1064–R1069.
808. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
809. Muchlinski, A. E., Baldwin, B. C., Padick, D. A., Lee, B. Y., Salguero, H. S. & 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.
810. Lee, T. M., Holmes, W. G. & Zucker, I. (1990). Temperature dependence of circadian rhythms in golden-mantled ground squirrels. *Journal of Biological Rhythms* 5: 25–34.
811. Ishii, K., Uchino, M., Kuwahara, M., Tsubone, H. & 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.

812. Ingram, D. L. & 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.
813. Haim, A., Downs, C. T. & 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.
814. Refinetti, R. & Menaker, M. (1992). Body temperature rhythm of the tree shrew, *Tupaia belangeri*. *Journal of Experimental Zoology* 263: 453–457.
815. Peters, D. G. & 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.

Part IV

Physical Substrates



Microphotograph of the ventral part of a Nissl-stained coronal section of the hamster brain featuring the suprachiasmatic and paraventricular nuclei. (Histology and photography by R. Refinetti.)

11 Receptors

CHAPTER OUTLINE

11.1 Sensory Input
11.2 Photic Receptors
11.3 Nonphotic Receptors

11.1 SENSORY INPUT

Part II covered the phenomenology of circadian rhythms, and Part III focused on the physiological mechanisms responsible for rhythmicity. Now, Part IV discusses the *physical substrates* of circadian rhythms — that is, the cellular and molecular phenomena that underlie physiological processes. This part does not detail basic interactions between the chemical elements (Figure 11.1), but it does examine that class of organic compounds (DNA and proteins) responsible for the generation of circadian rhythmicity and for life itself.

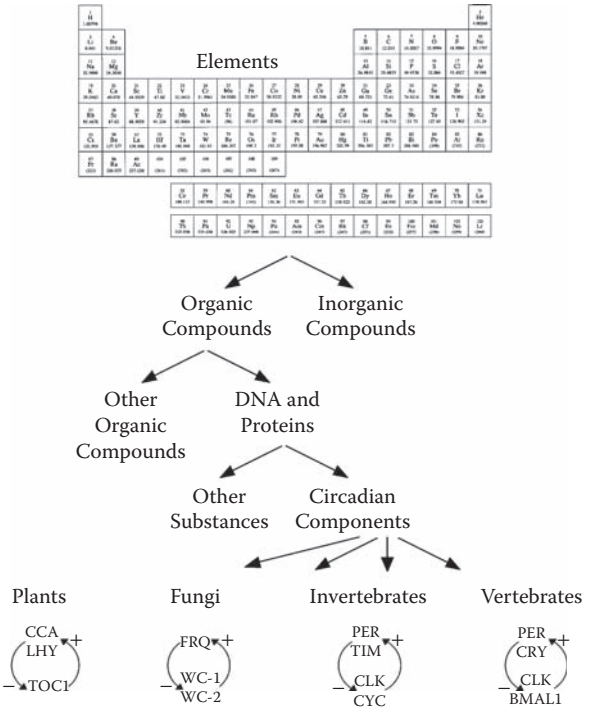


FIGURE 11.1 Physical substrate of life. Life is the result of a unique organization of basic elements, particularly carbon, oxygen, hydrogen, and nitrogen. DNA and proteins involved in translation/transcription loops are responsible for circadian rhythmicity and are discussed in Chapters 12 and 13.

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*). In terms of the circadian system, the 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.

Part III showed 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 muscles or glands, but in complex organisms, the brain serves as an additional effector organ for the generation of thoughts and emotions. This chapter covers the *receptors*. The *pacemaker* is dis-

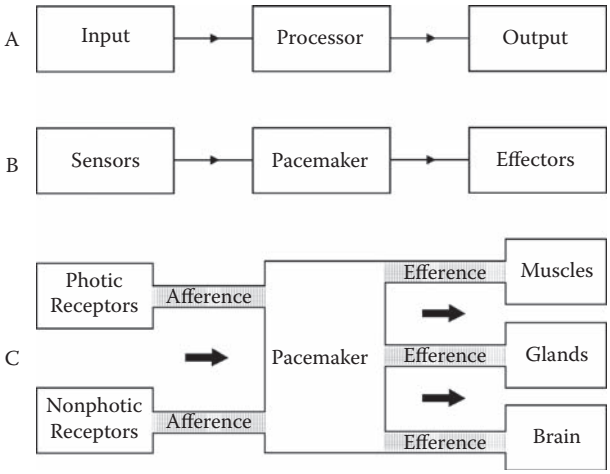


FIGURE 11.2 Sensory input. Sensory input in organisms can be conceptualized in cybernetic terms as the first member of the input–processor–output triad of information processing (A), which translates into the chronobiological triad of sensors-pacemaker-effectors (B). A more detailed diagram for the chronobiological triad is shown in Panel C. Photic and nonphotic receptors are discussed in this chapter. The pacemaker is discussed in Chapter 12. Chapter 13 discusses afference and efference.

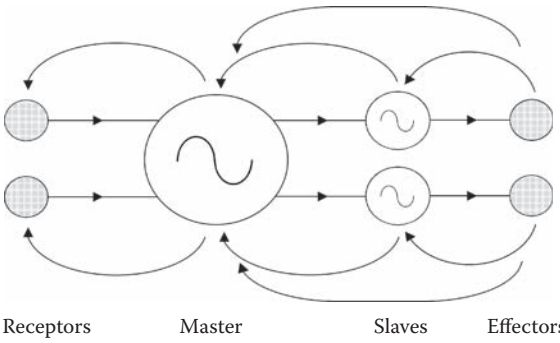


FIGURE 11.3 Recognizing the complexity of biological systems. This diagram emphasizes the complexity of the circadian system. Although the main flow of information may be conceptualized as the receptors–pacemaker–effectors triad, there seem to be many physical components of each member of the triad as well as multiple feedback loops among them.

cussed in Chapter 12, and *afference* and *efference* are discussed in Chapter 13. However, before you assume that the system is simpler than it really is, look at Figure 11.3. The main flow of information in the circadian system may be conceptualized as the receptors–pacemaker–effectors triad, but the actual system involves many physical components of each member of the triad, as well as multiple feedback loops among them.[1] Slave pacemakers are 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*,[2] you will be surprised to learn that humans have many more senses. As shown in Table 11.1, humans have at least nine somatic (conscious) senses as well as numerous autonomic senses. Different senses use different sensory receptors. *Sensory receptors* are specialized neural structures that transduce specific forms of energy (such as light, sound, and 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. 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). For the circadian pacemaker to be

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. (2001). *Review of Medical Physiology*, 20th Edition. New York: Lange; Schiffman, H. R. (1996). *Sensation and Perception*, 4th Edition. New York: Wiley.

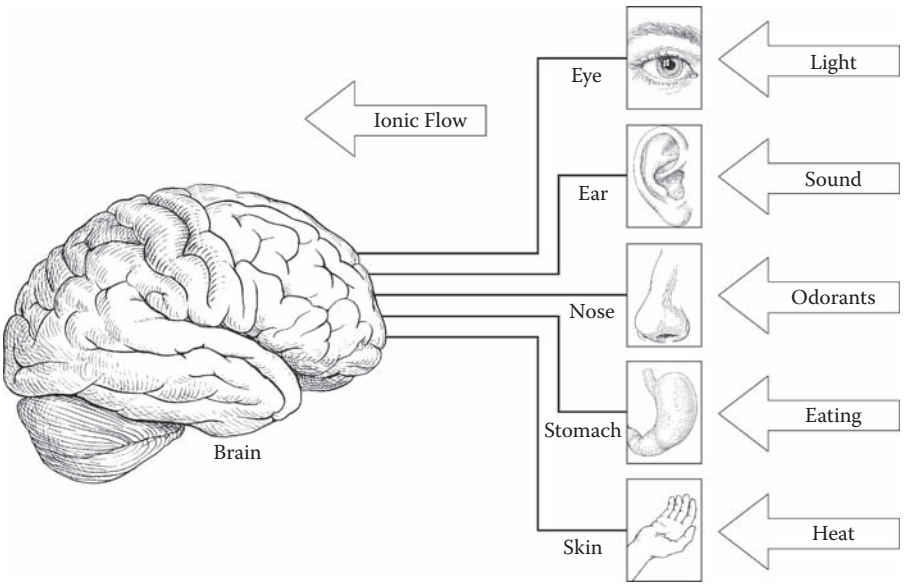


FIGURE 11.4 Sensory systems. Sensory systems convert different forms of energy into ionic flow in nerve cells. This diagram illustrates five of the many sensory systems of mammals: vision, hearing, olfaction, stomach fullness, and skin temperature.

affected by photic and nonphotic stimuli, these stimuli first must be sensed and transduced. Section 11.2 discusses the sensing and transduction of photic stimuli, and Section 11.3 covers nonphotic stimuli.

11.2 PHOTIC RECEPTORS

Photic receptors transduce light (electromagnetic radiation). Transduction of light is the domain of vision, and the eyes are the organs specialized in vision. It is natural, therefore, to start the discussion of circadian photic receptors by discussing the role of the eyes.

11.2.1 The Eyes

Plants, fungi, archea, and bacteria do not have eyes and must perceive light by other means. Most animals possess eyes. Eyes are thought of 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*. Because the eye also performs nonvisual functions, it is not surprising that the circadian system of visually blind humans can be affected by photic stimuli.[3] The circadian system of blind subterranean molerats, which have atrophied eyes underneath the skin, also can be affected by photic stimuli.[4] The eye, however, is the only photosensitive organ in mammals. Removal of the eyes eliminates all photic input to the circadian system,[5–10] as exemplified in Figure 11.6.

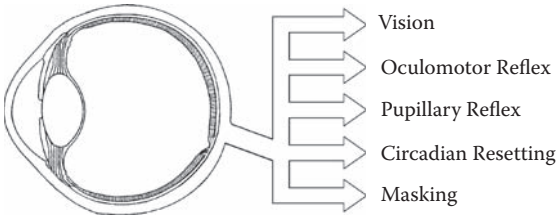


FIGURE 11.5 Sensory systems that use the eye as the receptor organ. The eye is the organ specialized in the transduction of electromagnetic radiation (light). It serves as the receptor organ for vision and many other functions, including circadian resetting.

Two golden hamsters (*Mesocricetus auratus*) were housed in a single cage with two running wheels and maintained under a 23.3-hour T cycle. If you don't know what a T cycle is, refer to Chapter 7. The eyes of one hamster were surgically removed, while the eyes of the other hamster were left intact. 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 tissue (Figure 11.7). Decades of research on the visual system have shown 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[11,12] (Figure 11.8). *Rods* are associated with nighttime (black-and-white) vision, while *cones* are associated with daytime (color) vision. The mechanism of color vision requires two or more distinct types of cones; humans have three types of cones, which were identified in the early 1960s.[13,14] Rods and cones make complex connections

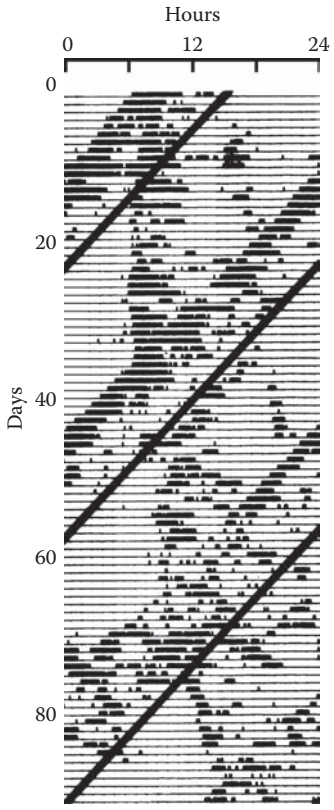


FIGURE 11.6 Missing the eyes. This actogram shows the combined rhythms of running-wheel activity of two male golden hamsters (*Mesocricetus auratus*) maintained under a skeleton light–dark cycle with a period of 23.3 hours (LD 1:22.3), as indicated by the oblique heavy lines. One of the hamsters had 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 free-ran with a period slightly longer than 24.0 hours. (Source: Adapted from Refinetti, R. et al. (1992). Social stimuli fail to act as entraining agents of circadian rhythms in the golden hamster. *Journal of Comparative Physiology A* 170: 181–187.)

with *bipolar cells*, which then 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 15 years on mice with genetic degeneration of rods and cones revealed that, while rods and cones are indeed needed for vision, they are not required for photic stimulation of the circadian system.[10,15–18] Evidently, some other retinal (or extra-retinal) structure must be able to carry out circadian photoreception. A few years ago, researchers discovered that some *ganglion cells* can function as photoreceptors.[19] An example of this type of ganglion cell is depicted in black in the diagram in Figure 11.8. Figure 11.9 shows a microphotograph of an actual photosensitive ganglion cell in the rat retina. These cells have sparse but wide dendritic fields (0.1 mm^2), and they *depolarize* in response to local photic stimulation.[19,20] Retinal photoreceptors normally

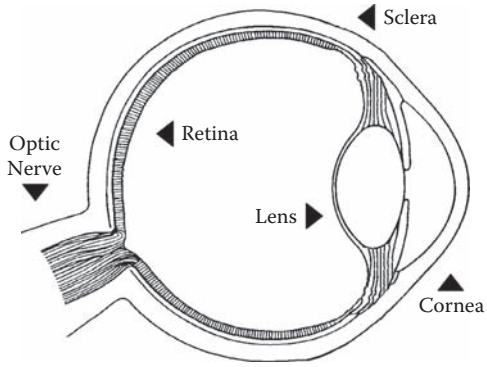


FIGURE 11.7 The human eye. This diagram of the human eye shows its major structures, including the retina (which contains photoreceptor cells) and the optic nerve (which takes information from the eye to the brain). (Source: Adapted from Goldstein, E. B. (1996). *Sensation and Perception*, 4th Edition. Pacific Grove, CA: Brooks/Cole.)

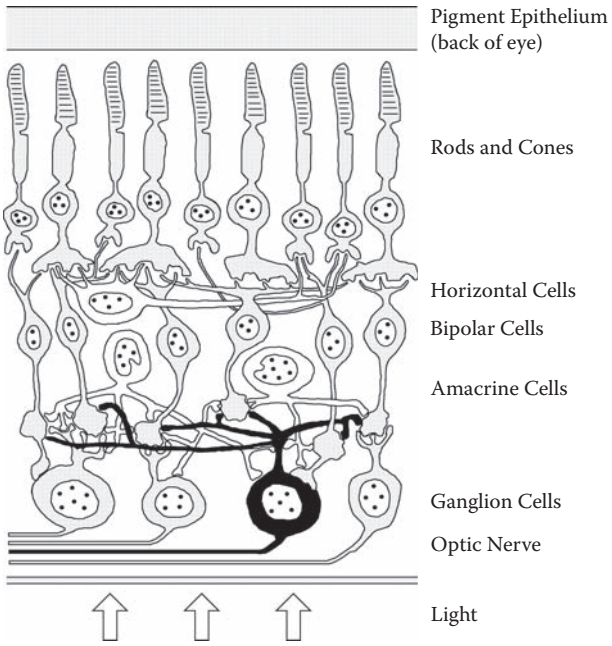


FIGURE 11.8 The retina. This diagram shows the cellular organization of the primate retina. Light traverses the various layers to stimulate the visual photoreceptors (rods and cones) next to the pigment epithelium. Some ganglion cells (such as the cell drawn in black in this diagram) are also photosensitive and provide additional photic input to nonvisual systems such as the circadian system and the system responsible for the pupillary reflex. (Source: Adapted from Dowling, J. E. & Boycott, B. B. (1966). Organization of the primate retina. *Proceedings of the Royal Society of London B* 166: 80–111.)

do not work this way. In most sensory modalities, receptors respond to stimulation in the same way as normal neurons — 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

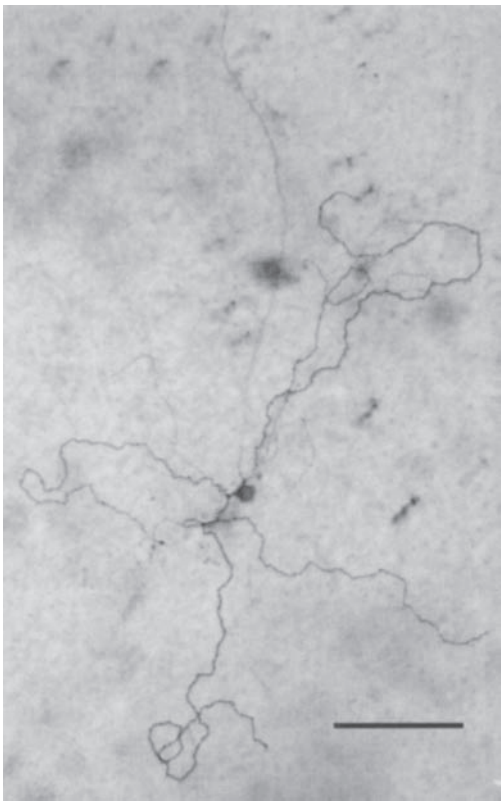


FIGURE 11.9 A close view of a photoreceptive ganglion cell. This microphotograph of the rat retina shows the typical morphology of a photoreceptive ganglion cell that projects to the circadian system. Scale bar: 100 μm. (Source: Warren, E. J. et al. (2003). Intrinsic light responses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735. © 2003 Blackwell Publishing. Reproduced with permission from the publisher and the authors.)

diagrammed in Figure 11.10, rods and cones in the vertebrate retina react in an opposite fashion: they hyperpolarize when stimulated. Note that photoreceptive retinal ganglion cells behave like afferent neurons, not like retinal photoreceptors.

The way in which photoreceptive ganglion cells sense light is a more complicated issue. The process of photic transduction in rods and cones has been studied extensively,[11,12] so it is discussed 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 change 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.

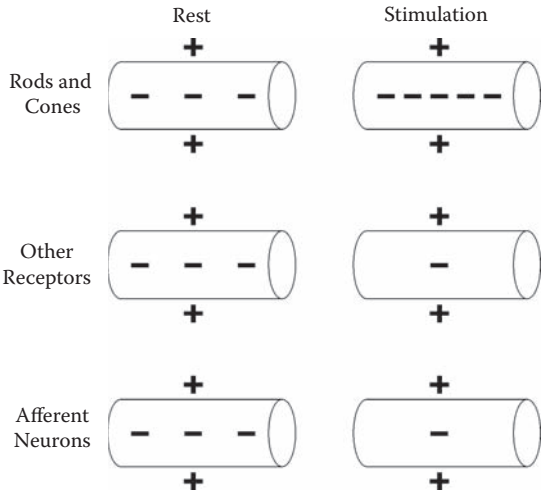


FIGURE 11.10 Changes in the polarization of nerve cells during stimulation. Unlike other sensory receptors and all afferent neurons, rods and cones in the vertebrate retina hyperpolarize in response to stimulation.

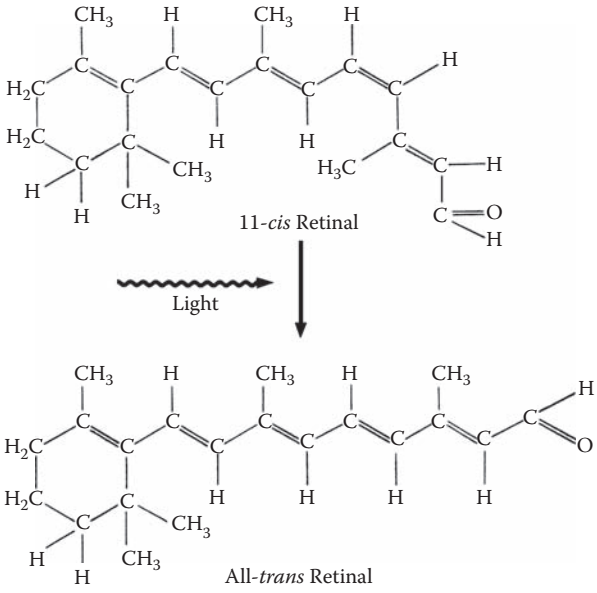


FIGURE 11.11 The chemistry of phototransduction. The photoisomerization of the chemical *retinal* is the key element in the transduction of photic energy in the retina. (Source: Adapted from Bailey, C. H. (1981). Visual system I: the retina. In: Kandel, E. R. & Schwartz, J. H. (Eds.). *Principles of Neural Science*. New York: Elsevier, pp. 213–225.)

The opsin in rhodopsin (encoded by the *rho* gene) is distinct from the opsins in cones (encoded by the *opn1* genes), so which opsin is present in the photoreceptive ganglion cells? In fact, the substance might not even be an opsin. Plants contain a blue-light photopigment (*cryptochrome*) that is based on vitamin B_2 rather than on vitamin A (as in opsin),[21] and ganglion cells in the mouse retina have been shown to contain cryptochrome.[22] This

finding means that cryptochrome could be the photopigment of photoreceptive ganglion cells. This possibility is 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[23] and that mice missing both cryptochrome and rods/cones exhibit reduced pupillary response to photic stimulation.[24] Also, mice missing both cryptochrome and rods/cones show reduced activation of the master pacemaker in the brain when they are photically stimulated.[25]

Evidence strongly supports the hypothesis that cryptochrome is the photopigment present in photoreceptive ganglion cells. However, the theory contains many holes. As discussed in greater detail in Chapter 12, cryptochrome is a major component of the cellular mechanism of the circadian clock.[26] Thus, the impairment of circadian function in mice with cryptochrome deletion could be the consequence of impairment in the clock itself, not in its photic input. Mice with deletion of the cryptochrome genes are arrhythmic in constant darkness but retain photic sensitivity.[27,28] Even more important, 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[29] and was found to be present in photoreceptive ganglion cells but not in nonphotoreceptive cells of the rat retina.[30–32] Furthermore, melanopsin knockout mice (mice with deletion of *opn4*) were found to exhibit reduced responsiveness to photic stimulation.[33–36] Figure 11.12 shows a microphotograph of a retinal ganglion cell containing melanopsin. Approximately 1% of ganglion cells in the retina of mice[37] and humans[38] contain melanopsin. Melanopsin-containing ganglion cells, just like regular ganglion cells, are innervated by bipolar cells.[39]

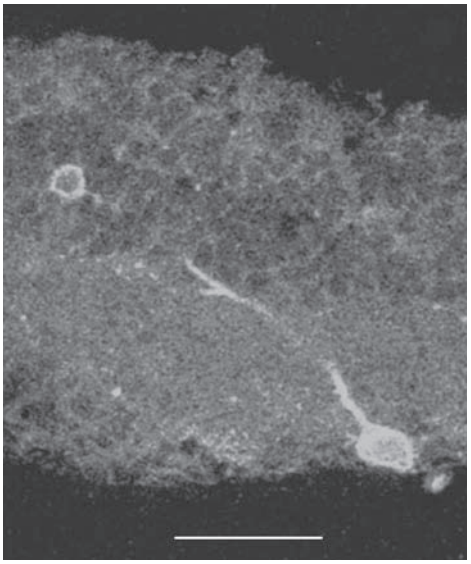


FIGURE 11.12 Melanopsin as a photopigment. This microphotograph of the mouse retina shows a melanopsin-immunopositive cell in the retinal ganglion cell layer with processes extending towards another melanopsin-immunopositive cell deeper in the retina. Scale bar: 50 μm. (Source: Image courtesy of Russell G. Foster, Department of Visual Neuroscience, Imperial College London, U.K.)

The fact that the circadian system of mice deprived of rods and cones can respond to photic stimulation[10,15–18,40,41] indicates that rhodopsin and the cone opsins are not necessary for circadian photoreception. Melanopsin may be 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.[42,43] 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[33,34] indicates that rods and cones also are used by the circadian system. Thus, all three of these opsins (*rho*, *opn1*, and *opn4*) may be involved in photoreception for the circadian system. A novel opsin (*opn5*, or neuropsin) has been identified recently in the eye (and other structures) of mice and humans.[44] Its potential role as a circadian photopigment has yet to be investigated.

One problem regarding the role of melanopsin as a circadian photopigment derives from the recent finding that melanopsin is expressed not only in photoreceptive ganglion cells but also in the pigment epithelium of the eye.[45,46] 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 finding may be inconsequential, but it also may mean that, similarly to cryptochrome, melanopsin is the central component of a different process that only secondarily affects photoreception.

The role of the various photoreceptors in the circadian system also can be investigated by comparing the *action spectra* of the photoreceptors with the action spectrum of the full circadian system. An action spectrum 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 possess two cone opsins; some mammals have only one, and several primates (including humans) have three.[47] The action spectrum of human scotopic vision follows closely the absorption response of rhodopsin, with peak sensitivity at 505 nanometers (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 sensitivity of human photopic vision peaks at 555 nm.

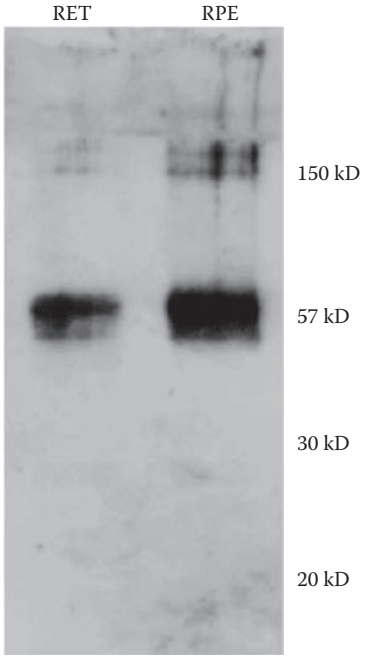


FIGURE 11.13 Melanopsin beyond the retina. This Western blot of protein extracts from the mouse eye show greater translation of melanopsin (57 kD band) in the retinal pigment epithelium (RPE) than in the retina itself (RET). For the location of the pigment epithelium, see Figure 11.8. (Source: Adapted from Peirson, S. N., Bovee-Geurts, P. H. M., Lupi, D., Jeffery, G., DeGrip, W. J. & Foster, R. G. (2004). Expression of the candidate circadian photopigment melanopsin (*Opn4*) in the mouse retinal pigment epithelium. *Molecular Brain Research* 123: 132–135.)

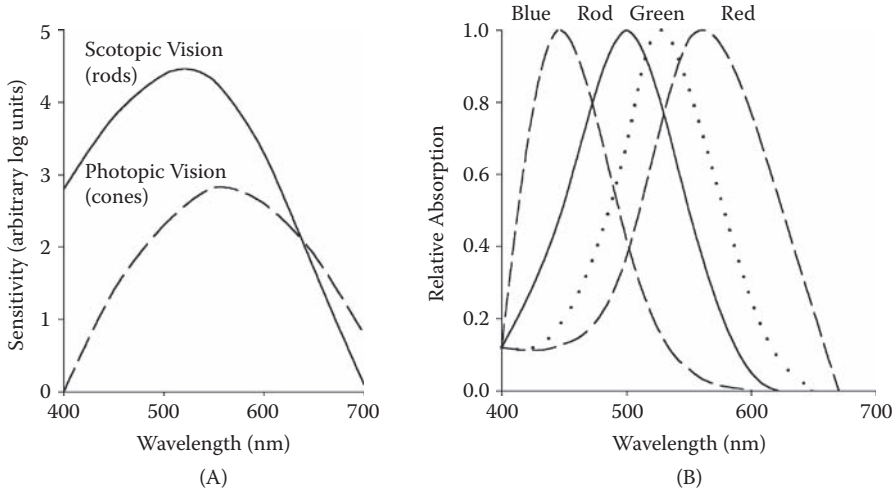


FIGURE 11.14 Spectral sensitivity of human visual receptors. Two processes can be distinguished in human vision (as well as in the vision of numerous other species): scotopic (or black-and-white) vision, which depends on retinal rods, and photopic (or color) vision, which depends on retinal cones (A). The spectral sensitivity of scotopic vision approximates that of rods, while the spectral sensitivity of photopic vision is a composite of the spectral sensitivities of the three types of cones (B). Note that in Panel B all functions are normalized to facilitate comparison of the spreads of spectral sensitivity. (Source: Adapted from Schiffman, H. R. (1996). *Sensation and Perception*, 4th Edition. New York: Wiley.)

Figure 11.15 shows action spectra for various photoreceptive processes (left panels) and for phase shifts of circadian rhythms evoked by photic stimuli of different wavelengths (right panels) 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 360 nm.[48,49] 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.[50–56] Presumably, circadian sensitivity in the ultraviolet range is due to the cones with peak sensitivity at 360 nm. However, the 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.

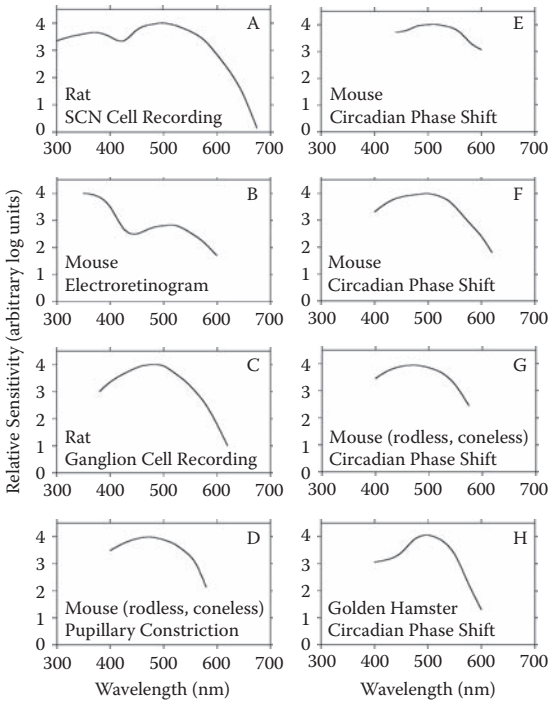


FIGURE 11.15 Comparing various action spectra. These eight action spectra, including four spectra for circadian resetting (E through H), provide useful information about the spectral sensitivity of the circadian system in rodents. A peak between approximately 480 and 510 nm ("green") is found consistently 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. (Source A: Aggelopoulos, N. C. & Meissl, H. (2000). Responses of neurones of the rat suprachiasmatic nucleus to retinal illumination under photopic and scotopic conditions. *Journal of Physiology* 523: 211–222. Source B: Jacobs, G. H., Neitz, J. & Deegan, J. F. (1991). Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature* 353: 655–656. Source C: Berson, D. M., Dunn, F. A. & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073. Source D: Lucas, R. J., Douglas, R. H. & Foster, R. G. (2001). Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nature Neuroscience* 4: 621–626. Source E: Provencio, I. & Foster, R. G. (1995). Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. *Brain Research* 694: 183–190. Source F: Yoshimura, T. & 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. Source G: Hattar, S., Lucas, R. J., Mrosovsky, N., Thompson, S., Douglas, R. H., Hankins, M. W., Lem, J., Biel, M., Hofmann, F., Foster, R. G. & Yau, K. W. (2003). Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424: 76–81. Source H: Takahashi, J. S., DeCoursey, P. J., Mauman, L. & Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.)

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).[48,49] The spectrum for photoreceptive ganglion cells (Panel C) peaks at approximately 480 nm,[19] which is also the peak wavelength for phase shifts and pupillary reflex in mice with degenerate retina[42,57,58] as well as for long-term retinal adaptation in the human visual system.[59] Furthermore, phase shifts of the human circadian system and melatonin suppression seem to be evoked more easily by light pulses of short wavelength (around 480 nm) than by pulses in the green or red regions.[60–65] Panels E through H (rodent phase-shift data) show that peak sensitivity is at about 480 nm for retinally degenerate mice[42,57] (Panel G) and at 500 to 515 nm in animals with intact rods and cones.[51,57,66] Thus, it seems that traditional photoreceptors, as well as 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 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.[66,67] The low sensitivity 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.[68]

11.2.2 Other Photic Receptors

Figure 11.16 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. Nothing appears 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 free-runs in constant darkness. However, the bird whose records are shown in the figure had *both eyes removed* prior to the beginning of the study. The circadian system of birds — unlike that of mammals — clearly can obtain photic information from organs other than the eyes.[69–72]

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 extra-ocular photoreceptors that have not been clearly characterized.[73,74] 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 fish), and additional deep brain photoreceptors.[75–77] Figure 11.17 depicts in diagrammatic form the various vertebrate photoreceptors. Cells with an outer segment resembling

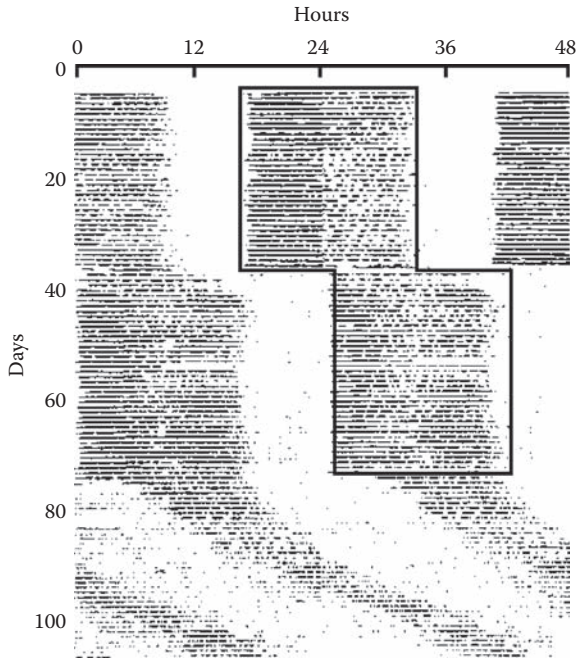


FIGURE 11.16 Blinded but not blind. This double-plotted actogram shows the rhythm of perching activity of a house sparrow (*Passer domesticus*) maintained under a light–dark cycle (as indicated by the single-plotted rectangles). The sparrow had both eyes removed prior to the beginning of the records. 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. (Source: Adapted from Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences U.S.A.* 59: 414–421.)

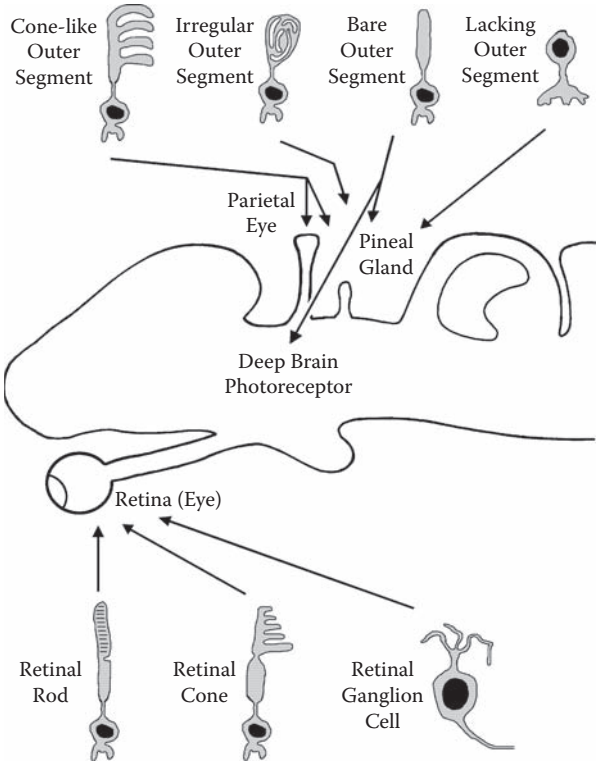


FIGURE 11.17 Vertebrate photoreceptive structures. This diagram of the vertebrate brain shows the approximate location of seven types of opsin-containing cells. Opsin-containing cells can be found in the retina, in the pineal gland, in the parietal eye, and deep in the brain. Although all vertebrates have eyes (retina), only some groups of vertebrates have other photoreceptive structures. The retina is the only photoreceptive structure in adult mammals. (Source: Adapted from Foster, R. G. & Menaker, M. (1993). Circadian photoreception in mammals and other vertebrates. In: Wetterberg, L. (Ed.). *Light and Biological Rhythms in Man*. Oxford, UK: Pergamon, pp. 73–91.)

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 located 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. One recent study in lizards identified 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).[78]

Most vertebrates, including humans, have a pineal gland (Figure 11.18). Section 5.3.3 showed that the pineal gland secretes the hormone melatonin; melatonin secretion is inhibited by photic stimulation in humans,[60,61,64,79–88] rodents,[9,89–93] and other vertebrates.[94–96] 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.[97] In all other vertebrates, the adult pineal gland is intrinsically photosensitive.[76,77,98] Figure 11.19 shows an example. 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 given discrete 4-hour light pulses (as indicated by the vertical grey lines). Note that the rhythm of melatonin secretion phase shifted in response to the light pulse, and pulses at different circadian times evoked different types of shifts (phase delay *versus* phase advance).[99]

The opsin used by photoreceptors in the pineal gland was identified in chickens[100,101] and in pigeons.[102] *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 shown above — is expressed in the retina, is also expressed in the chicken pineal gland[46] and may contribute to pineal photoreception.

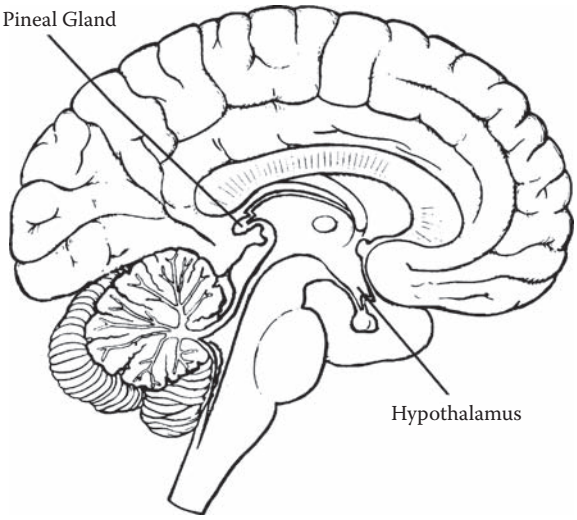


FIGURE 11.18 The pineal gland. This diagram of a midsagittal section of the human brain shows the location of the pineal gland and hypothalamus. Although the pineal gland is not photosensitive in humans, it is an important photosensitive structure in the circadian system of other vertebrates. (Source: Adapted from *Medical Illustration Library.* (1994). Baltimore, MD: Williams & Wilkins.)

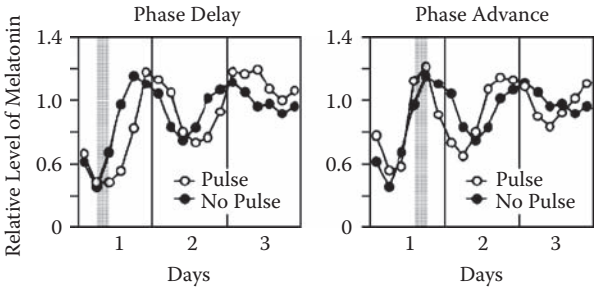


FIGURE 11.19 Intrinsic photosensitivity of the pineal gland in chicken. The graphs show 3-day segments of the records of melatonin secretion of cultures of dispersed pineal cells of young chicks (*Gallus domesticus*). After 10 days under a light–dark cycle, the cultures were placed in constant darkness and received a 4-hour light-pulse (300 lux) at the time indicated by the vertical grey lines. Each set of data points corresponds to the means of three cell cultures. Note that photic stimulation during early subjective night caused a phase delay of the rhythm, while stimulation during late subjective night caused a phase advance. (Source: Adapted from Kasahara, T., Okano, T., Haga, T. & Fukada, Y. (2002). Opsin-G11-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *Journal of Neuroscience* 22: 7321–7325.)

Researchers have not identified extra-ocular photoreceptors in mammals despite many attempts. 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).[103] These phase shifts were said to be comparable to those obtained by

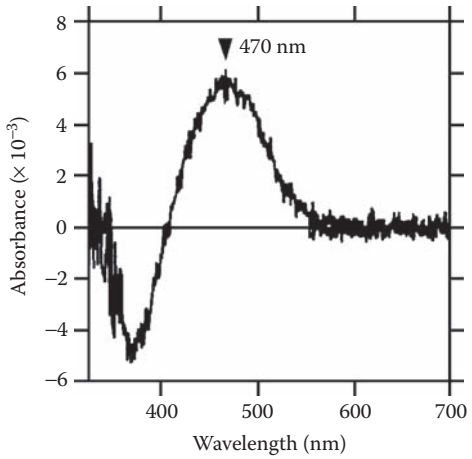


FIGURE 11.20 Pinopsin: the pineal photopigment. The graph shows the difference absorption spectrum (difference between the regenerated photopigment and its photoproducts) of pinopsin, a photopigment found only in the pineal gland. The peak absorption (λ_{max}) is at 470 nm. Pinopsin was extracted from pineal glands of young chicken chicks (*Gallus domesticus*). (Source: Adapted from Okano, T., Yoshizawa, T. & Fukada, Y. (1994). Pinopsin is a chicken pineal photoreceptive molecule. *Nature* 372: 94–97.)

ocular stimulation and to conform to a consistent PRC. Three years later, the same researchers reported that photic stimulation of the popliteal region could also enhance REM sleep in human subjects.[104] If this finding were true, it could greatly facilitate the treatment of medical conditions by phototherapy (see Chapters 15 and 16). Yet, seven studies on human subjects[60,84,105–109] and two studies on golden hamsters[9,110] conducted since then by other research teams have failed to detect circadian phase shifts or melatonin suppression in response to photic stimulation of the skin. Dermal photosensitivity has been documented in snails,[111] frogs,[112] and lizards.[113] However, the experimental evidence clearly indicates that, if there are photoreceptors in the mammalian skin, they do not provide input to the circadian system.

11.3 NONPHOTIC RECEPTORS

Chapter 8 discussed several nonphotic stimuli that are capable of phase shifting the circadian pacemaker: ambient temperature, food availability, physical activity (exercise), and social contact. Although researchers have not 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. This section reviews the vast body of knowledge on the sensory systems responsible for the skin temperature sense, the monitoring of body core temperature, and the monitoring of nutritional state. The sensory processes involved in exercise likely involve the monitoring of

muscle contraction, tendon distension, vasomotion, partial pressure of oxygen, pH, and many other variables not fully understood at this time. The receptors responsible for the monitoring of social interaction, which are probably even more numerous, are not known.

11.3.1 Skin Temperature

Chapter 10 mentioned that animals treat the temperature continuum as two separate continua: the cold continuum and the warmth continuum. This phenomenon occurs 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[114,115] and different sensitivities at suprathreshold intensities.[116–119] Although warm and cold sensations can oppose each other (as if the stimuli canceled each other out),[120,121] the opposition is only a cognitive combination of distinct sensations because subjects can tell the sensations apart if asked to do so.[122]

Electrophysiological studies of temperature receptors have been conducted since the early days of sensory neuroscience research. 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.[123] 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.[124] The electrophysiological properties of temperature receptors have been studied extensively since then in rats,[125–127] cats,[125,128–137] rabbits,[138,139] dogs,[125,126] and primates,[140–148] including humans.[149–151] 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 pain receptors that respond to various stimuli, including heat. They probably do not contribute to the pure sensations of nonnoxious cold and warmth, which are evoked by cold and warm receptors.[152] Although *cold receptors* generally yield a tonic response to thermal stimuli below normal skin temperature (peaking at 25°C), while *warm receptors* yield a tonic response to thermal stimuli above normal skin temperature (peaking at 42°C), the two types of receptors are distinguished more reliably 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, while warm receptors show an overshoot on warming steps and an undershoot on cooling steps.[152,153]

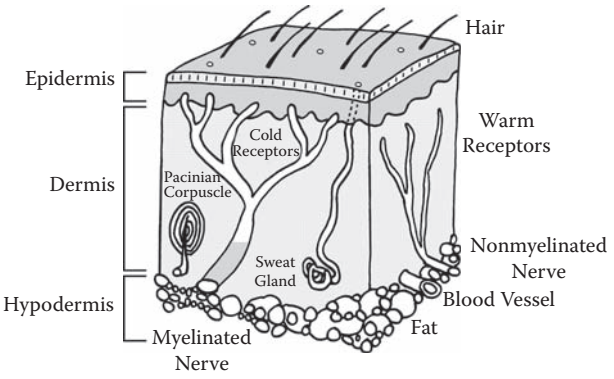


FIGURE 11.21 Skin receptors. This diagram of the human skin shows several specialized structures, including cold receptors in the epidermis and warm receptors in the dermis. Pacinian corpuscles are touch receptors. (Sources: Andrews, G. C. (1946). *Diseases of the Skin*, 3rd Edition. Philadelphia: Saunders; Hensel, H. (1981). *Thermoreception and Temperature Regulation*. New York: Academic Press.)

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, while 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 takes place at the level of particular ion channels in the membrane of the afferent fibers.[154–156] 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 studied more easily *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).[156] As shown in Figure 11.22, cells 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

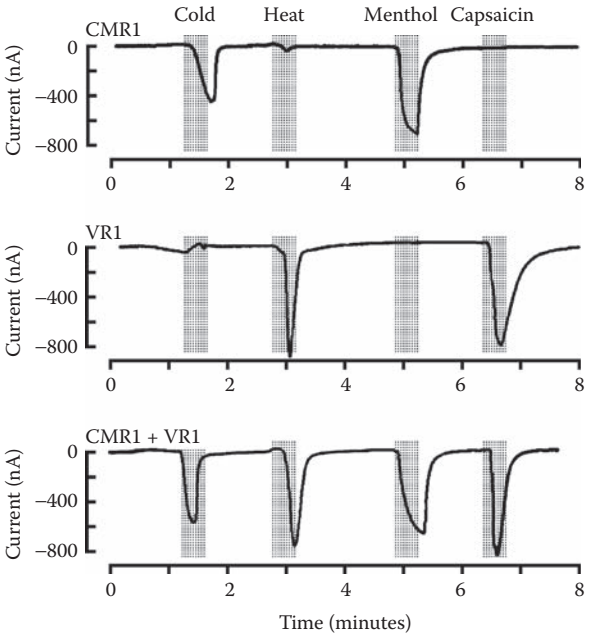


FIGURE 11.22 Transduction of skin temperature. The graphs show electrophysiological records of transmembrane current changes evoked by various stimuli in frog cells expressing rat genes for skin thermoreception. CMR1 stands for cold- and menthol-sensitive receptor. VR1 stands for vanilloid receptor (heat- and capsaicin-sensitive receptor). Stimulation is indicated by the grey vertical bars (cold: 8°C, heat: 50°C, menthol: 100 μM, capsaicin: 1 μM). (Source: Adapted from McKemy, D. D., Neuhausser, W. M. & Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416: 52–58.)

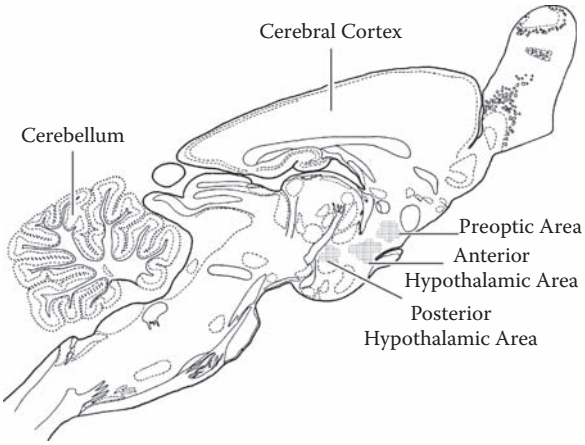


FIGURE 11.23 Intrinsic thermosensitivity in the brain. This diagram of the rodent brain (sagittal section) shows the location of thermosensitive structures: preoptic area, anterior hypothalamic area, and posterior hypothalamic area. (Source: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum.)

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. 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.[157] 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,[158–185] spinal cord,[186–196] and visceral organs.[197–200] In mammals brain thermosensitivity seems to be more important than spinal thermosensitivity, while in birds the opposite is true.[201] 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, while the preoptic area and anterior hypothalamic area (POAH) are important for both behavioral and autonomic responses.[202–204] Neurons in the POAH play a double role as thermoreceptors and as integrators of thermal information received from other parts of the body,[185,205–222] although only the thermoreceptor aspect is discussed in this section.

The electrophysiological properties of preoptic and anterior-hypothalamic thermoreceptors have been studied in birds,[223,224] rodents,[225–236] larger mammals,[167,237–243] and lower vertebrates.[244–246] However, the identification of thermoreceptors in the brain is not as simple as the identification of skin thermoreceptors. First, one cannot impose large variations in local temperature without damaging the tissue. More important, most body tissues are technically temperature-sensitive, as they have a Q_{10} of about 2. This temperature sensitivity 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, while firing rates with thermal coefficients smaller than –0.5 impulses $\cdot$ s^{-1} $\cdot$ $°C^{-1}$ characterize cold-sensitive cells.[235]

A further complication is the distinction between true thermoreceptors (i.e., cells that are intrinsically temperature-sensitive) and interneurons that receive excitatory or

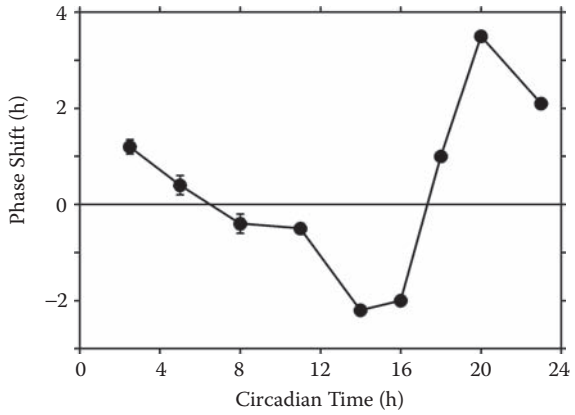


FIGURE 11.24 Intrinsic thermosensitivity of the circadian clock. The graph shows a phase response curve (PRC) for temperature pulses. Small slices of rat brain (*Rattus norvegicus*) containing the master circadian clock were kept *in vitro* for several days at 34°C. Electrophysiological recording of 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 PRC resembling the PRC of whole animals exposed to light pulses can be seen. Each data point corresponds to the mean (± SE) of two to six cells. (Source: Ruby, N. F., Burns, D. E. & 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.)

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.[230,235] However, if synaptic communication between cells is chemically blocked, the cold-sensitive cells lose thermosensitivity.[234] This loss implies that, while warm-sensitive cells are genuine warm receptors, cold-sensitive cells are actually interneurons synaptically modulated by warm receptors. Although several diencephalic areas contain warm- and cold-sensitive cells,[232] most genuine temperature receptors are located in the preoptic area.[233]

Very few studies have addressed the issue of intrinsic thermosensitivity of the circadian pacemaker (in the suprachiasmatic nucleus). Chapter 6 showed that the pacemaker is temperature compensated, meaning that its period is not significantly affected by variations in local temperature. However, variations in temperature *can* phase shift the pacemaker. Approximately 10% of suprachiasmatic cells are warm-sensitive, and 2% are cold-sensitive.[247] 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.[248] 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 finding 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. Chapter 9 discussed the issue of photic self-stimulation, but the implications of thermal self-stimulation to the process of entrainment remain to be determined.

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. This section briefly reviews 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.[249]

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*)[250] and satiety (the *ventromedial hypothalamus*).[251] 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 essential components

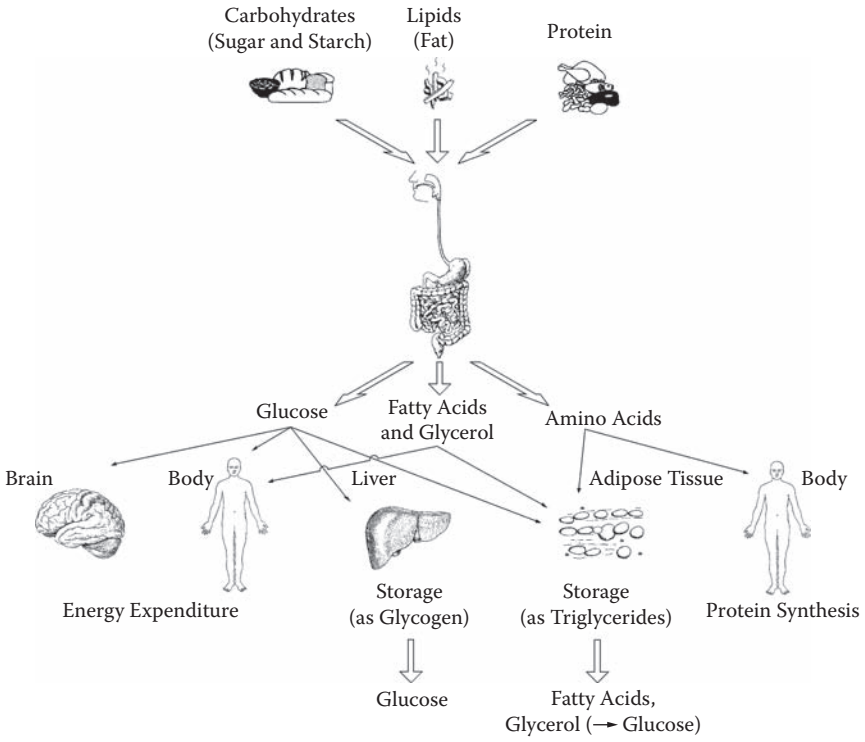


FIGURE 11.25 Origin and destination of nutrients. This diagram shows the three basic types of nutrients (carbohydrates, lipids, and protein), their breakdown by the digestive system, and their three main uses (energy expenditure, storage, and protein synthesis). For long-term storage, excess energy is stored as fat regardless of the nutrients ingested.

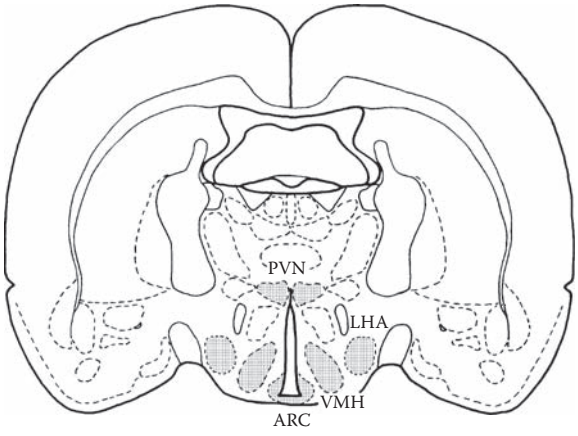


FIGURE 11.26 Brain regions important in the control of food intake. This diagram of the rodent brain (coronal section) shows four regions of the hypothalamus that are involved in the control of food intake: arcuate nucleus of the hypothalamus (ARC), ventromedial nucleus of the hypothalamus (VMH), lateral hypothalamic area (LHA), and paraventricular nucleus of the hypothalamus (PVN). (Source: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain, 2nd Edition*. New York: Plenum.)

of the neural circuit responsible for the control of hunger and satiety.[252–254] To appropriately generate hunger and satiety, these hypothalamic areas must be able to sense the nutritional state of the body. Pertinent information 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.[255] Long-term (hunger) signals must be related to the depletion of energy stores, while short-term (satiety) signals may depend on meal-associated factors. Because 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[256,257] — seems a likely candidate. Leptin is synthesized by adipocytes (fat cells) and released into the general circulation. Leptin is actually an anorexic hormone, in the sense that its action on the *arcuate nucleus* is to reduce food intake.[253,254] Presumably, the "default" state of organisms is hunger, 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 satiety — that is, it increases hunger. Thus, depletion of fat stores increases hunger, which 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

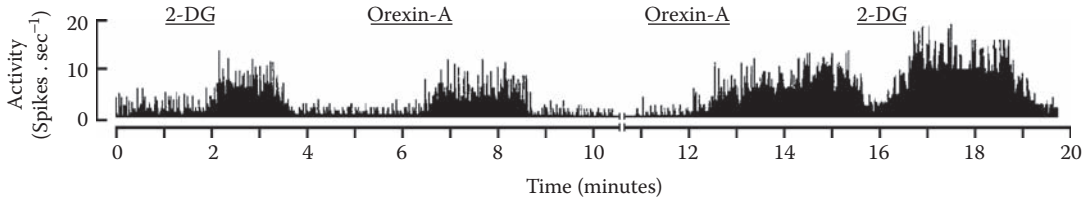


FIGURE 11.27 A glucose-sensitive hypothalamic cell. The graph shows a 20-minute segment of the electrophysiological records of extracellular activity of a glucose-sensitive neuron in the lateral hypothalamus of a rat (*Rattus norvegicus*). The cell was stimulated by microiontophoretic application of either 2-deoxy-glucose (2-DG), a nonmetabolizable analog of glucose, or orexin-A, a hypothalamic peptide previously known to stimulate feeding. (Source: Adapted from Shiraishi, T. et al. (2000). Effects of leptin and orexin-A on food intake and feeding related hypothalamic neurons. *Physiology and Behavior* 71: 251–261.)

stores might be used as a hunger signal. This signal does not seem to be used, however.[258] Fifty years ago it was suggested that the brain may directly monitor glucose content (or, more precisely, glucose utilization) in the general circulation.[259] This possibility is more likely, as infusion of glucose into cerebral ventricles inhibits food intake,[260] and neurons sensitive to local concentration of glucose can be found in the hypothalamus.[261,262] Figure 11.27 shows an example of a glucose-sensitive neuron. 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 known to stimulate feeding), and its electrical activity was monitored. Note that the firing rate of the cell was temporarily elevated after delivery of either stimulus.

Various short-term satiety signals, which are needed to terminate a meal so that the food can be digested and assimilated, also have 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.[263] The hormone *cholecystokinin* (CCK) is secreted by the intestine and acts both on vagal terminals and on the brain to inhibit feeding.[264,265] 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.[266] The hormone *ghrelin*, which also participates in the regulation of pituitary growth hormone release, is secreted by the stomach and acts on the arcuate nucleus to modulate feeding.[267,268] 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 uses 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 that the caloric content,[269] while the opposite seems to be true for the rat.[270]

The limited knowledge about how the circadian system obtains information on nonphotic stimuli may be somewhat frustrating, and the uncertainty about the exact photopigments involved in circadian photoreception is not encouraging. However, it is likely that knowledge in this area will increase considerably in the near future because of the recent increase in research in the field. Chapter 12 discusses the cellular and molecular characteristics of the pacemaker, and Chapter 13 examines how the information acquired by the receptors is transmitted to the pacemaker.

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, the parietal eye (or the parapineal organ), and 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* seems to be the photopigment used by photosensitive ganglion cells.
3. Little is known about the way in which the circadian pacemaker acquires the information about temperature and nutritional state required 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, intestines, and fat cells.

EXERCISES

Exercise 11.1 Perception of color

Although the circadian system seems to use more photoreceptors than those used for vision, it is still instructive to explore how light of different wavelengths evokes the visual perception of color. This exercise assumes that you can see color; that is, it assumes that you do not suffer from daltonism.

1. The CD-ROM that accompanies this book contains a short program (Color.exe) 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. Color.exe should be located in the folder where the other programs are stored (\Program Files\Circadian). To start the program, double-click on Color.exe.
2. The program has two sections. The section on Color Mixture is quite intuitive and easy to use. Any color can be produced by the combination of different proportions of the three basic colors (blue, green, and red), because the human visual system uses only three types of color receptors with maximal absorption in the blue, green, and red sections of the spectrum. Note 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 to use. Because of the peculiar way in which photoreceptors connect with bipolar and ganglion cells in the retina, human 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! You may need to blink your eyes briefly after the stimulus disappears to see the afterimage. Also, because computer monitors do not produce perfectly monochromatic stimuli, the afterimages may be slightly off-color. You should be able to see an afterimage in every case, however.

Exercise 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. This brief exercise examines a particular aspect of the sensitivity to ambient temperature. In mammals, as in many other animals, ambient temperature is sensed by specialized skin receptors. Although a physical continuum exists between low and high temperatures, 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.

1. You will need 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). Each container should be large enough for you to place your hand inside it.
2. Start by placing your right hand briefly in each of the containers. You should be able to distinguish easily among the three containers even if blindfolded.
3. 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 feel the difference easily.
4. Leave your hands in the two containers for a few minutes, then 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!
5. British philosopher John Locke called what you just experienced 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," important concepts in his theory of philosophical empiricism. Today, this paradox is explained 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 opposite is true for high temperatures below 40°C (104°F).

Exercise 11.3 Electroretinogram

The electrical activity of cells in the retina can be recorded by electrodes placed on the surface of the eye. The *electroretinogram* (ERG) 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 today, it requires sophisticated equipment 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 technician) at the facility can show you that retinal cells respond reliably to photic stimulation. Using previous knowledge of intraretinal processes, a researcher 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 also can be demonstrated easily.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Warren, E. J., Allen, C. N., Brown, R. L. & Robinson, D. W. (2003). Intrinsic light responses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735. This research article is an exceptionally good example of a well-written article with rigorous research methods and clear-cut results.

Fein, A. G. & Szuts, E. Z. (1982). *Photoreceptors: Their Role in Vision*. New York: Cambridge University Press. An intermediate-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 20 years old, it remains an exceptional summary of scientific research on the temperature senses and their role in body temperature regulation.

WEB SITES TO EXPLORE

Center for Perceptual Systems at the University of Texas:
http://www.cps.utexas.edu

Directory of Free Online Medical Journals:
http://www.freemedicaljournals.com

Photoreceptor Pages (University of Utah):
http://webvision.med.utah.edu/photo1.html

Sensory Physiology Site (University of Western Ontario):
http://www.med.uwo.ca/physiology/courses/sensesweb

LITERATURE CITED

1. Turek, F. W. (1994). Circadian rhythms. *Recent Progress in Hormone Research* 49: 43–90.
2. Fulghum, R. (2003). *All I Really Need to Know I Learned in Kindergarten*, 15th Anniversary Edition. New York: Ballantine.
3. Klerman, E. B., Shanahan, T. L., Brotman, D. J., Rimmer, D. W., Emens, J. S., Rizzo, J. F. & Czeisler, C. A. (2002). Photic resetting of the human circadian pacemaker in the absence of conscious vision. *Journal of Biological Rhythms* 17: 548–555.
4. Goldman, B. D., Goldman, S. L., Riccio, A. P. & Terkel, J. (1997). Circadian patterns of locomotor activity and body temperature in blind mole-rats, *Spalax ehrenbergi*. *Journal of Biological Rhythms* 12: 348–361.
5. 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 U.S.A.* 75: 6276–6280.
6. Sato, T. & 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.
7. Ruis, J. F., Rietveld, W. J. & 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.
8. Bobbert, A. C. & 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.
9. Yamazaki, S., Goto, M. & Menaker, M. (1999). No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *Journal of Biological Rhythms* 14: 197–201.

10. Freedman, M. S., Lucas, R. J., Soni, B., von Schantz, M., Muñoz, M., David-Gray, Z. & Foster, R. (1999). Regulation of mammalian circadian behavior ny non-rod, non-cone, ocular photoreceptors. *Science* 284: 502–504.
11. Kandel, E. R., Schwartz, J. H. & Jessell, T. M. (2000). *Principles of Neural Science*, 4th Edition. New York: McGraw-Hill.
12. Goldstein, E. B. (2002). *Sensation and Perception, 6th Edition*. Pacific Grove, Calif.: Wadsworth.
13. Marks, W. B., Dobelle, W. H. & MacNichol, E. F. (1964). Visual pigments of single primate cones. *Science* 143: 1181–1182.
14. Brown, P. K. & Wald, G. (1964). Visual pigments in single rods and cones of the human retina. *Science* 144: 45–51.
15. Foster, R. G., Provencio, I., Hudson, D., Fiske, S., De Grip, W. & Menaker, M. (1991). Circadian photoreception in the retinally degenerate mouse (*rd*/*rd*). *Journal of Comparative Physiology A* 169: 39–50.
16. Yoshimura, T., Nishio, M., Goto, M. & 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.
17. Yoshimura, T., Yokota, Y., Ishikawa, A., Yasuo, S., Hayashi, N., Suzuki, T., Okabayashi, N., Namikawa, T. & Ebihara, S. (2002). Mapping quantitative trait loci affecting circadian photosensitivity in retinally degenerate mice. *Journal of Biological Rhythms* 17: 512–519.
18. Mrosovsky, N. (2003). Aschoff's rule in retinally degenerate mice. *Journal of Comparative Physiology A* 189: 75–78.
19. Berson, D. M., Dunn, F. A. & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073.
20. Warren, E. J., Allen, C. N., Brown, R. L. & Robinson, D. W. (2003). Intrinsic light reponses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735.
21. Cashmore, A. R., Jarillo, J. A., Wu, Y. J. & Liu, D. (1999). Chryptochromes: blue light receptors for plants and animals. *Science* 284: 760–765.
22. Miyamoto, Y. & 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 U.S.A.* 95: 6097–6102.
23. Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P., Dawut, L., Smithies, O., Takahashi, J. S. & Sancar, A. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 282: 1490–1494.
24. Van Gelder, R. N., Wee, R., Lee, J. A. & Tu, D. C. (2003). Reduced pupillary light responses in mice lacking cryptochromes. *Science* 299: 222.
25. Thompson, C. L., Selby, C. P., Partch, C. L., Plante, D. T., Thresher, R. J., Araujo, F. & Sancar, A. (2004). Further evidence for the role of cryptochromes in retinohypothalamic photoreception/phototransduction. *Molecular Brain Research* 122: 158–166.
26. Griffin, E. A., Staknis, D. & Weitz, C. J. (1999). Light-independent role of CRY1 and CRY2 in the mammalian clock. *Science* 286: 768–771.
27. Van der Horst, G. T. J., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J., Verkerk, A., Eker, A. P. M., van Leenen, D., Buijs, R., Bootsma, D., Hoeijmakers, J. H. J. & Yasui, A. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* 398: 627–630.
28. Okamura, H., Miyake, S., Sumi, Y., Yamaguchi, S., Yasui, A., Muijtjens, M., Hoeijmakers, J. H. J. & 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.
29. Provencio, I., Rodriguez, I. R., Jiang, G., Hayes, W. P., Moreira, E. F. & Rollag, M. D. (2000). A novel human opsin in the inner retina. *Journal of Neuroscience* 20: 600–605.
30. Hattar, S., Liao, H. W., Takao, M., Berson, D. M. & Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* 295: 1065–1069.
31. Gooley, J. J., Lu, J., Fischer, D. & Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience* 23: 7093–7106.
32. Hannibal, J. & Fahrenkrug, J. (2004). Melanopsin-containing retinal ganglion cells are light responsive from birth. *NeuroReport* 15: 2317–2320.
33. Ruby, N. F., Brennan, T. J., Xie, X., Cao, V., Franken, P., Heller, H. C. & O'Hara, B. F. (2002). Role of melanopsin in circadian responses to light. *Science* 298: 2211–2213.
34. Panda, S., Sato, T. K., Castrucci, A. M., Rollag, M. D., DeGrip, W. J., Hogenesch, J. B., Provencio, I. & Kay, S. A. (2002). Melanopsin (*Opn4*) requirement for normal light-induced circadian phase shifting. *Science* 298: 2213–2216.
35. Lucas, R. J., Hattar, S., Takao, M., Berson, D. M., Foster, R. G. & Yau, K. W. (2003). Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* 299: 245–247.
36. Mrosovsky, N. & Hattar, S. (2003). Impaired masking responses to light in melanopsin-knockout mice. *Chronobiology International* 20: 989–999.
37. Sekaran, S., Foster, R. G., Lucas, R. J. & Hankins, M. W. (2003). Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Current Biology* 13: 1290–1298.
38. Hannibal, J., Hindersson, P., Østergaard, J., Georg, B., Heegaard, S., Larsen, P. J. & 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.

39. Belenky, M. A., Smeraski, C. A., Provencio, I., Sollars, P. J. & Pickard, G. E. (2003). Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *Journal of Comparative Neurology* 460: 380–393.
40. Semo, M., Peirson, S., Lupi, D., Lucas, R. J., Jeffery, G. & 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.
41. Semo, M., Lupi, D., Peirson, S. N., Butler, J. N. & 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.
42. Hattar, S., Lucas, R. J., Mrosovsky, N., Thompson, S., Douglas, R. H., Hankins, M. W., Lem, J., Biel, M., Hofmann, F., Foster, R. G. & Yau, K. W. (2003). Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* 424: 76–81.
43. Panda, S., Provencio, I., Tu, D. C., Pires, S. S., Rollag, M. D., Castrucci, A. M., Pletcher, M. T., Sato, T. K., Wiltshire, T., Andahazy, M., Kay, S. A., Van Gelder, R. N. & Hogenesch, J. B. (2003). Melanopsin is required for non-image-forming photic responses in blind mice. *Science* 301: 525–527.
44. Tarttelin, E. E., Bellingham, J., Hankins, M. W., Foster, R. G. & Lucas, R. J. (2003). Neuropsin (Opn5): a novel opsin identified in mammalian neural tissue. *FEBS Letters* 554: 410–416.
45. Peirson, S. N., Bovee-Geurts, P. H. M., Lupi, D., Jeffery, G., DeGrip, W. J. & Foster, R. G. (2004). Expression of the candidate circadian photopigment melanopsin (*Opn4*) in the mouse retinal pigment epithelium. *Molecular Brain Research* 123: 132–135.
46. Chaurasia, S. S., Rollag, M. D., Jiang, G., Hayes, W. P., Haque, R., Natesan, A., Zatz, M., Tosini, G., Liu, C., Korf, H. W., Iuvone, P. M. & Provencio, I. (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.
47. Rowe, M. H. (2002). Trichromatic color vision in primates. *News in Physiological Sciences* 17: 93–98.
48. Jacobs, G. H., Neitz, J. & Deegan, J. F. (1991). Retinal receptors in rodents maximally sensitive to ultraviolet light. *Nature* 353: 655–656.
49. Lyubarsky, A. L., Falsini, B., Pennesi, M. E., Valentini, P. & 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.
50. McGuire, R. A., Rand, W. M. & Wurtman, R. J. (1973). Entrainment of the body temperature rhythm in rats: effect of color and intensity of environmental light. *Science* 181: 956–957.
51. Provencio, I. & Foster, R. G. (1995). Circadian rhythms in mice can be regulated by photoreceptors with cone-like characteristics. *Brain Research* 694: 183–190.
52. Von Schantz, M., Argamaso-Hernan, S. M., Szél, A. & Foster, R. G. (1997). Photopigments and photoentrainment in the Syrian golden hamster. *Brain Research* 770: 131–138.
53. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & 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.
54. 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.
55. Hut, R. A., Scheper, A. & 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.
56. Refinetti, R. (2003). Effects of prolonged exposure to darkness on circadian photic responsiveness in the mouse. *Chronobiology International* 20: 417–440.
57. Yoshimura, T. & 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.
58. Lucas, R. J., Douglas, R. H. & Foster, R. G. (2001). Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nature Neuroscience* 4: 621–626.
59. Hankins, M. W. & Lucas, R. J. (2002). The primary visual pathway in humans is regulated according to long-term light exposure through the action of a non-classical photopigment. *Current Biology* 12: 191–198.
60. Skene, D. J., Lockley, S. W., Thapan, K. & Arendt, J. (1999). Effects of light on human circadian rhythms. *Reproduction Nutrition Development* 39: 295–304.
61. Brainard, G. C., Hanifin, J. P., Greeson, J. M., Byrne, B., Glickman, G., Gerner, E. & Rollag, M. D. (2001). Action spectrum for melatonin regulation in humans: evidence for a novel circadian photoreceptor. *Journal of Neuroscience* 21: 6405–6412.
62. Lack, L. & Wright, H. (2003). Shorter wavelength light is more effective for changing melatonin phase. *Chronobiology International* 20: 1179–1181.
63. Warman, V. L., Dijk, D. J., Warman, G. R., Arendt, J. & Skene, D. J. (2003). Phase advancing human circadian rhythms with short wavelength light. *Neuroscience Letters* 342: 37–40.
64. Lockley, S. W., Brainard, G. C. & 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.
65. Wright, H. R., Lack, L. C. & Kennaway, D. J. (2004). Differential effects of light wavelength in phase advancing the melatonin rhythm. *Journal of Pineal Research* 36: 140–144.

66. Takahashi, J. S., DeCoursey, P. J., Mauman, L. & Menaker, M. (1984). Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* 308: 186–188.
67. Nelson, D. E. & 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.
68. Sakamoto, K., Liu, C. & Tosini, G. (2004). Classical photoreceptors regulate *melanopsin* mRNA levels in the rat retina. *Journal of Neuroscience* 24: 9693–9697.
69. Menaker, M. (1968). Extraretinal light perception in the sparrow. I. Entrainment of the biological clock. *Proceedings of the National Academy of Sciences U.S.A.* 59: 414–421.
70. McMillan, J. P., Keatts, H. C. & 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.
71. Oshima, I., Yamada, H., Goto, M., Sato, K. & 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.
72. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
73. Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J. C. & Stanewsky, R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30: 249–261.
74. Rieger, D., Stanewsky, R. & 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.
75. Foster, R. G. & Menaker, M. (1993). Circadian photoreception in mammals and other vertebrates. In: Wetterberg, L. (Ed.). *Light and Biological Rhythms in Man*. Oxford, UK: Pergamon, pp. 73–91.
76. Underwood, H. (2001). Circadian organization in nonmammalian vertebrates. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 111–140.
77. Bertolucci, C. & Foà, A. (2004). Extraocular photoreception and circadian entrainment in nonmammalian vertebrates. *Chronobiology International* 21: 501–519.
78. Pasqualetti, M., Bertolucci, C., Ori, M., Innocenti, A., Magnone, M. C., De Grip, W. J., Nardi, I. & Foà, A. (2003). Identification of circadian brain photoreceptors mediating photic entrainment of behavioural rhythms in lizards. *European Journal of Neuroscience* 18: 364–372.
79. Leproult, R., Van Reeth, O., Byrne, M. M., Sturis, J. & 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.
80. Shanahan, T. L., Kronauer, R. E., Duffy, J. F., Williams, G. H. & 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.
81. Zeitzer, J. M., Dijk, D. J., Kronauer, R. E., Brown, E. N. & Czeisler, C. A. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *Journal of Physiology* 526: 695–702.
82. Hébert, M., Martin, S. K., Lee, C. & 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.
83. Glickman, G., Hanifin, J. P., Rollag, M. D., Wang, J., Cooper, H. & 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.
84. Rüger, M., Gordijn, M. C. M., Beersma, D. G. M., de Vries, B. & Daan, S. (2003). Acute and phase-shifting effects of ocular and extraocular light in human circadian physiology. *Journal of Biological Rhythms* 18: 409–419.
85. Smith, K. A., Schoen, M. W. & Czeisler, C. A. (2004). Adaptation of human pineal melatonin suppression by recent photic history. *Journal of Clinical Endocrinology and Metabolism* 89: 3610–3614.
86. Lewy, A. J., Wehr, T. A., Goodwin, F. K., Newsome, D. A. & Markey, S. P. (1980). Light suppresses melatonin secretion in humans. *Science* 210: 1267–1269.
87. Cagnacci, A., Soldani, R. & 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.
88. Lavoie, S., Paquet, J., Selmaoui, B., Rufiange, M. & Dumont, M. (2003). Vigilance levels during and after bright light exposure in the first half of the night. *Chronobiology International* 20: 1019–1038.
89. Maywood, E. S., Hastings, M. H., Max, M., Ampleford, E., Menaker, M. & 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.
90. Lucas, R. J., Freedman, M. S., Muñoz, M., Garcia-Fernández, J. M. & Foster, R. G. (1999). Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* 284: 505–507.
91. Nakahara, D., Nakamura, M., Iigo, M. & Okamura, H. (2003). Bimodal circadian secretion of melatonin from the pineal gland in a living CBA mouse. *Proceedings of the National Academy of Sciences U.S.A.* 100: 9584–9589.
92. Kennaway, D. J., Voultsios, A., Varcoe, T. J. & Moyer, R. W. (2002). Melatonin in mice: rhythms, response to light, adrenergic stimulation, and metabolism. *American Journal of Physiology* 282: R358–R365.

93. Bronstein, D. M., Jacobs, G. H., Haak, K. A., Neitz, J. & 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.
94. Bayarri, M. J., Muñoz-Cueto, J. A., López-Olmeda, J. F., Vera, L. M., Rol-de-Lama, M. A., Madrid, J. A. & Sánchez-Vázquez, F. J. (2004). Daily locomotor activity and melatonin rhythms in Senegal sole (*Solea senegalensis*). *Physiology and Behavior* 81: 577–583.
95. Masuda, T., Iigo, M., Mizusawa, K., Naruse, M., Oishi, T., Aida, K. & 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.
96. Hasegawa, M. & Ebihara, S. (1992). Circadian rhythms of pineal melatonin release in the pigeon measured by *in vivo* microdialysis. *Neuroscience Letters* 148: 89–92.
97. Torres, G. & 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.
98. Menaker, M., Moreira, L. F. & Tosini, G. (1997). Evolution of circadian organization in vertebrates. *Brazilian Journal of Medical and Biological Research* 30: 305–313.
99. Kasahara, T., Okano, T., Haga, T. & Fukada, Y. (2002). Opsin-G_{11}-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *Journal of Neuroscience* 22: 7321–7325.
100. Okano, T., Yoshizawa, T. & Fukada, Y. (1994). Pinopsin is a chicken pineal photoreceptive molecule. *Nature* 372: 94–97.
101. Max, M., McKinnon, P. J., Seidenman, K. J., Barrett, R. K., Applebury, M. L., Takahashi, J. S. & Margolskee, R. F. (1995). Pineal opsin: a nonvisual opsin expressed in chick pineal. *Science* 267: 1502–1506.
102. Okano, T., Takanaka, Y., Nakamura, A., Hirunagi, K., Adachi, A., Ebihara, S. & Fukada, Y. (1997). Immunocytochemical identification of pinopsin in pineal glands of chicken and pigeon. *Molecular Brain Research* 50: 190–196.
103. Campbell, S. S. & Murphy, P. J. (1998). Extraocular circadian phototransduction in humans. *Science* 279: 396–399.
104. Murphy, P. J. & Campbell, S. S. (2001). Enhancement of REM sleep during extraocular light exposure in humans. *American Journal of Physiology* 280: R1606–R1612.
105. Hébert, M., Martin, S. K. & Eastman, C. I. (1999). Nocturnal melatonin secretion is not suppressed by light exposure behind the knee in humans. *Neuroscience Letters* 274: 127–130.
106. Lindblom, N., Heiskala, H., Hätönen, T., Mustanoja, S., Alfthan, H., Alila-Johansson, A. & Laakso, M. L. (2000). No evidence for extraocular light induced phase shifting of human melatonin, cortisol and thyrotropin rhythms. *NeuroReport* 11: 713–717.
107. Jean-Louis, G., Kripke, D. F., Cole, R. J. & Elliott, J. A. (2000). No melatonin suppression by illumination of popliteal fossae or eyelids. *Journal of Biological Rhythms* 15: 265–269.
108. Lushington, K., Galka, R., Sassi, L. N., Kennaway, D. J. & Dawson, D. (2002). Extraocular light exposure does not phase shift saliva melatonin rhythms in sleeping subjects. *Journal of Biological Rhythms* 17: 377–386.
109. Wright, K. P. & Czeisler, C. A. (2002). Absence of circadian phase resetting in response to bright light behind the knees. *Science* 297: 571.
110. Meijer, J. H., Thio, B., Albus, H., Schaap, J. & Ruijs, A. C. J. (1999). Functional absence of extraocular photoreception in hamster circadian rhythm entrainment. *Brain Research* 831: 337–339.
111. Chono, K., Fujito, Y. & Ito, E. (2002). Non-ocular dermal photoreception in the pond snail *Lymnaea stagnalis*. *Brain Research* 951: 107–112.
112. Moriya, T., Miyashita, Y., Arai, J., Kusunoki, S., Abe, M. & 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.
113. Tosini, G. & Avery, R. A. (1996). Dermal photoreceptors regulate basking behavior in the lizard *Podarcis muralis*. *Physiology and Behavior* 59: 195–198.
114. Ebaugh, F. G. & Thauer, R. (1950). Influence of various environmental temperatures on the cold and warmth thresholds. *Journal of Applied Physiology* 3: 173–182.
115. Gray, L., Stevens, J. C. & Marks, L. E. (1982). Thermal stimulus thresholds: sources of variability. *Physiology and Behavior* 29: 355–360.
116. Stevens, J. C. & Stevens, S. S. (1960). Warmth and cold: dynamics of sensory intensity. *Journal of Experimental Psychology* 60: 183–192.
117. Stevens, S. S. (1965). Matching functions between loudness and ten other continua. *Perception and Psychophysics* 1: 5–8.
118. Banks, W. P. (1969). Temperature sensitivity: one subjective continuum or two? *Perception and Psychophysics* 6: 189–192.
119. Green, B. G. (1984). Thermal perception on lingual and labial skin. *Perception and Psychophysics* 36: 209–220.
120. Green, B. G. (1977). Localization of thermal sensation: an illusion and synthetic heat. *Perception and Psychophysics* 22: 331–337.
121. 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.
122. Refinetti, R. (1990). Is there modal opponency in the thermal senses? *Journal of Thermal Biology* 15: 255–258.
123. Adrian, E. D. & 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.

124. Zotterman, Y. (1935). Action potentials in the glossopharyngeal nerve and in the chorda tympani. *Skandinavishes Archiv für Physiologie* 72: 73–77.
125. Iriuchijima, J. & Zotterman, Y. (1960). The specificity of afferent cutaneous C fibres in mammals. *Acta Physiologica Scandinavica* 49: 267–278.
126. Iggo, A. (1969). Cutaneous thermoreceptors in primates and sub-primates. *Journal of Physiology* 200: 403–430.
127. Hellon, R. F., Hensel, H. & Schäfer, K. (1975). Thermal receptors in the scrotum of the rat. *Journal of Physiology* 248: 349–357.
128. Hensel, H. (1953). Das Verhalten der Thermoreceptoren bei Temperatursprüngen. *Pflügers Archiv* 256: 470–487.
129. Hensel, H. & Witt, I. (1959). Spatial temperature gradient and thermoreceptor stimulation. *Journal of Physiology* 148: 180–187.
130. Hensel, H., Iggo, A. & Witt, I. (1960). A quantitative study of sensitive cutaneous thermoreceptors with C afferent fibres. *Journal of Physiology* 153: 113–126.
131. Martin, H. F. & Manning, J. W. (1969). Rapid thermal cutaneous stimulation: peripheral nerve responses. *Brain Research* 16: 524–526.
132. Hensel, H. (1968). Spezifische Wärmeimpulse aus der Nasenregion der Katze. *Pflügers Archiv* 302: 374–376.
133. Hensel, H. & Wurster, R. D. (1969). Static behaviour of cold receptors in the trigeminal area. *Pflügers Archiv* 313: 153–154.
134. Hensel, H. & Kenshalo, D. R. (1969). Warm receptors in the nasal region of cats. *Journal of Physiology* 204: 99–112.
135. Kenshalo, D. R. & Brearley, E. A. (1970). Eletrophysiological measurements of the sensitivity of cat's upper lip to warm and cool stimuli. *Journal of Comparative and Physiological Psychology* 70: 5–14.
136. Hensel, H., Andres, K. H. & von Düring, M. (1974). Structure and function of cold receptors. *Pflügers Archiv* 352: 1–10.
137. Duclaux, R., Schäfer, K. & Hensel, H. (1980). Response of cold receptors to low skin temperatures in nose of cat. *Journal of Neurophysiology* 43: 1571–1577.
138. Ivanov, K., Konstantinov, V. & Danilova, N. (1982). Thermoreceptor localization in the deep and surface skin layers. *Journal of Thermal Biology* 7: 75–78.
139. Ivanov, K., Konstantinov, V., Danilova, N., Sleptchuck, N., & Rumiantsev, G. (1986). Thermoreceptor distribution in different skin layers and its significance for thermoregulation. *Journal of Thermal Biology* 11: 25–29.
140. Iggo, A. (1964). Temperature discrimination in the skin. *Nature* 204: 481–483.
141. Kenshalo, D. R. & Gallegos, E. S. (1967). Multiple temperature-sensitive spots innervated by single nerve fibers. *Science* 158: 1064–1065.
142. Hensel, H. (1969). Cutane Wärmereceptoren bei Primaten. *Pflügers Archiv* 313: 150–152.
143. Poulos, D. A. & Lende, R. A. (1970). Response of trigeminal ganglion neurons to thermal stimulation of oral-facial regions. *Journal of Neurophysiology* 33: 508–517.
144. Hensel, H. & Iggo, A. (1971). Analysis of cutaneous warm and cold fibres in primates. *Pflügers Archiv* 329: 1–8.
145. Kenshalo, D. R. & Duclaux, R. (1977). Response characteristics of cutaneous cold receptors in the monkey. *Journal of Neurophysiology* 40: 319–332.
146. Darian-Smith, I., Johnson, K. O., LaMotte, C., Shigenaga, Y., Kenins, P. & Champness, P. (1979). Warm fibers innervating palmar and digital skin of the monkey: responses to thermal stimuli. *Journal of Neurophysiology* 42: 1297–1315.
147. Duclaux, R. & Senshalo, D. R. (1980). Response characteristics of cutaneous warm receptors in the monkey. *Journal of Neurophysiology* 43: 1–15.
148. LaMotte, R. H. & Thalhammer, J. G. (1982). Response properties of high-threshold cutaneous cold receptors in the primate. *Brain Research* 244: 279–287.
149. Hensel, H. & Boman, K. K. A. (1960). Afferent impulses in cutaneous sensory nerves in human subjects. *Journal of Neurophysiology* 23: 564–578.
150. Konietzny, F. & Hensel, H. (1975). Warm fiber activity in human skin nerves. *Pflügers Archiv* 359: 265–267.
151. Konietzny, F. & Hensel, H. (1977). The dynamic response of warm units in human skin nerves. *Pflügers Archiv* 370: 111–114.
152. Hensel, H. (1983). Recent advances in thermoreceptor physiology. *Journal of Thermal Biology* 8: 3–6.
153. Pierau, F. K. (1996). Peripheral thermosensors. In: Fregly, M. J. & Blatteis, C. M. (Eds.). *Handbook of Physiology, Section 4: Environmental Physiology*, Volume 1. New York: Oxford University Press, pp. 85–104.
154. Caterina, M. J., Leffler, A., Malmberg, A. B., Martin, W. J., Trafton, J., Petersen-Zeitz, K. R., Koltzenburg, M., Basbaum, A. I. & Julius, D. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288: 306–313.
155. Peier, A. M., Reeve, A. J., Andersson, D. A., Moqrich, A., Earley, T. J., Hergarden, A. C., Story, G. M., Colley, S., Hogenesch, J. B., McIntyre, P., Bevan, S. & Patapoutian, A. (2002). A heat-sensitive TRP channel expressed in keratinocytes. *Science* 296: 2046–2049.
156. McKemy, D. D., Neuhausser, W. M. & Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416: 52–58.
157. Brück, K. & Hinckel, P. (1990). Thermoafferent networks and their adaptive modifications. In: Schönbaum, E. & Lomax, P. (Eds.). *Thermoregulation: Physiology and Biochemistry.* New York: Pergamon, pp. 129–152.
158. Magoun, H. W., Harrison, F., Brobeck, J. R. & Ranson, S. W. (1938). Activation of heat loss mechanisms by local heating of the brain. *Journal of Neurophysiology* 1: 101–114.
159. Folkow, B., Ström, G. & Uvnäs, B. (1949). Cutaneous vasodilatation elicited by local heating of the anterior hypothalamus in cats and dogs. *Acta Physiologica Scandinavica* 17: 317–326.

160. Forster, R. E. & Ferguson, T. B. (1952). Relationship between hypothalamic temperature and thermoregulatory effectors in unanesthetized cat. *American Journal of Physiology* 169: 255–269.
161. Chatonnet, J., Tanche, M. & 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.
162. Hammel, H. T., Hardy, J. D. & Fusco, M. M. (1960). Thermoregulatory responses to hypothalamic cooling in unanesthetized dogs. *American Journal of Physiology* 198: 481–486.
163. Fusco, M. M., Hardy, J. D. & Hammel, H. T. (1961). Interaction of central and peripheral factors in physiological temperature regulation. *American Journal of Physiology* 200: 572–580.
164. Satinoff, E. (1964). Behavioral thermoregulation in response to local cooling of the rat brain. *American Journal of Physiology* 206: 1389–1394.
165. Carlisle, H. J. (1966). Behavioural significance of hypothalamic temperature-sensitive cells. *Nature* 209: 1324–1325.
166. 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.
167. Cabanac, M. & 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.
168. Roberts, W. W., Bergquist, E. H. & 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.
169. Adair, E. R. (1970). Control of thermoregulatory behavior by brief displacements of hypothalamic temperature. *Psychonomic Science* 20: 11–13.
170. Adair, E. R., Casby, J. U. & 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.
171. Gale, C. C., Mathews, M. & Young, J. (1970). Behavioral thermoregulatory responses to hypothalamic cooling and warming in baboons. *Physiology and Behavior* 5: 1–6.
172. Mills, S. H. & 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.
173. Adair, E. R. & Rawson, R. O. (1974). Autonomic and behavioral temperature regulation: unilateral vs. bilateral preoptic thermal stimulation. *Pflügers Archiv* 352: 91–103.
174. Fuller, C. A., Horwitz, B. A. & Horowitz, J. M. (1975). Shivering and nonshivering thermogenic responses of cold-exposed rats to hypothalamic warming. *American Journal of Physiology* 228: 1519–1524.
175. McEwen, G. N. & Heath, J. E. (1975). Thermal responses to preoptic heating and ambient temperature in unrestrained rabbits. *Journal of Thermal Biology* 1: 23–27.
176. Simon, E., Simon-Oppermann, C., Hammel, H. T., Kaul, R. & Maggert, J. (1976). Effects of altering rostral brain stem temperature on temperature regulation in the Adelie penguin, *Pygoscelis adeliae*. *Pflügers Archiv* 362: 7–13.
177. Banet, M. & Hensel, H. (1976). Nonshivering thermogenesis induced by repetitive hypothalamic cooling in the rat. *American Journal of Physiology* 230: 522–526.
178. Schmidt, I. (1976). Effect of central thermal stimulation on the thermoregulatory behavior of the pigeon. *Pflügers Archiv* 363: 271–272.
179. Simon-Oppermann, C., Simon, E., Jessen, C. & Hammel, H. T. (1978). Hypothalamic thermosensitivity in conscious Pekin ducks. *American Journal of Physiology* 235: R130–R140.
180. Carlisle, H. J. & Laudenslager, M. L. (1979). Observations on the thermoregulatory effects of preoptic warming in rats. *Physiology and Behavior* 23: 723–732.
181. Schmidt, I. & Simon, E. (1982). Negative and positive feedback of central nervous system temperature in thermoregulation of pigeons. *American Journal of Physiology* 243: R363–R372.
182. Cabanac, M. & Dib, B. (1983). Behavioural responses to hypothalamic cooling and heating in the rat. *Brain Research* 264: 79–87.
183. Kanosue, K., Nakayama, T., Tanaka, H., Yanase, M. & Yasuda, H. (1990). Modes of action of local hypothalamic and skin thermal stimulation on salivary secretion in rats. *Journal of Physiology* 424: 459–471.
184. Klir, J. J. & 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.
185. Kanosue, K., Chen, X. M., Hosono, T., Yoda, T. & Liu, S. (1999). Inhibitory control of shivering and nonshivering thermogenesis by preoptic warm-sensitive neurons. *Journal of Thermal Biology* 24: 351–354.
186. Wünnenberg, W. & Brück, K. (1968). Zur Funktionsweise thermoreceptiver Strukturen im Cervicalmark des Meerschweinchens. *Pflügers Archiv* 299: 1–10.
187. Jessen, C. & 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.
188. Rautenberg, W., Necker, R. & May, B. (1972). Thermoregulatory responses of the pigeon to changes of the brain and spinal cord temperatures. *Pflügers Archiv* 338: 31–42.
189. Chai, C. Y. & 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.

190. Carlisle, H. J. & 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.
191. Banet, M. & 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.
192. Graf, R. (1980). Diurnal changes of thermoregulatory functions in pigeons. II. Spinal thermosensitivity. *Pflügers Archiv* 386: 181–185.
193. Avery, P. & Richards, S. A. (1983). Thermosensitivity of the hypothalamus and spinal cord in the domestic fowl. *Journal of Thermal Biology* 8: 237–239.
194. Wünnenberg, W. (1983). Thermosensitivity of the preoptic region and the spinal cord in the golden hamster. *Journal of Thermal Biology* 8: 381–384.
195. Barnas, G. M., Gleeson, M., Nomoto, S., & 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.
196. Graf, R., Heller, H. C., Sakaguchi, S. & Krishna, S. (1987). Influence of spinal and hypothalamic warming on metabolism and sleep in pigeons. *American Journal of Physiology* 252: R661–R667.
197. Rawson, R. O. & Quick, K. P. (1970). Evidence of deep-body thermoreceptor response to intra-abdominal heating of the ewe. *Journal of Applied Physiology* 28: 813–820.
198. Riedel, W., Siaplauras, G. & Simon, E. (1973). Intra-abdominal thermosensitivity in the rabbit as compared with spinal thermosensitivity. *Pflügers Archiv* 340: 59–70.
199. Jessen, C. & Feistkorn, G. (1984). Some characteristics of core temperature signals in the conscious goat. *American Journal of Physiology* 247: R456–R464.
200. Merver, J. B. & Simon, E. (1984). A comparison between total body thermosensitivity and local thermosensitivity in mammals and birds. *Pflügers Archiv* 400: 228–234.
201. Simon, E., Pierau, F. K. & Taylor, D. C. M. (1986). Central and peripheral thermal control of effectors in homeothermic temperature regulation. *Physiological Reviews* 66: 235–300.
202. Adair, E. R. (1974). Hypothalamic control of thermoregulatory behavior: preoptic-posterior hypothalamic interaction. In: Lederis, K. & Cooper, K. E. (Eds.). *Recent Studies of Hypothalamic Function*. Basel: Karger, pp. 341–358.
203. Refinetti, R. & Carlisle, H. J. (1986). Effects of anterior and posterior hypothalamic temperature changes on thermoregulation in the rat. *Physiology and Behavior* 36: 1099–1103.
204. Tanaka, H., Kanosue, K., Nakayama, T. & 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.
205. 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.
206. Satinoff, E., Valentino, D. & Teitelbaum, P. (1976). Thermoregulatory cold-defense deficits in rats with preoptic/anterior hypothalamic lesions. *Brain Research Bulletin* 1: 553–565.
207. Toth, D. M. (1973). Temperature regulation and salivation following preoptic lesions in the rat. *Journal of Comparative and Physiological Psychology* 82: 480–488.
208. Morimoto, A. & Murakami, N. (1985). [^{14}C]deoxyglucose incorporation into rat brain regions during hypothalamic or peripheral thermal stimulation. *American Journal of Physiology* 248: R84–R92.
209. Gilbert, T. M. & Blatteis, C. M. (1977). Hypothalamic thermoregulatory pathways in the rat. *Journal of Applied Physiology* 43: 770–777.
210. Han, P. W. & Brobeck, J. R. (1961). Deficits of temperature regulation in rats with hypothalamic lesions. *American Journal of Physiology* 200: 707–710.
211. Briese, E. (1989). Do medial preoptic lesions interfere with the set-point of temperature regulation? *Brain Research Bulletin* 23: 137–144.
212. Lipton, J. M. (1968). Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiology and Behavior* 3: 165–169.
213. Joyce, M. P. & 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.
214. Ishiwata, T., Hasegawa, H., Yasumatsu, M., Akano, F., Yazawa, T., Otokawa, M. & 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.
215. Bratincsák, A. & Palkovits, M. (2004). Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience* 127: 385–397.
216. Satinoff, E. (1983). Disturbances in circadian body temperature rhythms in rats with hypothalamic damage. *Journal of Thermal Biology* 8: 149–157.
217. Ott, I. (1887). The heat-center in the brain. *Journal of Nervous and Mental Disease* 14: 152–162.
218. Keller, A. D. & Hare, W. K. (1932). The hypothalamus and heat regulation. *Proceedings of the Society for Experimental Biology and Medicine* 29: 1069–1070.
219. 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.
220. Hori, T., Kiyohara, T., Oomura, Y., Nishino, H., Aou, S. & Fujita, I. (1987). Activity of thermosensitive neurons of monkey preoptic hypothalamus during thermoregulatory operant behavior. *Brain Research Bulletin* 18: 649–655.
221. Hagan, A. A. & Heath, J. E. (1980). Effects of preoptic lesions on thermoregulation in ducks. *Journal of Thermal Biology* 5: 141–150.

222. Lepkovsky, S., Snapir, N. & Furuta, F. (1968). Temperature regulation and appetitive behavior in chickens with hypothalamic lesions. *Physiology and Behavior* 3: 911–915.
223. Eissel, K. & 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.
224. Nakashima, T., Pierau, F. K., Simon, E. & Hori, T. (1987). Comparison between hypothalamic thermoresponsive neurons from duck and rat slices. *Pflügers Archiv* 409: 236–243.
225. Wünnenberg, W., Merker, G. & Speulda, E. (1976). Thermosensitivity of preoptic neurones in a hibernator (golden hamster) an a non-hibernator (guinea pig). *Pflügers Archiv* 363: 119–123.
226. Boulant, J. A. & Bignall, K. E. (1973). Determinants of hypothalamic neuronal thermosensitivity in ground squirrels and rats. *American Journal of Physiology* 225: 306–310.
227. Vasilenko, V. Y. & Gourine, V. N. (1992). Threshold temperature responses of thermosensitive neurons in guinea-pig hypothalamic slices. *Journal of Thermal Biology* 17: 367–374.
228. Glotzbach, S. F. & Heller, H. C. (1984). Changes in the thermal characteristics of hypothalamic neurons during sleep and wakefulness. *Brain Research* 309: 17–26.
229. Jahns, R. & Werner, J. (1974). Special aspects of firing rates and thermosensitivity of preoptic neurons. *Experimental Brain Research* 21: 107–112.
230. Silva, N. L. & Boulant, J. A. (1984). Effects of osmotic pressure, glucose, and temperature on neurons in preoptic tissue slices. *American Journal of Physiology* 247: R335–R345.
231. Baldino, F. & Geller, H. M. (1982). Electrophysiological analysis of neuronal thermosensitivity in rat preoptic and hypothalamic tissue cultures. *Journal of Physiology* 327: 173–184.
232. Dean, J. B. & Boulant, J. A. (1989). *In vitro* localization of thermosensitive neurons in the rat diencephalon. *American Journal of Physiology* 257: R57–R64.
233. Dean, J. B. & Boulant, J. A. (1989). Effects of synaptic blockade on thermosensitive neurons in rat diencephalon *in vitro*. *American Journal of Physiology* 257: R65–R73.
234. Kelso, S. R. & Boulant, J. A. (1982). Effect of synaptic blockade on thermosensitive neurons in hypothalamic tissue slices. *American Journal of Physiology* 243: R480–R490.
235. Kelso, S. R., Perlmutter, M. N. & Boulant, J. A. (1982). Thermosensitive single-unit activity of *in vitro* hypothalamic slices. *American Journal of Physiology* 242: R77–R84.
236. Sato, H. (1984). Midbrain neurons of rats responsive to hypothalamic temperature change and their local thermosensitivity. *Journal of Thermal Biology* 9: 39–45.
237. Parmeggiani, P. L., Azzaroni, A., Cevolani, D. & Ferrari, G. (1983). Responses of anterior hypothalamic-preoptic neurons to direct thermal stimulation during wakefulness and sleep. *Brain Research* 269: 382–385.
238. Nakayama, T., Eisenman, J. S. & Hardy, J. D. (1961). Single unit activity of anterior hypothalamus during local heating. *Science* 134: 560–561.
239. Eisenman, J. S. & Jackson, D. C. (1967). Thermal response patterns of septal and preoptic neurons in cats. *Experimental Neurology* 19: 33–45.
240. Hardy, J. D., Hellon, R. F. & Sutherland, K. (1964). Temperature-sensitive neurones in the dog's hypothalamus. *Journal of Physiology* 175: 242–253.
241. Cabanac, M., Stolwijl, J. A. J. & 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.
242. Nakayama, T. & 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.
243. 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.
244. Greer, G. L. & Gardner, D. R. (1970). Temperature-sensitive neurons in the brain of brook trout. *Science* 169: 1220–1222.
245. Nelson, D. O. & Prosser, C. L. (1981). Intracellular recordings from thermosensitive preoptic neurons. *Science* 213: 787–789.
246. Cabanac, M., Hammel, T. & Hardy, J. D. (1967). Tiliqua scincoides: temperature-sensitive units in lizard brain. *Science* 158: 1050–1051.
247. Derambure, P. S. & Boulant, J. A. (1994). Circadian thermosensitive characteristics of suprachiasmatic neurons *in vitro*. *American Journal of Physiology* 266: R1876–R1884.
248. Ruby, N. F., Burns, D. E. & 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.
249. Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment*, 5th Edition. New York: Cambridge University Press.
250. Anand, B. K. & 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.
251. Hetherington, A. W. & Ranson, S. W. (1940). Hypothalamic lesions and adiposity in the rat. *Anatomical Record* 78: 149–172.
252. Strubbe, J. H. (1994). Regulation of food intake. In: Westerterp-Plantenga, M. S., Fredrix, E. W. H. M. & Steffens, A. B. (Eds.). *Food Intake and Energy Expenditure*. Boca Raton, FL: CRC Press, pp. 141–154.
253. Woods, S. C., Seeley, R. J., Porte, D. & Schwartz, M. W. (1998). Signals that regulate food intake and energy homeostasis. *Science* 280: 1378–1383.
254. Funahashi, H., Takenoys, F., Guan, J. L., Kageyama, H., Yada, T. & Shioda, S. (2003). Hypothalamic neuronal networks and feeding-related peptides involved in the regulation of feeding. *Anatomical Science International* 78: 123–138.

255. Kreier, F., Kalsbeek, A., Ruiter, M., Yilmaz, A., Romijn, J. A., Sauerwein, H. P., Fliers, E. & 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.
256. Zhang, Y., Proença, R., Maffei, M., Barone, M., Leopold, L. & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425–432.
257. 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. & Kuijper, J. L. (1995). Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. *Journal of Clinical Investigation* 96: 2065–2070.
258. Inoue, S., Satoh, S., Saito, M., Naitoh, M., Suzuki, H. & 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.
259. 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.
260. Davis, J. D., Wirtshafter, D., Asin, K. E. & Brief, D. (1981). Sustained intracerebroventricular infusion of brain fuels reduces body weight and food intake in rats. *Science* 212: 81–82.
261. Boulant, J. A. & Silva, N. L. (1988). Neuronal sensitivities in preoptic tissue slices: interactions among homeostatic systems. *Brain Research Bulletin* 20: 871–878.
262. Ritter, S., Dinh, T. T. & Zhang, Y. (2000). Localization of hindbrain glucoreceptive sites controlling food intake and blood glucose. *Brain Research* 856: 37–47.
263. Phillips, R. J. & Powley, T. L. (1996). Gastric volume rather than nutrient content inhibits food intake. *American Journal of Physiology* 271: R766–R779.
264. Weller, A., Smith, G. P. & Gibbs, J. (1990). Endogenous cholecystokinin reduces feeding in young rats. *Science* 247: 1589–1591.
265. Della-Fera, M. A., Coleman, B. D. & 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.
266. Brüning, J. C., Gautam, D., Burks, D. J., Gillette, J., Schubert, M., Orban, P. C., Klein, R., Krone, W., Müller-Wieland, D. & Kahn, C. R. (2000). Role of brain insulin receptor in control of body weight and reproduction. *Science* 289: 2122–2125.
267. Tschöp, M., Smiley, D. L. & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature* 407: 908–913.
268. St.-Pierre, D. H., Wang, L. & 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.
269. Sánchez-Vásquez, F., Aranda, A. & 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.
270. Stephan, F. K. (1997). Calories affect zeitgeber properties of the feeding entrained circadian oscillator. *Physiology and Behavior* 62: 995–1002.

12 Pacemakers

CHAPTER OUTLINE

12.1 The Suprachiasmatic Nucleus
12.2 Cellular Processes
12.3 Molecular Processes
12.4 Other Pacemakers

12.1 THE SUPRACHIASMATIC NUCLEUS

If you have been reading this book from the beginning, you know that organisms have a circadian clock — but how do you locate this clock in the body? This question is not easy to answer, especially when dealing with complex organisms such as mammals. The brain controls most bodily functions, so you might want to examine it first. But where in the brain should you look? The brain contains about 100 billion nerve cells, which renders the trial-and-error approach useless. Serendipity may sometimes be the only available path. The discovery of a "pleasure center" in the brain by James Olds fifty years 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. The electrodes were implanted under anesthesia. The goal was to use them to pass minute electrical currents to the brain and simulate the natural electrical activity of nerve cells. Because he was inexperienced at this task, Olds inadvertently implanted the electrodes in the wrong area of the brain; in fact, he placed them on the side of the brain opposite to that intended by his professor. It ruined the professor's study. However, the research showed that direct electrical stimulation of that part of the brain (the *lateral hypothalamus*) resulted in the rats experiencing a high level of pleasure. 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 to reach the lever and stimulate their brains. They would forego food or a female in heat to self-stimulate. By pure luck, Olds seemed to have found the "pleasure center" in the brain. He made one of the first discoveries about neural mechanisms involved in motivation.

Luck and the ability to integrate fragmented pieces of evidence also were involved in the discovery of the anatomical site of the master circadian pacemaker in mammals. 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. 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.

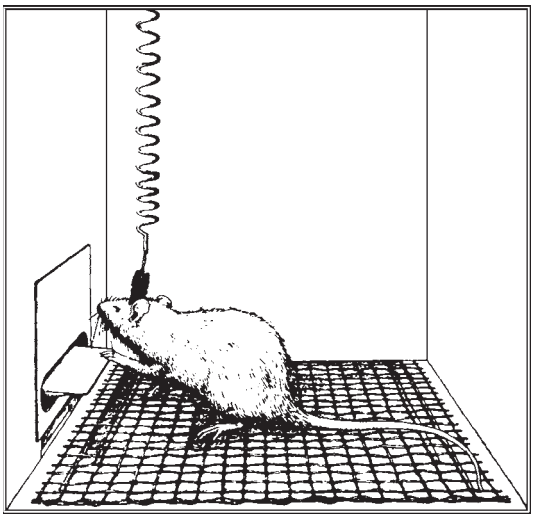


FIGURE 12.1 Having fun. The diagram shows the apparatus used for intracranial self-stimulation. When the rat presses the lever, it triggers a highly pleasurable electric stimulus to the brain. (Source: Adapted from Olds, J. (1956). Pleasure centers in the brain. *Scientific American* 195: 105–116.)

12.1.1 Lesion Studies

In the early 1970s, Friedrich Stephan — whose more recent work was 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, but Zucker's main interest was in circadian rhythms. Zucker's interest prevailed. Stephan's interest in reproductive physiology, however, proved useful. Reproductive physiologists had already discovered that the estrous cycle in rats (the equivalent of the menstrual cycle in humans) is dependent on a small part of the brain — that is, the rostral (frontal) part of the hypothalamus (Figure 12.2). After researchers established that the estrous cycle depended on circadian rhythms,[2] Stephan wondered whether the circadian

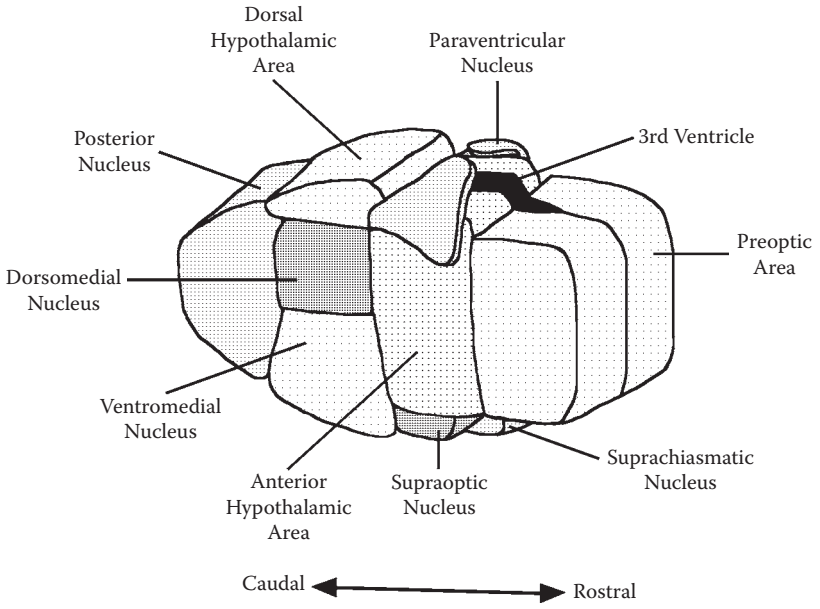


FIGURE 12.2 The hypothalamus. This diagram identifies the various subdivisions of the hypothalamus, a small structure at the base of the brain that controls most vital functions in vertebrates. One major subdivision, the lateral hypothalamic area, is not shown. (Source: Adapted from Kalat, J. W. (2001). *Biological Psychology*, 7th Edition. Belmont, CA: Wadsworth.)

pacemaker might be located in the rostral hypothalamus. The rostral hypothalamus is not that small, however, which made it difficult to find the pacemaker.

The next clue to locating the pacemaker came from research by neuroanatomist Robert Moore, then 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. It was not clear, however, whether a separate pathway was responsible for the entrainment of circadian rhythms. Moore discovered that there was indeed a second path from the retina of the eyes to the rostral hypothalamus; this path accordingly was named the *retino-hypothalamic tract* (see Chapter 13). The retino-hypothalamic tract terminates at the base of the brain at a small bilateral nucleus called the *suprachiasmatic nucleus* (SCN). As shown in Figure 12.3, the SCN sits above the optic chiasm (the crossing of the optic nerves). Note that the SCN is *supra-chiasmatic*, not *supercharismatic* (although circadian physiologists would not object to the characterization of the SCN as charismatic!). Moore's findings gave Stephan a specific area of the brain in which to look for the circadian pacemaker. The SCN had to be the physical substrate of the pacemaker.

If a brain structure houses the biological clock, then destruction of this structure must eliminate biological timing. How can you destroy a minute part of the brain of a living animal without damaging everything around it? This question faced researchers in the late 1800s when they started to systematically study the effects of the destruction of different brain areas on a variety of physiological

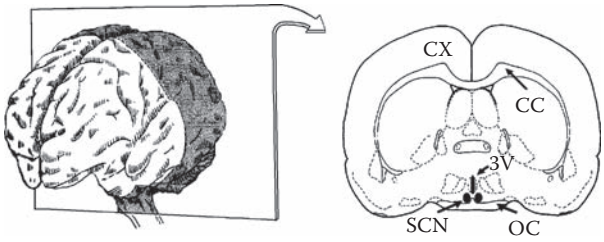


FIGURE 12.3 Locating the suprachiasmatic nucleus. This diagram shows the location of the suprachiasmatic nucleus in a coronal section of the mammalian brain. (Note: 3V = third ventricle, CX = cerebral cortex, CC = corpus callosum, OC = optic chiasm, SCN = suprachiasmatic nucleus)

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 not 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 located 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 a thin wire used to lesion the brain tissue, usually by passage of moderate electric current. Thus, the atlas gives you the location of the brain structure, the stereotaxic instrument allows you to reach the structure without

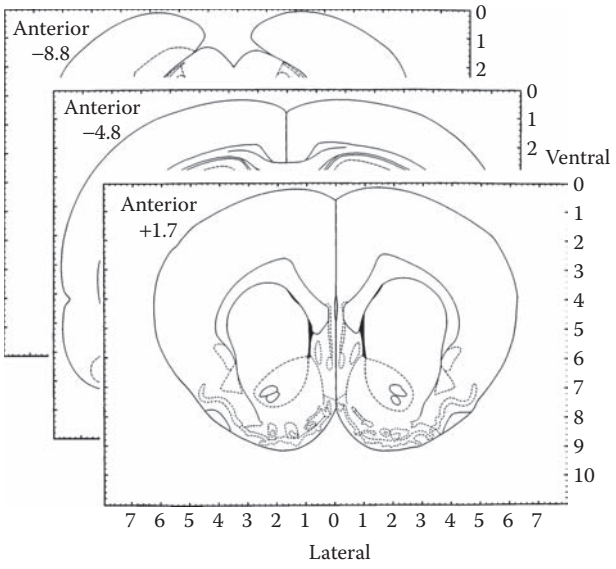


FIGURE 12.4 A stereotaxic atlas. These sample pages from a stereotaxic atlas of the rat brain indicate how brain structures can be located in a coordinate system with three axes: anterior, lateral, and ventral. Measurements in millimeters are given in reference to a skull landmark (bregma). (Source: Adapted from Paxinos, G. and Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates*, 2nd Edition. San Diego, CA: Academic Press.)

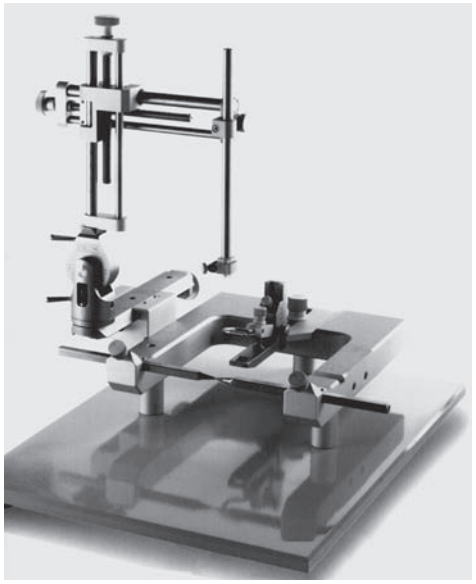


FIGURE 12.5 A sterotaxic instrument. A stereotaxic instrument allows precise access to brain structures through a small hole in the skull. (Source: Image of Stoelting stereotaxic instrument reproduced with permission of Stoelting Co., Wood Dale, IL.)

damaging the surrounding tissue, and the electrode provides the means to lesion the structure.

Using the method described in the previous paragraph, Stephan began studies to locate the circadian pacemaker in the SCN. He eventually discovered that complete lesions of the SCN (but not partial lesions) eliminated the circadian rhythms of behavioral activity and drinking in rats. He had found the physical substrate of the biological clock! He summarized his findings in an article and submitted it to the top science journal, *Science*. The editors of *Science* were not that impressed with his findings, however, and declined to publish his paper. In 1972, however, the *Proceedings of the National Academy of Sciences* published Stephan's article.[3] In that same year, Moore's team also inferred the role of the SCN in circadian rhythmicity and published similar results in *Brain Research*.[4]

Figure 12.6 shows the ventral parts of coronal slices of golden hamster brains without any lesions (A) and with a large lesion that completely destroyed the SCN (B). Lesions of this magnitude have been shown to eliminate the circadian rhythm of activity in lizards,[5–7] birds,[8,9] golden hamsters,[10–16] rats,[17–27] and various other rodents.[28–34] The effects of the procedure 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 also is eliminated by SCN lesions, as shown in Figure 12.8. This finding has been documented in golden hamsters,[16,35,36] Siberian hamsters,[32,33,37] rats,[18–20,23,25,38,39] and ground squirrels.[40] Several researchers, however, reported persistence of the temperature rhythm in animals whose activity rhythm had been eliminated by SCN lesions.[17,31,41–44] Incomplete lesions might have caused the discrepancy; this explanation became plausible 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,[17–19,25,30,45] gnawing,[46,47] heart rate,[23,26,47] blood pressure,[24] dentin increment,[27] intraocular pressure,[48] sleep,[29,38,49,50] and secretion of various hormones.[47,51–55] In contrast, circadian rhythmicity was found *not* to be eliminated by lesions of other hypothalamic and extrahypothalamic sites.[56–63]

12.1.2 Monitoring of SCN Activity

The following anecdote about the legless flea illustrates why lesion studies are not sufficient to settle the issue of the location of the circadian pacemaker:

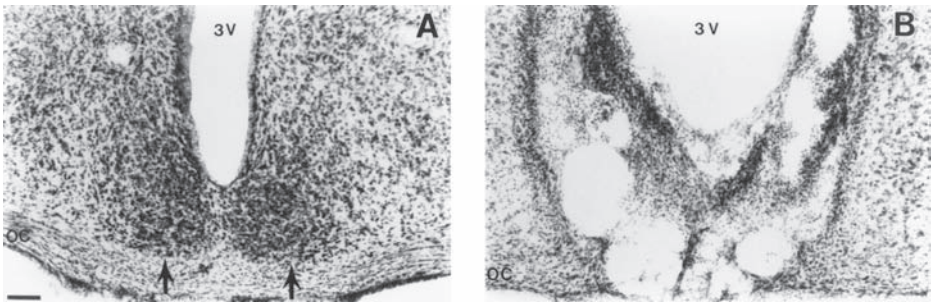


FIGURE 12.6 Lesioning the SCN. These microphotographs of Nissl-stained coronal sections of the brains of two golden hamsters (*Mesocricetus auratus*) show the region that normally contains the suprachiasmatic nuclei (dorsal to the optic chiasm and ventral to the third ventricle). Both nuclei (arrows) are intact in the brain on the left (A). The brain on the right (B) sustained a complete bilateral lesion of the SCN. (Note: 3V = third ventricle and OC = optic chiasm) (Source: Refinetti, R., Kaufman, C. M. & 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. © 1994 Springer Verlag. Reproduced with permission from the publisher and the authors.)

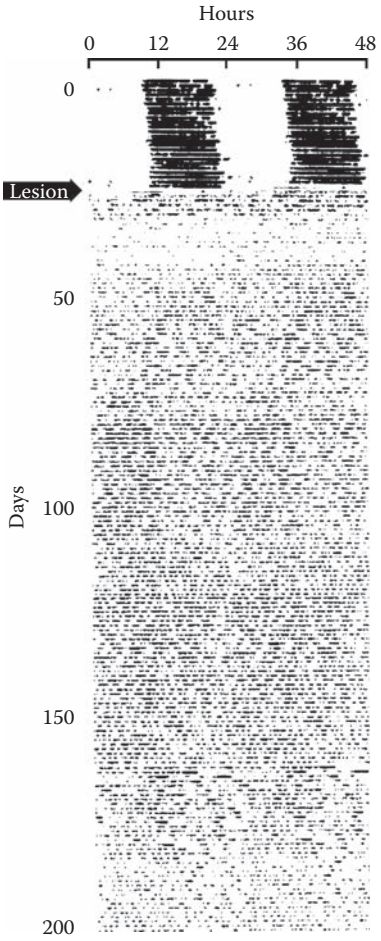


FIGURE 12.7 SCN lesion eliminates rhythm of locomotor activity. This double-plotted actogram shows the rhythm of running-wheel activity of a Siberian chipmunk (*Eutamias sibiricus*) maintained in constant light before and after a complete lesion of the SCN (arrow). Note the absence of any sign of recovery of circadian rhythmicity for 5 months after the lesion was produced. (Source: Adapted from Sato, T. & 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.)

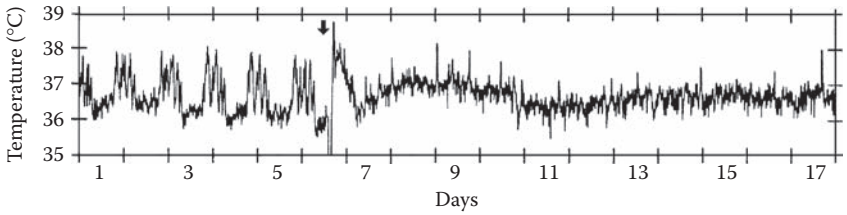


FIGURE 12.8 SCN lesion eliminates rhythm of body temperature. The graph shows a 17-day segment of the body temperature records of a golden hamster (*Mesocricetus auratus*), obtained by telemetry in 6-minute intervals. On the sixth day, the animal received a complete SCN lesion (arrow) that eliminated the body temperature rhythm. (Source: Osborne, A. R. & Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.)

> 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, six-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."

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. In a similar fashion, the SCN might be only part of the nervous pathway that connects the clock to the effector organs. If the SCN really contains a clock, it should display rhythmicity in isolation. Can the SCN be removed from the brain and studied in a dish? Yes, it 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. Unlike live animals, brain slices cannot be studied for several months, but clear circadian rhythmicity in the electrical activity of rat SCN cells was recorded continuously for several days by various research groups.[64–72] Although rats are active at night, SCN cells were found to be more active during what would have been subjective day had the animals not been killed. Similar results were obtained in studies using electrophysiological recording, as well as other methods, in rats and other species.[73–87]

Destruction of the SCN in live 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 hypothesis has been investigated in two ways. Some researchers replicated the *in vitro* studies *in vivo*. By implanting long-term recording electrodes, they were able to record the electrical activity of SCN tissue in freely-moving animals and identify robust circadian rhythmicity.[68,88–92] Figure 12.9 shows an example. Multiple-unit neural activity was recorded from various brain sites of freely moving golden hamsters. Note that the firing rate of SCN cells exhibits robust daily rhythmicity. Note 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. Chapter 2 showed that the 2-deoxy-glucose (2-DG) methodology can determine which parts of the brain are more active because 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 and is more active during the daytime.[93–98] Figure 12.10 shows autoradiographs of rat brains when the SCN was very active (top panel) and when it was rather inactive (bottom panel). Studies using the Fos methodology also documented *in vivo* rhythmicity of the SCN.[99–103] 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

Researchers sought further evidence to establish beyond doubt that the SCN is the site of the circadian pacemaker. If a brain structure houses the biological clock, destruction of this structure should eliminate rhythmicity, and 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.[46,77,104–109] Because 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

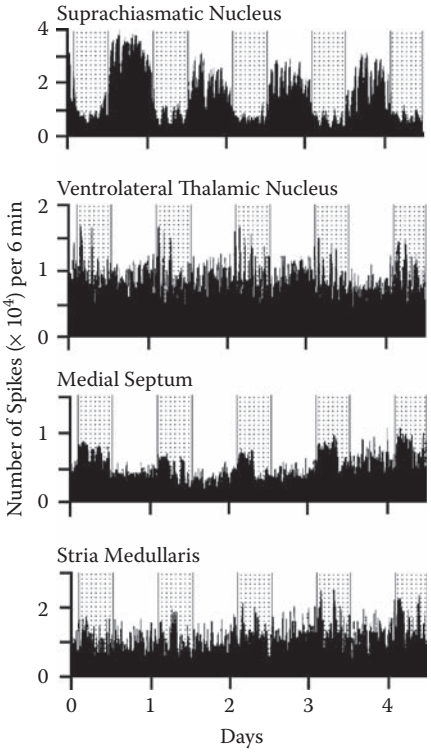


FIGURE 12.9 SCN cells exhibit circadian rhythmicity of electrical activity. The graphs show 4-day segments of the records of multi-unit neural activity of four brain regions obtained from freely moving golden hamsters (*Mesocricetus auratus*). The number of spikes (action potentials) per 6 minutes is shown for representative hamsters. The stippled vertical bars indicate the timing of the dark phase of the prevailing light–dark cycle (LD 14:10). Note 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. (Source: Adapted from Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D. & Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus *in vivo*. *Journal of Neuroscience* 18: 10709–10723.)

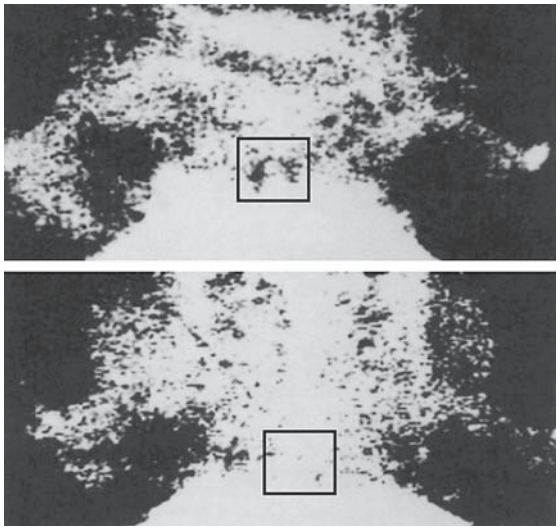


FIGURE 12.10 SCN cells exhibit circadian rhythmicity of metabolic activity. These microphotographs show the ventral sections of coronal slices of the rat brain (*Rattus norvegicus*) in the region of the SCN (indicated by the squares). The images are autoradiographs based on the assimilation of radioactive 2-deoxyglucose (2-DG) prior to euthanasia. High metabolic activity is associated with high assimilation of 2-DG (top), while low metabolic activity is associated with low assimilation (bottom). (Source: Adapted from Cassone, V. M. et al. (1988). Effects of melatonin on 2-deoxy-[1-^{14}C] glucose uptake within rat suprachiasmatic nucleus. *American Journal of Physiology* 255: R332–R337.)

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[110] and from rats and mice to hamsters.[111,112] Again, rhythmicity was restored, and the period of the rhythm matched, at least closely, that of the donor — not that of the host.

The results of transplants using the *tau*-mutant hamster were even more dramatic. 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 an SCN lesion and were implanted with the fetal SCN of a hamster with a period of 20 hours? 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.[12,113,114] Furthermore, when animals received implants after having experienced only partial lesion of the SCN, they showed alternating 20-hour and 24-hour rhythmicity![115,116] Figure 12.11 shows an example. 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 more impaired but, by Day 45, 24-hour rhythmicity clearly was seen again. Note that starting on Day 60, 20-hour rhythmicity predominates for a couple of weeks, is followed by a few days of 24-rhythmicity, and then returns 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.[117]

Based on the experimental evidence described so far, it seems justified to conclude that the SCN is the physical substrate of the circadian pacemaker. This conclusion may be limited in scope, however. For example, a few other parts of the brain — particularly in birds and other non-mammalian 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 organisms do not possess an SCN.

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, left panel). Each side of the brain contains an SCN, and each SCN houses approximately 8000 neurons with simple dendritic arbors and fine caliber axons.[118] A few glial cells are located in the center of the nucleus; many more glial cells are found in the periphery.[119]

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 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. In a similar fashion, 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 if less descriptive. Cells in the core send many axons to the shell, but little projection occurs in the opposite direction.[120] Projections to homologous contralateral areas (on the opposite side of the brain) are common.[118,120]

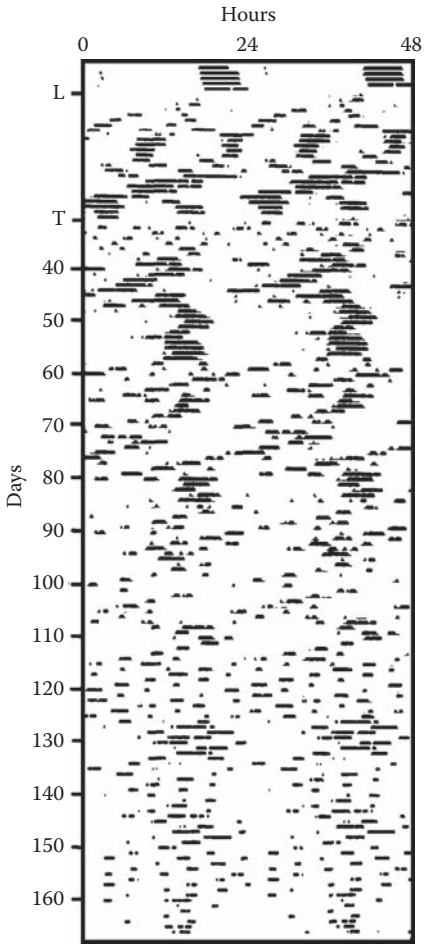


FIGURE 12.11 SCN transplant determines circadian period. This double-plotted actogram shows the rhythm of running-wheel activity of a golden hamster (*Mesocricetus auratus*) that received a partial lesion of the SCN (indicated by L on the left margin) followed by a transplant of fetal hypothalamic tissue 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. (Source: Vogelbaum, M. A. & Menaker, M. (1992). Temporal chimeras produced by hypothalamic transplants. *Journal of Neuroscience* 12: 3619–3627. © 1992 Society for Neuroscience. Reproduced with permission from the publisher and the authors.)

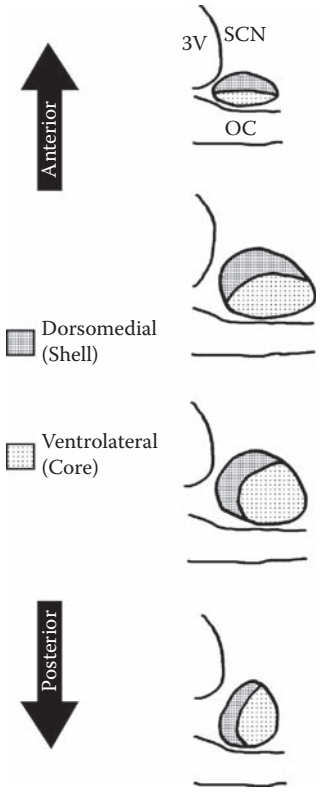


FIGURE 12.12 Major subdivisions of the SCN. Anatomically and functionally, the SCN can be subdivided into two main regions: the dorsomedial region (or shell) and the ventrolateral region (or core). The dorsomedial-ventrolateral designation inaccurately describes the anterior and posterior ends of the SCN. (Note: 3V = third ventricle, OC = optic chiasm, SCN = suprachiasmatic nucleus) (Source: Diagram based on Leak, R. K. & Moore, R. Y. (2001). Topographic organization of suprachiasmatic nucleus projection neurons. *Journal of Comparative Neurology* 433: 312–334.)

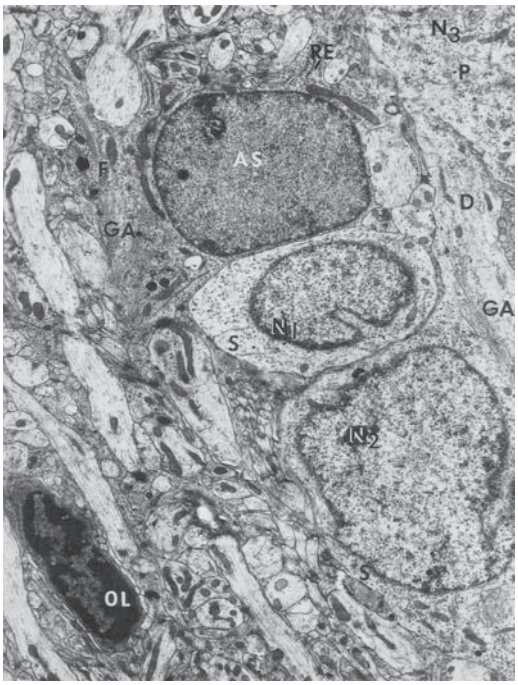


FIGURE 12.13 Cellular organization of the SCN. This electron microphotograph shows several cellular features of the SCN in the rat (*Rattus norvegicus*). (Note: AS = astrocyte, D = dendrite, F = glial filaments, GA = Golgi apparatus, N_1–N_3 = neuron, OL = oligodendrocyte, P = perikaryon, RE = rough endoplasmic reticulum, and S = synapse) (Width of microphotograph: 15 μm) (Source: Van den Pol, A. N. (1980). The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy. *Journal of Comparative Neurology* 191: 661–702. © 1980 Alan R. Liss, Inc. Reprinted by permission of Wiley-Liss, a subsidiary of John Wiley & Sons, Inc.)

Figure 12.13 provides a close look at cells in the SCN of the rat. This electron microphotograph shows a neuron (N2) that receives an axosomatic synapse (S) and that contains fewer organelles than a neighboring neuron (N1).[118] Although not evident in the figure, axodendritic synapses (i.e., synapses between the axon of a neuron and dendrites of another neuron) are more common than axosomatic ones (i.e., synapses between the axon of a neuron and the cell body of another neuron).[119] The figure also shows two glial cells — an astrocyte (AS) and an oligodendrocyte (OL) — both of which have greater nuclear density than the neurons. The neurotransmitters that mediate synaptic communication within the SCN are discussed later in this section, but it should be mentioned that functional and anatomical evidence indicates that SCN neurons can also communicate by electrical coupling.[121,122] 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.[122]

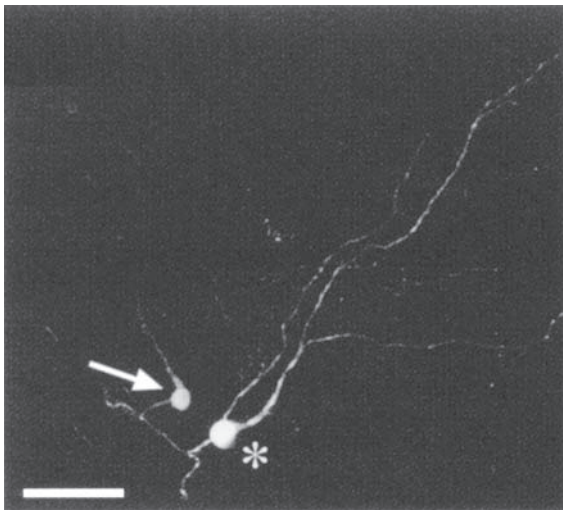


FIGURE 12.14 Gap junction between SCN neurons. Some neurons in the SCN communicate through gap junctions rather than through chemical synapses. For this confocal laser scanning microscope image, an individual SCN neuron (asterisk) in a coronal slice of golden hamster brain was microinjected with neurobiotin (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 make contact. (Scale bar: 50 μm) (Source: Jobst, E. E., Robinson, D. W. & Allen, C. N. (2004). Potential pathways for intercellular communication within the calbindin subnucleus of the hamster suprachiasmatic nucleus. *Neuroscience* 123: 87–99. © Elsevier Science Publishers. Reproduced with permission from Elsevier.)

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.[64–87] 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.[87]

Electrophysiological analysis of individual SCN cells generally has revealed a greater proportion of rhythmic cells in the dorsomedial (shell) region than in the ventrolateral (core) region.[67,72] These findings agree with *in vivo* studies using the Fos methodology.[99,102] 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

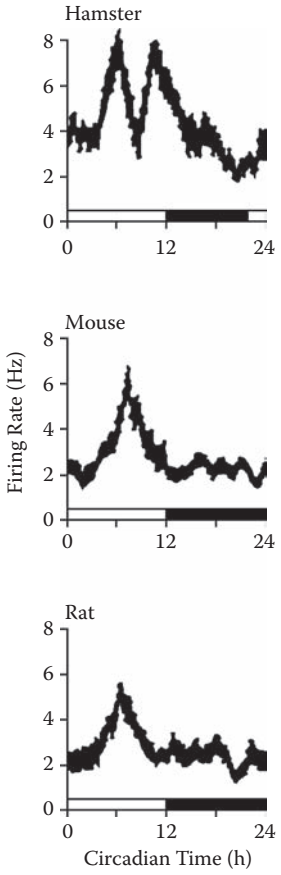


FIGURE 12.15 Circadian variation in firing rate of SCN neurons. The graphs show the circadian variation in mean firing rate of three to four single units recorded *in vitro* in horizontal brain slices from golden hamsters (*Mesocricetus auratus*), domestic mice (*Mus musculus*), and laboratory rats (*Rattus 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. Note that in horizontal slices the hamster SCN exhibits two peaks instead of one. (Source: Adapted from Burgoon, P. W., Lindberg, P. T. & 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.)

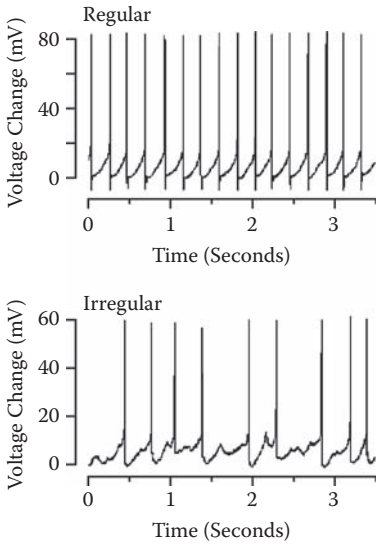


FIGURE 12.16 Firing patterns of SCN neurons. These electrophysiological records of single cell activity in brain slices of rats (*Rattus norvegicus*) exemplify the regular and irregular firing patterns of SCN neurons. At rest, cells of the regular type generate action potentials at a frequency of approximately 4 Hz, while cells of the irregular type fire irregularly at a frequency of approximately of 2 Hz. (Source: Adapted from Kononenko, N. I. & Dudek, F. E. (2004). Mechanism of irregular firing of suprachiasmatic nucleus neurons in rat hypothalamic slices. *Journal of Neurophysiology* 91: 267–273.)

day.[123–127] Most cells fire regularly, but some fire irregularly.[67,128] As shown in Figure 12.16, cells that fire irregularly tend to have lower average firing rates.[128]

In principle, the SCN could generate circadian rhythmicity by relying on rhythmic cells or by structuring the activity of cells that do not exhibit circadian rhythmicity (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,[129–131] studies conducted during the past decade clearly have 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 brains have 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.[132–135] 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.[132]

The range of periods of individual cells is much wider than that of the whole SCN. For example, in the rat, whose circadian period is approximately 24.4 hours, the standard deviation of 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), while 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).[134] This difference is easily explained by basic statistical properties of the arithmetic mean. Because the value of a mean is 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). Because the standard deviation of the distribution of means equals the standard deviation of the distribution of the population divided by

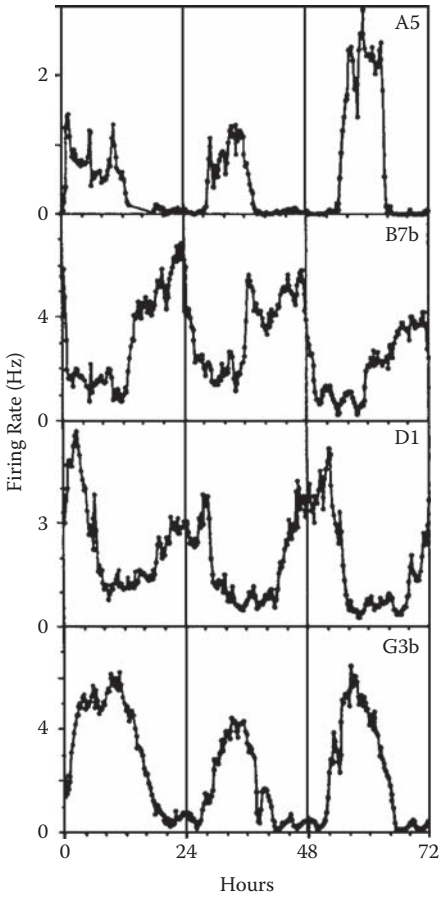


FIGURE 12.17 Activity of isolated SCN neurons. The graphs show the circadian variation in firing rate of four individual neurons in a dissociated culture system of SCN cells from the laboratory rat (*Rattus norvegicus*). Different neurons exhibit different circadian periods. (Source: Welsh, D. K., Logothetis, D. E., Meister, M. & Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14: 697–706. © Elsevier Science Publishers. Reproduced with permission from the publisher and the authors.)

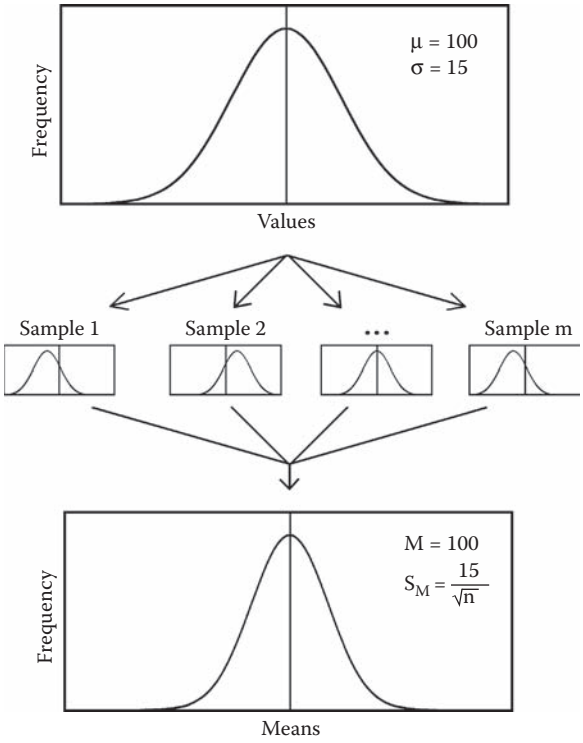


FIGURE 12.18 Variability of the means is smaller than variability of the population. Because the value of a mean is, by necessity, never as extreme as the most extreme values of its elements, the variability of sample means is always smaller than the variability of the original population of elements. As shown in this figure, if many samples of size n are drawn randomly 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 ($S_M = 15 \div a$, 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.

the square root of the group size ($S_M = \sigma / n^{0.5}$), 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. Based on the standard deviation of the individual cells (1.4 hours), one would expect the group of 16,000 SCN cells to exhibit a mean period with a standard deviation slightly larger than 0.01 hour. Obviously, 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.[136] Figure 12.19 shows an example. 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 Freerun, 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).

Average period is only part of the problem of coordination of multiple cells. If the cells were not mutually synchronized, the overall output of the SCN would be temporally uniform, not sinusoidal. Thus, the individual cells must be coupled somehow. Functional models indicate that intercommunication in a population of autonomous oscillators can yield a stable ensemble period.[137–140] Efforts to identify the coupling mechanism have suggested synaptic communication between neurons,[141–143] although glial cells may also be involved.[144,145] Figure 12.20 shows

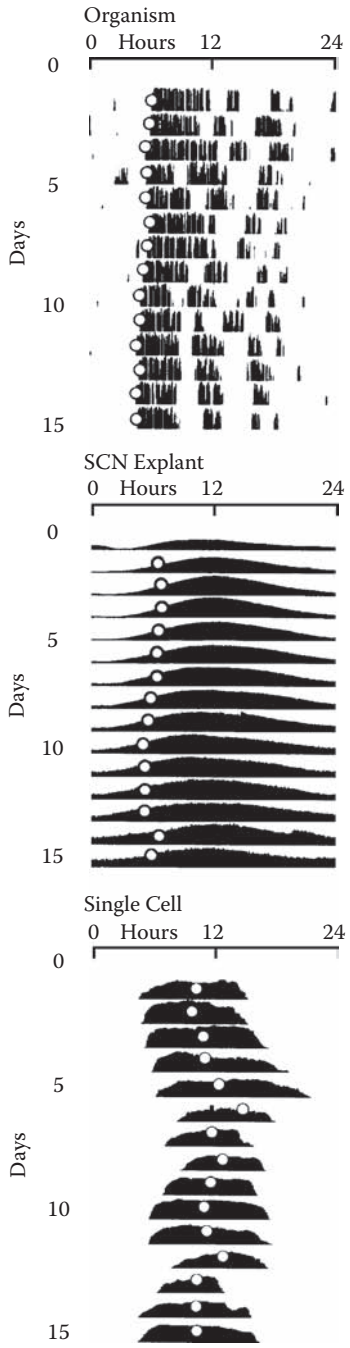


FIGURE 12.19 Precision of circadian period. Great variability is present in the circadian period of different SCN cells and in the circadian period of each individual cell. As indicated by the phase markers (open circles) in these three actograms, the circadian periods of the whole organism and of SCN explants are much more precise than that of a single SCN neuron. All three variables were recorded from domestic mice (*Mus musculus*). (Source: Adapted from Herzog, E. D., Aton, S. J., Numano, R., Sakaki, Y. & Tei, H. (2004). Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons. *Journal of Biological Rhythms* 19: 35–46.)

some of the evidence that supports synaptic communication. 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 sixth and seventh days are shown). The blockade of action potentials clearly disrupted the synchronization of the cells. Two days later, after TTX was washed out, synchronization resumed.[143]

12.2.3 Neurotransmitters in the SCN

Much has been learned recently about the neurotransmitters used for communication between nerve cells in the SCN. Three neurotransmitters are particularly abundant in the SCN: GABA (gamma-aminobutyric acid, a ubiquitous inhibitory amino-acid transmitter found throughout the central nervous system), AVP (arginine vasopressin, 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 VIP (vasoactive intestinal polypeptide, a peptide first identified by its action on the intestinal wall). The structural formulas of the three neurotransmitters are shown in Figure 12.21.

Anatomical studies using immunocytochemical methods (that is, methods that use especially 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.[141,146–149] GABA seems to be the most common neurotransmitter in the SCN.[147] GABA administration in hypothalamic slices or dissociated cell cultures was shown to inhibit the activity of SCN neurons.[150–152] Of the two main GABA receptors ($GABA_A$ and $GABA_B$), $GABA_A$ seems to be the most common one in SCN cells,[153] although functional evidence shows that $GABA_B$ also may be involved in the operation of the circadian clock.[154] Discrete administration of the $GABA_A$ agonist *muscimol* causes phase shifts in the activity rhythm of SCN cells *in vitro*,[152,155] as well as in the activity rhythm of the whole organism.[156,157] Administration of the $GABA_A$ antagonist *bicuculline* abolishes inhibitory postsynaptic currents[158,159] and increases the spontaneous firing rate of SCN cells.[128,141]

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, top panel).[146,149,160–164] Immunocytochemical studies, as well as microdialysis studies, in brain slices or cultures of SCN

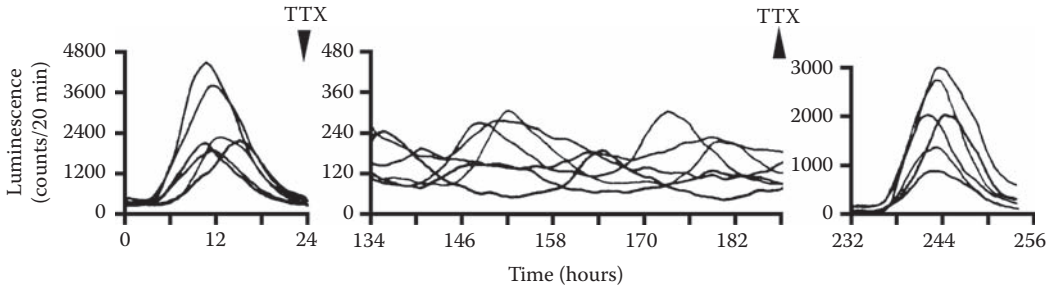


FIGURE 12.20 Synchronization of individual SCN neurons. Circadian rhythmicity of six individual SCN neurons in a slice of mouse brain (*Mus musculus*) was measured by variations in luminiscence (glow) induced by a luminiscence gene attached to a clock gene (*mPer1*-promoter-driven luciferase reporter gene). Luminiscence 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. (Source: Adapted from Yamaguchi, S., Isejima, H., Matsuo, T., Okura, R., Yagita, K., Kobayashi, M. & Okamura, H. (2003). Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302: 1408–1412.)

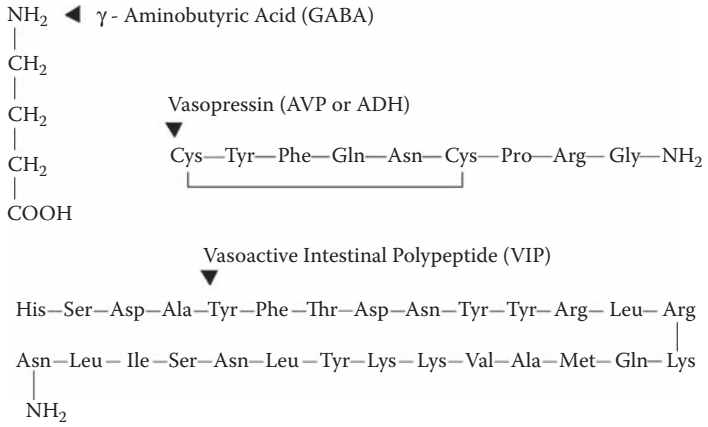


FIGURE 12.21 The main neurotransmitters in the SCN. Synapses within the SCN involve three main neurotransmitters: γ-aminobutyric acid (GABA), arginine vasopressin (AVP), and vasoactive intestinal polypeptide (VIP). (Sources: Feldman, R. S. & Quenzer, L. F. (1984). *Fundamentals of Neuropsychopharmacology.* Sunderland, MA: Sinauer; Cooper, J. R. et al. (1991). *The Biochemical Basis of Neuropharmacology,* 6th Edition. New York: Oxford University Press.)

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.[72,74,160,161,165–171] (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.) A study in voles indicated that animals with a smaller number of AVP immunoreactive cells have more robust rhythms of running-wheel activity than animals with a greater number of AVP immunoreactive cells.[172]

In contrast to AVP, VIP is present mostly in the ventrolateral (core) section of the SCN and in its dorsal projections (Figure 12.22, bottom panel).[149,160–162,164,173,174] (As shown 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,[72,74,161,164,170] presumably because of delayed rhythmic signals received from the dorsomedial (shell) region. VIP is an inhibitory neurotransmitter within the SCN.[159,175] Administration of VIP *in vivo*[176] and *in vitro*[177] causes 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.[178]

In summary, cells in the ventrolateral (core) region generally are not intrinsically rhythmic and use VIP (in addition to GABA) as their main neurotransmitter, while cells in the dorsomedial (shell) region are intrinsically

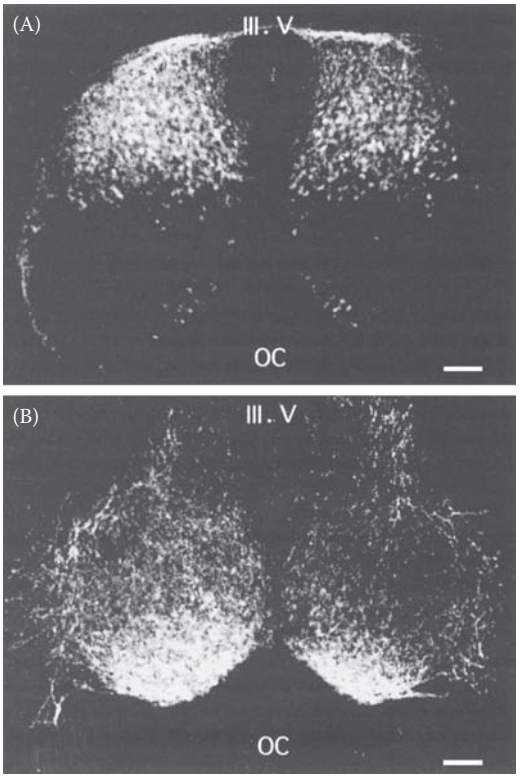


FIGURE 12.22 Location of neurotransmitters in the SCN. These immunofluorochemistry images of the SCN of newborn rats (*Rattus norvegicus*) show the concentration of AVP-containing neurons in the dorsal region of the nucleus (A) and the concentration of VIP-containing somata in the ventral region with fibers extending dorsally (B). (Note: III V = third ventricle, OC = optic chiasm.) (Source: Nakamura, W., Honma, S., Shirakawa, T. & Honma, K. (2001). Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *European Journal of Neuroscience* 14: 666–674. © 2003 Blackwell Publishing. Reproduced with permission from the publisher and the authors.)

rhythmic and use AVP (in addition to GABA) as their main neurotransmitter. Researchers have found evidence that other neurotransmitters are used in communication among SCN cells, although additional studies are necessary to better determine their function. These neurotransmitters include gastrin-releasing peptide,[71,146,161,162,170,176,179] somatostatin,[146,160] thyrotropin-releasing hormone,[180] angiotensin,[181] nitric oxide,[182] and neuromedin U.[183] Neurotransmitters used in the afferent pathways to the SCN are discussed in Chapter 13.

12.3 MOLECULAR PROCESSES

Little was known about molecular mechanisms of circadian rhythmicity until 1992. Since then, circadian physiology has benefited greatly from the rapid growth in molecular biology research that characterized the late 20th century and early 21st 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 in movies. Steven Spielberg's three motion pictures based on Michael Crichton's book, *Jurassic Park*,[184] 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.[185,186] Dolly, the ewe, made the headlines as the first demonstration that a fully functional mammal could be cloned from a single adult cell.[187] The rate of genetic mutation has been used as a "molecular clock" to measure archeological time and to determine branching of species during evolution.[188,189] DNA fingerprinting was used to prove that the stain in Ms. Lewinsky's dress was semen from President Bill Clinton.[190] The Human Genome Project has cataloged every gene in the human genome,[191] opening the doors for unlimited progress in human medicine.

The philosophical implications of molecular biology are just as impressive as its technical achievements. 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 finally has been provided for the claims of *logical positivism* (in the first half of the 20th century) — that complex phenomena can be reduced to simple elements[192] and that all sciences can ultimately be reduced to the physical sciences.[193] This idea can be expanded to include the solution of the old *mind–body dichotomy* — that is, that the mind is an expression of the operation of the brain cells.[194–197] Many readers may not agree with this "radical materialism," but it will become more acceptable to future generations as progress in molecular biology proceeds.

Note that the relative simplicity of the phenomena described here is the result of a careful presentation of the most significant features of the material and it does not reflect the complexity of actual research in the field. Because of the rapid advances in scientific research, a basic college education cannot possibly prepare the average citizen to fully understand specialized research conducted 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 that the molecular mechanisms of circadian rhythmicity would be identified more easily 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.[198] The gene — called *per*, for *period* — is located in the X chromosome

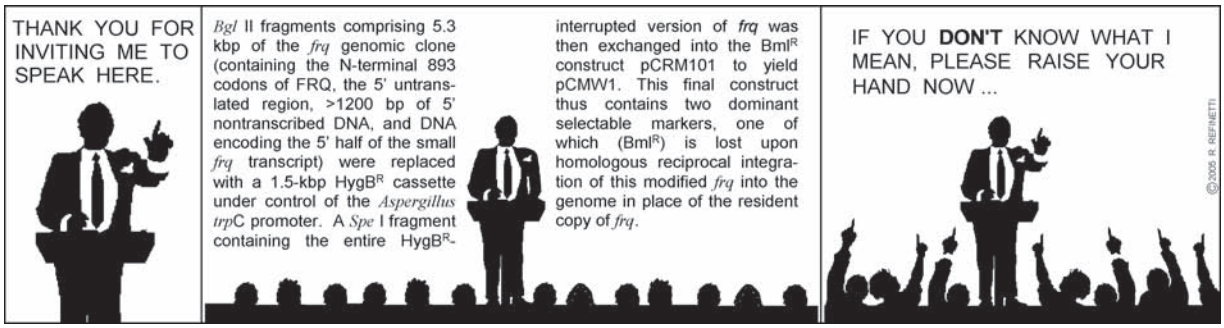


FIGURE 12.23 Raise your hand! This cartoon emphasizes the complexity of current research methods in molecular biology. (Source: Lecture contents quoted from Aronson, B. D., Johnson, K. A. & Dunlap, J. C. (1994). Circadian clock locus frequency: protein encoded by a single open reading frame defines period length and temperature compensation. *Proceedings of the National Academy of Sciences U.S.A.* 91: 7683–7687.)

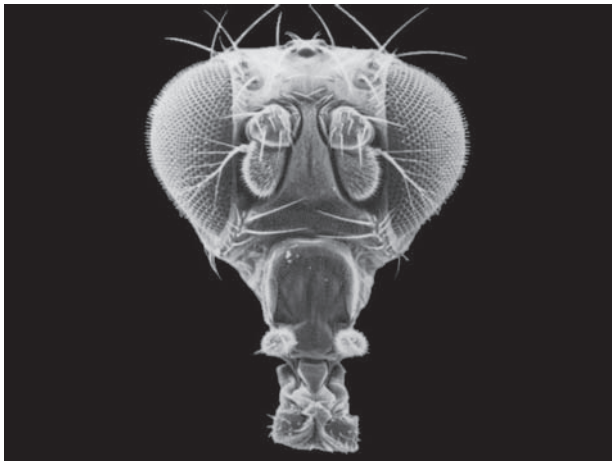


FIGURE 12.24 Close-up of a fly. This electron-microscope photograph shows the head of a fruit fly (*Drosophila melanogaster*) up close. (Source: FlyBase, Indiana University.)

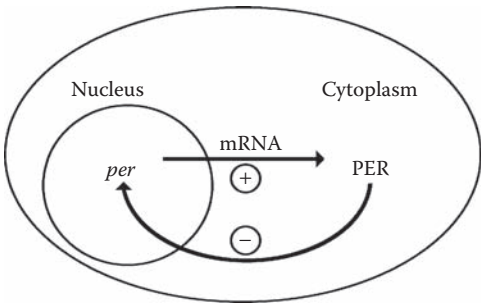


FIGURE 12.25 A simple molecular loop. This diagram of a eukaryotic cell illustrates the core mechanism of the circadian clock in animals: a feedback loop involving the *per* gene and its protein.

and exhibits multiple mutant alleles.[199] As with any other gene, *per* is present in cells of all organs of the fly. It also is activated in most organs, so that PER (the protein produced by the *per* gene) is produced rhythmically almost everywhere in the fly's body.[200,201] However, only *per* expression in nerve cells and glia of the brain is necessary for circadian rhythmicity of locomotor activity.[202] The *accessory medulla* (aMe) or a nearby group of cells called the *ventral lateral neurons* (LN_V) are considered to be the fly's equivalent of the mammalian SCN.[203] Recent research suggests that the function of the LN_V is not to generate rhythmicity (because other organs also can generate molecular rhythmicity) but to synchronize the rhythmicity of multiple oscillators.[204]

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).[205] Accordingly, transient experimenter-induced increases in PER production cause phase shifts of the activity rhythm.[206] This finding is exciting because the *per*–PER relationship constitutes the type of feedback loop 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. Thousands or millions of feedback loops exist in the body, but very few of them involve genes 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.

The *per* gene, like most 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.[207] Similar to *per*, *tim* RNA accumulates rhythmically, and the rhythmicity is dependent on the presence of both TIM and PER.[208] TIM binds to PER *in vitro*, and the TIM–PER

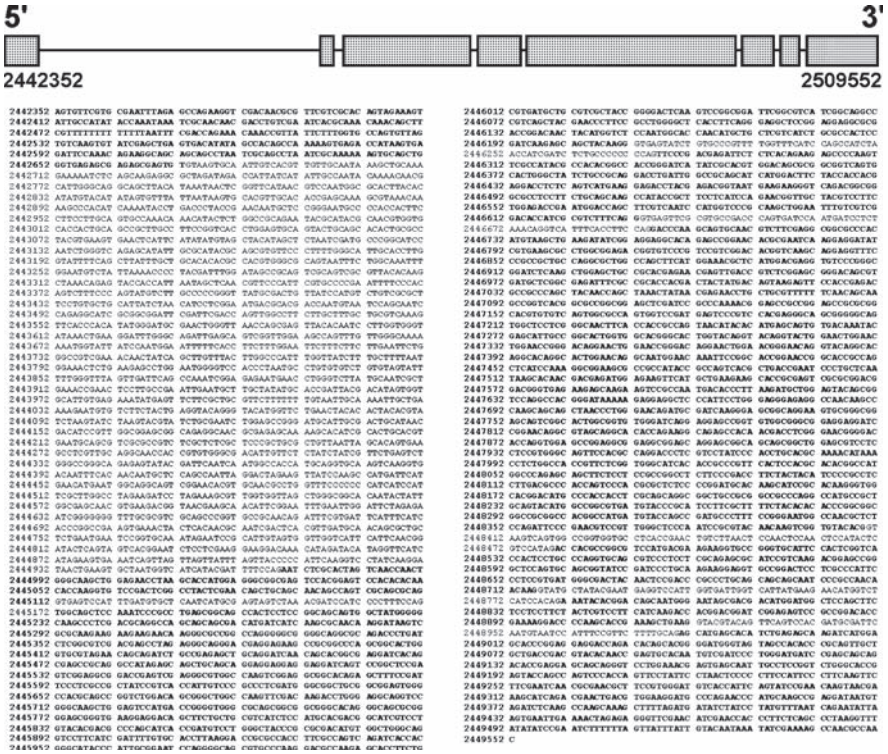


FIGURE 12.26 The *per* gene. This figure shows a positional diagram and the full sequence of bases of the *per* gene in the fruit fly (*Drosophila melanogaster*). Coding regions of the gene are shown in boldface. (A = adenine, C = cytosine, G = guanine, T = thymine) (Source: GenBank, U.S. National Center for Biotechnology Information, National Institutes of Health, 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, refer to Table 2.2 in Chapter 2. (Source: GenBank, U.S. National Center for Biotechnology Information, National Institutes of Health, accession number P07663.)

complex migrates to the nucleus to inhibit *per* transcription.[209] The *per*–PER loop, then, is not a one-gene loop. A loop involving a few genes and a few proteins, however, still became the top candidate for the basis of the biochemical mechanism of circadian rhythmicity.

Research on mammals did not lag behind research on fruit flies. Chapter 6 showed that two single-gene mutations had been identified in mammals: the *tau* gene in the golden hamster[210] and the *clock* gene in the mouse.[211] Other research teams identified three mammalian homologs of

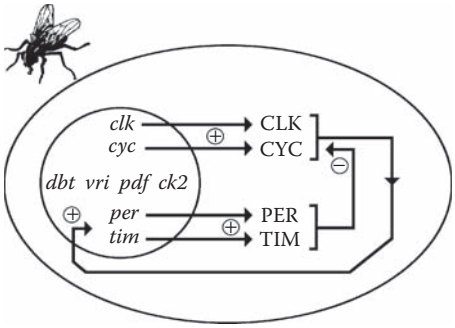


FIGURE 12.28 Molecular mechanism of the circadian clock in the fruit fly. This diagram illustrates the major players in the circadian clock of the fruit fly. For graphic purposes, the inhibition of CLK-CYC by PER-TIM is displayed in the cytoplasm although it actually takes place in the nucleus of the cell. (Sources: Williams, J. A. & Sehgal, A. (2001). Molecular components of the circadian system in Drosophila. *Annual Review of Physiology* 63: 729–755; Devlin, P. F. & Kay, S. A. (2001). Circadian photoreception. *Annual Review of Physiology* 63: 677–694; Lin, J. M. et al. (2002). A role for casein kinase 2α in the *Drosophila* circadian clock. *Nature* 420: 816–820.)

the *per* gene: *per1*,[212] *per2*,[213] and *per3*.[214] 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.[215,216] Soon after this discovery, researchers identified another clock gene in the mouse, *bmal1*.[217] It turned out that *bmal1*, *clock* (or just *clk*), and *per1* are co-expressed in the SCN, and the proteins encoded by the first two (BMAL1 and CLK) interact to drive the transcription of *per1*. Not long after this discovery, 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.[218,219]

12.3.2 The 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*.[220,221] 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*.[222,223] In more detail, *cyc* is constitutively (nonrhythmically) expressed, while *clk* is rhythmically expressed by a mechanism described later in this section. 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.[224–228] 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 down the loop to the circadian range (about 24 hours).

Figure 12.29 shows the amino-acid sequences of the CLK protein in the fruit fly as well as in the zebrafish 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 named because this sequence of approximately 270 amino acids was originally found in three distinct proteins: Per, ARNT (aryl hydrocarbon receptor nuclear transporter), and Sim (single-minded protein).[229] All of the major clock proteins in animals (including CLK) contain at least one PAS domain. Note, however, in the figure that the CLK sequences for the three species are very similar but not identical. For example, 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 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 Figure12.30. 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*.[226] In addition to the circadian transcriptional cycles, several points of posttranscriptional control are present, particularly a gradual phosphorylation of PER by the product of *dbt* (*doubletime*).[225,227,228] Additional genes that participate in the processing of afferent signals to the clock (and efferent signals from the clock) are discussed in Chapter 13.

A lingering question is whether all clock genes have been identified. One way to answer 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 generally have 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).[230–233] 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

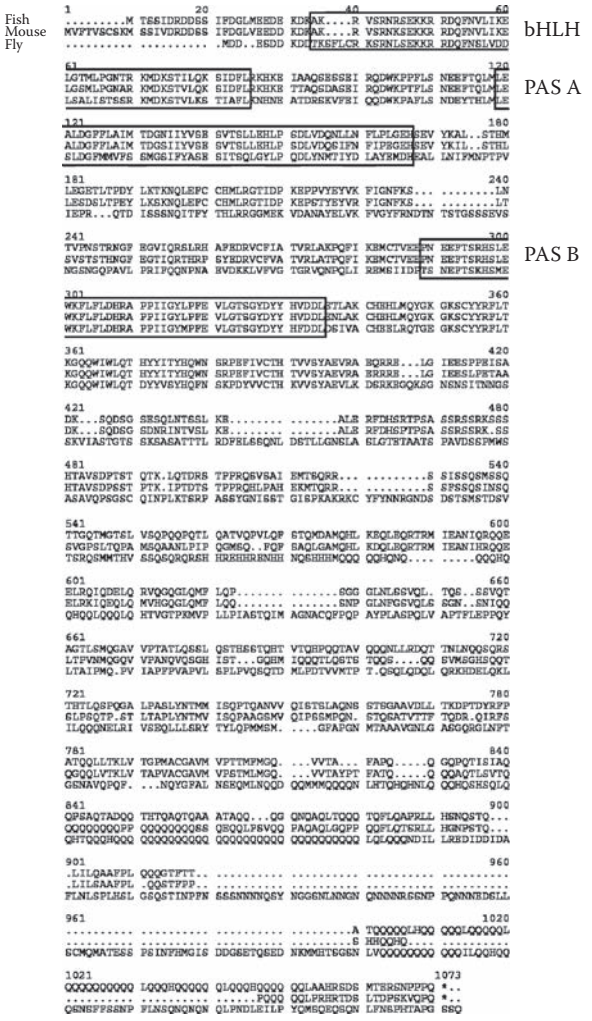


FIGURE 12.29 The Clock protein. The amino-acid sequence of the CLOCK protein is shown for the zebrafish (*Danio rerio*), the domestic mouse (*Mus musculus*), and the fruit fly (*Drosophila melanogaster*). The bHLH, PAS A, and PAS B regions are boxed and labeled. Dots indicate spaces inserted in the sequences to provide optimal alignment. The one-letter abbreviations of amino acids are listed in Table 2.2 in Chapter 2. (Source: Adapted from Whitmore, D., Foulkes, N. S., Strähle, U. & Sassone-Corsi, P. (1998). Zebrafish *Clock* rhythmic expression reveals independent peripheral circadian oscillators. *Nature Neuroscience* 1: 701–707.)

expression. Note that different genes peak at different times of the day across the entire day. A few of these genes are known clock genes (such as *per*, *tim*, and *clk*), but researchers do not know 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 found consistently to be rhythmically expressed in all the studies; the other 130 or so were detected in only or two of the studies.[233] The data clearly do not yet provide

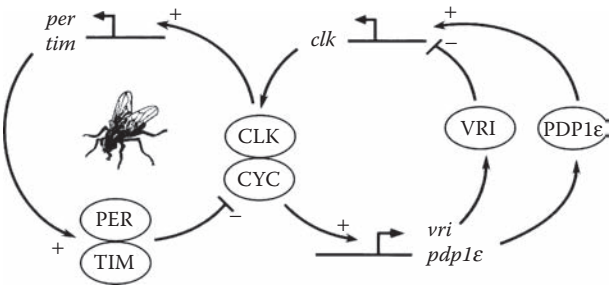


FIGURE 12.30 Molecular mechanism of the circadian clock in the fruit fly: double loop. If you look closely at the core clock mechanism in the fruit fly, you can see at least two feedback loops: the main one for *per* and *tim* activation and a secondary one for *clk* activation. (Source: Adapted from 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. & Blau, J. (2003). *Vrille*, *Pdp1*, and *dClock* form a second feedback loop in the *Drosophila* circadian clock. *Cell* 112: 329–341.)

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.[234]

12.3.3 The Molecular Clock of Other Simple Organisms

Negative feedback loops also were found to be at the core of the circadian clock in bacteria (*Synechococcus*), plants (the mustard plant), and fungi (bread mold), although the specific genes and proteins are not the same (Figure 12.32). 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.[235,236] In the mustard plant (*Arabidopsis thaliana*), the loop involves two sets of clock proteins: TOC1 (Timing of chlorophyll a/b-binding protein expression 1) and CCA1-LHY (Circadian clock associated 1 and Late elongation hypocotyl),[237,238] 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 (Gigantea),[239] ZTL (Zeitlupe),[240] and TIC (Time for coffee).[241] (Incidentally, if you think that these genes/proteins have silly names, you are right! With thousands of genes to be named, researchers quickly run out of elegant names.)

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.[242,243] The abundance of *frq* mRNA increases in response to photic stimulation, and the magnitude of this increase

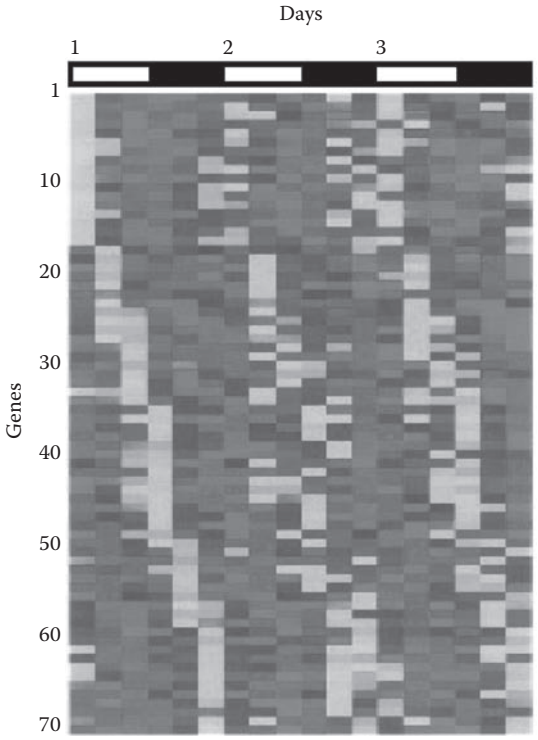


FIGURE 12.31 DNA array of *Drosophila* head. The graph shows a 72-gene segment of a DNA-array study of genes expressed in the head of the fruit fly (*Drosophila melanogaster*). Higher levels of gene expression are indicated by lighter shades of grey. Note that all genes depicted exhibit three bands of higher expression over the 3 days, 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. (Source: Adapted from Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N. & 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 U.S.A.* 99: 9562–9567.)

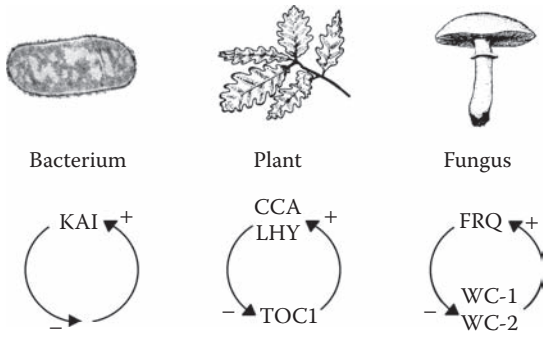


FIGURE 12.32 Molecular mechanisms of the circadian clock in nonanimals. The diagrams show the main proteins involved in the feedback loops that make up the circadian clock in bacteria, plants, and fungi. (Sources: Johnson, C. H. (2001). Endogenous timekeepers in photosynthetic organisms. *Annual Review of Physiology* 63: 695–728; Schultz, T. F. & Kay, S. A. (2003). Circadian clocks in daily and seasonal control of development. *Science* 301: 326–328; Cheng, P. et al. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 98: 7408–7413.)

correlates with the magnitude of phase shifts of the "activity" rhythm (production of asexual spores).[244] 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[245–247] (Figure 12.32). A microarray study involving 1300 genes (14% of the genome) identified 145 cycling genes,[248] but no external criterion exists to judge how many of these are false positives or how many are clock genes or clock-controlled genes.

12.3.4 The Mammalian Molecular Clock

The main loop in the mouse is similar to the loop in the fruit fly (Figure 12.33). BMAL1 (also known as MOP3) is homologous to CYC in the fly and forms a heterodimer with CLK.[249] Although TIM may play a similar role in the rat as it does in the fly,[250] the bulk of the available evidence suggests that TIM is not an important player in the mammalian clock.[251] Instead of TIM, PER combines with CRY (cryptochrome).[252–254] In addition, there are three forms of PER (PER1, PER2, and PER3) and two forms of CRY (CRY1 and CRY2).[255] The abundance of the various PER and CRY proteins has been shown to oscillate daily in the SCN.[256–264]

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. Note that PER1 is present in much higher concentration between circadian time (CT) 10 and CT 18 than at other circadian times. CLOCK was constitutively expressed, and BMAL1 showed weak rhythmicity.[265] Constitutive expression of CLOCK and rhythmic expression of BMAL1 have been reported in various other studies in mice.[254,258,261] Figure 12.35 shows similar results in sheep.

As diagrammed in Figure 12.33, the current view of the molecular clock in the mouse is that the feedback loop starts with *clk* and *bmal1* being transcribed in the nucleus and translated 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. In the fly, CYC (the homolog of BMAL1) is constitutively

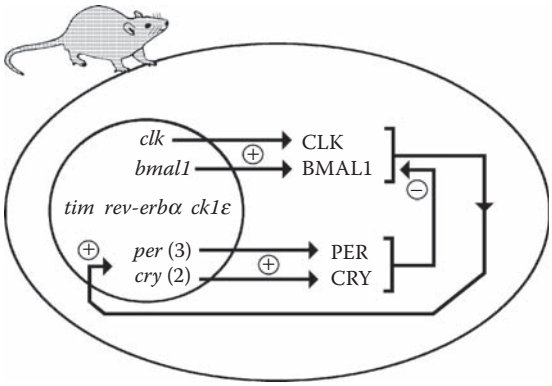


FIGURE 12.33 Molecular mechanism of the circadian clock in the mouse. This diagram illustrates the major components of the circadian clock of the mouse. For graphic purposes, the inhibition of CLK-BMAL1 by PER-CRY is displayed in the cytoplasm although it actually takes place in the nucleus of the cell. BMAL1 is also known as MOP3. (Sources: Reppert, S. M. & Weaver, D. R. (2001). Molecular analysis of mammalian circadian rhythms. *Annual Review of Physiology* 63: 647–676; Devlin, P. F. & Kay, S. A. (2001). Circadian photoreception. *Annual Review of Physiology* 63: 677–694; Barnes, J. W., Tischkau, S. A., Barnes, J. A., Mitchell, J. W., Burgoon, P. W., Hickok, J. R. & Gillette, M. U. (2003). Requirement of mammalian timeless for circadian rhythmicity. *Science* 302: 439–442.)

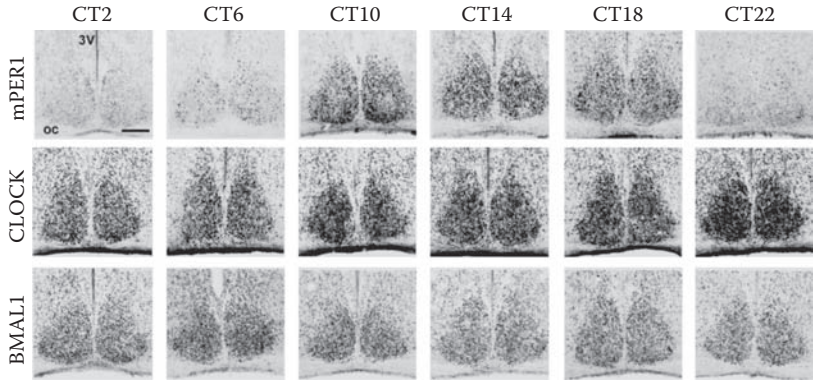


FIGURE 12.34 Tracking the molecular clock in mice. These series of coronal slices of mouse brain in the region of the suprachiasmatic nucleus show the circadian variation in abundance of three clock proteins: mPER1, CLOCK (CLK), and BMAL1. The mice were maintained in constant darkness (dim red light) on the day of euthanasia. The brain slices were stained by antibodies against the three proteins (with diaminobenzidine as the chromogen). Note that abundance of PER1 varies according to circadian time, while abundance of CLK and BMAL1 is relatively constant throughout the circadian cycle. (Note: 3V = third ventricle, CT = circadian time, OC = optic chiasm) (Scale bar: 200 μm) (Source: Adapted from Von Gall, C., Noton, E., Lee, C. & 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.)

expressed, while CLK is rhythmically expressed because of a second loop that involves *vri* and *pdp1* (compare with Figure 12.30). In the mouse, however, BMAL1 is rhythmically expressed, and this rhythmicity seems to be due to a second loop in which REV–ERBα is stimulated by CLK–BMAL1 and later inhibits the transcription of *bmal1*.[266] As mentioned earlier, *clk* seems to be constitutively expressed in the mouse, but its expression pattern may be more complicated. As indicated in Figure 12.36, the abundance of CLK *is* cyclic in both 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.[267] More research clearly is needed on this issue.

As for the *tau* gene in the golden hamster, it eventually was found to be an allele of the *ck1ε* gene (*casein kinase 1ε*), a homolog of the *Drosophila*'s *dbt* gene.[268] The mutated (*tau*) form of *ck1* is deficient in its ability to phosphorylate PER, so that the PER–CRY dimer can inhibit CLK–BMAL1 sooner than in wild types, resulting in the shorter endogenous period.[268] The fact that *ck1* is a secondary component in the mechanism of the circadian clock explains why homozygous *tau*-mutant hamsters have a shortened circadian period but are otherwise fully

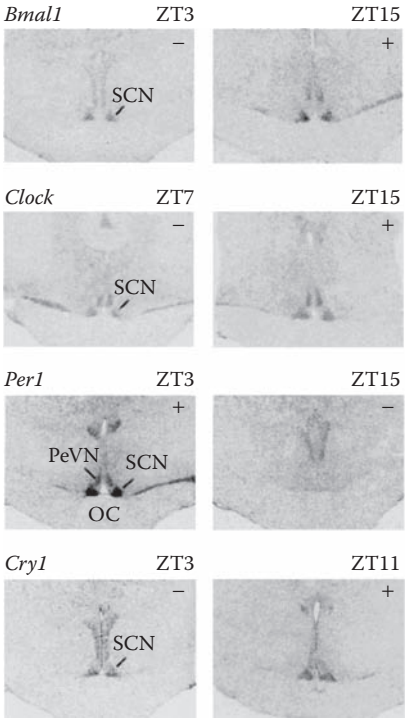


FIGURE 12.35 Tracking the molecular clock in sheep. These autoradiograms of coronal slices of sheep brain in the region of the suprachiasmatic nucleus show the daily variation in the expression of four clock genes: *bmal1*, *clock*, *per1*, and *cry1*. The sheep were maintained under a light–dark cycle (LD 16:8) prior to euthanasia. The brain slices were hybridized to radioactively labeled probes (*in situ* hybridization). Note that *per1* is expressed more strongly during the early part of the day (ZT 3) than later in the day (ZT 15), while expression of the other three genes shows much less daily variation. (Note: OC = optic chiasm, PeVN = periventricular nucleus, SCN = suprachiasmatic nucleus, ZT = zeitgeber time) (Source: Lincoln, G. et al. (2002). Temporal expression of seven clock genes in the suprachiasmatic nucleus and pars tuberalis of the sheep. *Proceedings of the National Academy of Sciences U.S.A.* 99: 13890–13895. © 2002 National Academy of Sciences U.S.A. Reproduced with permission from the publisher and the authors.)

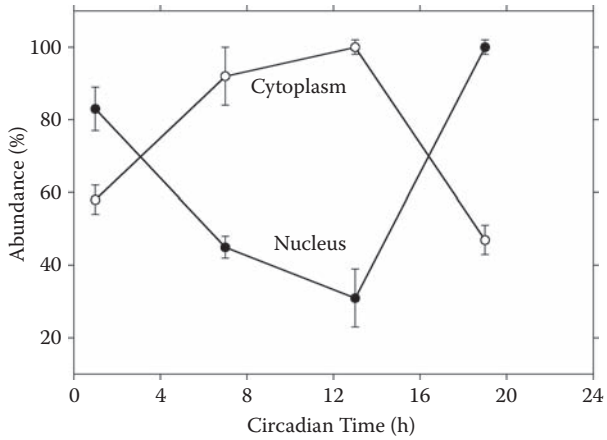


FIGURE 12.36 Clock in nucleus and cytoplasm of mouse cells. The graph shows that in the SCN of the mouse abundance of clock protein exhibits circadian oscillation if abundance is measured separately in the nucleus and cytoplasm of cells. Mice were maintained in constant darkness prior to euthanasia. SCN extracts were fractionated and analyzed by Western blotting using clock antibodies. Each data point corresponds to the mean (± SE) of three mice. Note that the rhythm of clock abundance in the cytoplasm is in antiphase to the rhythm in the cell nucleus. (Source: Kondratov, R. V., Chernov, M. V., Kondratova, A. A., Gorbacheva, V. Y., Gudkov, A. V. & 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.)

rhythmic.[210] In contrast, the fact that *clk* is a central component of the clock mechanism explains why homozygous *clock*-mutant mice lose rhythmicity in constant darkness.[211]

Figure 12.37 summarizes the clock loops in bacteria, the mustard plant, bread mold, the fruit fly, and the mouse. Fundamental differences in the molecular machinery of circadian pacemakers exist between bacteria and eukaryotes, and the proteins involved in the loops are not homologous in plants, fungi, and animals.[269] However, one small component (*casein kinase 2*) is conserved among all eukaryotic (animal, plant, and fungal) circadian systems.[225,270] Within the animal kingdom, different sets of clock genes are used by different taxa, but the *per* gene is found in all animal taxa studied so far,[271] including mollusks,[272] insects,[273–276] birds,[9,277] and mammals.[212,278–282] *Per*, *clk*, and *cyc* (or *bmal1*) seem to be common to all arthropods and chordates, including insects,[222,283] fish,[284] birds,[277,285,286] rodents,[287–292] ruminants,[293] and humans.[294,295] 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. These comparisons may be possible in the very near future.

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. The aMe or the LN_V are the fruit fly's equivalent of the mammalian SCN.[203] However, even in vertebrates — which possess an SCN — there are intrinsically rhythmic extra-SCN structures.

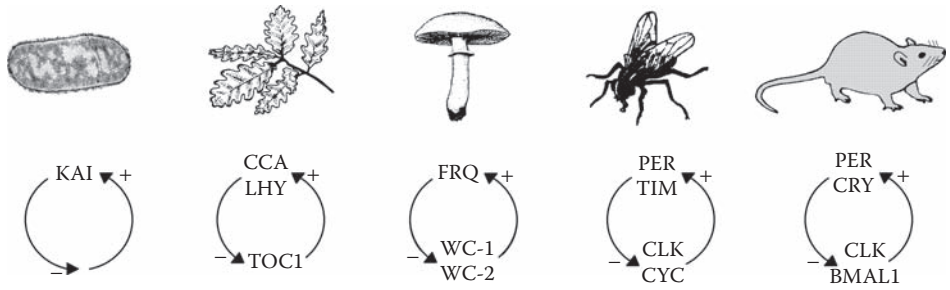


FIGURE 12.37 Summary of molecular mechanisms of the circadian clock. The diagrams show the main proteins involved in the feedback loops that make up the circadian clock in the five main groups of living beings (bacteria, plants, fungi, invertebrate animals, and vertebrate animals). (Sources: Johnson, C. H. (2001). Endogenous timekeepers in photosynthetic organisms. *Annual Review of Physiology* 63: 695–728; Devlin, P. F. and Kay, S. A. (2001). Circadian photoreception. *Annual Review of Physiology* 63: 677–694.)

12.4.1 The Pineal Gland

The 17th-century French philosopher René Descartes (Figure 12.38), who was also a skilled mathematician and anatomist, proposed that the pineal gland was the brain structure where the soul merged with the body.[296] Contemporary scientists do not consider the concept of the *soul* in their work. In a metaphorical sense, however, the pineal is the "seat of the soul" of the circadian system in some nonmammalian vertebrates. Consider Figure 12.39, 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 fish,[297] lizards,[298,299] and birds.[300–302]

Ablation studies cannot prove the pacemaker role of a structure. The pineal gland of nonmammalian vertebrates could be necessary only for motor behavior and have nothing to do with circadian rhythmicity. Figure 12.40 shows the mean pattern of melatonin release from the pineal glands of house sparrows *in vitro*. Robust rhythmicity is evident. The pineal gland is intrinsically

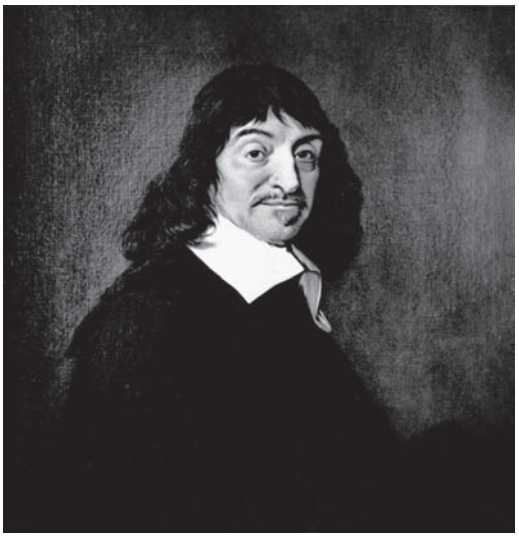


FIGURE 12.38 René Descartes (1596–1650). This French philosopher and mathematician, universally recognized as the father of modern rationalism, conjectured that the pineal gland might be the seat of the soul in the human brain. (Source: © ArtToday, Tucson, AZ.)

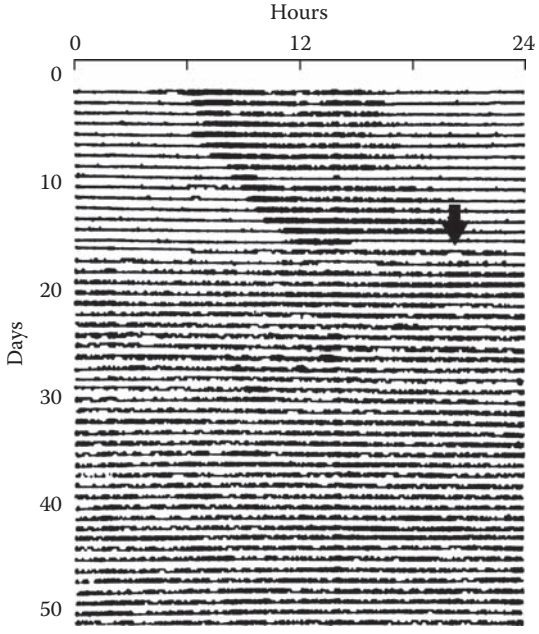


FIGURE 12.39 Sparrow needs the pineal gland to remain rhythmic. This actogram shows the rhythm of perching activity of a house sparrow (*Passer domesticus*) maintained in constant darkness. Pinealectomy was performed at the time indicated by the arrow. Note that the circadian pattern of activity observed in the intact animal vanished after the pineal gland was removed. (Source: Adapted from Gaston, S. & Menaker, M. (1968). Pineal function: the biological clock in the sparrow? *Science* 160: 1125–1127.)

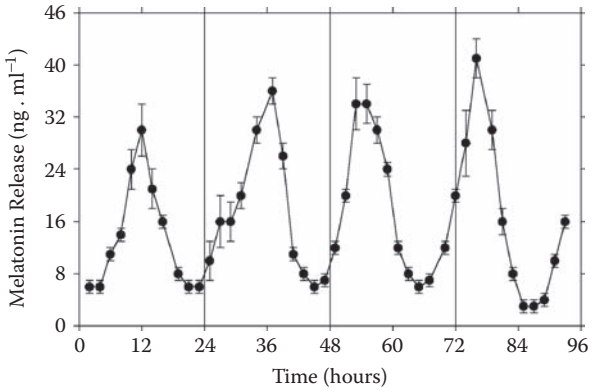


FIGURE 12.40 Sparrow's pineal gland is rhythmic *in vitro*. The graph shows the circadian oscillation in melatonin release from pineal glands *in vitro*. The data points correspond to the means (± SE) of eight pineal glands from house sparrows (*Passer domesticus*). The glands were maintained in constant darkness. (Source: Brandstätter, R. et al. (2000). Photoperiodic information acquired and stored *in vivo* is retained *in vitro* by a circadian oscillator, the avian pineal gland. *Proceedings of the National Academy of Sciences U.S.A.* 97: 12324–12328.)

FIGURE 12.41 Timed look. In some organisms, the eyes contain circadian pacemaking cells. (Source: © ArtToday, Tucson, AZ.)

photosensitive (see Chapter 11), so 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 fish,[284,303] lizards,[304,305] and birds.[306,307]

The role of the pineal gland as a circadian pacemaker is modest in many animals. In mammals, pineal ablation is inconsequential.[308–314] The armadillo (*Dasypus novemcinctus*) does not have a pineal gland, but this animal exhibits circadian rhythmicity.[315] In various studies on fish,[316] lizards[317–320] and birds,[321–324] pineal ablation disrupted behavioral rhythmicity but did not consistently abolish it. The pineal gland may be an important component of

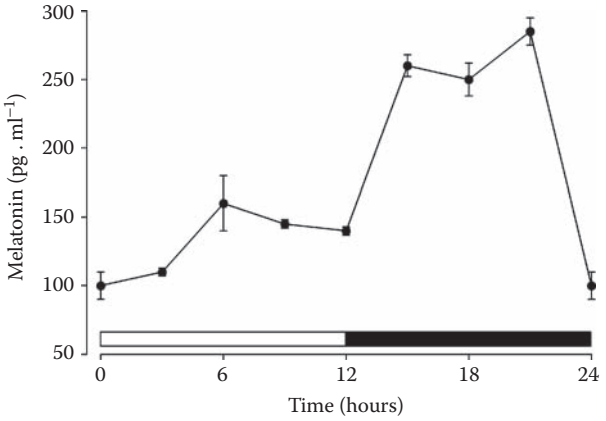


FIGURE 12.42 Chicken's retina is rhythmic *in vitro*. The graph shows daily rhythmicity in melatonin secretion of cultured retinal cells. The data points correspond to the means (± SE) of three cell cultures (retinospheroids) of chicken embryos (*Gallus domesticus*). Note that much more melatonin is 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). (Source: Willbold, E., Huhn, J., Korf, H. W., Voisin, P. & 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.)

the circadian system of some nonmammalian vertebrates,[325,326] but it does not rival the SCN as the master pacemaker.

12.4.2 The Eyes

The eyes (Figure 12.41) are photosensitive organs — even more photosensitive than the pineal gland. Like the pineal gland, they also are 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[284,304,327,328] but also in birds and rodents.[329–333] Figure 12.42 shows that cell cultures of the chick retina release melatonin rhythmically *in vitro*. Retinal cells were dissociated and then allowed to re-aggregate. By the eighth 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.[329]

In the quail (*Coturnix coturnix*), the intrinsic rhythmicity of the eyes seems to be important for the organism's circadian system because ocular enucleation (i.e., removal of the eyes) abolishes the circadian rhythm of body temperature.[322,334] 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.[321,335] In mammals, the eyes are not necessary for organismal rhythmicity,[18,29,310,336–338] although the absence of the eyes may have a small effect on the free-running period of the locomotor activity rhythm[339] and may affect cellular rhythmicity in a small region of the SCN.[340]

12.4.3 The Liver

Researchers recently have become greatly interested in the liver because of the liver's potential role as the food-entrainable oscillator (FEO). As shown in Chapter 8, evidence suggests there is a damped extra-SCN pacemaker that responds to food restriction. Because no brain structure has been identified unequivocally as the site of the FEO,[57,341,342] and because the liver is directly involved in food processing, many researchers have reasoned that the liver might be the sought-after FEO.

Does liver function display daily or circadian rhythmicity? The answer to this question is a straightforward *yes*. Chapter 5 showed that hepatic function, as measured by enzymatic activity and cholesterol synthesis, exhibits daily rhythmicity.[343–348] 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[287,349] or in constant darkness or constant dim light.[285,350,351] Microarray studies identified hundreds of cyclic genes in the livers of rats and mice.[352–356]

Rhythmicity in the liver could be driven by the SCN. The next question, then, 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.[353] Figure 12.43 shows examples for *per2* and *bmal1*. Note that, for both genes, livers from intact controls exhibit robust rhythmicity (lower panels), while 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.[31,357] Evidently, *in vitro* studies are needed to settle the issue. At least three studies have been conducted using the technique of genetically engineered bioluminescence (luciferase reporter gene). All three identified intrinsic rhythmicity of gene expression in liver explants;[357–359] an example is shown in Figure 12.44. Because the culture conditions were not ideal, SCN data are shown for comparison. Note 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. Chapter 13 discusses the afferent pathways of the circadian system in greater detail, but this section describes how the rhythmicity in the liver is affected by food restriction. Several studies have addressed the issue of the dependence of the temporal

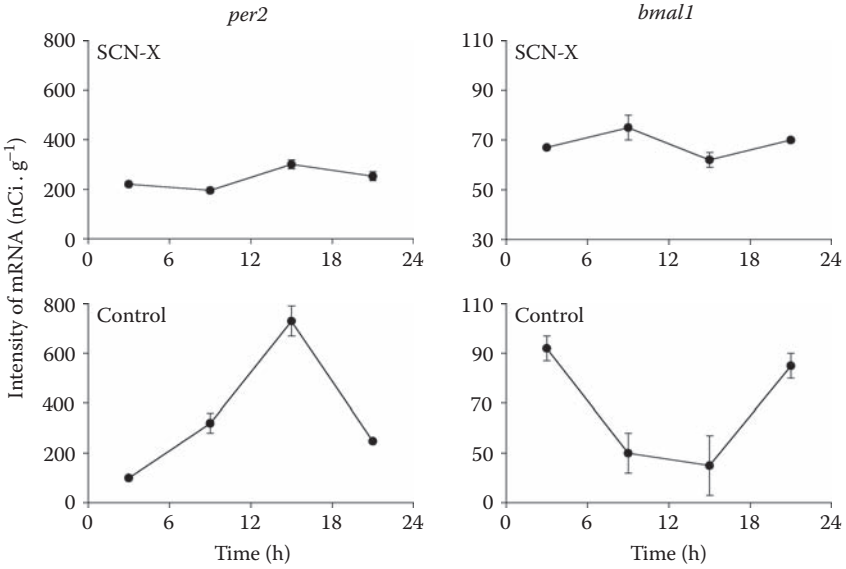


FIGURE 12.43 Liver's clock depends on SCN clock. The graphs show the daily variation in the expression of two clock genes (*per2* and *bmal1*) in the liver of previously intact mice (Control) and of mice that had been given SCN-lesions many days earlier (SCN-X). Each data point corresponds to the mean (± SE) of three mice (*Mus musculus*). Note that rhythmicity in gene expression in the liver is absent in mice devoid of SCN. (Source: Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H. & 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.)

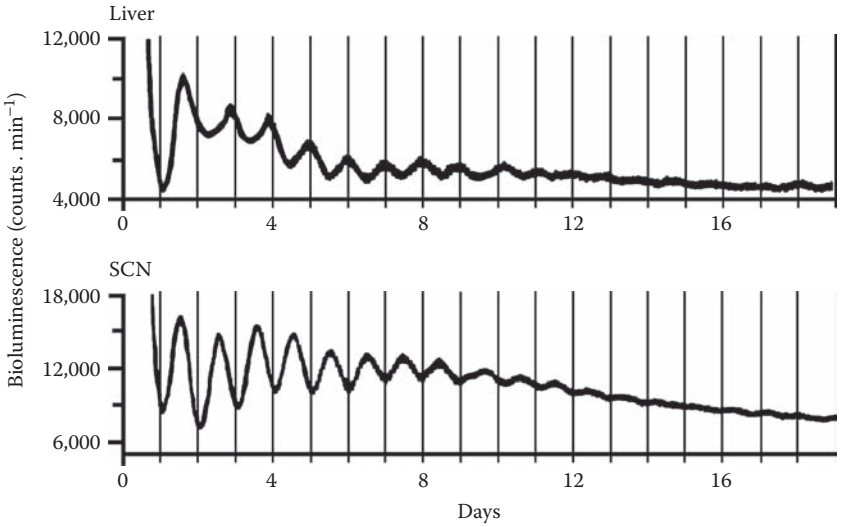


FIGURE 12.44 Is the SCN really necessary? By tracking the expression of the *per2* gene instead of the *per1* gene in mice (*Mus musculus*), investigators were able to measure persistent daily rhythmicity of liver tissue *in vitro*. The top graph shows that hepatic function — as estimated by variations in luminiscence (glow) induced by an *mPer2*-promoter-driven luciferase reporter gene — is rhythmic for at least a week *in vitro*. Rhythmicity declines over time, but the decline is similar to that observed in SCN tissue (bottom graph), suggesting that the liver tissue would have remained rhythmic if the culture conditions were more favorable. (Source: Adapted from Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M., Hong, H. K., Oh, W. J., Yoo, O. J., Menaker, M. & Takahashi, J. S. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences U.S.A.* 101: 5339–5346.)

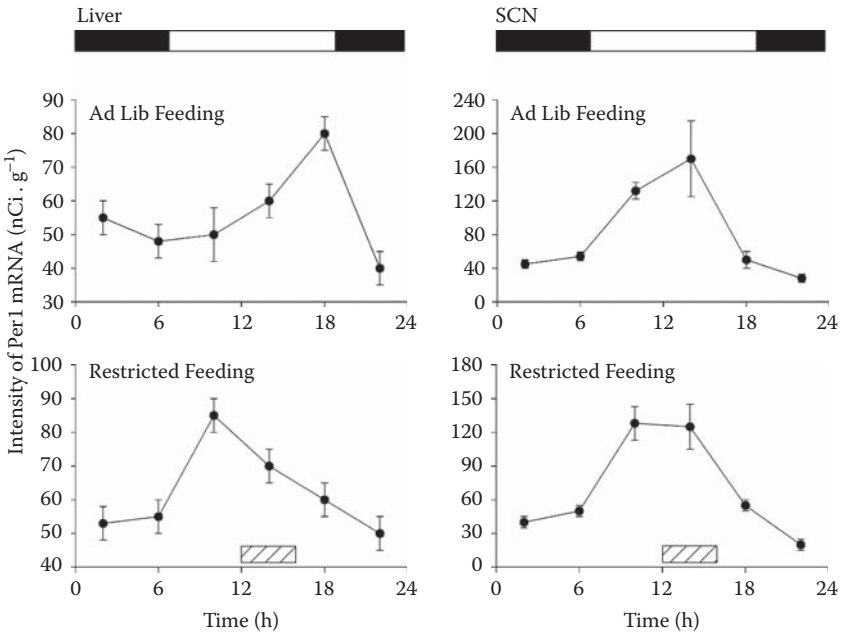


FIGURE 12.45 Is the liver the food-entrainable pacemaker? The graphs show the daily variation in the expression of a clock gene (*per1*) in the liver and the SCN of mice previously maintained under a light–dark cycle (indicated by the horizontal bars at the top) with food freely available (Ad Lib Feeding) and with food access restricted to 4 hours per day (Restricted Feeding, as indicated by the hatched bars at the bottom). Each data point corresponds to the mean (± SE) of three or four mice (*Mus musculus*). Note 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. (Source: Hara, R., Wan, K., Wakamatsu, H., Aida, R., Moriya, T., Akiyama, M. & Shibata, S. (2001). Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes to Cells* 6: 269–278.)

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, while 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.[259,358–360] An example is shown in Figure 12.45. Note 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 also has been shown in animals maintained in constant darkness.[259,360] This finding supports the distinction made between food-entrainable oscillator (liver) and light-entrainable oscillator (SCN). However, as shown in Chapter 8, behavioral evidence suggests that the SCN can be entrained by photic stimuli *and* 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.[361] In the other studies, the failure to detect effects of restricted feeding on the SCN could have been 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 is whether the pattern of gene expression measured in the liver is related to liver function. 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. This chapter mentioned earlier that in the fruit fly the *per* gene is expressed in nearly every organ of the body — yet, behavioral rhythmicity is dependent on *per* expression in the brain. 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.[362] Also, rats with dysfunctional livers (chemically induced cirrhosis) exhibit normal anticipatory activity to a restricted-feeding schedule.[363]

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 procedure technically is difficult to perform in small rodents but can be accomplished easily in larger mammals. Figure 12.46 shows data from a study conducted on goats. 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). Note 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.[348] Thus, at least in goats, the liver does not seem to be entrained by restricted feeding and, therefore, is *not* the site of the food-entrainable oscillator.

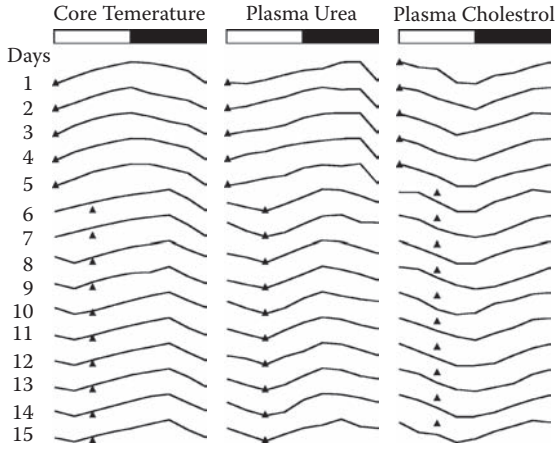


FIGURE 12.46 No evidence of liver clock in actual organ function. These actogram-style graphs show the average rhythms of body core temperature, plasma urea concentration, and plasma cholesterol concentration of five goats (*Capra hircus*) maintained under a light–dark cycle (as indicated by the horizontal bars at the top) and a restricted-feeding schedule (with food provided 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. (Source: Adapted from Piccione, G., Caola, G. & 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.)

12.4.4 Other Peripheral Clocks

In addition to the SCN, the pineal gland, 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, muscle, oral mucosa, pancreas, pituitary, spleen, stomach, testis, and thyroid.[260,263,284,285,287,293,350,352,354,360,362,364–367] In principle, all of these organs could derive their rhythmicity from the rhythmicity of the SCN. However, *in vitro* studies have described rhythmicity of gene expression in fibroblast (collagen) cell cultures[368–372] as well as in the kidney, lung, muscle, olfactory bulb, and pituitary.[357–359,373] It is clear, therefore, that peripheral organs can exhibit intrinsic rhythmicity. The question, again, is 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,[374] that cultured pancreatic islets secrete insulin rhythmically,[375] that cultured muscle and fat cells consume glucose rhythmically,[376] and that explanted olfactory bulbs fire action potentials rhythmically.[377] The fact that an organ possesses intrinsic circadian rhythmicity does not imply that it has an impact on the operation of the organism. For example, the olfactory bulb is intrinsically rhythmic, but its ablation has no effect on the circadian rhythm of locomotor activity of rats.[377]

In invertebrates and plants, various reports of multiple pacemakers in individual organisms have been published. For example, in studies of the common bean plant (*Phaseolus vulgaris*), individual plants maintained in constant light exhibited 27-hour rhythmicity in leaflet movement at the same time as 24-hour rhythmicity in photosynthesis. The ability to maintain this dual rhythmicity requires the presence of at least two distinct circadian pacemakers.[378] In the mustard plant (*Arabidopsis thaliana*), the epidermal cell layer and the mesophyll layers express genes with periods differing by up to 1.5 hours.[379] 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.[380] The bioluminescence rhythm at 18°C exhibits a 22-hour glow component simultaneously with a 24-hour flash component.[381] Particularly interesting is the olfactory response of the fruit fly (*Drosophila melanogaster*). Olfactory responses can be measured as the electrical activity of neurons in the antenna (*electroantennogram*, or EAG) following stimulation by an odorant substance (such as ethyl acetate). As shown in the upper panel of Figure 12.47, 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 (middle panel). If, instead, the clock genes are rendered dysfunctional in the head, rhythmicity is not disturbed (bottom panel). Thus, circadian gene expression in the antenna is both necessary and sufficient to produce circadian oscillation in the EAG response.[382] Olfactory rhythmicity can be maintained by a clock distinct from the clock that controls rhythmicity of locomotor behavior. Researchers do not know whether the brain clock supervises the operation of the antennal clock in normal flies, but clearly the antenna can operate independently of the brain. Circadian physiologists also do not know whether a similar autonomy of pacemakers can be found in mammals, although they do know that various autonomous processes in nonmammalian organisms are *not* found in mammals. For example, urodele amphibians can regenerate lost limbs, while mammals cannot.[383,384]

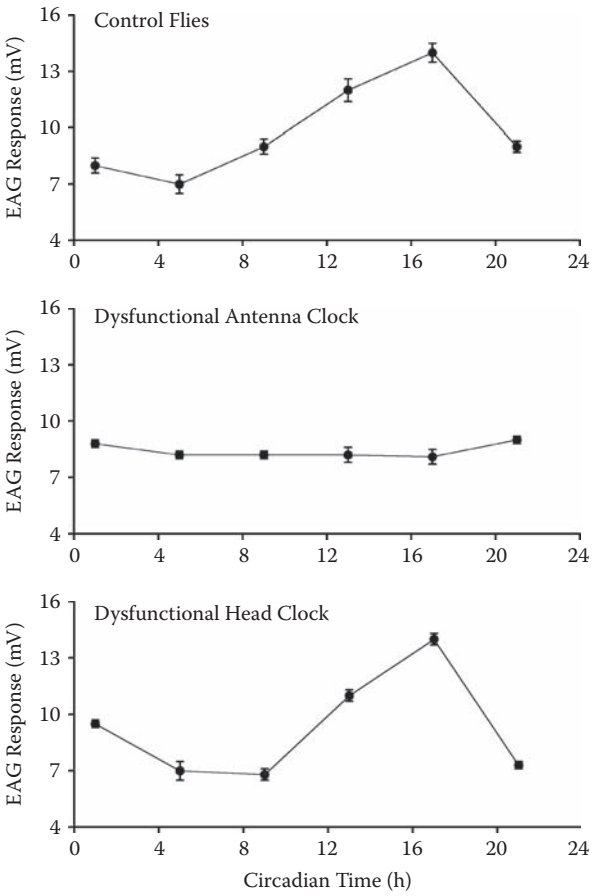


FIGURE 12.47 A true peripheral clock in the fruit fly. The graphs show the circadian variation in the electroantennogram (EAG) response of the fruit fly to an odor (ethyl acetate) presented to the antenna of control flies, flies with a dysfunctional molecular clock in the antenna, and flies with a dysfunctional molecular clock in the head. The data points correspond to the means (± SE) of approximately 24 flies. Note that a dysfunctional antenna clock eliminates rhythmicity of the EAG response but a dysfunctional head clock does not. (Source: Tanoue, S., Krishnan, P., Krishnan, B., Dryer, S. E. & Hardin, P. E. (2004). Circadian clocks in antennal neurons are necessary and sufficient for olfaction rhythms in *Drosophila*. *Current Biology* 14: 638–649.)

Recently, investigators proposed that two other structures, this time in the brain, might be the site of the mammalian FEO. One candidate is the *paraventricular nucleus of the thalamus* (PVT). A study in rats showed 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.[385] This finding does not prove that the PVT is the FEO, but it strongly suggests that the PVT is at least a major component of the brain circuit responsible for food anticipatory activity.

The evidence in support of the other FEO candidate is limited but worth mentioning. CLK is a major component in the mammalian molecular clock. A paralog of CLK, NPAS2 (neuronal PAS domain protein 2, also known as MOP4), is expressed in the forebrain but not in the SCN,[386] and it interacts there with BMAL1 (or MOP3) just like CLK does in the SCN.[387,388] NPAS2-knockout mice respond faster to a shift in the light–dark cycle than do wild-type mice (as if the master pacemaker were no longer disrupted by the FEO) and respond poorly to restricted feeding (as if the FEO had been eliminated).[389] In contrast, homozygous *clock*-mutant mice (with dysfunctional CLK) respond normally to food restriction.[390] 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.[391] As diagrammed in Figure 12.48, 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 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 speculation is 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 adapted easily 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). A recent study in orexin-knockout mice helps support the proposal that NPAS2 cells are the seat of the FEO. Orexin (also called hypocretin) is a neurotransmitter used in brain circuits involved in the regulation of feeding and sleep.[392] Orexin-knockout mice show reduced food-anticipatory activity under restricted-feeding conditions and also have reduced *npas2* expression in the forebrain.[393] This correlation between deficient *npas2* expression and deficient food-anticipatory activity is suggestive of a causal link.

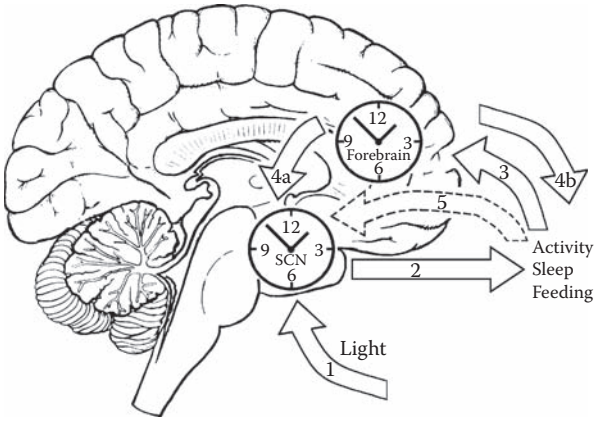


FIGURE 12.48 A circadian pacemaker in the forebrain? If the recent discovery of a molecular clock in the forebrain — which responds to restricted feeding — is confirmed at the organism level, a new conceptualization of the circadian system may be justified. As indicated in the diagram, it is possible that environmental light entrains the circadian clock in the 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. (Source: Adapted from Green, C. B. & Menaker, M. (2003). Clocks on the brain. *Science* 301: 319–320.)

Great variety exists in the organization of the circadian systems of different taxonomic groups. Within the vertebrates, and particularly in mammals, there is compelling evidence that multiple organs exhibit intrinsic circadian rhythmicity and 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,"[394] 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 generally are not intrinsically rhythmic and use VIP (in addition to GABA) as their main neurotransmitter, while 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 decade. The circadian clock is 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.
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 controlled by the master circadian pacemaker located in the SCN.

EXERCISES

Exercise 12.1 Simulation of free-running rhythms

This exercise uses the program Freerun. Freerun 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. Double-click on the Circadian icon to open the program banner, then click Freerun (the tenth icon from the left).
2. In the top panel, select Two pacemakers.
3. Set the Period and Strength of the two pacemakers to any values you choose and click on Plot. For example, if you set the values for one pacemaker to a period of 24 hours and a strength of 60% and the other pacemaker to a period of 20 hours and a strength of 40%, the actogram of organismal activity will look like a series of horizontal lines that is dense at the center. If you look closely, however, you will be able to identify both the 24-hour and the 20-hour components.
4. Repeat the procedure using other combinations of periods and strengths. It may be difficult to identify 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.
5. When you are finished entering different values, select One pacemaker in the top panel.
6. Accept 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 resulting actogram has a circadian period that may range from 22 to 26 hours. Click on Plot repeatedly and observe the various periods.
8. Change the No. of cells to 100 and run several simulations again. Note how the range of periods is greatly reduced. Because 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. Note 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 set to the default value (Oscillator), 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 occurs 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. 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. Note 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 appears that in real animals, integration does not occur at the effector organs. 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, but if your computer speed 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.

EXERCISE 12.2 *IN VITRO* ACTIVITY OF SUPRACHIASMATIC NEURONS

Perhaps the most convincing evidence that the suprachiasmatic nucleus (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). You cannot conduct this exercise on your own, because sophisticated techniques are required to maintain a brain slice healthy *in vitro* and to record the activity of nerve cells in the preparation. If you have access to a cellular electrophysiology laboratory, the main investigator (or one of his/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 exhibits only random oscillations in activity.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M. & Allada, R. (2002). A role for casein kinase 2-alpha in the *Drosophila* circadian clock. *Nature* 420: 816–820. This research article is an exceptionally good example of a well-written article with rigorous research methods and clear-cut results.

Klein, D. C., Moore, R. Y. & 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 suprachiasmatic nucleus. Research advances during the last decade 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.

Van Esseveldt, L. E., Lehman, M. N. & Boer, G. J. (2000). The suprachiasmatic nucleus and the circadian timekeeping system revisited. *Brain Research Reviews* 33: 34–77. A review article dealing with the anatomy, physiology, and molecular biology of the suprachiasmatic nucleus.

WEB SITES TO EXPLORE

Biological Clocks Lectures (Howard Hughes Medical Institute):
http://www.hhmi.org/biointeractive/clocks/lectures.html

Brain Catalog:
http://braininfo.rprc.washington.edu

Carl Johnson's Laboratory (Vanderbilt University):
http://www.cas.vanderbilt.edu/johnsonlab/

Circadian Mouse Genes:
http://expression.gnf.org/circadian/

Society for Neuroscience:
http://apu.sfn.org

LITERATURE CITED

1. Olds, J. (1958). Self-stimulation of the brain. *Science* 127: 315–324.
2. Alleva, J. J., Waleski, M. V. & Alleva, F. R. (1971). A biological clock controlling the estrous cycle of the hamster. *Endocrinology* 88: 1368–1379.
3. Stephan, F. K. & 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 U.S.A.* 69: 1583–1586.
4. Moore, R. Y. & 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. & 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. & 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. & 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. & 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. & Ebihara, S. (2001). Identification of the suprachiasmatic nucleus in birds. *American Journal of Physiology* 280: R1185–R1189.
10. Redlin, U. & 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. & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247: 975–978.
13. Pickard, G. E. & 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. & Watson-Whitmyre, M. (1976). Nucleus suprachiasmaticus: the biological clock in the hamster? *Science* 191: 197–199.

15. Harrington, M. E., Rahmani, T. & 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. & 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. Satinoff, E. & 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.
18. Ruis, J. F., Rietveld, W. J. & 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.
19. Stephan, F. K. & 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.
20. Honma, S., Honma, K., Shirakawa, T. & Hiroshige, T. (1988). Rhythms in behavior, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. *Physiology and Behavior* 44: 247–255.
21. Stephan, F. K., Swann, J. M. & Sisk, C. L. (1979). Entrainment of circadian rhythms by feeding schedules in rats with suprachiasmatic lesions. *Behavioral and Neural Biology* 25: 545–554.
22. Kurumiya, S. & 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.
23. Warren, W. S., Champney, T. H. & 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.
24. Sano, H., Hayashi, H., Makino, M., Takezawa, H., Hirai, M., Saito, H. & 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.
25. Wachulec, M., Li, H., Tanaka, H., Peloso, E. & Satinoff, E. (1997). Suprachiasmatic nuclei lesions do not eliminate homeostatic thermoregulatory responses in rats. *Journal of Biological Rhythms* 12: 226–234.
26. Scheer, F. A. J. L., Ter Horst, G. J., van der Vliet, J. & 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.
27. Ohtsuka-Isoya, M., Hayashi, H. & Shinoda, H. (2001). Effect of suprachiasmatic nucleus lesion on circadian dentin increment in rats. *American Journal of Physiology* 280: R1364–R1370.
28. Mahoney, M., Bult, A. & 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.
29. Sato, T. & 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.
30. Gerkema, M. P., Groos, G. A. & 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.
31. Filipski, E., King, V. M., Etienne, M. C., Li, X. M., Claustrat, B., Granda, T. G., Milano, G., Hastings, M. H. & 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.
32. Ruby, N. F. & Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *American Journal of Physiology* 263: R353–R362.
33. Paul, M. J., Kauffman, A. S. & Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
34. Zucker, I., Boshes, M. & Dark, J. (1983). Suprachiasmatic nuclei influence circannual and circadian rhythms of ground squirrels. *American Journal of Physiology* 244: R472–R480.
35. Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.
36. Osborne, A. R. & Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.
37. Ruby, N. F., Ibuka, N., Barnes, B. M. & Zucker, I. (1989). Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *American Journal of Physiology* 257: R210–R215.
38. Eastman, C. I., Mistlberger, R. E. & Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology and Behavior* 32: 357–368.
39. Liu, S., Chen, X. M., Yoda, T. Nagashima, K., Fukuda, Y. & Kanosue, K. (2002). Involvement of the suprachiasmatic nucleus in body temperature modulation by food deprivation in rats. *Brain Research* 929: 26–36.
40. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. & Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
41. Dunn, J. D., Castro, A. J. & McNulty, J. A. (1977). Effect of suprachiasmatic ablation on the daily temperature rhythm. *Neuroscience Letters* 6: 345–348.

42. Powell, E. W., Halberg, F., Pasley, J. N., Lubanovic, W., Ernsberger, P. & Scheving, L. E. (1980). Suprachiasmatic nucleus and circadian core temperature rhythm in the rat. *Journal of Thermal Biology* 5: 189–196.
43. Halberg, F., Lubanovic, W. A., Sothern, R. B., Brockway, B., Powell, E. W., Pasley, J. N. & 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.
44. Fuller, C. A., Lydic, R., Sulzman, F. M., Albers, H. E., Tepper, B. & 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.
45. Aalto, J. & 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.
46. LeSauter, J. & Silver, R. (1994). Suprachiasmatic nucleus lesions abolish and fetal grafts restore circadian gnawing rhythms in hamsters. *Restorative Neurology and Neuroscience* 6: 135–143.
47. Kriegsfeld, L. J., LeSauter, J. & Silver, R. (2004). Targeted microlesions reveal novel organization of the hamster suprachiasmatic nucleus. *Journal of Neuroscience* 24: 2449–2457.
48. Liu, J. H. K. & 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.
49. Mistlberger, R. E., Bergmann, B. M., Waldenar, W. & Rechtschaffen, A. (1983). Recovery sleep following sleep deprivation in intact and suprachiasmatic nuclei-lesioned rats. *Sleep* 6: 217–233.
50. Trachsel, L., Edgar, D. M., Seidel, W. F., Heller, H. C. & Dement, W. C. (1992). Sleep homeostasis in suprachiasmatic nuclei-lesioned rats: effects of sleep deprivation and triazolam administration. *Brain Research* 589: 253–261.
51. Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J. & 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.
52. Palm, I. F., van der Beek, E. M., Swarts, H. J. M., van der Vliet, J., Wiegant, V. M., Buijs, R. M. & Kalsbeek, A. (2001). Control of the estradiol-induced prolactin surge by the suprachiasmatic nucleus. *Endocrinology* 142: 2296–2302.
53. Kalsbeek, A., Fliers, E., Romijn, J. A., la Fleur, S. E., Wortel, J., Bakker, O., Endert, E. & Buijs, R. M. (2001). The suprachiasmatic nucleus generates the diurnal changes in plasma leptin levels. *Endocrinology* 142: 2677–2685.
54. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N. & Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
55. Ruiter, M., La Fleur, S. E., van Heijningen, C., van der Vliet, J., Kalsbeek, A. & 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.
56. Hastings, M. H., Roberts, A. C. & 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.
57. Mistlberger, R. E. & Rusak, B. (1988). Food-anticipatory circadian rhythms in rats with paraventricular and lateral hypothalamic ablations. *Journal of Biological Rhythms* 3: 277–291.
58. Harrington, M. E., Eskes, G. A., Dickson, P. & Rusak, B. (1990). Lesions dorsal to the suprachiasmatic nuclei abolish split activity rhythms of hamsters. *Brain Research Bulletin* 24: 593–597.
59. Pieper, D. R. & Lobocki, C. A. (1991). Olfactory bulbectomy lengthens circadian period of locomotor activity in golden hamsters. *American Journal of Physiology* 261: R973–R978.
60. Goodless-Sanchez, N., Moore, R. Y. & Morin, L. P. (1991). Lateral hypothalamic regulation of circadian rhythm phase. *Physiology and Behavior* 49: 533–537.
61. Ruby, N. F. (1995). Paraventricular nucleus ablation disrupts daily torpor in Siberian Hamsters. *Brain Research Bulletin* 37: 193–198.
62. Marcilhac, A., Maurel, D., Anglade, G., Ixart, G., Mekaouche, M., Héry, F. & 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.
63. Moore, R. Y. and Danchenko, R. L. (2002). Paraventricular-subparaventricular hypothalamic lesions selectively affect circadian function. *Chronobiology International* 19: 345–360.
64. Shibata, S. & Moore, R. Y. (1987). Development of neuronal activity in the rat suprachiasmatic nucleus. *Brain Research* 431: 311–315.
65. Satinoff, E., Li, H., Tcheng, T. K., Liu, C., McArthur, A. J., Medanic, M. & 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.
66. Shibata, S. & Moore, R. Y. (1994). Calmodulin inhibitors produce phase shifts of circadian rhythms *in vivo* and *in vitro*. *Journal of Biological Rhythms* 9: 27–41.
67. Derambure, P. S. & Boulant, J. A. (1994). Circadian thermosensitive characteristics of suprachiasmatic neurons *in vitro*. *American Journal of Physiology* 266: R1876–R1884.
68. Meijer, J. H., Schaap, J., Watanabe, K. & Albus, H. (1997). Multi-unit activity recordings in the suprachiasmatic nuclei: *in vivo* versus *in vitro* models. *Brain Research* 753: 322–327.
69. 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.

70. Chen, D., Buchanan, G. F., Ding, J. M., Hannibal, J. & 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 U.S.A.* 96: 13468–13473.
71. McArthur, A. J., Coogan, A. N., Ajpru, S., Sugden, D., Biello, S. M. & Piggins, H. D. (2000). Gastrin-releasing peptide phase-shifts suprachiasmatic nuclei neuronal rhythms *in vitro*. *Journal of Neuroscience* 20: 5496–5502.
72. Nakamura, W., Honma, S., Shirakawa, T. & Honma, K. (2001). Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *European Journal of Neuroscience* 14: 666–674.
73. Zlomanczuk, P., Margraf, R. R. & 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.
74. Shinohara, K., Honma, S., Katsuno, Y., Abe, H. & Honma, K. (1995). Two distinct oscillators in the rat suprachiasmatic nucleus *in vitro*. *Proceedings of the National Academy of Sciences U.S.A.* 92: 7396–7400.
75. Ruby, N. F. & Heller, H. C. (1996). Temperature sensitivity of the suprachiasmatic nucleus of ground squirrels and rats *in vitro*. *Journal of Biological Rhythms* 11: 126–136.
76. Hall, A. C., Hoffmater, R. M., Stern, E. L., Harrington, M. E. & Bickar, D. (1997). Suprachiasmatic nucleus neurons are glucose sensitive. *Journal of Biological Rhythms* 12: 388–400.
77. Earnest, D. J., Liang, F. Q., Ratcliff, M. & Cassone, V. M. (1999). Immortal time: circadian clock properties of rat suprachiasmatic cell lines. *Science* 283: 693–695.
78. Abe, M., Herzog, E. D. & Block, G. D. (2000). Lithium lengthens the circadian period of individual suprachiasmatic nucleus neurons. *NeuroReport* 11: 3261–3264.
79. Jagota, A., de la Iglesia, H. O. & Schwartz, W. J. (2000). Morning and evening circadian oscillations in the suprachiasmatic nucleus *in vitro*. *Nature Neuroscience* 3: 372–376.
80. Mrugala, M., Zlomanczuk, P., Jagota, A. & Schwartz, W. J. (2000). Rhythmic multi-unit neural activity in slices of hamster suprachiasmatic nucleus reflect prior photoperiod. *American Journal of Physiology* 278: R987–R994.
81. Allen, G., Rappe, J., Earnest, D. J. & 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.
82. Albus, H., Bonnefont, X., Chaves, I., Yasui, A., Diczy, J., van der Horst, G. T. J. & Meijer, J. H. (2002). Cryptochrome-deficient mice lack circadian electrical activity in the suprachiasmatic nuclei. *Current Biology* 12: 1130–1133.
83. Quintero, J. E., Kuhlman, S. J. & McMahon, D. G. (2003). The biological clock nucleus: a multiphasic oscillator network regulated by light. *Journal of Neuroscience* 23: 8070–8076.
84. Herzog, E. D. & Huckfeldt, R. M. (2003). Circadian entrainment to temperature, but not light, in the isolated suprachiasmatic nucleus. *Journal of Neurophysiology* 90: 763–770.
85. Sugiyama, T., Yoshioka, T. & 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.
86. Aton, S. J., Block, G. D., Tei, H., Yamazaki, S. & 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.
87. Burgoon, P. W., Lindberg, P. T. & 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.
88. Kurumiya, S. & Kawamura, H. (1988). Circadian oscillation of the multiple unit activity in the guinea pig suprachiasmatic nucleus. *Journal of Comparative Physiology A* 162: 301–308.
89. Meijer, J. H., Watanabe, K., Détári, L. & Schaap, J. (1996). Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats. *Brain Research* 741: 352–355.
90. Meijer, J. H., Watanabe, K., Schaap, J., Albus, H. & Détári, L. (1998). Light responsiveness of the suprachiasmatic nucleus: long-term multi-unit and single-unit recordings in freely moving rats. *Journal of Neuroscience* 18: 9078–9087.
91. Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D. & Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus *in vivo*. *Journal of Neuroscience* 18: 10709–10723.
92. Deboer, T., Vansteensel, M. J., Détári, L. & Meijer, J. H. (2003). Sleep states alter activity of suprachiasmatic nucleus neurons. *Nature Neuroscience* 6: 1086–1090.
93. Schwartz, W. J., Davidsen, L. C. & Smith, C. B. (1980). *In vivo* metabolic activity of a putative circadian oscillator, the rat suprachiasmatic nucleus. *Journal of Comparative Neurology* 189: 157–167.
94. Schwartz, W. J., Reppert, S. M., Eagan, S. M. & Moore-Ede, M. C. (1983). *In vivo* metabolic activity of the suprachiasmatic nuclei: a comparative study. *Brain Research* 274: 184–187.
95. McEachron, D. L., Kripke, D. F., Sharp, F. R., Lewy, A. J. & McClellan, D. E. (1985). Lithium effects on selected circadian rhythms in rats. *Brain Research Bulletin* 15: 347–350.
96. Room, P., Tielemans, A. J. P. C., De Boer, T., Tonnaer, J. A. D. M., Wester, J., Van den Broek, J. H. M. & 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.
97. Room, P. & Tielemans, A. J. P. C. (1989). Circadian variations in local cerebral glucose utilization in freely moving rats. *Brain Research* 505: 321–325.

98. Servière, J., Gendrot, G., LeSauter, J. & Silver, R. (1994). Host resets phase of grafted suprachiasmatic nucleus: a 2-DG study of time course of entrainment. *Brain Research* 655: 168–176.
99. Sumová, A., Trávnícková, Z., Mikkelsen, J. D. & Illnerová, H. (1998). Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research* 801: 254–258.
100. Guido, M. E., Guido, L. B. de, Goguen, D., Robertson, H. A. & Rusak, B. (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *Journal of Biological Rhythms* 14: 275–280.
101. Nunez, A. A., Bult, A., McElhinny, T. L. & 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.
102. Sumová, A., Trávnícková, Z. & Illnerová, H. (2000). Spontaneous c-Fos rhythm in the rat suprachiasmatic nucleus: location and effect of photoperiod. *American Journal of Physiology* 279: R2262–R2269.
103. Schwartz, M. D., Nunez, A. A. & Smale, L. (2004). Differences in the suprachiasmatic nucleus and lower subparaventricular zone of diurnal and nocturnal rodents. *Neuroscience* 127: 13–23.
104. Romero, M. T., Lehman, M. N. & Silver, R. (1993). Age of donor influences ability of suprachiasmatic nucleus grafts to restore circadian rhythmicity. *Developmental Brain Research* 71: 45–52.
105. Viswanathan, N. & Davis, F. C. (1995). Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. *Brain Research* 686: 10–16.
106. Grosse, J. & 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.
107. Boer, G. J., van Esseveldt, K. E., van der Geest, B. A. M., Duindam, H. & Rietveld, W. J. (1999). Vasopressin-deficient suprachiasmatic nucleus grafts re-instate circadian rhythmicity in suprachiasmatic nucleus lesioned arrhythmic rats. *Neuroscience* 89: 375–385.
108. Zlomanczuk, P., Mrugala, M., de la Iglesia, H. O., Ourednik, V., Quesenberry, P. J., Snyder, E. Y. & 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.
109. Tousson, E. & Meissl, H. (2004). Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus. *Journal of Neuroscience* 24: 2983–2988.
110. Saitoh, Y., Matsui, Y., Nihonmatsu, I. & 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.
111. Sollars, P. J., Kimble, D. P. & Pickard, G. E. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *Journal of Neuroscience* 15: 2109–2122.
112. Lehman. M. N., LeSauter, J. & Silver, R. (1998). Fiber outgrowth from anterior hypothalamic and cortical xenografts in the third ventricle. *Journal of Comparative Neurology* 391: 133–145.
113. Kaufman, C. M. & 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.
114. LeSauter, J. & Silver, R. (1999). Localization of a suprachiasmatic nucleus subregion regulating locomotor rhythmicity. *Journal of Neuroscience* 19: 5574–5585.
115. Vogelbaum, M. A. & Menaker, M. (1992). Temporal chimeras produced by hypothalamic transplants. *Journal of Neuroscience* 12: 3619–3627.
116. Vogelbaum, M. A., Galef, J. & Menaker, M. (1993). Factors determining the restoration of circadian behavior by hypothalamic transplants. *Journal of Neural Transplantation and Plasticity* 4: 239–256.
117. Low-Zeddies, S. S. & Takahashi, J. S. (2001). Chimera analysis of the *Clock* mutation in mice shows that complex cellular integration determines circadian behavior. *Cell* 105: 25–42.
118. Van den Pol, A. N. (1980). The hypothalamic suprachiasmatic nucleus of rat: intrinsic anatomy. *Journal of Comparative Neurology* 191: 661–702.
119. Okamura, H., Tominaga, K., Ban, Y., Tanaka, M., Tamada, Y., Inouye, S. T. & 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.
120. Leak, R. K., Card, J. P. & Moore, R. Y. (1999). Suprachiasmatic pacemaker organization analyzed by viral transynaptic transport. *Brain Research* 819: 23–32.
121. Colwell, C. S. (2000). Rhythmic coupling among cells in the suprachiasmatic nucleus. *Journal of Neurobiology* 43: 379–388.
122. Jobst, E. E., Robinson, D. W. & Allen, C. N. (2004). Potential pathways for intercellular communication within the calbindin subnucleus of the hamster suprachiasmatic nucleus. *Neuroscience* 123: 87–99.
123. Burgoon, P. W. & Boulant, J. A. (2001). Temperature-sensitive properties of rat suprachiasmatic nucleus neurons. *American Journal of Physiology* 281: R706–R715.
124. Pennartz, C. M. A., de Jeu, M. T. G., Bos, N. P. A., Schaap, J. & Geurtsen, A. M. S. (2002). Diurnal modulation of pacemaker potentials and calcium current in the mammalian circadian clock. *Nature* 416: 286–290.
125. Saeb-Parsy, K. & 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.
126. Kuhlman, S. J. & 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.

127. Jackson, A. C., Yao, G. L. & Bean, B. P. (2004). Mechanism of spontaneous firing in dorsomedial suprachiasmatic nucleus neurons. *Journal of Neuroscience* 24: 7985–7998.
128. Kononenko, N. I. & Dudek, F. E. (2004). Mechanism of irregular firing of suprachiasmatic nucleus neurons in rat hypothalamic slices. *Journal of Neurophysiology* 91: 267–273.
129. Dowse, H. B. & 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.
130. Miller, J. D. & Fuller, C. A. (1992). Isoperiodic neuronal activity in suprachiasmatic nucleus of the rat. *American Journal of Physiology* 263: R51–R58.
131. Barrio, R. A., Zhang, L. & Maini, P. K. (1997). Hierarchically coupled ultradian oscillators generating robust circadian rhythms. *Bulletin of Mathematical Biology* 59: 517–532.
132. Welsh, D. K., Logothetis, D. E., Meister, M. & Reppert, S. M. (1995). Individual neurons dissociated from rat suprachiasmatic nucleus express independently phased circadian firing rhythms. *Neuron* 14: 697–706.
133. Liu, C., Weaver, D. R., Strogatz, S. H. & Reppert, S. M. (1997). Cellular construction of a circadian clock: period determination in the suprachiasmatic nuclei. *Cell* 91: 855–860.
134. Honma, S., Shirakawa, T., Katsuno, Y., Namihira, M. & Honma, K. (1998). Circadian periods of single suprachiasmatic neurons in rats. *Neuroscience Letters* 250: 157–160.
135. Honma, S., Nakamura, W., Shirakawa, T. & 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.
136. Herzog, E. D., Aton, S. J., Numano, R., Sakaki, Y. & Tei, H. (2004). Temporal precision in the mammalian circadian system: a reliable clock from less reliable neurons. *Journal of Biological Rhythms* 19: 35–46.
137. 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.
138. Bouskila, Y. & 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.
139. Kunz, H. & 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.
140. Antle, M. C., Foley, D. K., Foley, N. C. & Silver, R. (2003). Gates and oscillators: a network model of the brain clock. *Journal of Biological Rhythms* 18: 339–350.
141. Shirakawa, T., Honma, S., Katsuno, Y., Oguchi, H. & Honma, K. (2000). Synchronization of circadian firing rhythms in cultured rat suprachiasmatic neurons. *European Journal of Neuroscience* 12: 2833–2838.
142. Honma, S., Shirakawa, T., Nakamura, W. & Honma, K. (2000). Synaptic communication of cellular oscillations in the rat suprachiasmatic neurons. *Neuroscience Letters* 294: 113–116.
143. Yamaguchi, S., Isejima, H., Matsuo, T., Okura, R., Yagita, K., Kobayashi, M. & Okamura, H. (2003). Synchronization of cellular clocks in the suprachiasmatic nucleus. *Science* 302: 1408–1412.
144. Prosser, R. A., Edgar, D. M., Heller, H. C. & Miller, J. D. (1994). A possible glial role in the mammalian circadian clock. *Brain Research* 643: 296–301.
145. Bennett, M. R. & Schwartz, W. J. (1994). Are glia among the cells that express immunoreactive c-Fos in the suprachiasmatic nucleus? *NeuroReport* 5: 1737–1740.
146. 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.
147. Moore, R. Y. & Speh, J. C. (1993). GABA is the principal neurotransmitter of the circadian system. *Neuroscience Letters* 150: 112–116.
148. Morin, L. P. & 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.
149. Belenky, M. A. & 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.
150. Liou, S. Y., Shibata, S., Albers, H. E. & 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.
151. Wagner, S., Castel, M., Gainer, H. & Yarom, Y. (1997). GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature* 387: 598–603.
152. Liu, C. & Reppert, S. M. (2000). GABA synchronizes clock cells within the suprachiasmatic circadian clock. *Neuron* 25: 123–128.
153. Belenky, M. A., Sagiv, N., Fritschy, J. M. & Yarom, Y. (2003). Presynaptic and postsynaptic $GABA_A$ receptors in rat suprachiasmatic nucleus. *Neuroscience* 118: 909–923.
154. Biggs, K. R. & Prosser, R. A. (1998). $GABA_B$ receptor stimulation phase-shifts the mammalian circadian clock *in vitro*. *Brain Research* 807: 250–254.
155. Tominaga, K., Shibata, S., Hamada, T. & 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.
156. Novak, C. M. & 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.
157. Novak, C. M. & 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.

158. Strecker, G. J., Wuarin, J. P. & Dudek, F. E. (1997). $GABA_A$-mediated local synaptic pathways connect neurons in the rat suprachiasmatic nucleus. *Journal of Neurophysiology* 78: 2217–2220.
159. Itri, J. & 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.
160. Okamura, H., Tominaga, K., Ban, Y., Fukuhara, C., Yanaihara, N., Ibata, Y. & 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.
161. Schilling, J. & 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.
162. Smale, L. & 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.
163. Van der Zee, E. A., Oklejewicz, M., Jansen, K., Daan, S. & 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.
164. Hofman, M. A. (2003). Circadian oscillations of neuropeptide expression in the human biological clock. *Journal of Comparative Physiology A* 189: 823–831.
165. Murakami, N., Takamure, M., Takahashi, K., Utunomiya, K., Kuroda, H. & 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.
166. Hofman, M. A. & Swaab, D. F. (1993). Diurnal and seasonal rhythms of neuronal activity in the suprachiasmatic nucleus of humans. *Journal of Biological Rhythms* 8: 283–295.
167. Tominaga, K., Shinohara, K., Otori, Y., Fukuhara, C. & Inouye, S. T. (1992). Circadian rhythms of vasopressin content in the suprachiasmatic nucleus of the rat. *NeuroReport* 3: 809–812.
168. Kalsbeek, A., van Heerikhuize, J. J., Wortel, J. & Buijs, R. M. (1998). Restricted daytime feeding modifies suprachiasmatic nucleus vasopressin release in rats. *Journal of Biological Rhythms* 13: 18–29.
169. Watanabe, K., Vanecek, J. & 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.
170. Dardente, H., Menet, J. S., Challet, E., Tournier, B. B., Pévet, P. & 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.
171. Noguchi, T., Watanabe, K., Ogura, A. & Yamaoka, S. (2004). The clock in the dorsal suprachiasmatic nucleus runs faster than that in the ventral. *European Journal of Neuroscience* 20: 3199–3202.
172. Gerkema, M. P., van der Zee, E. A. & 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.
173. Kaufman, C. M. & Menaker, M. (1994). Ontogeny of light-induced Fos-like immunoreactivity in the hamster suprachiasmatic nucleus. *Brain Research* 633: 162–166.
174. Abizaid, A., Horvath, B., Keefe, D. L., Leranth, C. & Horvath, T. L. (2004). Direct visual and circadian pathways target neuroendocrine cells in primates. *European Journal of Neuroscience* 20: 2767–2776.
175. Reed, H. E., Cutler, D. J., Brown, T. M., Coen, C. W. & Piggins, H. D. (2002). Effects of vasoactive intestinal polypeptide on neurones of the rat suprachiasmatic nuclei *in vitro*. *Journal of Neuroendocrinology* 14: 639–646.
176. Piggins, H. D., Antle, M. C. & Rusak, B. (1995). Neuropeptides phase shift the mammalian circadian pacemaker. *Journal of Neuroscience* 15: 5612–5622.
177. Reed, H. E., Meyer-Spasche, A., Cutler, D. J., Coen, C. W. & Piggins, H. D. (2001). Vasoactive intestinal polypeptide (VIP) phase-shifts the rat suprachiasmatic nucleus clock *in vitro*. *European Journal of Neuroscience* 13: 839–843.
178. Hughes, A. T., Fahey, B., Cutler, D. J., Coogan, A. N. & 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.
179. Tanaka, M., Hayashi, S., Tamada, Y., Ikeda, T., Hisa, Y., Takamatsu, T. & Ibata, Y. (1997). Direct retinal projections to GRP neurons in the suprachiasmatic nucleus of the rat. *NeuroReport* 8: 2187–2191.
180. Gary, K. A., Sollars, P. J., Lexow, N., Winokur, A. & Pickard, G. E. (1996). Thyrotropin-releasing hormone phase shifts circadian rhythms in hamsters. *NeuroReport* 7: 1631–1634.
181. Thomas, M. A., Fleissner, G., Stöhr, M., Hauptfleisch, S. & 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.
182. Mitome, M., Shirakawa, T., Oshima, S., Nakamura, W. & Oguchi, H. (2001). Circadian rhythm of nitric oxide production in the dorsal region of the suprachiasmatic nucleus in rats. *Neuroscience Letters* 303: 161–164.
183. Nakahara, K., Hanada, R., Murakami, N., Teranishi, H., Ohgusu, H., Fukushima, N., Moriyama, M., Ida, T., Kangawa, K. & 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.
184. Crichton, M. (1990). *Jurassic Park*. New York: Knopf.
185. Cherfas, J. (1991). Ancient DNA: still busy after death. *Science* 253: 1354–1356.

186. Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D. A. & Cooper, A. (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* 300: 791–795.
187. Krauthammer, C. (1997). A special report on cloning. *Time* 149(10).
188. Doolittle, R. F., Feng, D. F., Tsang, S., Cho, G. & Little, E. (1996). Determining divergence times of the major kingdoms of living organisms with a protein clock. *Science* 271: 470–477.
189. Wray, G. A., Levinton, J. S. & Shapiro, L. H. (1996). Molecular evidence for Deep Precambrian divergences among metazoan phyla. *Science* 274: 568–573.
190. Starr, K. (1998). *The Starr Report: The Official Report of the Independent Counsel's Investigation of the President*. Rocklin, CA: Prima.
191. International Human Genome Sequencing Consortium (2001). Initial sequencing and analysis of the human genome. *Nature* 409: 860–921.
192. Ayer, A. J. (1959). *Logical Positivism*. Chicago: Free Press.
193. Carnap, R. (1966). *Philosophical Foundations of Physics*. New York: Basic Books.
194. Quine, W. V. (1995). *From Stimulus to Science*. Cambridge, MA: Harvard University Press.
195. Crick, F. (1995). *The Astonishing Hypothesis: The Scientific Search for the Soul*. New York: Simon & Schuster.
196. Metzinger, T. (2003). *Being No One: The Self-Model Theory of Subjectivity*. Cambridge, MA: MIT Press.
197. Koch, C. (2004). *The Quest for Consciousness: A Neurobiological Approach*. Englewood, CO: Roberts & Company.
198. Konopka, R. J. & Benzer, S. (1971). Clock mutants of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences U.S.A.* 68: 2112–2116.
199. Hall, J. C. (1990). Genetics of circadian rhythms. *Annual Review of Genetics* 24: 659–697.
200. Plautz, J. D., Kaneko, M., Hall, J. C. & Kay, S. A. (1997). Independent photoreceptive circadian clocks throughout *Drosophila*. *Science* 278: 1632–1635.
201. Cheng, Y., Gvakharia, B. & 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.
202. Ewer, J., Frisch, B., Hamblen-Coyle, M. J., Rosbash, M. & 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.
203. 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.
204. Peng, Y., Stoleru, D., Levine, J. D., Hall, J. C. & Rosbash, M. (2003). *Drosophila* free-running rhythms require intercellular communication. *PLoS Biology* 1: 32–40.
205. Hardin, P. E., Hall, J. C. & Rosbash, M. (1992). Circadian oscillations in period gene mRNA levels are transcriptionally regulated. *Proceedings of the National Academy of Sciences U.S.A.* 89: 11711–11715.
206. Edery, I., Rutila, J. E. & Rosbash, M. (1994). Phase shifting of the circadian clock by induction of the *Drosophila period* protein. *Science* 263: 237–240.
207. Sehgal, A., Price, J. L., Man, B. & Young, M. W. (1994). Loss of circadian behavioral rhythms and *per* RNA oscillations in the *Drosophila* mutant *timeless*. *Science* 263: 1603–1606.
208. Sehgal, A., Rothenfluh-Hilfiker, A., Hunter-Ensor, M., Chen, Y., Myers, M. P. & Young, M. W. (1995). Rhythmic expression of *timeless*: a basis for promoting circadian cycles in *period* gene autoregulation. *Science* 270: 808–810.
209. Gekakis, N., Saez, L., Delahaye-Brown, A. M., Myers, M. P., Sehgal, A., Young, M. W. & 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.
210. Ralph, M. R. & Menaker, M. (1988). A mutation of the circadian system in golden hamsters. *Science* 241: 1225–1227.
211. 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. & Takahashi, J. S. (1994). Mutagenesis and mapping of a mouse gene, *Clock*, essential for circadian behavior. *Science* 264: 719–725.
212. Tei, H., Okamura, H., Shigeyoshi, Y., Fukuhara, C., Ozawa, R., Hirose, M. & Sakaki, Y. (1997). Circadian oscillation of a mammalian homologue of the *Drosophila period* gene. *Nature* 389: 512–516.
213. Shearman, L. P., Zylka, M. J., Weaver, D. R., Kolakowski, L. F. & Reppert, S. M. (1997). Two *period* homologs: circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron* 19: 1261–1269.
214. Takumi, T., Taguchi, K., Miyake, S., Sakakida, Y., Takashima, N., Matsubara, C., Maebayashi, Y., Okumura, K., Takekida, S., Yamamoto, S., Yagita, K., Yan, L., Young, M. W. & Okamura, H. (1998). A light-independent oscillatory gene *mPer3* in mouse SCN and OVLT. *EMBO Journal* 17: 4753–4759.
215. King, D. P., Zhao, Y., Sangoram, A. M., Wilsbacher, L. D., Tanaka, M., Antoch, M. P., Steeves, T. D. L., Vitaterna, M. H., Kornhauser, J. M., Lowrey, P. L., Turek, F. W. & Takahashi, J. S. (1997). Positional cloning of the mouse circadian *Clock* gene. *Cell* 89: 641–653.
216. 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. & Takahashi, J. S. (1997). Functional identification of the mouse circadian *Clock* gene by transgenic BAC rescue. *Cell* 89: 655–667.
217. Gekakis, N., Staknis, D., Nguyen, H. B., Davis, F. C., Wilsbacher, L. D., King, D. P., Takahashi, J. S. & Weitz, C. J. (1998). Role of the CLOCK protein in the mammalian circadian mechanism. *Science* 280: 1564–1569.

218. Koike, N., Hida, A., Numano, R., Hiose, M., Sakaki, Y. & Tei, H. (1998). Identification of the mammalian homologues of the *Drosophila timeless* gene, *Timeless1*. *FEBS Letters* 441: 427–431.
219. Zylka, M. J., Shearman, L. P., Levine, J. D., Jin, X., Weaver, D. R. & Reppert, S. M. (1998). Molecular analysis of mammalian *Timeless*. *Neuron* 21: 1115–1122.
220. Bae, K., Lee, C., Sidote, D., Chuang, K. & 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.
221. Lee, C., Bae, K. & Edery, I. (1998). The *Drosophila* CLOCK protein undergoes daily rhythms in abundance, phosphorylation, and interactions with the PER-TIM complex. *Neuron* 21: 857–867.
222. Glossop, N. R. J., Lyons, L. C. & Hardin, P. E. (1999). Interlocked feedback loops within the *Drosophila* circadian oscillator. *Science* 286: 766–768.
223. Bae, K., Lee, C., Hardin, P. E. & 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.
224. Renn, S. C. P., Park, J. H., Rosbash, M., Hall, J. C. & 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.
225. Akten, B., Jauch, E., Genova, G. K., Kim, E. Y., Edery, I., Raabe, T. & Jackson, F. R. (2003). A role for CK2 in the *Drosophila* circadian oscillator. *Nature Neuroscience* 6: 251–257.
226. 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. & Blau, J. (2003). *Vrille*, *Pdp1*, and *dClock* form a second feedback loop in the *Drosophila* circadian clock. *Cell* 112: 329–341.
227. Preuss, F., Fan, J. Y., Kalive, M., Bao, S., Schuenemann, E., Bjes, E. S. & 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.
228. Nawathean, P. & Rosbash, M. (2004). The Doubletime and CKII kinases collaborate to potentiate Drosophila PER transcriptional repressor activity. *Molecular Cell* 13: 213–223.
229. Takahashi, J. S. (1992). Circadian clock genes are ticking. *Science* 258: 238–239.
230. McDonald, M. J. & Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* 107: 567–578.
231. Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N. & Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32: 657–671.
232. Ceriani, M. F., Hogenesch, J. B., Yanovsky, M., Panda, S., Straume, M. & Kay, S. A. (2002). Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *Journal of Neuroscience* 22: 9305–9319.
233. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N. & 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 U.S.A.* 99: 9562–9567.
234. Marshall, E. (2004). Getting the noise out of gene arrays. *Science* 306: 630–631.
235. Kitayama, Y., Iwasaki, H., Nishiwaki, T. & Kondo, T. (2003). KaiB functions as an attenuator of KaiC phosporilation in the cyanobacterial circadian clock system. *EMBO Journal* 22: 2127–2134.
236. Nakahira, Y., Katayama, M., Miyashita, H., Kutsuna, S., Iwasaki, H., Oyama, T. & Kondo, T. (2004). Global gene repression by KaiC as a master process of prokaryotic circadian system. *Proceedings of the National Academy of Sciences U.S.A.* 101: 881–885.
237. Schultz, T. F. & Kay, S. A. (2003). Circadian clocks in daily and seasonal control of development. *Science* 301: 326–328.
238. Salomé, P. A. & McClung, C. R. (2004). The *Arabidopsis thaliana* clock. *Journal of Biological Rhythms* 19: 425–435.
239. Park, D. H., Somers, D. E., Kim, Y. S., Choy, Y. H., Lim, H. K., Soh, M. S., Kim, H. J., Kay, S. A. & Nam, H. G. (1999). Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science* 285: 1579–1582.
240. Más, P., Kim, W. Y., Somers, D. & Kay, S. A. (2003). Targeted degradation of TOC1 by ZTL modulates circadian function in *Arabidopsis thaliana*. *Nature* 426: 567–570.
241. Hall, A., Bastow, R. M., Davis, S. J., Hanano, S., McWatters, H. G., Hibberd, V., Doyle, M. R., Sung, S., Halliday, K. J., Amasino, R. M. & Millar, A. J. (2003). The *TIME FOR COFFEE* gene maintains the amplitude and timing of Arabidopsis circadian clocks. *Plant Cell* 15: 2719–2729.
242. Aronson, B. D., Johnson, K. A., Loros, J. L. & Dunlap, J. C. (1994). Negative feedback defining a circadian clock: autoregulation of the clock gene *frequency*. *Science* 263: 1578–1584.
243. Aronson, B. D., Johnson, K. A. & 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 U.S.A.* 91: 7683–7687.
244. Crosthwaite, S. K., Loros, J. J. & Dunlap, J. C. (1995). Light-induced resetting of a circadian clock is mediated by a rapid increase in *frequency* transcript. *Cell* 81: 1003–1012.

245. Crosthwaite, S. K., Dunlap, J. C. & Loros, J. J. (1997). *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. *Science* 276: 763–769.
246. Refinetti, R. (1999). Amplitude of the daily rhythm of body temperature in eleven mammalian species. *Journal of Thermal Biology* 24: 477–481.
247. Cheng, P., Yang, Y. & Liu, Y. (2001). Interlocked feedback loops contribute to the robustness of the *Neurospora* circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 98: 7408–7413.
248. Correa, A., Lewis, Z. A., Greene, A. V., March, I. J., Gomer, R. H. & Bell-Pedersen, D. (2003). Multiple oscillators regulate circadian gene expression in *Neurospora*. *Proceedings of the National Academy of Sciences U.S.A.* 100: 13597–13602.
249. Bunger, M. K., Wilsbacher, L. D., Moran, S. M., Clendenin, C., Radcliffe, L. A., Hogenesch, J. B., Simon, M. C., Takahashi, J. S. & Bradfield, C. A. (2000). *Mop3* is an essential component of the master circadian pacemaker in mammals. *Cell* 103: 1009–1017.
250. Barnes, J. W., Tischkau, S. A., Barnes, J. A., Mitchell, J. W., Burgoon, P. W., Hickok, J. R. & Gillette, M. U. (2003). Requirement of mammalian *timeless* for circadian rhythmicity. *Science* 302: 439–442.
251. Hardin, P. E. (2004). Transcription regulation within the circadian clock: the E-box and beyond. *Journal of Biological Rhythms* 19: 348–360.
252. Van der Horst, G. T. J., Muijtjens, M., Kobayashi, K., Takano, R., Kanno, S., Takao, M., de Wit, J., Verkerk, A., Eker, A. P. M., van Leenen, D., Buijs, R., Bootsma, D., Hoeijmakers, J. H. J. & Yasui, A. (1999). Mammalian Cry1 and Cry2 are essential for maintenance of circadian rhythms. *Nature* 398: 627–630.
253. Okamura, H., Miyake, S., Sumi, Y., Yamaguchi, S., Yasui, A., Muijtjens, M., Hoeijmakers, J. H. J. & 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.
254. Shearman, L. P., Sriram, S., Weaver, D. R., Maywood, E. S., Chaves, I., Zheng, B., Kume, K., Lee, C. C., van der Horst, G. T. J., Hastings, M. H. & Reppert, S. M. (2000). Interacting molecular loops in the mammalian circadian clock. *Science* 288: 1013–1019.
255. Reppert, S. M. & Weaver, D. R. (2001). Molecular analysis of mammalian circadian rhythms. *Annual Review of Physiology* 63: 647–676.
256. Miyamoto, Y. & 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 U.S.A.* 95: 6097–6102.
257. Abe, H., Honma, S., Namihira, M., Masubichi, S., Ikeda, M., Ebihara, S. & 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.
258. Bae, K., Jin, X., Maywood, E. S., Hastings, M. H., Reppert, S. M. & Weaver, D. R. (2001). Differential functions of *mPer1*, *mPer2*, and *mPer3* in the SCN circadian clock. *Neuron* 30: 525–536.
259. Hara, R., Wan, K., Wakamatsu, H., Aida, R., Moriya, T., Akiyama, M. & Shibata, S. (2001). Restricted feeding entrains liver clock without participation of the suprachiasmatic nucleus. *Genes to Cells* 6: 269–278.
260. Abe, H., Honma, S., Namihira, M., Masubuchi, S. & 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.
261. Maywood, E. S., O'Brien, J. A. & Hastings, M. H. (2003). Expression of mCLOCK and other circadian clock-relevant proteins in the mouse suprachiasmatic nuclei. *Journal of Neuroendocrinology* 15: 329–334.
262. Butler, M. P., Honma, S., Fukumoto, T., Kawamoto, T., Fujimoto, K., Noshiro, M., Kato, Y. & Honma, K. (2004). *Dec1* and *Dec2* expression is disrupted in the suprachiasmatic nuclei of *Clock* mutant mice. *Journal of Biological Rhythms* 19: 126–134.
263. Bittman, E. L., Doherty, L., Huang, L. & Paroskie, A. (2003). *Period* gene expression in mouse endocrine tissues. *American Journal of Physiology* 285: R561–R569.
264. Nagano, M., Adachi, A., Nakahama, K., Nakamura, T., Tamada, M., Meyer-Bernstein, E., Sehgal, A. & Shigeyoshi, Y. (2003). An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *Journal of Neuroscience* 23: 6141–6151.
265. Von Gall, C., Noton, E., Lee, C. & 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.
266. Preitner, N., Damiola, F., Lopez-Molina, L., Zakany, J., Duboule, D., Albrecht, U. & Schibler, U. (2002). The orphan nuclear receptor REV-ERB-α controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110: 251–260.
267. Kondratov, R. V., Chernov, M. V., Kondratova, A. A., Gorbacheva, V. Y., Gudkov, A. V. & 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.
268. Lowrey, P. L., Shimomura, K., Antoch, M. P., Yamazaki, S., Zemenides, P. D., Ralph, M. R., Menaker, M. & Takahashi, J. S. (2000). Positional syntenic cloning and functional characterization of the mammalian circadian mutation *tau*. *Science* 288: 483–491.
269. Hardin, P. E. (2000). From biological clock to biological rhythms. *Genome Biology* 1: art. 1023.
270. Lin, J. M., Kilman, V. L., Keegan, K., Paddock, B., Emery-Le, M., Rosbash, M. & Allada, R. (2002). A role for casein kinase 2-α in the *Drosophila* circadian clock. *Nature* 420: 816–820.
271. Van Gelder, R. N., Herzog, E. D., Schwartz, W. J. & Taghert, P. H. (2003). Circadian rhythms: in the loop at last. *Science* 300: 1534–1535.

272. Constance, C. M., Green, C. B., Tei, H. & Block, G. D. (2002). *Bulla gouldiana period* exhibits unique regulation at the mRNA and protein levels. *Journal of Biological Rhythms* 17: 413–427.
273. Regier, J. C., Fang, Q. Q., Mitter, C., Peigler, R. S., Friedlander, T. P. & Solis, M. A. (1998). Evolution and phylogenetic utility of the *period* gene in Lepidoptera. *Molecular Biology and Evolution* 15: 1172–1182.
274. Toma, D. P., Bloch, G., Moore, D. & 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 U.S.A.* 97: 6914–6919.
275. Shimizu, I., Kawai, Y., Taniguchi, M. & Aoki, S. (2001). Circadian rhythm and cDNA cloning of the clock gene *period* in the honeybee *Apis cerana japonica*. *Zoological Science* 18: 779–789.
276. Závodská, R., Sauman, I. & 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.
277. Yoshimura, T., Suzuki, Y., Makino, E., Suzuki, T., Kuroiwa, A., Matsuda, Y., Namikawa, T. & Ebihara, S. (2000). Molecular analysis of avian circadian clock genes. *Molecular Brain Research* 78: 207–215.
278. Messager, S., Ross, A. W., Barrett, P. & Morgan, P. J. (1999). Decoding photoperiodic time through *Per1* and ICER gene amplitude. *Proceedings of the National Academy of Sciences U.S.A.* 96: 9938–9943.
279. Mrosovsky, N., Edelstein, K., Hastings, M. H. & 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.
280. Oster, H., Avivi, A., Joel, A., Albrecht, U. & 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.
281. Sumová, A., Sládek, M., Ják, M. & 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.
282. Beaulé, C., Houle, L. M. & 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.
283. Chang, D. C., McWatters, H. G., Williams, J. A., Gotter, A. L., Levine, J. D. & Reppert, S. M. (2003). Constructing a feedback loop with circadian clock molecules from the silkmoth, *Antheraea pernyi*. *Journal of Biological Chemistry* 278: 38149–38158.
284. Whitmore, D., Foulkes, N. S., Strähle, U. & Sassone-Corsi, P. (1998). Zebrafish *Clock* rhythmic expression reveals independent peripheral circadian oscillators. *Nature Neuroscience* 1: 701–707.
285. Chong, N. W., Chaurasia, S. S., Haque, R., Klein, D. C. & 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.
286. Fidler, A. E. & 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.
287. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U. & 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 U.S.A.* 99: 11718–11723.
288. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U. & 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.
289. Sumová, A., Ják, M., Sládek, M., Sauman, I. & 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.
290. Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H. & Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *Journal of Biological Rhythms* 18: 159–169.
291. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P. & 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.
292. Shieh, K. R. (2003). Distribution of the rhythm-related genes *rPeriod1*, *rPeriod2*, and *rClock* in the rat brain. *Neuroscience* 118: 831–843.
293. Lincoln, G., Messager, S., Andersson, H. & 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 U.S.A.* 99: 13890–13895.
294. Griffin, E. A., Staknis, D. & Weitz, C. J. (1999). Light-independent role of CRY1 and CRY2 in the mammalian clock. *Science* 286: 768–771.
295. Chen, Y. & Tan, E. C. (2004). Identification of human *Clock* gene variants by denaturing high-performance liquid chromatography. *Journal of Human Genetics* 49: 209–214.
296. Carlson, N. R. (1994). *Physiology of Behavior*, 5th Edition. Boston: Allyn and Bacon.
297. Garg, S. K. & 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.

298. Underwood, H. (1983). Circadian organization of the lizard *Anolis carolinensis*: a multioscillator system. *Journal of Comparative Physiology* 152: 265–274.
299. Molina-Borja, M. (1996). Pineal gland and circadian locomotor activity rhythm in the lacertid *Gallotia eisentrauti*: pinealectomy induces arrhthmicity. *Biological Rhythm Research* 27: 1–11.
300. Gaston, S. & Menaker, M. (1968). Pineal function: the biological clock in the sparrow? *Science* 160: 1125–1127.
301. Binkley, S., Kluth, E. & Menaker, M. (1971). Pineal function in sparrows: circadian rhythms and body temperature. *Science* 174: 311–314.
302. Heigl, S. & Gwinner, E. (1994). Periodic melatonin in the drinking water synchronizes circadian rhythms in sparrows. *Naturwissenschaften* 81: 83–85.
303. Iigo, M., Fujimoto, Y., Gunji-Suzuki, M., Yokosuka, M., Hara, M., Ohtani-Kaneko, R., Tabata, M., Aida, K. & 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.
304. Tosini, G. & Menaker, M. (1998). Multioscillatory circadian organization in a vertebrate, *Iguana iguana*. *Journal of Neuroscience* 18: 1105–1114.
305. Bertolucci, C., Wagner, G., Foà, A., Gwinner, E. & 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.
306. Brandstätter, R., Kumar, V., Abraham, U. & 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 U.S.A.* 97: 12324–12328.
307. Schenda, J. & 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.
308. Cheung, P. W. & McCormack, C. E. (1982). Failure of pinealectomy or melatonin to alter circadian activity rhythm of the rat. *American Journal of Physiology* 242: R261–R264.
309. Spencer, F., Shirer, H. W. & Yochim, J. M. (1976). Core temperature in the female rat: effect of pinealectomy or altered lighting. *American Journal of Physiology* 231: 355–360.
310. Bobbert, A. C. & 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.
311. Warren, W. S. & Cassone, V. M. (1995). The pineal gland: photoreception and coupling of behavioral, metabolic, and cardiovascular circadian outputs. *Journal of Biological Rhythms* 10: 64–79.
312. Morin, L. P. (1993). Age, but not pineal status, modulates circadian periodicity of golden hamsters. *Journal of Biological Rhythms* 8: 189–197.
313. Schuhler, S., Pitrosky, B., Kirsch, R. & Pévet, P. (2002). Entrainment of locomotor activity rhythm in pinealectomized adult Syrian hamsters by daily melatonin infusion. *Behavioural Brain Research* 133: 343–350.
314. Redman, J. R. & 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.
315. Harlow, H. J., Phillips, J. A. & 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.
316. Sánchez-Vázquez, F. J., Iigo, M., Madrid, J. A. & 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.
317. Underwood, H. (1983). Circadian pacemakers in lizards: phase-response curves and effects of pinealectomy. *American Journal of Physiology* 244: R857–R864.
318. Innicenti, A., Minutini, L. & Foà, A. (1993). The pineal and circadian rhythms of temperature selection and locomotion in lizards. *Physiology and Behavior* 53: 911–915.
319. Tosini, G. & 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.
320. Bartell, P. A., Miranda-Anaya, M. & Menaker, M. (2004). Period and phase control in a multioscillatory circadian system (*Iguana iguana*). *Journal of Biological Rhythms* 19: 47–57.
321. Oshima, I., Yamada, H., Goto, M., Sato, K. & 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.
322. 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.
323. Underwood, H. & Edmonds, K. (1995). The circadian rhythm of thermoregulation in Japanese quail. II. Multioscillator control. *Journal of Biological Rhythms* 10: 234–247.
324. Heigl, S. & Gwinner, E. (1999). Periodic food availability synchronizes locomotor and feeding activity in pinealectomized house sparrows. *Zoology* 102: 1–9.
325. Menaker, M., Moreira, L. F. & Tosini, G. (1997). Evolution of circadian organization in vertebrates. *Brazilian Journal of Medical and Biological Research* 30: 305–313.
326. Underwood, H. (2001). Circadian organization in nonmammalian vertebrates. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 111–140.

327. Michel, S., Geusz, M. E., Zaritsky, J. J. & Block, G. D. (1993). Circadian rhythm in membrane conductance expressed in isolated neurons. *Science* 259: 239–241.
328. Page, T. L., Wassmer, G. T., Fletcher, J. & 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.
329. Willbold, E., Huhn, J., Korf, H. W., Voisin, P. & 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.
330. Zhang, Y., Coleman, J. E., Fuchs, G. E. & Semple-Rowland, S. L. (2003). Circadian oscillator function in embryonic retina and retinal explant cultures. *Molecular Brain Research* 114: 9–19.
331. Tosini, G. & Menaker, M. (1996). Circadian rhythms in cultured mammalian retina. *Science* 272: 419–421.
332. Tosini, G. & Menaker, M. (1998). The *tau* mutation affects temperature compensation of hamster retinal circadian oscillators. *NeuroReport* 9: 1001–1005.
333. Sakamoto, K., Liu, C. & Tosini, G. (2004). Circadian rhythms in the retina of rats with photoreceptor degeneration. *Journal of Neurochemistry* 90: 1019–1024.
334. Steele, C. T., Zivkovic, B. D., Siopes, T. & 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.
335. Chabot, C. C. & Menaker, M. (1992). Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons. *Journal of Comparative Physiology A* 170: 615–622.
336. 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 U.S.A.* 75: 6276–6280.
337. Yamazaki, S., Goto, M. & Menaker, M. (1999). No evidence for extraocular photoreceptors in the circadian system of the Syrian hamster. *Journal of Biological Rhythms* 14: 197–201.
338. Freedman, M. S., Lucas, R. J., Soni, B., von Schantz, M., Muñoz, M., David-Gray, Z. & Foster, R. (1999). Regulation of mammalian circadian behavior ny non-rod, non-cone, ocular photoreceptors. *Science* 284: 502–504.
339. Yamazaki, S., Alones, V. & Menaker, M. (2002). Interaction of the retina with suprachiasmatic pacemakers in the control of circadian behavior. *Journal of Biological Rhythms* 17: 315–329.
340. Lee, H. S., Nelms, J. L., Nguyen, M., Silver, R. & Lehman, M. N. (2003). The eye is necessary for a circadian rhythm in the suprachiasmatic nucleus. *Nature Neuroscience* 6: 111–112.
341. Wakamatsu, H., Yoshinobu, Y., Aida, R., Moriya, T., Akiyama, M. & 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.
342. Angeles-Castellanos, M., Aguilar-Roblero, R. & Escobar, C. (2004). c-Fos expression in hypothalamic nuclei of food-entrained rats. *American Journal of Physiology* 286: R158–R165.
343. Slakey, L. L., Craig, M. C., Beytia, E., Briedis, A., Feldbruegge, D. H., Dugan, R. E., Qureshi, A. A., Subbarayan, C. & 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.
344. Dugan, R. E., Slakey, L. L., Briedis, A. V. & 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.
345. Lanza-Jacoby, S., Stevenson, N. R. & 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.
346. Wasan, K. M., Brunner, L. J., Berens, K. L., Meltzer, A. A. & Luke, D. R. (1989). Circadian assessment of lipids in the hyperphagic obese rat compared with lean litter-mates. *Chronobiology International* 6: 223–228.
347. Jurevics, H., Hostettler, J., Barrett, C., Morell, P. & 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.
348. Piccione, G., Caola, G. & 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.
349. Brown, S. A., Zumbrunn, G., Fleury-Olela, F, Preitner, N. & Schibler, U. (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Current Biology* 12: 1574–1583.
350. Tong, Y., Guo, H., Brewer, J. M., Lee, H., Lehman, M. N. & 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.
351. Lee, C., Weaver, D. R. & Reppert, S. M. (2004). Direct association between mouse PERIOD and CKI-ε is critical for a functional circadian clock. *Molecular and Cellular Biology* 24: 584–594.
352. Kita, Y., Shiozawa, M., Jin, W., Majewski, R. R., Besharse, J. C., Greene, A. S. & Jacob, H. J. (2002). Implications of circadian gene expression in kidney, liver and the effects of fasting on pharmacogenomic studies. *Pharmacogenetics* 12: 55–65.
353. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H. & 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.

354. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H. & Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.

355. Oishi, K., Miyazaki, K., Kadota, K., Kikuno, R., Nagase, T., Atsumi, G., Ohkura, N., Azama, T., Mesaki, M., Yukimasa, S., Kobayashi, H., Iitaka, C., Umehara, T., Horikoshi, M., Kudo, T., Shimizu, Y., Yano, M., Monden, M., Machida, K., Matsuda, J., Horie, S., Todo, T. & Ishida, N. (2003). Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. *Journal of Biological Chemistry* 278: 41519–41527.

356. Desai, V. G., Moland, C. L., Branham, W. S., Delongchamp, R. R., Fang, H., Duffy, P. H., Peterson, C. A., Beggs, M. L. & 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.

357. Yoo, S. H., Yamazaki, S., Lowrey, P. L., Shimomura, K., Ko, C. H., Buhr, E. D., Siepka, S. M., Hong, H. K., Oh, W. J., Yoo, O. J., Menaker, M. & Takahashi, J. S. (2004). PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proceedings of the National Academy of Sciences U.S.A.* 101: 5339–5346.

358. Yamazaki, S., Numano, R., Abe, M., Hida, A., Takahashi, R., Ueda, M., Block, G. D., Sakaki, Y., Menaker, M. & Tei, H. (2000). Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685.

359. Stokkan, K. A., Yamazaki, S., Tei, H., Sakaki, Y. & Menaker, M. (2001). Entrainment of the circadian clock in the liver by feeding. *Science* 291: 490–493.

360. Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F. & 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.

361. Miki, H., Yano, M., Iwanaga, H., Tsujinaka, T., Nakayama, M., Kobayashi, M., Oishi, K., Shiozaki, H., Ishida, N., Nagai, K. & Monden, M. (2003). Total parenteral nutrition entrains the central and peripheral circadian clocks. *NeuroReport* 14: 1457–1461.

362. Davidson, A. J., Poole, A. S., Yamazaki, S. & Menaker, M. (2003). Is the food-entrainable circadian oscillator in the digestive system? *Genes, Brain and Behavior* 2: 32–39.

363. Escobar, C., Mendoza, J. Y., Salazar-Juárez, A., Ávila, J., Hernández-Muñoz, R., Díaz-Muñoz, M. & 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.

364. Sakamoto, K. & 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.

365. Masubuchi, S., Honma, S., Abe, H., Ishizaki, K., Namihira, M., Ikeda, M. & 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.

366. Mühlbauer, E., Wolgast, S., Finckh, U., Peschke, D. & Peschke, E. (2004). Indication of circadian oscillations in the rat pancreas. *FEBS Letters* 564: 91–96.

367. Bjarnason, G. A., Jordan, R. C. K., Wood, P. A., Li, Q., Lincoln, D. W., Sothern, R. B., Hrushesky, W. J. M. & Ben-David, Y. (2001). Circadian expression of clock genes in human oral mucosa and skin. *American Journal of Pathology* 158: 1793–1801.

368. Yagita, K., Tamanini, F., van der Horst, G. T. J. & Okamura, H. (2001). Molecular mechanisms of the biological clock in cultured fibroblasts. *Science* 292: 278–281.

369. Grundschober, C., Delaunay, F., Pühlhofer, A., Triqueneaux, G., Laudet, V., Bartfai, T. & Nef, P. (2001). Circadian regulation of diverse gene products revealed by mRNA expression profiling of synchronized fibroblasts. *Journal of Biological Chemistry* 276: 46751–46758.

370. Duffield, G. E., Best, J. D., Meurers, B. H., Bittner, A., Loros, J. J. & 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.

371. Tamaru, T., Isojima, Y., van der Horst, G. T. J., Takei, K., Nagai, K. & Takamatsu, K. (2003). Nucleocytoplasmic shuttling and phosphorylation of BMAL1 are regulated by circadian clock in cultured fibroblasts. *Genes to Cells* 8: 973–983.

372. Izumo, M., Johnson, C. H. & 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 U.S.A.* 100: 16089–16094.

373. Granados-Fuentes, D., Prolo, L. M., Abraham, U. & Herzog, E. D. (2004). The suprachiasmatic nucleus entrains, but does not sustain, circadian rhythmicity in the olfactory bulb. *Journal of Neuroscience* 24: 615–619.

374. Andrews, R. V. (1968). Temporal secretory responses of cultured hamster adrenals. *Comparative Biochemistry and Physiology* 26: 179–193.

375. Peschke, E. & Peschke, D. (1998). Evidence for a circadian rhythm of insulin release from perifused rat pancreatic islets. *Diabetologia* 41: 1085–1092.

376. Feneberg, R. & 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.

377. Granados-Fuentes, D., Saxena, M. T., Prolo, L. M., Aton, S. J. & Herzog, E. D. (2004). Olfactory bulb neurons express functional, entrainable circadian rhythms. *European Journal of Neuroscience* 19: 898–906.

378. Hennessey, T. L. & Field, C. B. (1992). Evidence of multiple circadian oscillators in bean plants. *Journal of Biological Rhythms* 7: 105–113.

379. Thain, S. C., Murtas, G., Lynn, J. R., McGrath, R. B. & Millar, A. J. (2002). The circadian clock that controls gene expression in Arabidopsis is tissue specific. *Plant Physiology* 130: 102–110.
380. Roenneberg, T. & Morse, D. (1993). Two circadian oscillators in one cell. *Nature* 362: 362–364.
381. Von der Heide, F., Wilkens, A. & 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.
382. Tanoue, S., Krishnan, P., Krishnan, B., Dryer, S. E. & Hardin, P. E. (2004). Circadian clocks in antennal neurons are necessary and sufficient for olfaction rhythms in *Drosophila*. *Current Biology* 14: 638–649.
383. 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.
384. Nye, H. L., Cameron, J. A., Chernoff, E. A. & Stocum, D. L. (2003). Regeneration of the urodele limb: a review. *Developmental Dynamics* 226: 280–294.
385. Nakahara, K., Fukui, K. & 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.
386. Garcia, J. A., Zhang, D., Estill, S. J., Michnoff, C., Rutter, J., Reick, M., Scott, K., Diaz-Arrastia, R. & McKnight, S. L. (2000). Impaired cued and contextual memory in NPAS2-deficient mice. *Science* 288: 2226–2230.
387. Reick, M., Garcia, J. A., Dudley, C. & McKnight, S. L. (2001). NPAS2: an analog of Clock operative in the mammalian forebrain. *Science* 293: 506–509.
388. Rutter, J., Reick, M., Wu, L. C. & McNight, S. L. (2001). Regulation of Clock and NPAS2 DNA binding by the redox state of NAD cofactors. *Science* 293: 510–514.
389. Dudley, C. A., Erbel-Sieler, C., Estill, S. J., Reick, M., Franken, P., Pitts, S. & McKnight, S. L. (2003). Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science* 301: 379–383.
390. Pitts, S., Perone, E. & Silver, R. (2003). Food-entrained circadian rhythms are sustained in arrhythmic *Clk*/*Clk* mutant mice. *American Journal of Physiology* 285: R57–R67.
391. Green, C. B. & Menaker, M. (2003). Clocks on the brain. *Science* 301: 319–320.
392. Willie, J. T., Chemelli, R. M., Sinton, C. M. & Yanagisawa, M. (2001). To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annual Review of Neuroscience* 24: 429–458.
393. Akiyama, M., Yuasa, T., Hayasaka, N., Horikawa, K., Sakurai, T. & Shibata, S. (2004). Reduced food anticipatory activity in genetically orexin (hypocretin) neuron-ablated mice. *European Journal of Neuroscience* 20: 3054–3062.
394. Davidson, A. J., Yamazaki, S. & 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

CHAPTER OUTLINE

13.1 Afferent Pathways
13.2 Efferent Pathways

13.1 AFFERENT PATHWAYS

Chapter 11 discussed the sensory *receptors* that provide environmental information to the circadian system, while Chapter 12 described the cells and molecules that make up the master *pacemaker* and the multiple peripheral pacemakers. This chapter discusses 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*). Figure 13.1 lists some technical terms used frequently in this chapter. 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), while the term *antidromic* refers to the movement of impulses in the opposite direction (that is, from the axon to the cell body). Note that the physical direction of movement is reversed in the efferent pathway as compared with 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, while retrograde transport refers to movement of substances from the axon to the cell body.

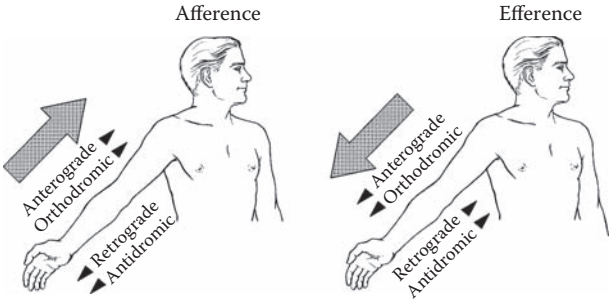


FIGURE 13.1 A few more technical terms. Afference refers to the flow of information from the periphery of the body to the central nervous system, while efference refers to the flow of information from the central nervous system to the periphery. Flow in the normal direction is called orthodromic (for nerve impulses) or anterograde (for everything else), while flow in a reverse direction is called antidromic (for nerve impulses) or retrograde (for everything else).

13.1.1 Anatomy

Chapter 11 showed that in mammals all photic input to the circadian system comes from the eyes. The eyes provide input to four different systems (Figure 13.2). Information destined for the visual system proceeds to the lateral geniculate nucleus (LGN) of the thalamus, 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 (SC) in the midbrain.[1,2] Photic information destined for the circadian system (for entrainment and probably also masking) proceeds mainly to the suprachiasmatic nucleus (SCN), although an alternate route through the thalamus also exists and is discussed later in this section.[3]

Ganglion cells in the eyes make monosynaptic contact with the SCN through the *retino-hypothalamic tract* (RHT). Originally described in the rat,[4] the RHT has been characterized in various other studies in rats,[5–11] golden hamsters,[12–17] moles,[18] Nile grass rats,[19,20] squirrels,[21] tree shrews,[22,23] monkeys,[23,24] and humans.[25] The various studies consistently indicate that the retinal fibers arrive predominantly in the ventral (core) region of the SCN.[4–6,11,19,22,24,25] 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, and most fibers proceed 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 towards the contralateral side — in mammals of various orders.[4,5,7,11,16,19,21,24] Bias towards *ipsilateral* afference has been described in tree shrews (*Tupaia belangeri*),[22] but the usual bias toward *contralateral* afference was described in a similar species (*Tupaia glis*).[23]

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. To visualize neural pathways, neuroanatomists use a few

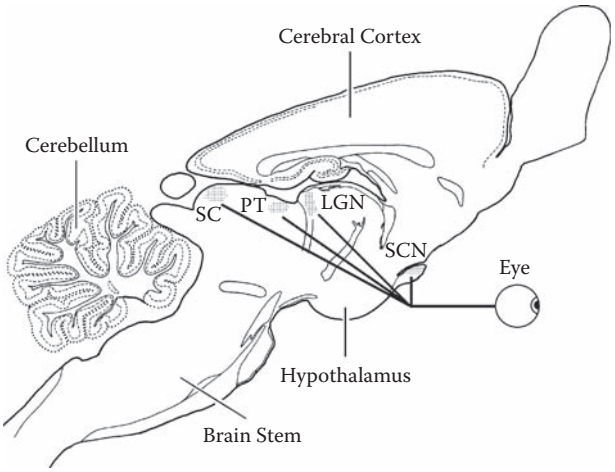


FIGURE 13.2 The nervous pathways from the eye to the brain. In mammals, retinal ganglion cells have four major projections to the brain, which correspond to the four sensory systems served by the eye. The visual afferent pathway passes through the lateral geniculate nucleus of the thalamus (LGN). The 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 travels directly to the suprachiasmatic nucleus (SCN) in the hypothalamus and indirectly through the thalamus (pathway not shown). (Sources: Kelly, J. P. (1981). Visual system II: anatomy of the central visual pathways. In: Kandel, E. R. & Schwartz, J. H. *Principles of Neural Science*. New York: Elsevier, pp. 226–235; Card, J. P. & Moore, R. Y. (1991). 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*. New York: Oxford University Press, pp. 51–76. Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum.)

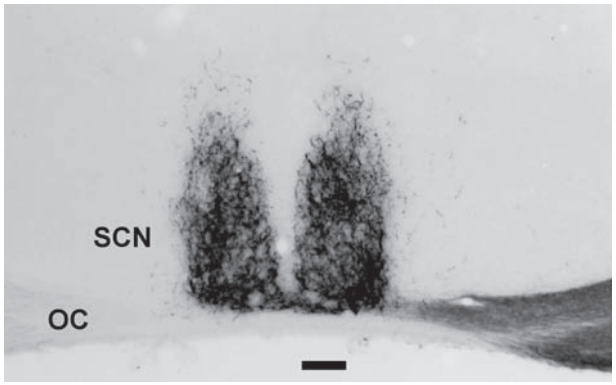


FIGURE 13.3 From the eye to the SCN: mouse. This microphotograph of the ventral region of a coronal section of the brain of a domestic mouse (*Mus musculus*) shows a typical result of anterograde tracing studies using cholera toxin subunit B (CTB). Injection of CTB in one eye results in staining of both the ipsilateral and the contralateral SCN (the right side of the section is the contralateral side). (Note: OC = optic chiasm, SCN = suprachiasmatic nucleus) (Scale bar: 100 μm) (Source: Image courtesy of Daniela Lupi, Department of Visual Neuroscience, Imperial College London, UK.)

carefully 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. A protein derived from the bean plant (*Phaseolus vulgaris* leucoagglutinin, or PHAL) is used commonly for *anterograde* tracing. For *retrograde* tracing, FluoroGold is the substance currently most often used. A third very popular substance is *cholera toxin subunit B* (CTB), which can be used for either anterograde or retrograde tracing. Figure 13.3 shows an example of the use of CTB for tracing the RHT. 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. Note that CTB advanced through the optic chiasm and invaded both the contralateral and the ipsilateral SCN. Figure 13.4 provides another example, in a tree shrew. 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). Figure 13.5 shows a third example, in a rat. CTB was injected in the right eye (left side of the figure). Projection to the SCN was heavier to the left SCN (right side of the figure), but strong ipsilateral projection also occurred. Contralaterally, the staining extended into the ventral subparaventricular zone (vSPZ), indicating unilateral innervation of this area. The brain slice was double-stained for vasoactive intestinal polypeptide (VIP), which is visible as sparse staining of the right vSPZ (left side of the figure) that corresponds to dorsal projections of the SCN (discussed in Section 13.2).

The SCN also 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[6,7,10,11,15,16,21,22,26] and projects directly to the SCN.[27,28] The projection from the IGL to the SCN is the GHT. While the RHT and the GHT are the pathways of photic input to the SCN, the SCN must receive *nonphotic* input through other pathways. Information from all regions of the body ascends to the brain through a variety of nerves (Figure 13.6), but the exact

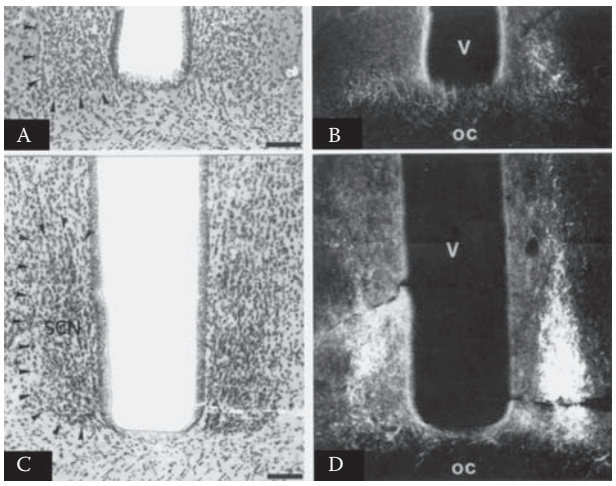


FIGURE 13.4 From the eye to the SCN: tree shrew. These microphotographs of the ventral region of coronal sections of the brain of a tree shrew (*Tupaia belangeri*) show the results of anterograde tracing studies using cholera toxin subunit B (CTB). The top panels (A and B) show sections from the anterior 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). (Note: OC = optic chiasm, V = third ventricle) (Scale bar: 100 μm) (Source: Reuss, S. & Fuchs, E. (2000). Anterograde tracing of retinal afferents to the tree shrew hypothalamus and raphe. *Brain Research* 874: 66–74. © Elsevier Science Publishers. Reproduced with permission from the publisher and the authors.)

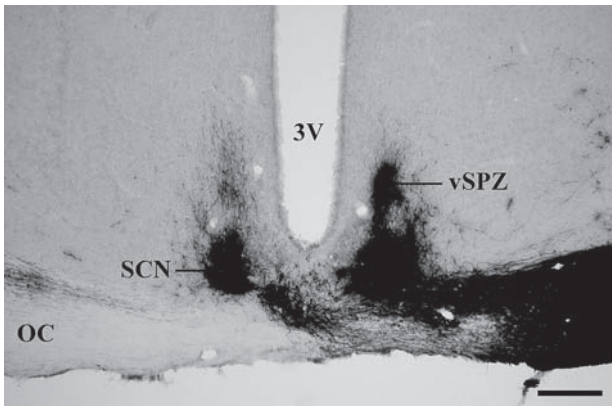


FIGURE 13.5 From the eye to the SCN: rat. This microphotograph of the ventral region of a coronal section of the brain of a laboratory rat (*Rattus norvegicus*) shows the result of an anterograde tracing study using cholera toxin subunit B (CTB). Injection of CTB in one eye resulted in bilateral staining of the SCN with slightly higher contralateral staining (right side of the section). Contralaterally, the staining extended into the vSPZ, indicating unilateral projection to this area. The section was double-stained for VIP, which is visible as sparse staining dorsal to the SCN on the left side of the section. (Note: 3V = third ventricle, OC = optic chiasm, SCN = suprachiasmatic nucleus, vSPZ = ventral subparaventricular zone) (Scale bar: 200 μm) (Source: Gooley, J. J., Lu, J., Fischer, D. & Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience* 23: 7093–7106. © 2003 Society for Neuroscience. Reproduced with permission from the publisher and the authors.)

pathways through which nonphotic information reaches the SCN are not known. Possible pathways identified in functional studies are described later in this section.

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.[6] Figure 13.7 summarizes these findings. In addition to the retina and the 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. 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 investigated thoroughly by a different group of researchers.[29]

13.1.2 Function

Of the seven sources of input to the SCN that were identified in anatomical studies (Figure 13.7), three are functionally significant: the retina, the IGL, and the raphe nuclei. These 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.

Electrophysiological recording of neuronal activity in the SCN indicates that SCN cells respond to photic stimulation of the eyes[30–37] as well as to electrical stimulation of the optic nerve.[38–41] Figure 13.9 shows the response of SCN neurons to electrical stimulation of the optic nerve. Instead of using traditional electrical recording techniques, the researchers recorded data optically (that is, they 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).[42] The short latency of the

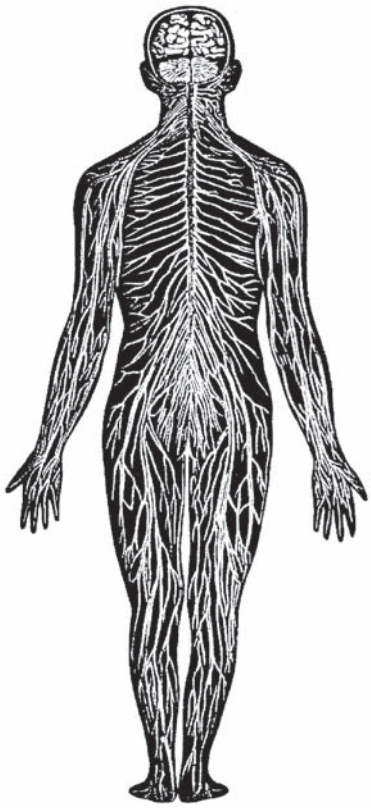


FIGURE 13.6 Nervous man. Information flows to and from the brain via a complex network of nerves. (Source: © ArtToday, Tucson, AZ.)

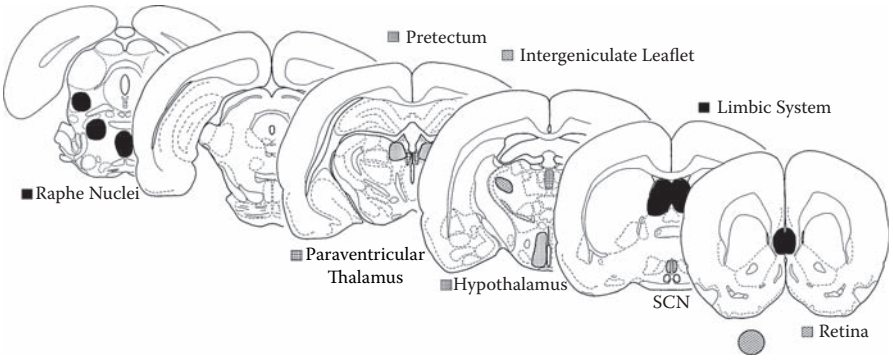


FIGURE 13.7 Brain afferents to the SCN. The diagrams identify the seven major brain structures with terminal fields in the suprachiasmatic nucleus (SCN) of the laboratory rat (*Rattus norvegicus*). (Sources: Brain diagrams adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of structures after Moga, M. M. & Moore, R. Y. (1997). Organization of neural inputs to the suprachiasmatic nucleus in the rat. *Journal of Comparative Neurology* 389: 508–534.)

response indicates that the afference is mediated by the direct RHT instead of by an indirect pathway such as the GHT. A study in which minute knife cuts were used to selectively transect retinal axons innervating the SCN, while sparing the projections to the midbrain and thalamus, demonstrated that the RHT is the main pathway of photic information to the SCN. Golden hamsters that underwent this procedure lost the ability to be entrained by light–dark cycles.[13] Electrical stimulation of the optic nerve also has been shown to cause phase shifts of the rhythm of firing rate of rat SCN cells[43] as well as of the rhythm of locomotor activity of golden hamsters.[44] Genetically engineered atrophy of the optic nerve in mice prevented behavioral entrainment to light–dark cycles.[45]

The action potentials that reach the SCN through the RHT consistently increase the firing rate of the majority

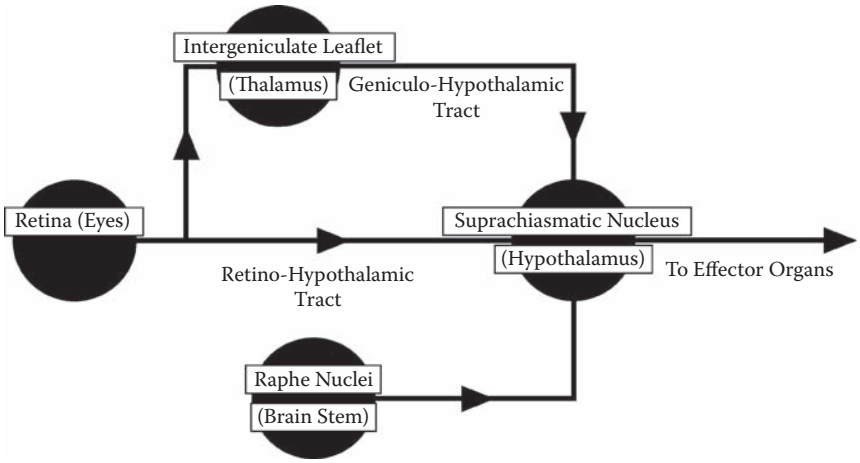


FIGURE 13.8 Functional afference to the circadian system. Functional studies have identified three main neural pathways to the suprachiasmatic nucleus (SCN): the retino-hypothalamic tract (RHT), the geniculo-hypothalamic tract (GHT), and a raphe-hypothalamic pathway.

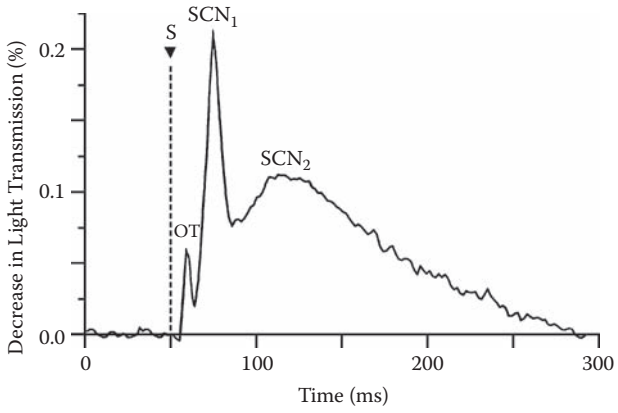


FIGURE 13.9 Recording afferent signals. The graph is a representative recording of neural activity in golden hamster (*Mesocricetus auratus*) brain slices with optic nerves attached. Optical, rather than electrical, recording was used (that is, the investigators used a voltage-sensitive dye that changes 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). (Source: Adapted from Senseman, D. M. & 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.)

of responsive SCN cells in rats, mice, and hamsters.[30,31,33,35,36] In contrast, studies on degus (*Octodon degus*) found that the majority of responsive SCN cells were inhibited by impulses arriving through the RHT.[34,41] 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. 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 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.[46–62] 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 (PRC) for behavioral phase shifts evoked by equivalent light pulses. In many — although not all — studies, the ventral region of the SCN responded to photic stimulation of the eyes much more strongly than the dorsal region responded.[46,47,50,54,56,59,61] This finding agrees with the anatomical data described above 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, while cells in the dorsal (shell) region of the SCN tend to be intrinsically rhythmic but *not* to be responsive to remote photic stimulation.[54,63–65] One research team found that the dorsal region is inhibited by light pulses that activate the ventral region,[66,67] although the findings have not been confirmed in other laboratories.

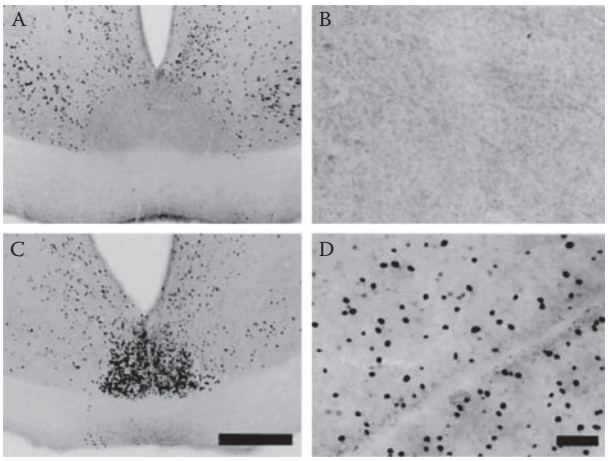


FIGURE 13.10 Photic stimulation induces *fos* expression in the SCN. These microphotographs show sections of the brain (left) and retina (right) of small naked-soled gerbils (*Taterillus petteri*) processed for Fos immunoreactivity. The top panels (A and B) refer to a control animal, while the bottom panels (C and D) refer to an animal that received a 15-minute light-pulse at CT 16. Both animals were killed at CT 17. Note robust Fos-immunoreactivity in the suprachiasmatic nucleus (SCN) and retina of the animal that received the light pulse. (Scale bars: 200 μm [left] and 40 μm [right]) (Source: Dkhissi-Benyahya, O., Sicard, B. & 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. © 2000 Society for Neuroscience. Reproduced with permission.)

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,[68] 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).[69] Studies involving surgical ablation of the 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.[70–76] 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).[77] 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.[78–83] Conversely, nonphotic stimuli that cause phase shifts of behavioral rhythms did not evoke Fos expression in the SCN, but did evoke Fos expression in the IGL.[84–86] This finding suggests that nonphotic afference to the SCN may travel through the GHT, while photic information travels primarily through the RHT.

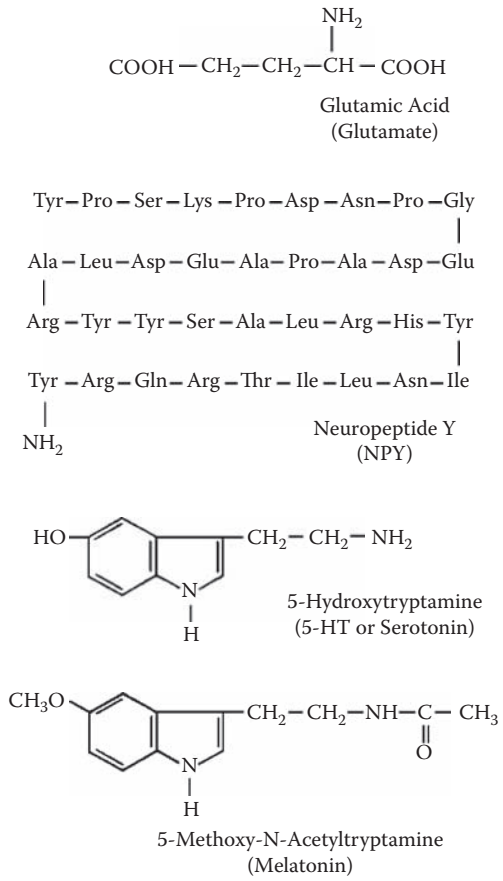


FIGURE 13.11. The main neurotransmitters in the afference to the SCN. Four main chemicals are used by afferent pathways to the suprachiasmatic nucleus (SCN). Three chemicals are neurotransmitters: glutamate, neuropeptide Y (NPY), and serotonin (5-HT). The fourth chemical is a hormone (melatonin). (Sources: Feldman, R. S. & Quenzer, L. F. (1984). *Fundamentals of Neuropsychopharmacology.* Sunderland, MA: Sinauer; Cooper, J. R. et al. (1991). *The Biochemical Basis of Neuropharmacology*, 6th Edition. New York: Oxford University Press.)

13.1.3 Pharmacology

Pharmacological studies provide much of the evidence for the involvement of the three pathways (RHT, GHT, and raphe-hypothalamic pathway) in the functional afference to the SCN. 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). Figure 13.11 shows the structural formulas of these four substances.

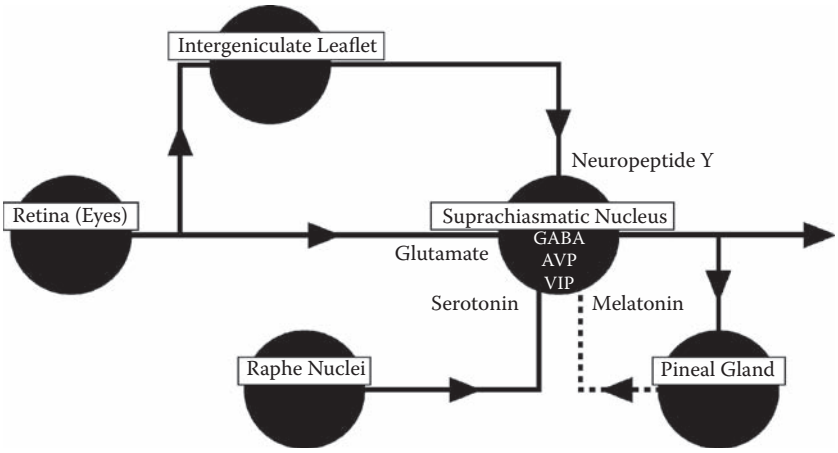


FIGURE 13.12 Neurochemistry of the SCN. The diagram illustrates the four main afferent pathways to the suprachiasmatic nucleus (SCN) (as determined by functional studies) and the chemicals that they use for signal transmission. As detailed in Chapter 12, three main neurotransmitters are involved in signal transmission within the SCN: γ-aminobutyric acid (GABA), arginine vasopressin (AVP), and vasoactive intestinal polypeptide (VIP).

Glutamate appears 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.[87] Stimulation of glutamatergic synapses by microinjection of glutamate or glutamatergic agonists causes phase shifts both in the rhythm of neuronal activity of SCN cells *in vitro*[43,88–91] and in behavioral rhythms *in vivo*.[92–95] 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*[38,41–43] and attenuates behavioral phase shifts evoked by photic stimulation *in vivo*.[44,96,97] Figure 13.13 compares the PRCs for the resetting of the locomotor activity rhythm of Nile grass rats caused by normal photic stimulation (bottom panel) and by microinjection of NMDA (*N*-methyl-D-aspartate, a glutamate agonist) into the SCN (top panel). Although the amplitude of the PRC for NMDA microinjection is smaller than that of the PRC for photic stimulation (probably because of unequal doses), the shape of the curves is nearly 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: PACAP (pituitary adenylate cyclase activating polypeptide). By performing double immunostaining for PACAP and CTB (the anterograde tracer mentioned above), the team found that PACAP-containing retinal ganglion cells project preferentially to the SCN in rats.[11,98] PACAP also is co-expressed with melanopsin (the main circadian photopigment) in retinal ganglion cells of hamsters and humans.[99,100] PACAP microinjected *in vivo* into the lateral ventricle of the hamster brain causes a small but significant phase shift of the behavioral activity rhythm.[99] The extent to which PACAP is actually involved in the operation of the circadian system is not clear, however. Another research group observed that PACAP-deficient mice exhibit only minor impairment in circadian photic sensitivity.[101] 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).[102] 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.[90] 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 appears to be *neuropeptide Y* (NPY). Immunocytochemical studies have shown that NPY-containing cells from the IGL project to the ventral SCN.[19,28,103] Microinjection of NPY into the SCN (*in vivo* or *in vitro*) evokes phase shifts during subjective day.[104–107] Note that subjective day is the circadian phase when nonphotic stimuli cause behavioral phase shifts. Because NPY administration causes phase shifts in accordance with the *nonphotic* PRC (in contrast to glutamate injections, which cause phase shifts in accordance with the photic PRC), the GHT may serve as the terminal link in the pathway responsible for entrainment by nonphotic stimuli. Figure 13.15 compares the PRCs

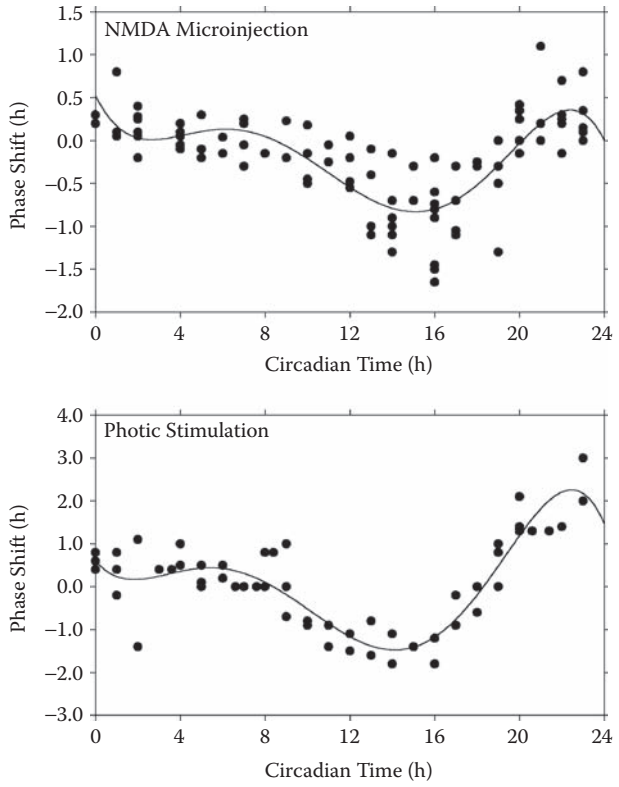


FIGURE 13.13 Glutamate and photic resetting. The graphs depict phase-response curves (PRCs) for the resetting of the rhythm of running-wheel activity of the Nile grass rat (*Arvicanthis niloticus*). Phase shifts were evoked either by microinjection of N-methyl-D-aspartate (NMDA, a glutamate agonist) into the suprachiasmatic nucleus (SCN) (top panel) or by the presentation of 4-hour-long light pulses (bottom panel). 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. (Sources: Novak, C. M. & 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; 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.)

for the resetting of the rhythm of locomotor activity of golden hamsters caused by exercise in a novel wheel (bottom panel) or by microinjection of NPY into the SCN (top panel). The shapes of the two PRCs 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, as discussed in Chapter 8, 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

FIGURE 13.14 Jens Hannibal. This circadian physiologist from the University of Copenhagen (Denmark) is the leading researcher of the role of PACAP in the retino-hypothalamic tract (RHT). (Source: Photograph courtesy of Jens Hannibal.)

in vitro.[91,95,108] 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*, also may 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.[109–116] Accordingly, electrical stimulation of the raphe nuclei increases serotonin release in the SCN.[117] Administration of serotonin or serotonergic agonists evokes phase shifts during subjective day both *in vivo*[107,118–122] and *in vitro*.[111,123] Figure 13.16 shows the PRC 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 mid subjective day. Thus, serotonergic activation causes phase shifts according to the nonphotic PRC, and the raphe-hypothalamic pathway might be a terminal link in the pathway responsible for entrainment by nonphotic stimuli. This interpretation is partially contradicted by a study in golden hamsters in which four different serotonergic antagonists failed to block phase shifts caused by nonphotic stimuli.[124] Considerable evidence suggests, however, that the raphe-hypothalamic pathway can modulate activity in the RHT. Serotonergic agonists attenuate[125,126] or enhance[127,128] behavioral phase shifts evoked by photic stimuli. Serotonergic antagonists potentiate photically evoked shifts[129,130] and prevent the attenuation of photically evoked shifts caused by simultaneous nonphotic stimulation.[131] Serotonergic activation *in vitro* reduced the response of SCN cells to optic nerve stimulation.[42,112] A variety of other effects of serotonergic activation on photic input has been

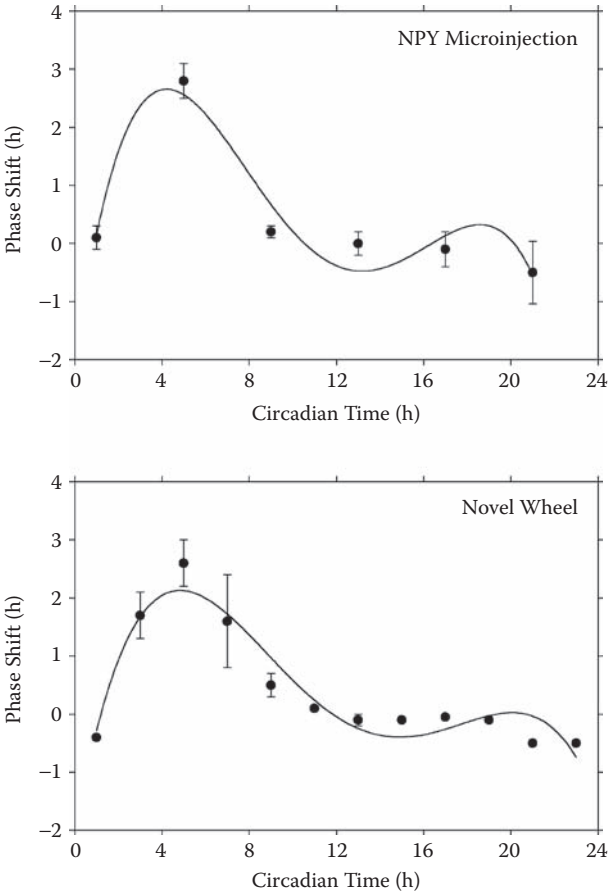


FIGURE 13.15 Neuropeptide Y and nonphotic resetting. The graphs depict phase-response curves (PRCs) for the resetting of the rhythm of running-wheel activity of the golden hamster (*Mesocricetus auratus*). Phase shifts were evoked either by microinjection of neuropeptide Y (NPY) into the suprachiasmatic nucleus (SCN) (top panel) or by 3-hour-long access to a novel wheel (bottom panel). The two PRCs are very similar, which suggests that activation of NPY receptors is the mechanism by which nonphotic stimulation resets the circadian clock. (Sources: Biello, S. M. & Mrosovsky, N. (1996). Phase response curves to neuropeptide Y in wildtype and tau mutant hamsters. *Journal of Biological Rhythms* 11: 27–34; Mrosovsky, N. et al. (1992). Nonphotic phase shifting in hamster clock mutants. *Journal of Biological Rhythms* 7: 41–49.)

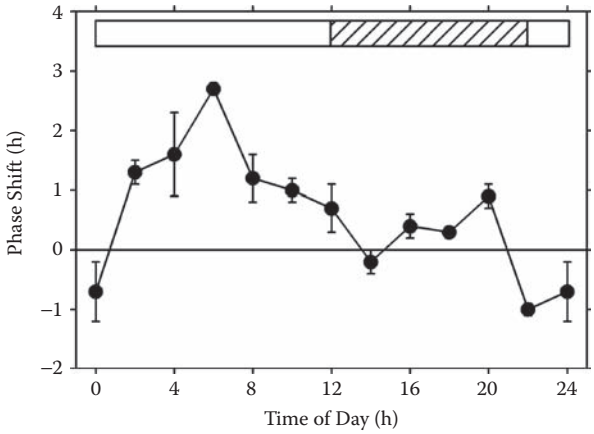


FIGURE 13.16 Serotonin and phase resetting *in vitro*. The graph depicts a phase-response curve (PRC) for the resetting of the rhythm of electrical activity of single cells in cultured brain slices from domestic mice (*Mus musculus*). Phase shifts were evoked by addition of serotonin (10 μM) to the culture 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 mid-subjective day and modest phase delays during early subjective day. (Source: Prosser, R. A. (2003). Serotonin phase-shifts the mouse suprachiasmatic circadian clock *in vitro*. *Brain Research* 966: 110–115.)

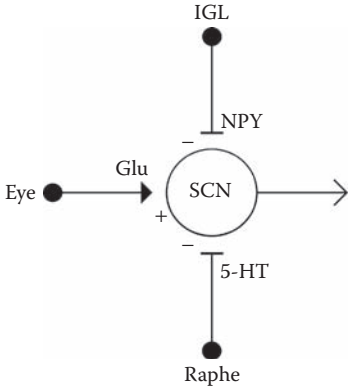


FIGURE 13.17 Neurotransmitters in action. This diagram summarizes the action of the three main neurotransmitters involved in the afferent pathways to the suprachiasmatic nucleus (SCN). See text for details. (Note: 5-HT = serotonin, Glu = glutamate, IGL = intergeniculate leaflet, NPY = neuropeptide Y)

reported as well.[132–135] 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 probably not 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 mediate 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.

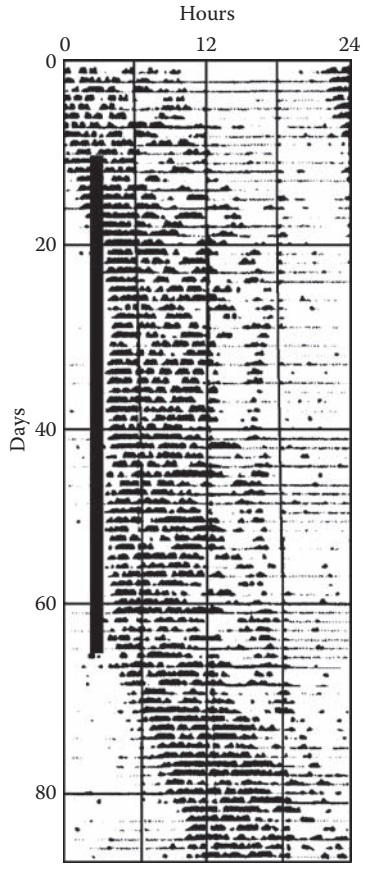


FIGURE 13.18 Melatonin and phase resetting *in vivo*. This actogram shows the rhythm of running-wheel activity of a laboratory rat (*Rattus norvegicus*) maintained in constant darkness and infused with melatonin subcutaneously for 1 hour each day (thick vertical bar). Note that the daily cycle of melatonin administration entrained the activity rhythm. When the daily infusions stopped, the rhythm freeran from the appropriate phase. (Source: Adapted from Slotten, H. A., Pitrosky, B. & 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.)

Melatonin (which should not be confused with *melanopsin*) is also a major component 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. Note that the running-wheel activity rhythm was free-running 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,[136–139] entrainment has been documented in amphibians,[140] birds,[141–145] rodents,[146–155] and humans.[156–161] Presence of the pineal gland is not required for the entraining effect of exogenous melatonin,[142,143,146,149,151,153] and immunocytochemical and autoradiographical studies demonstrated the presence of melatonin receptors in the SCN.[162–166] Various studies also documented phase shifts evoked by discrete pulses of melatonin administration,[167–175] and the PRC was found to be of the nonphotic type (that is, with phase advances during subjective day).[167,168,171]

Other hormones 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,[176–179] thyroid hormones,[180–182] corticosterone,[183] leptin,[184] and other hormones. In addition, several neurotransmitters that have not been mentioned yet have been implicated in the afference to the circadian system. They include enkephaline,[185–187] histamine,[188,189] adenosine,[190,191] substance P,[43] and norepinephrine.[192] In particular, *acetylcholine* (the main neurotransmitter of the peripheral nervous system that also is 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 PRC,[193–197] and treatment with a cholinergic toxin attenuates photically induced phase shifts.[198] As was the case for NPY and serotonin, acetylcholine seems to modulate the action of glutamate, as NMDA receptor antagonists block phase shifts induced by carbachol.[199]

13.1.4 Molecular Mechanisms

Most organisms (including invertebrate animals) are small enough to be translucent — that is, 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. Chapter 12 discussed the molecular mechanism of circadian rhythmicity, and the diagram in Figure 13.19 shows how the mechanism is affected by light in the fruit fly. 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 observed that light exposure degrades TIM and decreases its association with PER.[200,201] Soon after this discovery, 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.[202–205] Thus, light phase shifts the pacemaker by facilitating the binding of CRY to TIM,

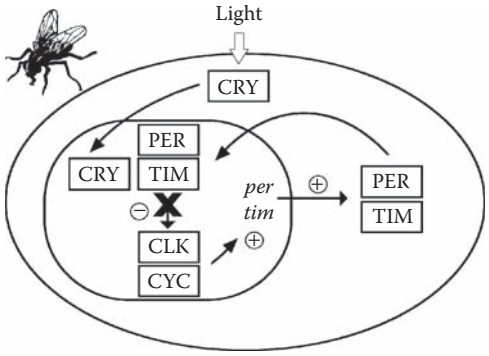


FIGURE 13.19 Molecular mechanism of photic resetting in the fruit fly. This simplified diagram illustrates the molecular mechanism of photic resetting in the fruit fly. Light promotes the binding of CRY with TIM. This binding reduces the inhibitory action of the PER–TIM dimer on CLK–CYC, which alters the timing of activation of the genes *per* and *tim*. (Sources: Williams, J. A. & Sehgal, A. (2001). Molecular components of the circadian system in *Drosophila*. *Annual Review of Physiology* 63: 729–755; Devlin, P. F. & Kay, S. A. (2001). Circadian photoreception. *Annual Review of Physiology* 63: 677–694.)

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).

The fruit fly's circadian system also receives photic information through the eyes.[206] In fact, the fruit fly has three to five different photic afferent pathways.[207,208] Although these pathways seem to affect different aspects of photic entrainment, only the molecular mechanisms of the CRY pathway (described above) are known in detail at this time. In contrast, plants and fungi have no eyes, and mammals can perceive light only through the eyes, as discussed in Chapter 11. Figure 13.20 summarizes the molecular mechanisms of photic entrainment in plants, fungi, flies, and mice.[209] In plants (the mustard plant, *Arabidopsis thaliana*), a family of four red-light-activated phytochromes (PHYs) and two blue-light-activated CRYs act as photoreceptors that affect the clock loop by inhibiting the transcription of *cca* and *lhy*.[210] In fungi (bread mold, *Neurospora crassa*), researchers observed early on that light interrupts the *frq* negative auto-regulation,[211] and that *wc-1* is needed for photoinduction.[212] 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.[213,214] Thus, the *Neurospora* clock is a neat clock in which light acts directly on one of the main components of the feedback loop.

Figure 13.20 also shows 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 involves calcium/calmodulin kinase and mitogen-activated protein kinase[215] 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. The process is actually much more complex because, as shown in Chapter 12, three different versions of PER exist. When the mouse *per* genes were first identified, it was already apparent that *per1* and *per2*, but not *per3*, are over-expressed in SCN cells following photic stimulation during subjective night.[216–222] This phenomenon was later found to be true also in laboratory rats, golden hamsters, grass rats, and mole-rats.[223–226] Recent 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.[227,228]

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 (Figure 13.21). Thus, *per1* expression in the ventral SCN reflects merely the

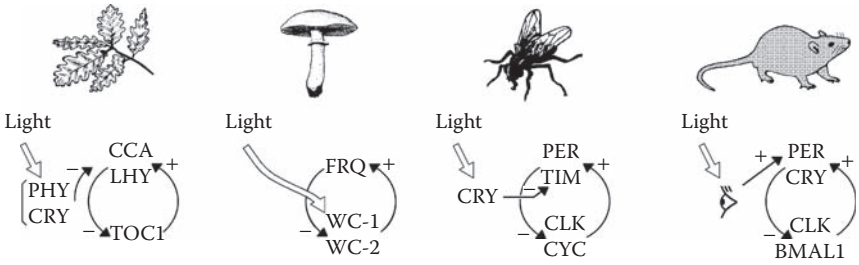


FIGURE 13.20 Molecular mechanisms of photic resetting in plants, fungi, and animals. These diagrams illustrate the basic mechanisms of photic resetting in plants, fungi, invertebrate animals, and vertebrate animals. See text for details. (Sources: Schultz, T. F. & Kay, S. A. (2003). Circadian clocks in daily and seasonal control of development. *Science* 301: 326–328; Devlin, P. F. & Kay, S. A. (2001). Circadian photoreception. *Annual Review of Physiology* 63: 677–694; Reppert, S. M. & Weaver, D. R. (2001). Molecular analysis of mammalian circadian rhythms. *Annual Review of Physiology* 63: 647–676.)

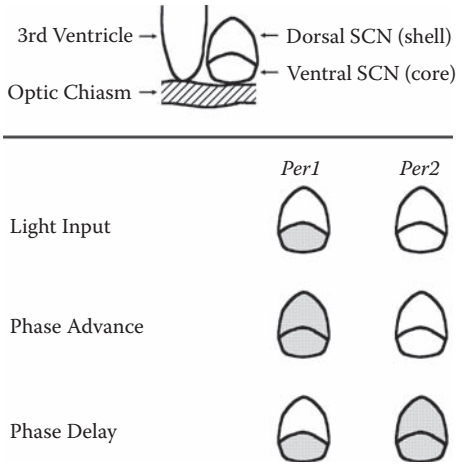


FIGURE 13.21 Molecular differentiation of phase advances and phase delays. These diagrams illustrate the finding that in the domestic mouse (*Mus musculus*), photic stimulation has a different effect on the expression of the genes *per1* and *per2* in different subdivisions of the suprachiasmatic nucleus (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 SCN. (Source: Yan, L. & Silver, R. (2002). Differential induction and localization of mPer1 and mPer2 during advancing and delaying phase shifts. *European Journal of Neuroscience* 16: 1531–1540.)

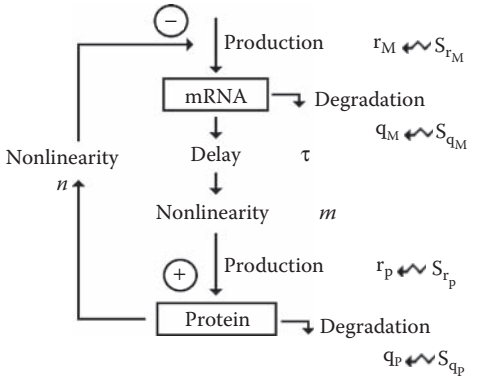


FIGURE 13.22 An early molecular model of the circadian clock. This diagram describes a simple mathematical model of the molecular mechanism of the circadian clock, including phase resetting by an environmental stimulus (S). The model assumes the involvement of only one gene and its protein, an unknown delay mechanism (τ), and unspecified sources of nonlinearity (*n* and *m*). (Source: Adapted from Olde Scheper, T., Klinkenberg, D., Pennartz, C. & van Pelt, J. (1999). A mathematical model for the intracellular circadian rhythm generator. *Journal of Neuroscience* 19: 40–47.)

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 described in relative terms. Chapter 12 showed that the *per* genes are expressed rhythmically in the SCN, and the expression is higher during subjective day than during subjective night. The pattern of activation indicated in Figure 13.21 refers to *increases* in gene expression above 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 also were observed by a different research team.[229] Silver's study is noteworthy because it showed 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 could be described within a single cell. While this assumption must be true in unicellular organisms, Silver's study demonstrated that it is not true in mammals. Chapter 12 showed 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 a network of cells *and* the differentiation of two networks of cells.

Several researchers have used mathematical models to explain the molecular mechanisms of photic resetting. Figure 13.22 presents a schematic representation of a model developed by Jaap van Pelt and colleagues at the Netherlands Institute for Brain Research (in Amsterdam) in 1999. 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 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, the researchers obtained excellent results in the simulation of freeruns and phase shifts.[230] Today circadian physiologists 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 PRC generated by the model under this assumption (Figure 13.23, top panel) does not closely approximate the actual PRC of the fruit fly (bottom panel). A better approximation is obtained by inhibition of parameter r_P (middle panel), but it has little biological significance. A more sophisticated model clearly is needed. Various models have been developed,[231–234] and one that deserves special mention was proposed by Daniel Forger and Charles Peskin (from New York University) in 2003.[235] This model, proposed as a detailed predictive model of the

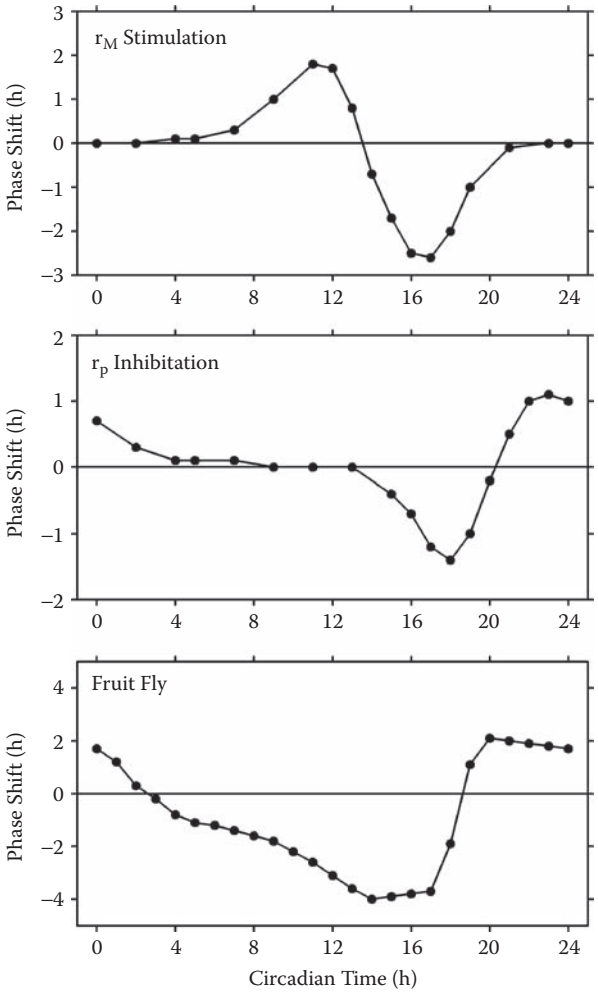


FIGURE 13.23 Testing the model. The graphs show two simulations of the photic phase-response curve (PRC) of the fruit fly based on the model described in Figure 13.22 and a PRC obtained from actual flies (as averaged from two independent studies). Note that the simulation based on r_M stimulation is unsatisfactory. The simulation based on r_P inhibition is better but not very accurate. (Sources: r_M and r_P data from Olde Scheper, T., Klinkenberg, D., Pennartz, C. & van Pelt, J. (1999). A mathematical model for the intracellular circadian rhythm generator. *Journal of Neuroscience* 19: 40–47. Fruit fly data from Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A. & Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740; Rothenfluh, A. et al. (2000). Isolation and analysis of six timeless alleles that cause short- or long-period circadian rhythms in *Drosophila*. *Genetics* 156: 665–675.)

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

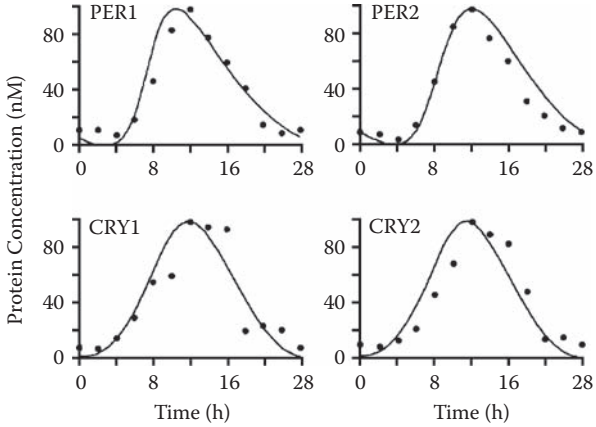


FIGURE 13.24 A recent molecular model of the circadian clock. The graphs illustrate the performance of a model of the circadian clock with 74 variables corresponding to chemical reactions in a cell of the suprachiasmatic nucleus (SCN), including processes of translation, transcription, degradation, dimerization, and so on. The curves are generated by the model. The data points correspond to actual measurements in real animals. Note that the model generates good predictions of daily oscillation in the concentration of circadian-clock proteins. (Source: Adapted from Forger, D. B. & Peskin, C. S. (2003). A detailed predictive model of the mammalian circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 100: 14806–14811.)

the modulation of PER1 and PER2 abundance. The model does not address the issue of how the multiple proteins interact to ultimately drive organismal rhythms.

13.2 EFFERENT PATHWAYS

A circadian pacemaker and its afferent pathways would be of little use if there were no efferent pathways to take the pacemaker's commands to the various organs in the body. Until very recently, much less was known about circadian efferent mechanisms than about sensory processes and afferent mechanisms. In the last 5 years, considerable knowledge has been gained. This section reviews the information available 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). In mammals and many other vertebrates, peripheral nerves are either part of the *somatic nervous system*, which controls striated muscles responsible for

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 and enzymes	Sweat glands (sweat)
Brain	Limbic system	Emotions	Happiness, sadness
	Reticular formation	Awareness	Wake, sleep
	Hypothalamus	Appetite, sex drive	Hunger, lust
	Cerebral cortex	Thought	$E = m \cdot c^2$

Note: 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.

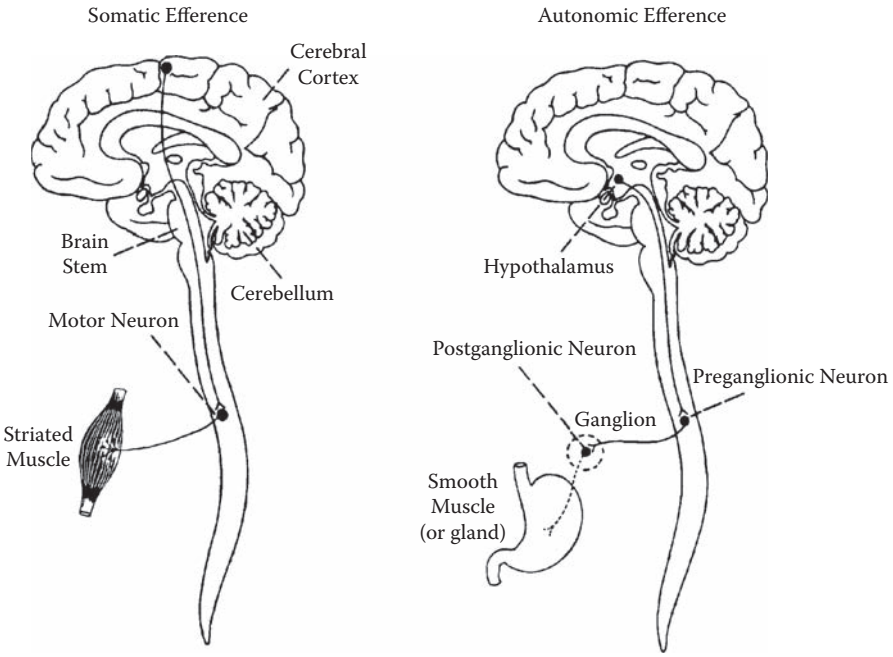


FIGURE 13.25 Efference. The major efferent pathways in higher vertebrates are part of either the somatic motor system (controlling striated muscles responsible for movement of body parts) or the autonomic motor system (controlling smooth muscles, cardiac muscle, and glands responsible for vegetative functions). Autonomic efferent pathways include a ganglion outside the central nervous system. (Source: Adapted from Machado, A. (1977). *Neuroanatomia Funcional*. Rio de Janeiro: Atheneu.)

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 extra-pyramidal 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.

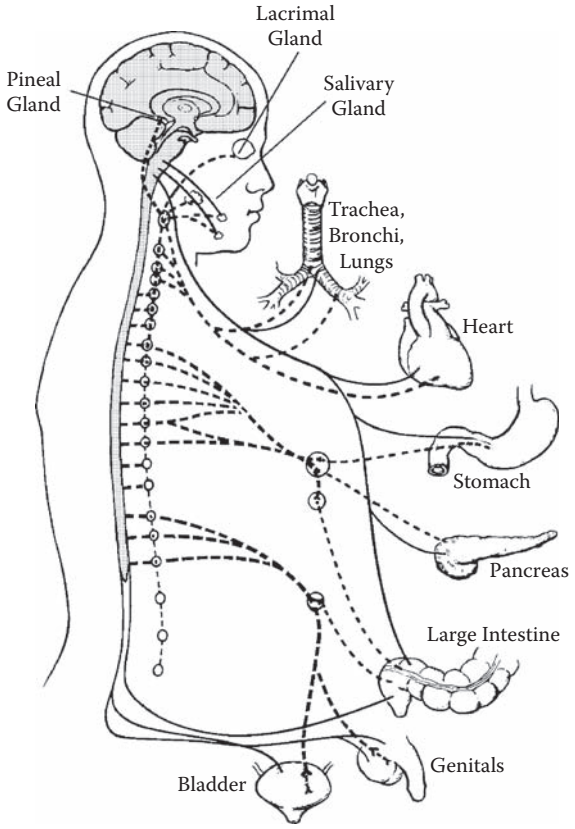


FIGURE 13.26 Autonomic nervous system. The diagram illustrates the innervation of major organs by the two branches of the autonomic nervous system: the sympathetic (dashed lines) and the parasympathetic (continuous lines). The sympathetic nervous system mobilizes the body's resources for action, while the parasympathetic nervous system promotes the conservation of bodily resources. (Source: Adapted from *Medical Illustration Library.* (1994). Baltimore, MD: Williams & Wilkins.)

The autonomic nervous system traditionally is 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 example, the pineal gland is innervated exclusively by the sympathetic system. The sympathetic nervous system generally is involved in energy-spending processes (elevated heart rate, increased blood flow to skeletal muscles, stimulation of adrenal gland, and so on), while the parasympathetic nervous system is involved in restorative processes (reduced heart rate, active salivation, increased blood flow to gastrointestinal system, and so on). Both systems use acetylcholine (through nicotinic receptors) as the neurotransmitter at the ganglionic synapses, but the sympathetic system generally uses norepinephrine at the postganglionic synapses, while the parasympathetic system uses acetylcholine (through muscarinic receptors) at the postganglionic synapses. The sympathetic ganglia generally are arranged in a row of ganglia parallel to the spinal cord, while the parasympathetic ganglia are located close to each target organ. In addition, sympathetic fibers leave the spinal cord at the thoracic or lumbar levels, while parasympathetic fibers leave the central nervous system at the cranial or sacral levels.

Researchers do not thoroughly understand how the SCN connects to the somatic and autonomic nervous systems. A series of recent studies conducted by the research team led by Ruud Buijs at the Netherlands Institute for Brain Research has elucidated much of the SCN efference to the autonomic nervous system.[236–240] 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.[241] Figure 13.27 shows a summary of the findings. 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).[241,242] 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).[241,242] 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.[243] Anterograde studies in vervet monkeys concurred in identifying SCN projections to the PVN, SPV, POA, and DMH.[24]

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 shown 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 subparaventricular zone (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. In some studies, the projection involved predominantly VIP cells,[236,244] which indicates efference from the ventral SCN, but in others it involved predominantly AVP (arginine vasopressin) cells,[237,239] 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

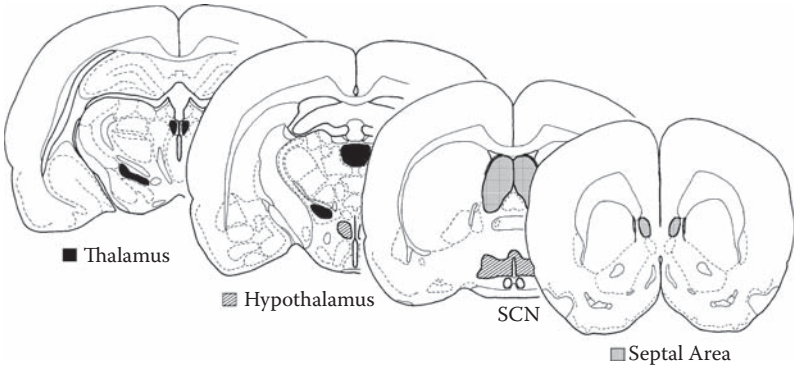


FIGURE 13.27 SCN efference within the brain. The diagrams identify the three major brain structures to which the suprachiasmatic nucleus (SCN) projects in the laboratory rat (*Rattus norvegicus*). (Source: Brain diagrams adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of structures after Leak, R. K. & Moore, R. Y. (2001). Topographic organization of suprachiasmatic nucleus projection neurons. *Journal of Comparative Neurology* 433: 312–334.)

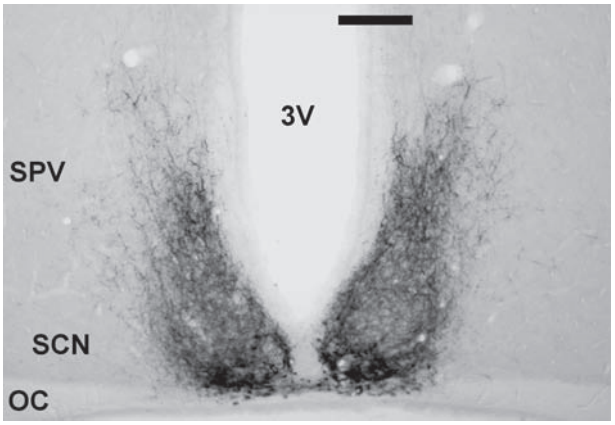


FIGURE 13.28 From SCN to SPV. This microphotograph of a coronal section of the brain of a domestic mouse (*Mus musculus*) shows SCN neurons that project dorsally to the SPV. The section was stained by immunohistochemistry for peptide histidine isoleucine (PHI). (Note: 3V = third ventricle, OC = optic chiasm, SCN = suprachiasmatic nucleus, SPV = subparaventricular zone) (Scale bar: 100 μm) (Source: Image courtesy of Daniela Lupi, Department of Visual Neuroscience, Imperial College London, UK.)

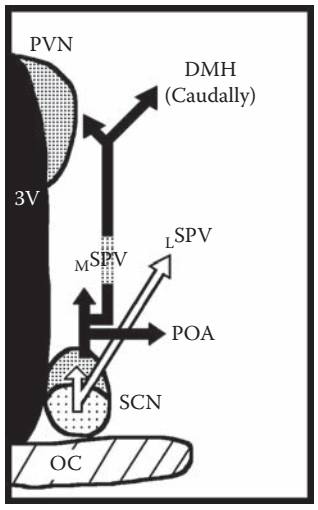


FIGURE 13.29 Out of the SCN. This diagram of a coronal section of the rodent brain in the vicinity of the SCN shows the major hypothalamic efferent routes from the ventral (core) and dorsal (shell) SCN. (Note: 3V = third ventricle, DMH = dorsomedial hypothalamic nucleus, OC = optic chiasm, POA = preoptic area, PVN = paraventricular nucleus of the hypothalamus, SCN = suprachiasmatic nucleus, $_{L}$SPV = lateral subparaventricular zone, $_{M}$SPV = medial subparaventricular zone.)

nerves, Buijs' team found that the SCN has separate projections to the PVN for the sympathetic and parasympathetic branches[240] (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 itermediolateral column of the spinal cord (IML), which projects to the sympathetic ganglia. Figure 13.31 shows cells in the PVN that were labeled retrogradely 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 also traced the efferent pathways from the dorsal SCN (through the PVN) to other organs, including the heart,[239] the pineal gland,[237] and the thyroid gland.[238] 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 to the PVN, from the PVN to the IML, and from the IML to the sympathetic ganglia.[245]

Several other research teams investigated the SCN efference to areas of the brain involved in the control of sleep and wakefulness.[246] Two of these areas are the ventrolateral preoptic area (VLPO) and the nucleus of the locus coeruleus (LC). The SCN does not project directly

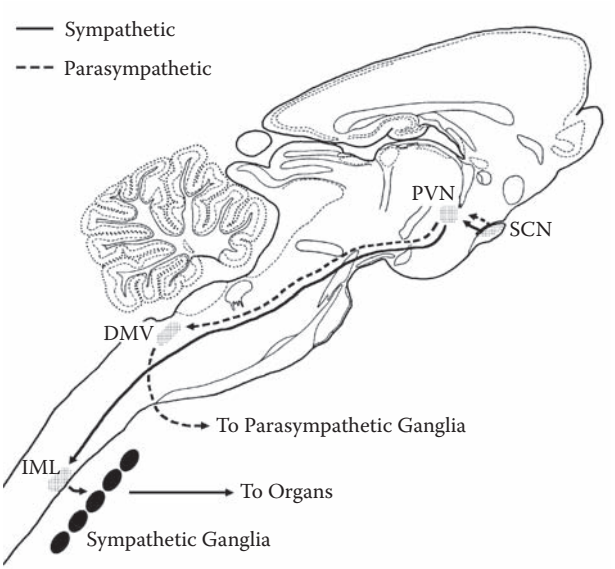


FIGURE 13.30 The SCN and the autonomic nervous system. This diagram of a sagittal section of the rat brain indicates that the SCN has separate projections to the PVN for sympathetic and parasympathetic efferences. (Note: DMV = dorsal motor nucleus of the vagus nerve, IML = intermediolateral column of the spinal cord, PVN = paraventricular nucleus of the hypothalamus, SCN = suprachiasmatic nucleus) (Sources: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of structures after Buijs, R. M., La Fleur, S. E., Wortel, J., van Heyningen, C., Zuiddam, L., Mettenleiter, T. C., Kalsbeek, A., Nagai, K. & 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.)

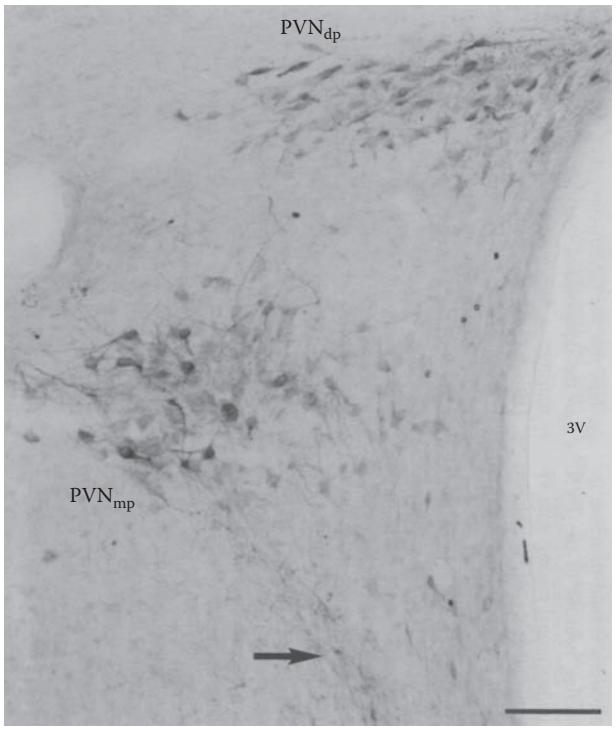


FIGURE 13.31 From PVN to spinal cord. This microphotograph of a coronal section of the brain of a laboratory rat (*Rattus norvegicus*) shows cells in the paraventricular nucleus (PVN) that project to the spinal cord. Large cell bodies and thick fibers were labeled retrogradely after injection of FluoroGold in the upper thoracic spinal cord. Thin fibers (indicated by the arrow) are VIP-immunoreactive fibers projecting dorsally from the SCN to the PVN. (Note: 3V = third ventricle, PVN_{dp} = dorsal paraventricular nucleus, PVN_{mp} = medial paraventricular nucleus.) (Scale bar: 200 μm) (Source: Teclemariam-Mesbah, R., Kalsbeek, A., Pévet, P. & 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. © Elsevier Science Publishers. Reproduced with permission from the publisher and the authors.)

to the VLPO,[247] but it does project to the DMH, which then projects to the VLPO.[248,249] The connection between the SCN and the LC is also disynaptic, involving a relay at the DMH.[250] 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

Functional studies have generally agreed, with small deviations, with anatomical studies of the SCN efference. For example, 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).[251] This finding 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.[250] 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).

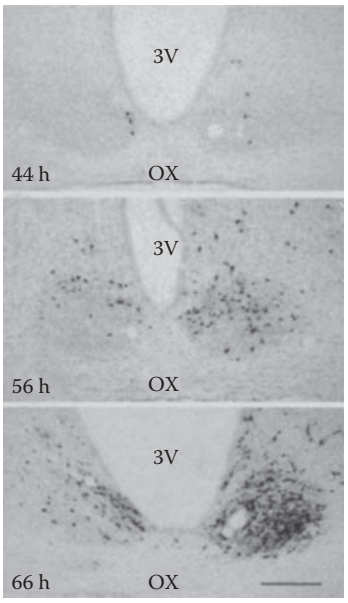


FIGURE 13.32 Backward from nucleus locus coeruleus. These microphotographs of coronal sections of the brain of a laboratory rat (*Rattus norvegicus*) show the temporal progression of SCN labeling after injection of pseudorabies virus (PRV) in the nucleus of the locus coeruleus (LC, a brain area involved 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). (Note: 3V = third ventricle, OX = optic chiasm) (Scale bar: 200 μm) (Source: Aston-Jones, G., Chen, S., Zhu, Y. & Oshinsky, M. L. (2001). A neural circuit for circadian regulation of arousal. *Nature Neuroscience* 4: 732–738. © Nature Publishing Group. Reproduced with permission from the publisher and the authors.)

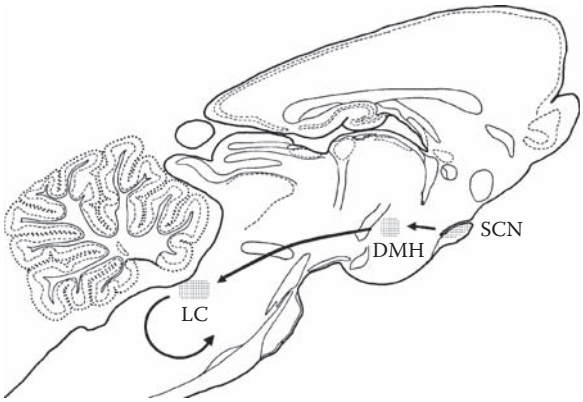


FIGURE 13.33 SCN efference to locus coeruleus. This diagram of a sagittal section of the rat brain illustrates the finding that the suprachiasmatic nucleus (SCN) sends signals to the locus coeruleus (LC) through the dorsomedial hypothalamic nucleus (DMH). The locus coeruleus is involved in the control of arousal. (Sources: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of structures after Aston-Jones, G., Chen, S., Zhu, Y. & Oshinsky, M. L. (2001). A neural circuit for circadian regulation of arousal. *Nature Neuroscience* 4: 732–738.)

Regarding the SCN efference to the 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.[252] The fact that neural activity peaks during the day in a nocturnal animal *is* surprising. Chapter 12 showed that the activity rhythm of SCN cells does peak during the day (regardless of whether the animal is diurnal or nocturnal), but 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 closely connected to the SCN functionally and that the VLPO may be a first-order efferent target.

A different group of researchers conducted electrophysiological recordings in *in vitro* brain slices and observed that about 30% of VLPO cells respond monosynaptically to electrical stimulation of the SCN.[253] 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.[249] As diagrammed in Figure 13.34, the researchers found that the VLPO receives minor input directly from the SCN but 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 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. Note, however, that all main pathways identified pass through the DMH, which, accordingly, they consider to be a major hub in the SCN efference.[249]

There is some disagreement among the results of functional studies of dorsal SCN projections to the PVN and the SPV. In a study conducted almost 30 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 — as if the SCN had been ablated.[254] 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.[255] The existence of 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

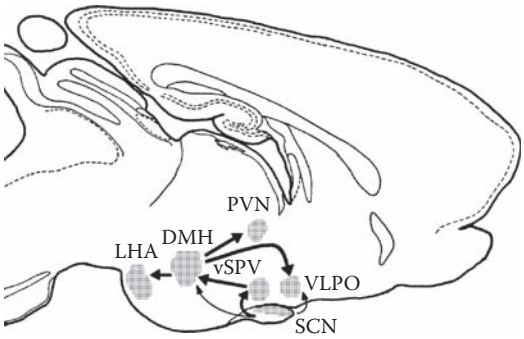


FIGURE 13.34 Functional role of dorsomedial hypothalamic nucleus. This diagram of a sagittal section of the rat brain illustrates the role of the dorsomedial hypothalamic nucleus (DMH) in the functional efferent pathways of the suprachiasmatic nucleus (SCN). Thick arrows represent main pathways. Thin arrows represent secondary pathways. The ventrolateral preoptic area (VLPO) is involved in the control of sleep, the lateral hypothamalic area (LHA) in the control of feeding and activity, and the paraventricular nucleus (PVN) in the control corticosteroid release. (Note: $_{v}$SPV = ventral subparaventricular zone) (Sources: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of structures after Chou, T. C., Scammell, T. E., Gooley, J. J., Gaus, S. E., Saper, C. B. & Lu, J. (2003). Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *Journal of Neuroscience* 23: 10691–10702.)

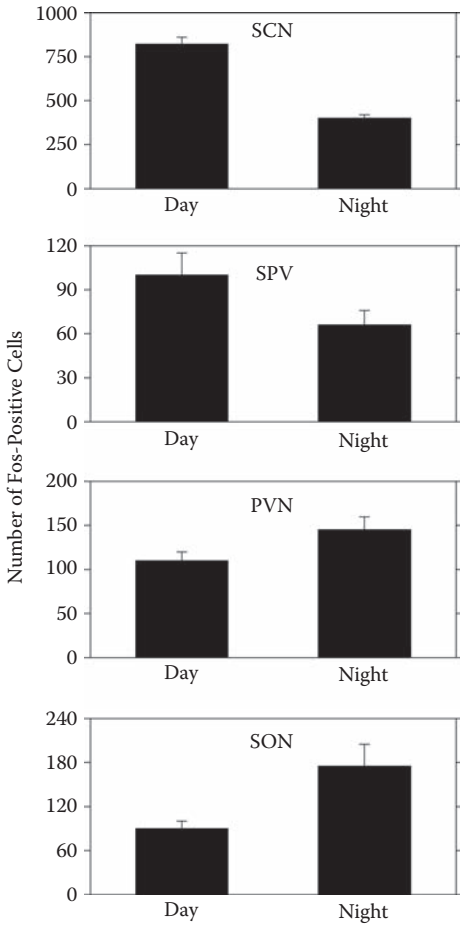


FIGURE 13.35 Functional role of subparaventricular zone. The graphs show the mean day–night differences in Fos immunoreactivity (an index of cellular activity) of neurons in four areas of the rat brain: suprachiasmatic nucleus (SCN), subparaventricular zone (SPV), paraventricular nucleus (PVN), and supraoptic 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. Note that the SPV, like the SCN, is more active during the day. (Source: Nunez, A. A., Bult, A., McElhinny, T. L. & 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.)

knife cuts inadvertently damaged the SCN.[256] These early studies clearly 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.[257–259] Lesions of the ventral SPV were found to disrupt rhythmicity of locomotor activity (but not of melatonin secretion or body temperature).[258,260] While one group of researchers observed disruption of the body temperature rhythm after lesions of the dorsal SPV,[260] another group did not note disruption.[258]

The idea 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 (SON) was also expected. Note, however, that Fos expression in the SPV matched that of the SCN, not that of the other extra-SCN sites.[261] This finding suggests that the SPV is 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.[262–264] Response latencies in the order of 10 milliseconds suggested a monosynaptic connection, which is consistent with the anatomical data discussed previously. Various functional studies have confirmed the full pathway from the SCN to the pineal gland through the PVN.[257,259,265] As diagrammed in Figure 13.36, the SCN and the pineal gland, which are located relatively close to each other, are connected by a long pathway that includes relays in the PVN, the intermediolateral column of the thoracic spinal cord, and the superior cervical sympathetic ganglion.

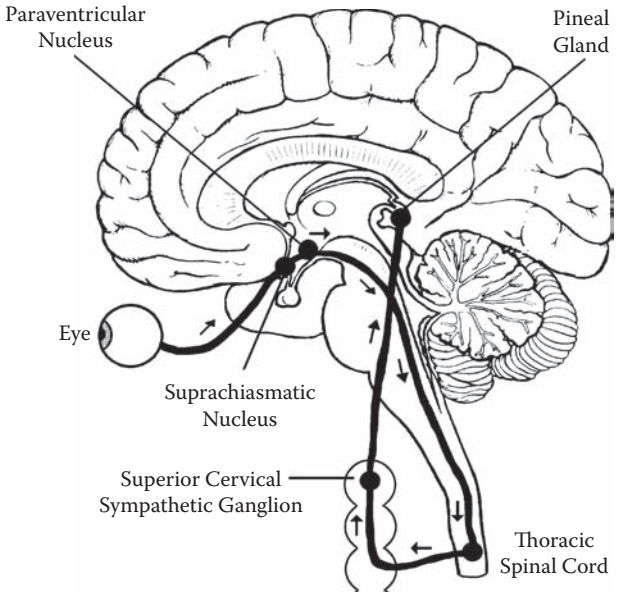


FIGURE 13.36 The retinal-pineal pathway. This diagram of the brain illustrates the long circadian-controlled pathway from the eyes to the pineal gland. Photic information is sent from the retina to the suprachiasmatic nucleus. The efferent pathway includes the paraventricular nucleus and the sympathetic branch of the autonomic nervous system. (Sources: Klein, D. C. et al. (1983). Lesions of the paraventricular nucleus area of the hypothalamus disrupt the suprachiasmatic-spinal cord circuit in the melatonin rhythm generating system. *Brain Research Bulletin* 10: 647-652; Buijs, R. M., La Fleur, S. E., Wortel, J., van Heyningen, C., Zuiddam, L., Mettenleiter, T. C., Kalsbeek, A., Nagai, K. & 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.)

The efferent pathway responsible for the rhythm of body temperature has not been identified. Chapter 11 showed that temperature receptors are present in the brain, the spinal cord, the skin, and many internal organs. The POA serves a dual 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 in which POA lesions caused serious impairment of thermoregulatory responses.[266–273] In addition, glucose utilization and Fos expression were observed to be increased in the POA during thermoregulation.[274–276] POA lesions impair autonomic but not behavioral thermoregulatory responses,[266,268–270,272,277] which implies that another brain site must control behavior.[278,279] However, the fact that homeothermic animals exhibit rhythmicity of body temperature even when deprived of opportunities for behavioral action indicates that behavioral mechanisms are not necessary for the generation or modulation of the body temperature rhythm.

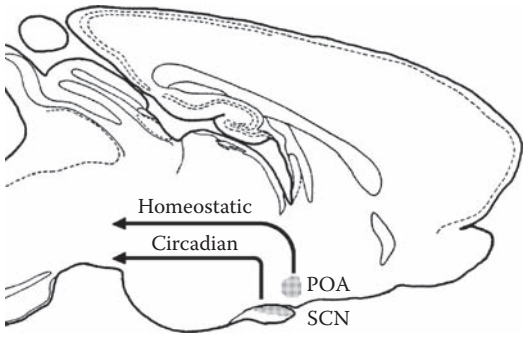


FIGURE 13.37 Efferent pathways for the control of body temperature. This diagram of a sagittal section of the rat brain illustrates that although the anatomical details about the efferent pathways for the control of body temperature are not yet known, functional studies indicate that the homeostatic and circadian components of temperature regulation are separately controlled by the preoptic area (POA) and suprachiasmatic nucleus (SCN), respectively. (Sources: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of structures after Osborne, A. R. & Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.)

As discussed in Chapter 12, ablation of the SCN eliminated circadian rhythmicity of body temperature in numerous studies on a variety of rodents.[254,280–292] Although *rhythmicity* is eliminated, *homeostatic control* is not affected.[280,281,290] That is, animals with full SCN lesions can thermoregulate normally. Homeostatic impairment (without loss of rhythmicity), however, results from lesions of the POA.[282,293,294] 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 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 suggest that at least one more SCN target, the bed nucleus of the stria terminalis (BST), exists. In one study, 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.[295] 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 appears to be the actual target: the oval nucleus of the BST (Figure 13.38). This

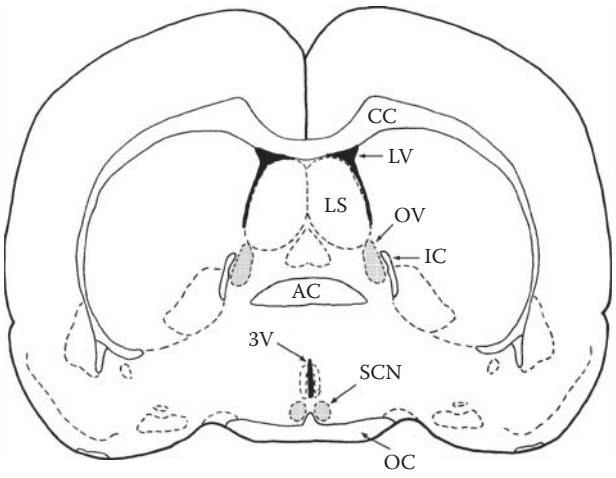


FIGURE 13.38 The oval nucleus of the bed nucleus of the stria terminalis. This diagram of a coronal section of the rat brain shows the location of the oval nucleus of the bed nucleus of the stria terminalis in reference to other major structures of the rat brain. (Note: 3V = third ventricle, AC = anterior comissure, CC = corpus calossum, IC = internal capsule, LS = lateral septum, LV = lateral ventricle, OV = oval nucleus of the bed nucleus, SCN = suprachiasmatic nucleus) (Sources: Brain diagram adapted from Pellegrino, L. J. et al. (1979). *A Stereotaxic Atlas of the Rat Brain*, 2nd Edition. New York: Plenum. Identification of oval nucleus after Amir, S. et al. (2004). A circadian rhythm in the expression of PERIOD2 protein reveals a novel SCN-controlled oscillator in the oval nucleus of the bed nucleus of the stria terminalis. *Journal of Neuroscience* 24: 781–790.)

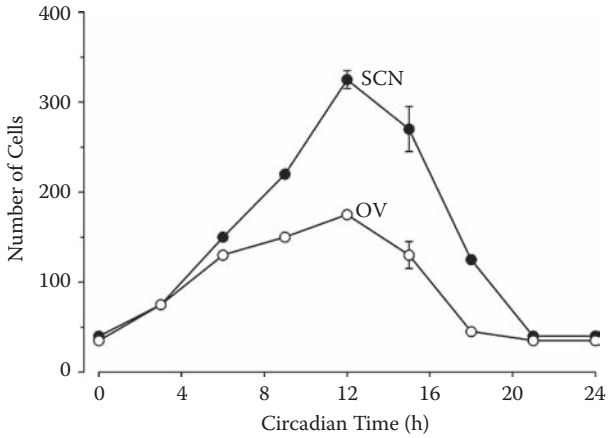


FIGURE 13.39 Cycling in phase with the SCN. The graph shows that activity in the oval nucleus of the bed nucleus of the stria terminalis (OV), as assessed by Per2 immunoreactivity, oscillates daily in phase with activity in the suprachiasmatic nucleus (SCN). Each data point corresponds to the mean cell count (± SE) in the brains of four rats maintained in constant darkness before euthanasia. (Source: Amir, S. et al. (2004). A circadian rhythm in the expression of PERIOD2 protein reveals a novel SCN-controlled oscillator in the oval nucleus of the bed nucleus of the stria terminalis. *Journal of Neuroscience* 24: 781–790.)

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).

Now that I've reviewed the evidence for anatomical and functional connections of the SCN with other brain areas, I must introduce a surprise. Chapter 12 showed that animals rendered arrhythmic by SCN ablation can regain circadian rhythmicity if they receive transplants of fetal hypothalamic tissue containing the SCN.[296–306] At that time, I allowed readers to assume 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 that restore rhythmicity of the locomotor activity rhythm show very limited integration with the host's tissue.[307,308] An example is shown in Figure 13.40, where fibers from the adjacent POA are seen to project contralaterally without penetrating the grafted tissue in the third ventricle. In fact, researchers have shown that outgrowth from the SCN grafts is equivalent to the outgrowth of control cortical grafts (which do not restore behavioral rhythmicity).[307] In addition, behavioral rhythmicity *is* restored by SCN grafts encapsulated in a semipermeable container that prevents neural outgrowth but allows the diffusion of hormones.[309] The restoration of behavioral rhythmicity clearly depends 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 co-cultured in the same medium but physically separated, the fibroblast cells became rhythmic.[310] 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.[306] Figure 13.41 provides examples of the recordings. 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).

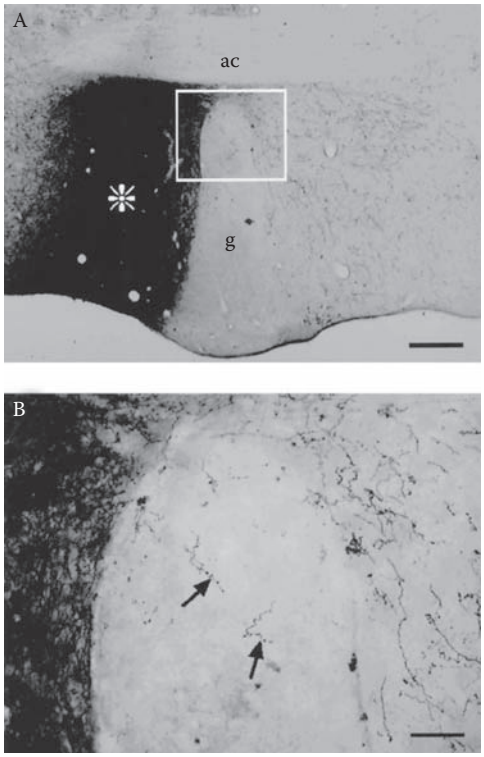


FIGURE 13.40 Alive and well, but not connected. These microphotographs of a coronal section of the brain of a golden hamster (*Mesocricetus auratus*) show a graft of fetal hypothalamic tissue transplanted into the third ventricle after ablation of the SCN. As seen several months after the transplant, 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 (asterisk) of the anterograde tracer PHAL 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). (Note: ac = anterior commissure, g = graft) (Scale bars: 100 μm [A] and 50 μm [B]) (Source: Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N. & Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218. © The Endocrine Society. Reproduced with permission from the copyright owner.)

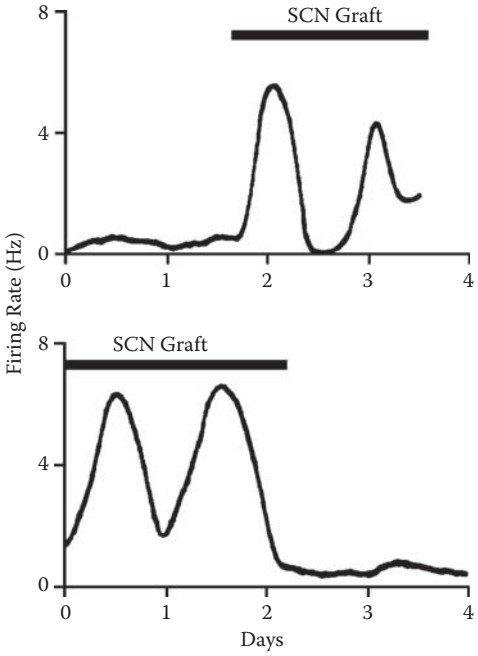


FIGURE 13.41 SCN efference: neural or humoral? The graphs show representative records of the electrical activity of single neurons in the paraventricular nucleus (PVN) as recorded in mouse brain slices. The slices did not contain the suprachiasmatic nuclei (SCN) and did not exhibit circadian rhythmicity 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 to exhibit circadian rhythmicity shortly after the addition of the SCN graft (top) and lost rhythmicity when the graft was removed (bottom panel). (Source: Adapted from Tousson, E. & Meissl, H. (2004). Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus. *Journal of Neuroscience* 24: 2983–2988.)

Finally, a few words must be added regarding the molecular mechanisms of efference. Chapter 12 discussed the feedback loops of the molecular clock and showed that microarray studies had identified about 100 or so genes that are rhythmically expressed in fruit flies[311–314] and rodents.[315–320] Most of these genes are probably *clock-controlled genes*, although some of them may be clock genes that have not been characterized yet. The lack of replicability of results in different studies[314,321] prevents researchers from drawing 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 usually are 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.[313] 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 SCN. The RHT connects retinal ganglion cells to the SCN via a monosynaptic pathway that uses glutamate as its main neurotransmitter. The GHT connects the intergeniculate islet of the thalamus to the SCN via a monosynaptic pathway that uses NPY 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, while 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 PVN is a main target of the SCN efference associated with circadian rhythmicity of the autonomic nervous system. The 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

Exercise 13.1 Body dissection

It is difficult 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 dissected the body of an animal recently, you should complete this exercise.

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. 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. If you want to inspect the brain and spinal cord, you also will need bone rongeurs to break off the skull and spinal vertebrae.
3. If you use a preserved specimen, you may want to wear a face mask to reduce the odor of formaldehyde. In the United States, all major suppliers currently use an odorless preservative instead of formalin.
4. 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.
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. Then explore the body using the diagram in Figure 13.42 as your reference.

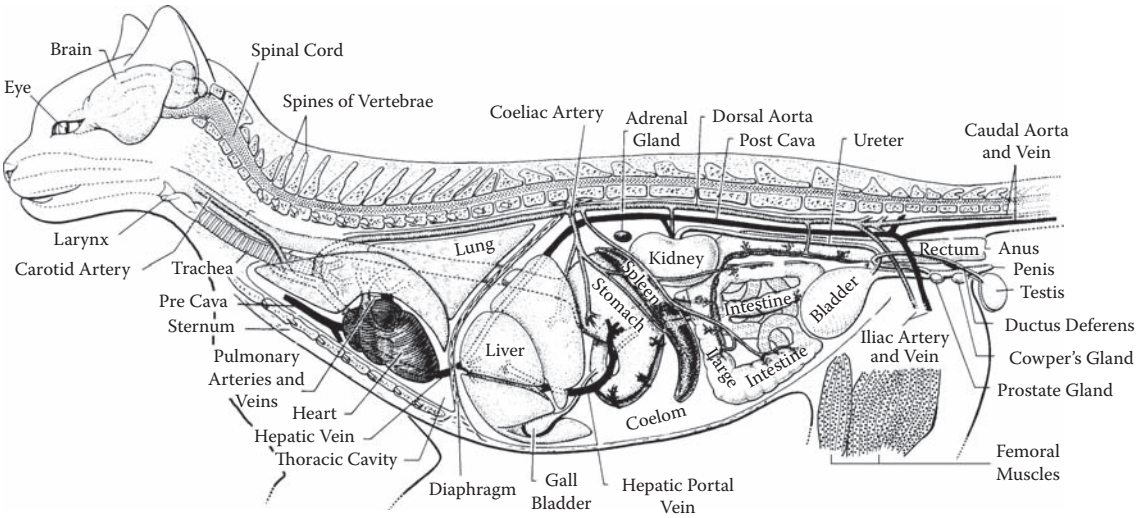


FIGURE 13.42 The inner cat. This diagram of the internal structure of a male cat can serve as a template for gross dissection of mammalian specimens. (Source: Adapted from Storer, T. I. et al. (1977). *Elements of Zoology*, 4th Edition. New York: McGraw-Hill.)

Exercise 13.2 Brain dissection

In the previous exercise, you dissected the entire body of an animal. In this exercise, you will dissect a brain.

1. 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). You also can prepare a fresh brain for dissection by freezing it and later thawing it in a formalin solution.
2. To conduct the dissection, you will need 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 face mask to reduce the odor of formaldehyde.
3. Figure 13.43 provides three diagrammatic views of the ruminant brain (e.g., cow, sheep, or goat). Try to identify all the structures shown in the diagrams. Start with the lateral view and then move to the ventral view. The brain you are dissecting may still have meninges, blood vessels, and 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 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, explore the brain on your own. Make multiple lateral sagittal cuts to reveal central structures in the temporal lobe, such as the hippocampus and the caudate. You probably will 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).

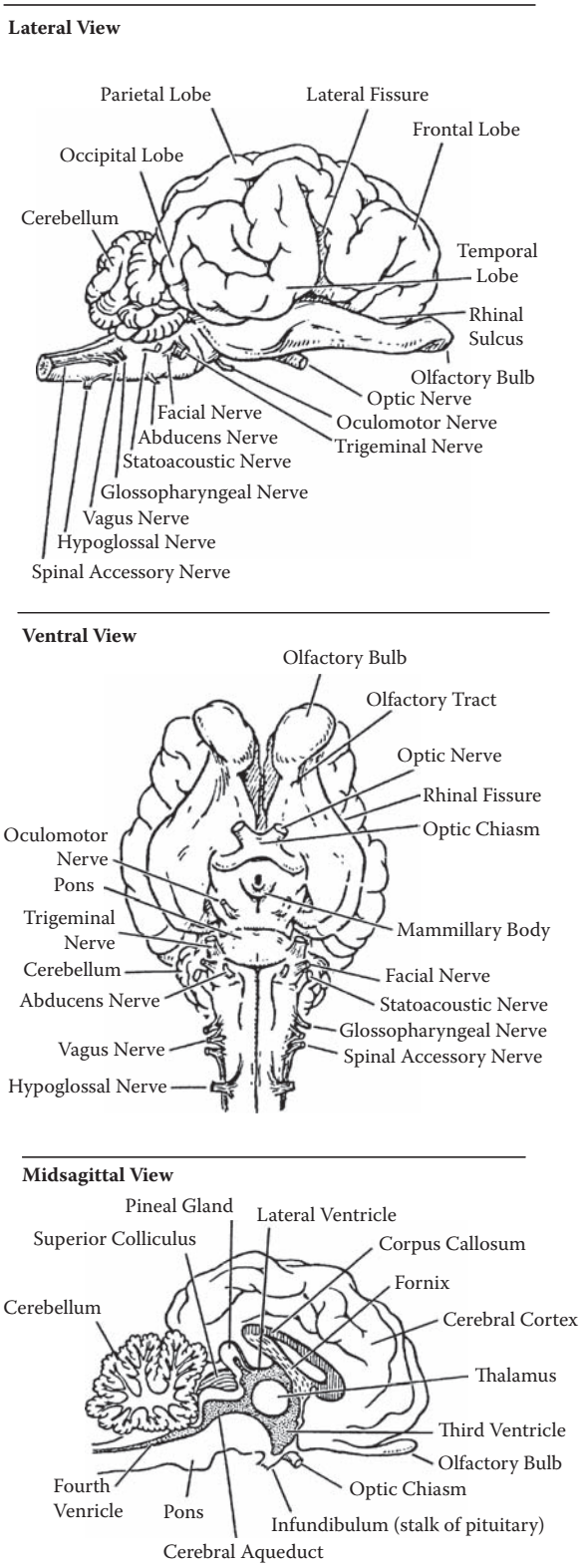


FIGURE 13.43 Ruminant brain. These diagrams of the ruminant brain can serve as templates for gross dissection of cow or sheep brains. (Source: Adapted from Hart, B. L. (1969). *Experimental Neuropsychology: A Laboratory Manual*. San Francisco: W. H. Freeman.)

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Butler, A. B. & Hodos, W. (1996). *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation.* New York: Wiley. No single book focuses only on 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. (2002). *Neuroanatomy* (3rd Edition). Baltimore, MD: Williams & Wilkins. A review of human neuroanatomy for medical students. This book covers afferent and efferent pathways, as well as related information.

Janig, W. & 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.

WEB SITES TO EXPLORE

Fundamentals of Human Anatomy:
http://www.innerbody.com/htm/body.html

Whole Brain Atlas (Harvard University):
http://www.med.harvard.edu/AANLIB/home.html

Visible Human Project at the U.S. National Library of Medicine:
http://www.nlm.nih.gov/research/visible/visible_human.html

LITERATURE CITED

1. Kandel, E. R., Schwartz, J. H. & Jessell, T. M. (2000). *Principles of Neural Science*, 4th Edition. New York: McGraw-Hill.
2. Ganong, W. F. (2001). *Review of Medical Physiology,* 20th Edition. New York: McGraw-Hill.
3. Card, J. P. & Moore, R. Y. (1991). The organization of visual circuits influencing the circadian activity of the suprachiasmatic nucleus. In: Klein, D. C., Moore, R. Y. & Reppert, S. M. (Eds.). *Suprachiasmatic Nucleus: The Mind's Clock*. New York: Oxford University Press, pp. 51–76.
4. Moore, R. Y. & Lenn, N. J. (1972). A retinohypothalamic projection in the rat. *Journal of Comparative Neurology* 146: 1–14.
5. Tanaka, M., Hayashi, S., Tamada, Y., Ikeda, T., Hisa, Y., Takamatsu, T. & Ibata, Y. (1997). Direct retinal projections to GRP neurons in the suprachiasmatic nucleus of the rat. *NeuroReport* 8: 2187–2191.
6. Moga, M. M. & Moore, R. Y. (1997). Organization of neural inputs to the suprachiasmatic nucleus in the rat. *Journal of Comparative Neurology* 389: 508–534.
7. Hattar, S., Liao, H. W., Takao, M., Berson, D. M. & Yau, K. W. (2002). Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science* 295: 1065–1069.
8. Berson, D. M., Dunn, F. A. & Takao, M. (2002). Phototransduction by retinal ganglion cells that set the circadian clock. *Science* 295: 1070–1073.
9. Warren, E. J., Allen, C. N., Brown, R. L. & Robinson, D. W. (2003). Intrinsic light reponses of retinal ganglion cells projecting to the circadian system. *European Journal of Neuroscience* 17: 1727–1735.
10. Gooley, J. J., Lu, J., Fischer, D. & Saper, C. B. (2003). A broad role for melanopsin in nonvisual photoreception. *Journal of Neuroscience* 23: 7093–7106.
11. Hannibal, J. & Fahrenkrug, J. (2004). Target areas innervated by PACAP-immunoreactive retinal ganglion cells. *Cell Tissue Research* 316: 99–113.
12. Pickard, G. E. & Zucker, I. (1986). Influence of deuterium oxide on circadian activity rhythms of hamsters: role of the suprachiasmatic nuclei. *Brain Research* 376: 149–154.
13. Johnson, R. F., Moore, R. Y. & Morin, L. P. (1988). Loss of entrainment and anatomical plasticity after lesions of the hamster retinohypothalamic tract. *Brain Research* 460: 297–313.
14. Bryant, D. N., LeSauter, J., Silver, R. & 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.
15. Morin, L. P., Blanchard, J. H. & 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.
16. Muscat, L., Huberman, A. D., Jordan, C. L. & Morin, L. P. (2003). Crossed and uncrossed retinal projections to the hamster circadian system. *Journal of Comparative Neurology* 466: 513–524.
17. Sollars, P. J., Smeraski, C. A., Kaufman, J. D., Ogilvie, M. D., Provencio, I. & Pickard, G. E. (2003). Melanopsin and nonmelanopsin expressing retinal ganglion cells innervate the hypothalamic suprachiasmatic nucleus. *Visual Neuroscience* 20: 601–610.
18. Kudo, M., Yamamoto, M. & Nakamura, Y. (1991). Suprachiasmatic nucleus and retinohypothalamic projections in moles. *Brain, Behavior and Evolution* 38: 332–338.
19. Smale, L. & 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.
20. Novak, C. M., Smale, L. & 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.
21. Major, D. E., Rodman, H. R., Libedinsky, C. & 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.

22. Reuss, S. & Fuchs, E. (2000). Anterograde tracing of retinal afferents to the tree shrew hypothalamus and raphe. *Brain Research* 874: 66–74.
23. Murakami, D. M. & 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.
24. Abizaid, A., Horvath, B., Keefe, D. L., Leranth, C. & Horvath, T. L. (2004). Direct visual and circadian pathways target neuroendocrine cells in primates. *European Journal of Neuroscience* 20: 2767–2776.
25. Dai, J., van der Vliet, J., Swaab, D. F. & Buijs, R. M. (1998). Human retinohypothalamic tract as revealed by *in vitro* postmortem tracing. *Journal of Comparative Neurology* 397: 357–370.
26. Vrang, N., Mrosovsky, N. & Mikkelsen, J. D. (2003). Afferent projections to the hamster intergeniculate leaflet demonstrated by retrograde and anterograde tracing. *Brain Research Bulletin* 59: 267–288.
27. Moore, R. Y., Weis, R. & 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.
28. Morin, L. P. & 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.
29. Hay-Schmidt, A., Vrang, N., Larsen, P. J. & Mikkelsen, J. D. (2003). Projections from the raphe nuclei to the suprachiasmatic nucleus of the rat. *Journal of Chemical Neuroanatomy* 25: 293–310.
30. Meijer, J. H., Rusak, B. & 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.
31. Meijer, J. H., Watanabe, K., Détári, L. & Schaap, J. (1996). Circadian rhythm in light response in suprachiasmatic nucleus neurons of freely moving rats. *Brain Research* 741: 352–355.
32. Roig, J. A., Granados-Fuentes, D. & Aguilar-Roblero, R. (1997). Neuronal subpopulations in the suprachiasmatic nuclei based on their response to retinal and intergeniculate leaflet stimulation. *NeuroReport* 8: 885–890.
33. Meijer, J. H., Watanabe, K., Schaap, J., Albus, H. & Détári, L. (1998). Light responsiveness of the suprachiasmatic nucleus: long-term multi-unit and single-unit recordings in freely moving rats. *Journal of Neuroscience* 18: 9078–9087.
34. Jiao, Y. Y., Lee, T. M. & Rusak, B. (1999). Photic responses of suprachiasmatic area neurons in diurnal degus (*Octodon degus*) and nocturnal rats (*Rattus norvegicus*). *Brain Research* 817: 93–103.
35. Schaap, J. & Meijer, J. H. (2001). Opposing effects of behavioural activity and light on neurons of the suprachiasmatic nucleus. *European Journal of Neuroscience* 13: 1955–1962.
36. Kuhlman, S. J., Silver, R., LeSauter, J., Bult-Ito, A. & 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.
37. Nakamura, T. J., Fujimura, K., Ebihara, S. & Shinohara, K. (2004). Light response of the neuronal firing activity in the suprachiasmatic nucleus of mice. *Neuroscience Letters* 371: 244–248.
38. Shibata, S., Liou, S. Y. & 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.
39. Kim, Y. I. & Dudek, F. E. (1992). Intracellular electrophysiological study of suprachiasmatic nucleus neurons in rodents: inhibitory synaptic mechanisms. *Journal of Physiology* 458: 247–260.
40. Pennartz, C. M. A., Hamstra, R. & 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.
41. Jiao, Y. Y. & Rusak, B. (2003). Electrophysiology of optic nerve input to suprachiasmatic nucleus neurons in rats and degus. *Brain Research* 960: 142–151.
42. Senseman, D. M. & 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.
43. Kim, D. Y., Kang, H. C., Shin, H. C., Lee, K. J., Yoon, Y. W., Han, H. C., Na, H. S., Hong, S. K. & 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.
44. De Vries, M. J., Treep, J. A., de Pauw, E. S. D. & 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.
45. Wee, R., Castrucci, A. M., Provencio, I., Gan, L. & Van Gelder, R. N. (2002). Loss of photic entrainment and altered free-running circadian rhythms in *math5*$^{-/-}$ mice. *Journal of Neuroscience* 22: 10427–10433.
46. Sutin, E. L. & 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.
47. Kaufman, C. M. & Menaker, M. (1994). Ontogeny of light-induced Fos-like immunoreactivity in the hamster suprachiasmatic nucleus. *Brain Research* 633: 162–166.
48. Teclemariam-Mesbah, R., Vuillez, P., van Rossum, A. & Pévet, P. (1995). Time course of neuronal sensitivity to light in the circadian timing system of the golden hamster. *Neuroscience Letters* 201: 5–8.
49. Sumová, A., Trávnícková, Z. & Illnerová, H. (1995). Memory on long but not on short days is stored in the rat suprachiasmatic nucleus. *Neuroscience Letters* 200: 191–194.

50. Grosse, J., Loudon, A. S. I. & 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.
51. Vuillez, P., Jacob, N., Teclemariam-Mesbah, R. & 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.
52. Shimomura, K., Kornhauser, J. M., Wisor, J. P., Umezu, T., Yamazaki, S., Ihara, N. L., Takahashi, J. S. & 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.
53. Morris, M. E., Viswanathan, N., Kuhlman, S., Davis, F. C. & Weitz, C. J. (1998). A screen for genes induced in the suprachiasmatic nucleus by light. *Science* 279: 1544–1547.
54. Sumová, A., Trávnícková, Z., Mikkelsen, J. D. & Illnerová, H. (1998). Spontaneous rhythm in c-Fos immunoreactivity in the dorsomedial part of the rat suprachiasmatic nucleus. *Brain Research* 801: 254–258.
55. Edelstein, K. & Amir, S. (1998). Glutamatergic antagonists do not attenuate light-induced Fos protein in rat intergeniculate leaflet. *Brain Research* 810: 264–268.
56. Guido, M. E., de Guido, L. B., Goguen, D., Robertson, H. A. & Rusak, B. (1999). Daily rhythm of spontaneous immediate-early gene expression in the rat suprachiasmatic nucleus. *Journal of Biological Rhythms* 14: 275–280.
57. Best, J. D., Maywood, E. S., Smith, K. L. & Hastings, M. H. (1999). Rapid resetting of the mammalian circadian clock. *Journal of Neuroscience* 19: 828–835.
58. Dkhissi-Benyahya, O., Sicard, B. & 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.
59. Amy, S. P., Chari, R. & 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.
60. Kriegsfeld, L. J., Drazen, D. L. & 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.
61. Mahoney, M., Bult, A. & 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.
62. Edelstein, K., de la Iglesia, H. O., Schwartz, W. J. & 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.
63. Derambure, P. S. & Boulant, J. A. (1994). Circadian thermosensitive characteristics of suprachiasmatic neurons *in vitro*. *American Journal of Physiology* 266: R1876–R1884.
64. Nakamura, W., Honma, S., Shirakawa, T. & Honma, K. (2001). Regional pacemakers composed of multiple oscillator neurons in the rat suprachiasmatic nucleus. *European Journal of Neuroscience* 14: 666–674.
65. Sumová, A., Trávnícková, Z. & Illnerová, H. (2000). Spontaneous c-Fos rhythm in the rat suprachiasmatic nucleus: location and effect of photoperiod. *American Journal of Physiology* 279: R2262–R2269.
66. Beaulé, C., Arvanitogiannis, A. & 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.
67. Beaulé, C., Barry-Shaw, J. & 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.
68. Rusak, B., Meijer, J. H. & Harrington, M. E. (1989). Hamster circadian rhythms are phase-shifted by electrical stimulation of the geniculo-hypothalamic tract. *Brain Research* 493: 283–291.
69. Treep, J. A., Abe, H., Rusak, B. & Goguen, D. M. (1995). Two distinct retinal projections to the hamster suprachiasmatic nucleus. *Journal of Biological Rhythms* 10: 299–307.
70. Harrington, M. E. & 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.
71. Kuroda, H., Fukushima, M., Nakai, M., Katayama, T. & 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.
72. Redlin, U., Vrang, N. & Mrosovsky, N. (1999). Enhanced masking response to light in hamsters with IGL lesions. *Journal of Comparative Physiology A* 184: 449–456.
73. Jacob, N., Vuillez, P., Lakdhar-Ghazal, N. & 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.
74. Edelstein, K. & Amir, S. (1999). The role of the intergeniculate leaflet in entrainment of circadian rhythms to a skeleton photoperiod. *Journal of Neuroscience* 19: 372–380.
75. Goel, N., Governale, M. M., Jechura, T. J. & Lee, T. M. (2000). Effects of intergeniculate leaflet lesions on circadian rhythms in *Octodon degus*. *Brain Research* 877: 306–313.
76. Menet, J., Vuillez, P., Jacob, N. & 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.
77. Morin, L. P. & 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.

78. Zhang, Y., Van Reeth, O., Zee, P. C., Takahashi, J. S. & 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.
79. Edelstein, K., Pfaus, J. G., Rusak, B. & Amir, S. (1995). Neonatal monosodium glutamate treatment prevents effects of constant light on circadian temperature rhythms of adults rats. *Brain Research* 675: 135–142.
80. Krajnak, K., Dickenson, L. & 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.
81. Amir, S. & Stewart, J. (1998). Conditioned fear suppresses light-induced resetting of the circadian clock. *Neuroscience* 86: 345–351.
82. Caldelas, I., Salazar-Juárez, A., Granados-Fuentes, D., Escobar, C. & 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.
83. Marchant, E. G. & Morin, L. P. (1999). The hamster circadian rhythm system includes nuclei of the subcortical visual shell. *Journal of Neuroscience* 19: 10482–10493.
84. Janik, D. & Mrosovsky, N. (1992). Gene expression in the geniculate induced by a nonphotic circadian phase shifting stimulus. *NeuroReport* 3: 575–578.
85. Mead, S., Ebling, F. J. P., Maywood, E. S., Humbly, T., Herbert, J. & 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.
86. Mikkelsen, J. D., Vrang, N. & Mrosovsky, N. (1998). Expression of Fos in the circadian system following nonphotic stimulation. *Brain Research Bulletin* 47: 367–376.
87. Liou, S. Y., Shibata, S., Iwasaki, K. & 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.
88. Shirakawa, T. & 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.
89. Franken, P., Cao, V., Heller, H. C. & Miller, J. D. (1999). The glutamate induced phase shift in the SCN slice: a two pulse study. *Brain Research* 818: 34–40.
90. Chen, D., Buchanan, G. F., Ding, J. M., Hannibal, J. & 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 U.S.A.* 96: 13468–13473.
91. Yannielli, P. C. & 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.
92. Mintz, E. M., Marvel, C. L., Gillespie, C. F., Price, K. M. & 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.
93. Mintz, E. M., Jasnow, A. M., Gillespie, C. F., Huhman, K. L. & Albers, H. E. (2002). GABA interacts with photic signaling in the suprachiasmatic nucleus to regulate circadian phase shifts. *Neuroscience* 109: 773–778.
94. Novak, C. M. & 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.
95. Gamble, K. L., Novak, C. M. & 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.
96. Colwell, C. S., Ralph, M. R. & Menaker, M. (1990). Do NMDA receptors mediate the effects of light on circadian behavior? *Brain Research* 523: 117–120.
97. Colwell, C. S. & Menaker, M. (1992). NMDA as well as nonNMDA 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.
98. Hannibal, J., Ding, J. M., Chen, D., Fahrenkrug, J., Larsen, P. J., Gillette, M. U. & 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.
99. Bergström, A. L., Hannibal, J., Hindersson, P. & 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.
100. Hannibal, J., Hindersson, P., Østergaard, J., Georg, B., Heegaard, S., Larsen, P. J. & 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.
101. Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Lelièvre, V., Hu, Z. & Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology* 287: R1194–R1201.
102. Minami, Y., Furuno, K., Akiyama, M., Moriya, T. & 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.

103. Stopa, E. G., Johnson, J. K., Friedman, D. I., Ryer, H. I., Reidy, J., Kuo-LeBlanc, V. & 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.
104. Huhman, K. L. & Albers, H. E. (1994). Neuropeptide Y microinjected into the suprachiasmatic region phase shifts circadian rhythms in constant darkness. *Peptides* 15: 1475–1478.
105. Biello, S. M. & Mrosovsky, N. (1996). Phase response curves to neuropeptide Y in wildtype and *tau* mutant hamsters. *Journal of Biological Rhythms* 11: 27–34.
106. Harrington, M. E. & 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.
107. Knoch, M. E., Gobes, S. M. H., Pavlovska, I., Su, C., Mistlberger, R. E. & 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.
108. Yannielli, P. C. & Harrington, M. E. (2000). Neuropeptide Y applied *in vitro* can block the phase shifts induced by light *in vivo*. *NeuroReport* 11: 1587–1591.
109. 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.
110. Morin, L. P. (1992). Serotonergic reinnervation of the hamster suprachiasmatic nucleus and intergeniculate leaflet without functional circadian rhythm recovery. *Brain Research* 599: 98–104.
111. Prosser, R. A., Dean, R. R., Edgar, D. M., Heller, H. C. & 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.
112. Pickard, G. E., Smith, B. N., Belenky, M., Rea, M. A., Dudek, F. E. & Sollars, P. J. (1999). 5-HT_{1B} receptor-mediated presynaptic inhibition of retinal input to the suprachiasmatic nucleus. *Journal of Neuroscience* 19: 4034–4045.
113. Manrique, C., Héry, F., Faudon, M. & 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.
114. Belenky, M. A. & 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.
115. Sanggaard, K. M., Hannibal, J. & 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.
116. Moore, R. Y. & Speh, J. C. (2004). Serotonin innervation of the primate suprachiasmatic nucleus. *Brain Research* 1010: 169–173.
117. Glass, J. D., Grossman, G. H., Farnbauch, L. & 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.
118. Edgar, D. M., Miller, J. D., Prosser, R. A., Dean, R. R. & 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.
119. Cutrera, R. A., Saboureau, M. & 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.
120. Bobrzynska, K. J., Godfrey, M. H. & Mrosovsky, N. (1996). Serotonergic stimulation and nonphotic phase-shifting in hamsters. *Physiology and Behavior* 59: 221–230.
121. Challet, E., Scarbrough, K., Penev, P. D. & 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.
122. Horikawa, K. & 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.
123. Prosser, R. A. (2003). Serotonin phase-shifts the mouse suprachiasmatic circadian clock *in vitro*. *Brain Research* 966: 110–115.
124. Antle, M. C., Marchant, E. G., Niel, L. & 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.
125. Challet, E., Turek, F. W., Laute, M. A. & 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.
126. Antle, M. C., Ogilvie, M. D., Pickard, G. E. & 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.
127. Moriya, T., Yoshinobu, Y., Ikeda, M., Akiyama, M. & 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.
128. Byku, M. & 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.
129. Rea, M. A., Barrera, J., Glass, J. D. & Gannon, R. L. (1995). Serotonergic potentiation of photic phase shifts of the circadian activity rhythm. *NeuroReport* 6: 1289–1292.

130. 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.
131. Mistlberger, R. E. & Antle, M. C. (1998). Behavioral inhibition of light-induced circadian phase resetting is phase and serotonin dependent. *Brain Research* 786: 31–38.
132. Challet, E., Pévet, P. & 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.
133. Mistlberger, R. E., Bossert, J. M., Holmes, M. M. & Marchant, E. G. (1998). Serotonin and feedback effects of behavioral activity on circadian rhythms in mice. *Behavioural Brain Research* 96: 93–99.
134. 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.
135. Sollars, P. J., Ogilvie, M. D., Rea, M. A. & Pickard, G. E. (2002). 5-HT$_{1B}$ receptor knockout mice exhibit an enhanced response to constant light. *Journal of Biological Rhythms* 17: 428–437.
136. Folkard, S., Arendt, J., Aldhous, M. & Kennett, H. (1990). Melatonin stabilises sleep onset time in a blind man without entrainment of cortisol or temperature rhythms. *Neuroscience Letters* 113: 193–198.
137. Hastings, M. H., Mead, S. M., Vindlacheruvu, R. R., Ebling, F. J. P., Maywood, E. S. & Grosse, J. (1992). Nonphotic phase shifting of the circadian activity rhythm of Syrian hamsters: the relative potency of arousal and melatonin. *Brain Research* 591: 20–26.
138. Grosse, J., Velickovic, A. & Davis, F. C. (1996). Entrainment of Syrian hamster circadian activity rhythms by neonatal melatonin injections. *American Journal of Physiology* 270: R533–R540.
139. Hao, H. & Rivkees, S. (2000). Melatonin does not shift circadian phase in baboons. *Journal of Clinical Endocrinology and Metabolism* 85: 3618–3622.
140. Chiba, A., Kikuchi, M. & 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.
141. Oshima, I., Yamada, H., Goto, M., Sato, K. & 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.
142. Chabot, C. C. & Menaker, M. (1992). Effects of physiological cycles of infused melatonin on circadian rhythmicity in pigeons. *Journal of Comparative Physiology A* 170: 615–622.
143. Heigl, S. & Gwinner, E. (1994). Periodic melatonin in the drinking water synchronizes circadian rhythms in sparrows. *Naturwissenschaften* 81: 83–85.
144. Lumineau, S., Guyomarc'h, C., Vivien-Roels, B. & Houdelier, C. (2002). Cyclic melatonin synchronises the circadian rhythm of feeding activity in Japanese quail, *Coturnix c. japonica*. *Comptes Rendus Biologies* 325: 205–212.
145. Houdelier, C., Guyomarc'h, C., Lumineau, S. & 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.
146. Warren, W. S., Hodges, D. B. & 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.
147. Duffield, G. E., Hastings, M. H. & 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.
148. Kirsch, R., Belgnaoui, S., Gourmelen, S. & 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.
149. Schuhler, S., Pitrosky, B., Kirsch, R. & Pévet, P. (2002). Entrainment of locomotor activity rhythm in pinealectomized adult Syrian hamsters by daily melatonin infusion. *Behavioural Brain Research* 133: 343–350.
150. Sharma, V. K. & 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.
151. Redman, J. R. & 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.
152. Slotten, H. A., Pitrosky, B. & Pévet, P. (1999). Influence of the mode of daily melatonin administration on entrainment of rat circadian rhythms. *Journal of Biological Rhythms* 14: 347–353.
153. Pitrosky, B., Kirsch, R., Malan, A., Mocaer, E. & 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.
154. Slotten, H. A., Pitrosky, B. & 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.
155. Slotten, H. A., Pitrosky, B., Krekling, S. & Pévet, P. (2002). Entrainment of circadian activity rhythms in rats to melatonin administered at T cycles different from 24 hours. *Neurosignals* 11: 73–80.
156. Sack, R. L., Lewy, A. J., Blood, M. L., Stevenson, J. & Keith, L. D. (1991). Melatonin administration to blind people: phase advances and entrainment. *Journal of Biological Rhythms* 6: 249–261.
157. Middleton, B., Arendt, J. & Stone, B. M. (1997). Complex effects of melatonin on human circadian rhythms in constant dim light. *Journal of Biological Rhythms* 12: 467–477.

158. Lockley, S. W., Skene, D. J., James, K., Thapan, K., Wright, J. & Arendt, J. (2000). Melatonin administration can entrain the free-running circadian system of blind subjects. *Journal of Endocrinology* 164: R1–R6.
159. Sack, R. L., Brandes, R. W., Kendall, A. R. & Lewy, A. J. (2000). Entrainment of free-running circadian rhythms by melatonin in blind people. *New England Journal of Medicine* 343: 1070–1077.
160. Hack, L. M., Lockley, S. W., Arendt, J. & 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.
161. Lewy, A. J., Emens, J. S., Bernert, R. A. & 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.
162. Weaver, D. R., Rivkees, S. A. & Reppert, S. M. (1989). Localization and characterization of melatonin receptors in rodent brain by *in vitro* autoradiography. *Journal of Neuroscience* 9: 2581–2590.
163. Weaver, D. R. & Reppert, S. M. (1996). The Mel_{1a} melatonin receptor gene is expressed in human suprachiasmatic nuclei. *NeuroReport* 8: 109–112.
164. Gauer, F., Schuster, C., Poirel, V. J., Pévet, P. & 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.
165. Hunt, A. E., Al-Ghoul, W. M., Gillette, M. U. & 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.
166. Jin, X., von Gall, C., Pieschl, R. L., Gribkoff, V. K., Stehle, J. H., Reppert, S. M. & Weaver, D. R. (2003). Targeted disruption of the mouse Mel_{1b} melatonin receptor. *Molecular and Cellular Biology* 23: 1054-1060.
167. Benloucif, S. & 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.
168. Sharma, V. K., Chandrashekaran, M. K., Singaravel, M. & 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.
169. Sumová, A. & Illnerová, H. (1996). Melatonin instantaneously resets intrinsic circadian rhythmicity in the rat suprachiasmatic nucleus. *Neuroscience Letters* 218: 181–184.
170. Weaver, D. R., Liu, C. & 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.
171. Lewy, A. J., Ahmed, S., Jackson, J. M. L. & Sack, R. L. (1992). Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiology International* 9: 380–392.
172. Deacon, S., English, J. & Arendt, J. (1994). Acute phase-shifting effects of melatonin associated with suppression of core body temperature in humans. *Neuroscience Letters* 178: 32–34.
173. Attenburrow, M. E. J., Dowling, B. A., Sargent, P. A., Sharpley, A. L. & Cowen, P. J. (1995). Melatonin phase advances circadian rhythm. *Psychopharmacology* 121: 503–505.
174. Rajaratnam, S. M. W., Dijk, D. J., Middleton, B., Stone, B. M. & 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.
175. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C. & 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.
176. Morin, L. P., Fitzgerald, K. M. & Zucker, I. (1977). Estradiol shortens the period of hamster circadian rhythms. *Science* 196: 305–307.
177. Takahashi, J. S. & Menaker, M. (1980). Interaction of estradiol and progesterone: effects on circadian locomotor rhythm of female golden hamsters. *American Journal of Physiology* 239: R497–R504.
178. Zucker, I., Fitzgerald, K. M. & Morin, L. P. (1980). Sex differentiation of the circadian system in the golden hamster. *American Journal of Physiology* 238: R97–R101.
179. Lumineau, S., Guyomarc'h, C., Boswell, T., Richard, J. P. & 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.
180. Schull, J., McEachron, D. L., Adler, N. T., Fiedler, L., Horvitz, J., Noyes, A., Olson, M. & Shack, J. (1988). Effects of thyroidectomy, parathyroidectomy and lithium on circadian wheelrunning in rats. *Physiology and Behavior* 42: 33–39.
181. Bauer, M. S., Soloway, A., Dratman, M. B. & Kreider, M. (1992). Effects of hypothyroidism on rat circadian activity and temperature rhythms and their response to light. *Biological Psychiatry* 32: 411–425.
182. Duncan, W. C. & 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.
183. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O. & Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
184. Prosser, R. A. & Bergeron, H. E. (2003). Leptin phase-advances the rat suprachiasmatic circadian clock *in vitro*. *Neuroscience Letters* 336: 139–142.
185. Byku, M. & Gannon, R. L. (2000). Opioid induced nonphotic phase shifts of hamster circadian activity rhythms. *Brain Research* 873: 189–196.

186. Byku, M. & Gannon, R. L. (2000). SNC 80, a delta-opioid agonist, elicits phase advances in hamster circadian activity rhythms. *NeuroReport* 11: 1449–1452.
187. Tierno, A., Fiore, P. & Gannon, R. L. (2002). Delta opioid inhibition of light-induced phase advances in hamster circadian activity rhythms. *Brain Research* 937: 66–73.
188. Cote, N. K. & Harrington, M. E. (1993). Histamine phase shifts the circadian clock in a manner similar to light. *Brain Research* 613: 149–151.
189. Meyer, J. L., Hall, A. C. & Harrington, M. E. (1998). Histamine phase shifts the hamster circadian pacemaker via an NMDA dependent mechanism. *Journal of Biological Rhythms* 13: 288–295.
190. Elliott, K. J., Weber, E. T. & 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.
191. Antle, M. C., Steen, N. M. & Mistlberger, R. E. (2001). Adenosine and caffeine modulate circadian rhythms in the Syrian hamster. *NeuroReport* 12: 2901–2905.
192. Cagampang, F. R. A., Okamura, H. & Inouye, S. T. (1994). Circadian rhythms of norepinephrine in the rat suprachiasmatic nucleus. *Neuroscience Letters* 173: 185–188.
193. Earnest, D. J. & 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 U.S.A.* 82: 4277–4281.
194. Meijer, J. H., van der Zee, E. & 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.
195. Wee, B. E. F., Anderson, K. D., Kouchis, N. S. & 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.
196. Liu, C. & Gillette, M. U. (1996). Cholinergic regulation of the suprachiasmatic nucleus circadian rhythm via a muscarinic mechanism at night. *Journal of Neuroscience* 16: 744–751.
197. Bina, K. G. & Rusak, B. (1996). Muscarinic receptors mediate carbachol-induced phase shifts of circadian activity rhythms in Syrian hamsters. *Brain Research* 743: 202–211.
198. Erhardt, C., Galani, R., Jeltsch, H., Cassel, J. C., Klosen, P., Menet, J. S., Pévet, P. & 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.
199. Colwell, C. S., Kaufman, C. M. & Menaker, M. (1993). Phase-shifting mechanisms in the mammalian circadian system: new light on the carbachol paradox. *Journal of Neuroscience* 13: 1454–1459.
200. Myers, M. P., Wager-Smith, K., Rothenfluh-Hilfiker, A. & Young, M. W. (1996). Light-induced degradation of TIMELESS and entrainment of the *Drosophila* circadian clock. *Science* 271: 1736–1740.
201. Lee, C., Parikh, V., Itsukaichi, T., Bae, K. & Edery, I. (1996). Resetting the Drosophila clock by photic regulation of PER and a PER-TIM complex. *Science* 271: 1740–1744.
202. Emery, P., So, W. V., Kaneko, M., Hall, J. C. & 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.
203. Ceriani, M. F., Darlington, T. K., Staknis, D., Más, P., Petti, A. A., Weitz, C. J. & Kay, S. A. (1999). Light-dependent sequestration of TIMELESS by CRYPTOCHROME. *Science* 285: 553–556.
204. Ivanchenko, M., Stanewsky, R. & Giebultowicz, J. M. (2001). Circadian photoreception in *Drosophila*: functions of cryptochrome in peripheral and central clocks. *Journal of Biological Rhythms* 16: 205–215.
205. Lin, F. J., Song, W., Meyer-Bernstein, E., Naidoo, N. & Sehgal, A. (2001). Photic signaling by cryptochrome in the *Drosophila* circadian system. *Molecular and Cellular Biology* 21: 7287–7294.
206. Klarsfeld, A., Malpel, S., Michard-Vanhée, C., Picot, M., Chélot, E. & Rouyer, F. (2004). Novel features of cryptochrome-mediated photoreception in the brain circadian clock of *Drosophila*. *Journal of Neuroscience* 24: 1468–1477.
207. Helfrich-Förster, C., Winter, C., Hofbauer, A., Hall, J. C. & Stanewsky, R. (2001). The circadian clock of fruit flies is blind after elimination of all known photoreceptors. *Neuron* 30: 249–261.
208. Rieger, D., Stanewsky, R. & 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.
209. Devlin, P. F. and Kay, S. A. (2001). Circadian photoperception. *Annual Review of Physiology* 63: 677–694.
210. Salomé, P. A. & McClung, C. R. (2004). The *Arabidopsis thaliana* clock. *Journal of Biological Rhythms* 19: 425–435.
211. Crosthwaite, S. K., Loros, J. J. & Dunlap, J. C. (1995). Light-induced resetting of a circadian clock is mediated by a rapid increase in *frequency* transcript. *Cell* 81: 1003–1012.
212. Crosthwaite, S. K., Dunlap, J. C. & Loros, J. J. (1997). *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. *Science* 276: 763–769.
213. Froehlich, A. C., Liu, Y., Loros, J. J. & Dunlap, J. C. (2002). White collar-1, a circadian blue light photoreceptor, binding to the *frequency* promoter. *Science* 297: 815–819.
214. He, Q., Cheng, P., Yang, Y., Wang, L., Gardner, K. H. & Liu, Y. (2002). White collar-1, a DNA binding transcription factor and a light sensor. *Science* 297: 840–842.

215. Butcher, G. Q., Dziema, H., Collamore, M., Brugoon, P. W. & 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.
216. Shearman, L. P., Zylka, M. J., Weaver, D. R., Kolakowski, L. F. & Reppert, S. M. (1997). Two *period* homologs: circadian expression and photic regulation in the suprachiasmatic nuclei. *Neuron* 19: 1261–1269.
217. Shigeyoshi, Y., Taguchi, K., Yamamoto, S., Takekida, S., Yan, L., Tei, H., Moriya, T., Shibata, S., Loros, J. J., Dunlap, J. C. & Okamura, H. (1997). Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the *mPer1* transcript. *Cell* 91: 1043–1053.
218. Thresher, R. J., Vitaterna, M. H., Miyamoto, Y., Kazantsev, A., Hsu, D. S., Petit, C., Selby, C. P., Dawut, L., Smithies, O., Takahashi, J. S. & Sancar, A. (1998). Role of mouse cryptochrome blue-light photoreceptor in circadian photoresponses. *Science* 282: 1490–1494.
219. Okamura, H., Miyake, S., Sumi, Y., Yamaguchi, S., Yasui, A., Muijtjens, M., Hoeijmakers, J. H. J. & 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.
220. Wakamatsu, H., Takahashi, S. Moriya, T., Inouye, S. T., Okamura, H., Akiyama, M. & Shibata, S. (2001). Additive effect of *mPer1* and *mPer2* antisense oligonucleotides on light-induced phase shift. *NeuroReport* 12: 127–131.
221. Asai, M., Yamaguchi, S., Isejima, H., Janouchi, M., Moriya, T., Shibata, S., Kobayashi, M. & 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.
222. Von Gall, C., Noton, E., Lee, C. & 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.
223. Avivi, A., Oster, H., Joel, A., Beiles, A., Albrecht, U. & 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 U.S.A.* 99: 11718–11723.
224. Caldelas, I., Poirel, V. J., Sicard, B., Pévet, P. & 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.
225. Beaulé, C., Houle, L. M. & 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.
226. Kolker, D. E., Fukuyama, H., Huang, D. S., Takahashi, J. S., Horton, T. H. & Turek, F. W. (2003). Aging alters circadian and light-induced expression of clock genes in golden hamsters. *Journal of Biological Rhythms* 18: 159–169.
227. Yan, L. & Silver, R. (2002). Differential induction and localization of mPer1 and mPer2 during advancing and delaying phase shifts. *European Journal of Neuroscience* 16: 1531–1540.
228. Yan, L. & 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.
229. Albrecht, U., Zheng, B., Larkin, D., Sun, Z. S. & Lee, C. C. (2001). *mPer1* and *mPer2* are essential for normal resetting of the circadian clock. *Journal of Biological Rhythms* 16: 100–104.
230. Olde Scheper, T., Klinkenberg, D., Pennartz, C. & van Pelt, J. (1999). A mathematical model for the intracellular circadian rhythm generator. *Journal of Neuroscience* 19: 40–47.
231. Leloup, J. C. & 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.
232. Petri, B. & 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.
233. Smolen, P., Baxter, D. A. & Byrne, J. H. (2001). Modeling circadian oscillations with interlocking positive and negative feedback loops. *Journal of Neuroscience* 21: 6644–6656.
234. Gonze, D., Halloy, J. & Goldbeter, A. (2002). Robustness of circadian rhythms with respect to molecular noise. *Proceedings of the National Academy of Sciences U.S.A.* 99: 673–678.
235. Forger, D. B. & Peskin, C. S. (2003). A detailed predictive model of the mammalian circadian clock. *Proceedings of the National Academy of Sciences U.S.A.* 100: 14806–14811.
236. Teclemariam-Mesbah, R., Kalsbeek, A., Pévet, P. & 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.
237. Teclemariam-Mesbah, R., Ter Horst, G. J., Postema, F., Wortel, J. & Buijs, R. M. (1999). Anatomical demonstration of the suprachiasmatic nucleus-pineal pathway. *Journal of Comparative Neurology* 406: 171–182.
238. Kalsbeek, A., Fliers, E., Franke, A. N., Wortel, J. & 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.
239. Scheer, F. A. J. L., Ter Horst, G. J., van der Vliet, J. & 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.

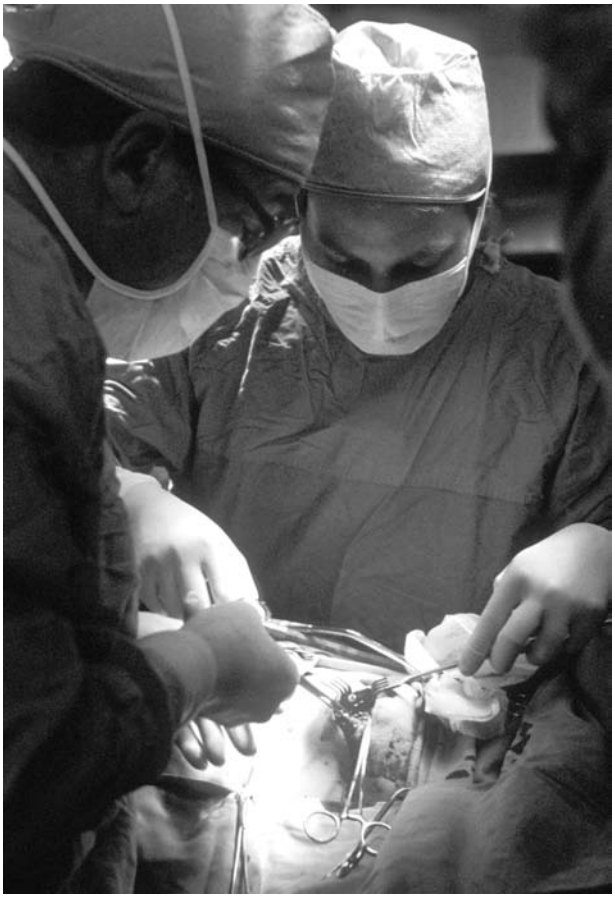
240. Buijs, R. M., La Fleur, S. E., Wortel, J., van Heyningen, C., Zuiddam, L., Mettenleiter, T. C., Kalsbeek, A., Nagai, K. & Niijima, A. (2003). The suprachiasmatic nucleus balances sympathetic and parasympathetic output to peripheral organs through separate preautonomic neurons. *Journal of Comparative Neurology* 464: 36–48.
241. Leak, R. K. & Moore, R. Y. (2001). Topographic organization of suprachiasmatic nucleus projection neurons. *Journal of Comparative Neurology* 433: 312–334.
242. Leak, R. K., Card, J. P. & Moore, R. Y. (1999). Suprachiasmatic pacemaker organization analyzed by viral transynaptic transport. *Brain Research* 819: 23–32.
243. Kriegsfeld, L. J., Leak, R. K., Yackulic, C. B., LeSauter, J. & 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.
244. Munch, I. C., Møller, M., Larsen, P. J. & 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.
245. Smeraski, C. A., Sollars, P. J., Ogilvie, M. D., Enquist, L. W. & 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.
246. Mistlberger, R. E. (in press). Circadian regulation of sleep in mammals: role of the suprachiasmatic nucleus. *Brain Research Reviews*.
247. Novak, C. M. & 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.
248. Deurveilher, S., Burns, J. & 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.
249. Chou, T. C., Scammell, T. E., Gooley, J. J., Gaus, S. E., Saper, C. B. & Lu, J. (2003). Critical role of dorsomedial hypothalamic nucleus in a wide range of behavioral circadian rhythms. *Journal of Neuroscience* 23: 10691–10702.
250. Aston-Jones, G., Chen, S., Zhu, Y. & Oshinsky, M. L. (2001). A neural circuit for circadian regulation of arousal. *Nature Neuroscience* 4: 732–738.
251. Noguchi, T., Watanabe, K., Ogura, A. & Yamaoka, S. (2004). The clock in the dorsal suprachiasmatic nucleus runs faster than that in the ventral. *European Journal of Neuroscience* 20: 3199–3202.
252. Novak, C. M. & 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.
253. Sun, X., Whitefield, S., Rusak, B. & Semba, K. (2001). Electrophysiological analysis of suprachiasmatic nucleus projections to the ventrolateral preoptic area in the rat. *European Journal of Neuroscience* 14: 1257–1274.
254. Stephan, F. K. & 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.
255. Honma, S., Honma, K. & Hiroshige, T. (1984). Dissociation of circadian rhythms in rats with a hypothalamic island. *American Journal of Physiology* 246: R949–R954.
256. Hakim, H., DeBernardo, A. P. & 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.
257. Klein, D. C., Smoot, R., Weller, J. L., Higa, S., Markey, S. P., Creed, G. J. & 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.
258. Moore, R. Y. and Danchenko, R. L. (2002). Paraventricular-subparaventricular hypothalamic lesions selectively affect circadian function. *Chronobiology International* 19: 345–360.
259. Perreau-Lenz, S., Kalsbeek, A., Garidou, M. L., Wortel, J., van der Vliet, J., van Heijningen, C., Simonneaux, V., Pévet, P. & Buijs, R. M. (2003). Suprachiasmatic control of melatonin synthesis in rats: inhibitory and stimulatory mechanisms. *European Journal of Neuroscience* 17: 221–228.
260. Lu, J., Zhang, Y. H., Chou, T. C., Gaus, S. E., Elmquist, J. K., Shiromani, P. & 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.
261. Nunez, A. A., Bult, A., McElhinny, T. L. & 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.
262. Hermes, M. L. H., Coderre, E. M., Buijs, R. M. & 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.
263. Cui, L., Coderre, E. & Renaud, L. P. (2000). $GABA_B$ presynaptically modulates suprachiasmatic input to hypothalamic paraventricular magnocellular neurons. *American Journal of Physiology* 278: R1210–R1216.
264. Cui, L. N., Coderre, E. & Renaud, L. P. (2001). Glutamate and GABA mediate suprachiasmatic nucleus inputs to spinal-projecting paraventricular neurons. *American Journal of Physiology* 281: R1283–R1289.
265. Kalsbeek, A., Garidou, M. L., Palm, I. F., van der Vliet, J., Simonneauz, V., Pévet, P. & 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.
266. Lipton, J. M. (1968). Effects of preoptic lesions on heat-escape responding and colonic temperature in the rat. *Physiology and Behavior* 3: 165–169.

267. Lepkovsky, S., Snapir, N. & Furuta, F. (1968). Temperature regulation and appetitive behavior in chickens with hypothalamic lesions. *Physiology and Behavior* 3: 911–915.
268. 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.
269. Satinoff, E. & Rutstein, J. (1970). Behavioral thermoregulation in rats with anterior hypothalamic lesions. *Journal of Comparative and Physiological Psychology* 71: 77–82.
270. Toth, D. M. (1973). Temperature regulation and salivation following preoptic lesions in the rat. *Journal of Comparative and Physiological Psychology* 82: 480–488.
271. Satinoff, E., Valentino, D. & Teitelbaum, P. (1976). Thermoregulatory cold-defense deficits in rats with preoptic/anterior hypothalamic lesions. *Brain Research Bulletin* 1: 553–565.
272. Van Zoeren, J. G. & 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.
273. Hagan, A. A. & Heath, J. E. (1980). Effects of preoptic lesions on thermoregulation in ducks. *Journal of Thermal Biology* 5: 141–150.
274. Morimoto, A. & Murakami, N. (1985). [^{14}C]deoxyglucose incorporation into rat brain regions during hypothalamic or peripheral thermal stimulation. *American Journal of Physiology* 248: R84–R92.
275. Joyce, M. P. & 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.
276. Bratincsák, A. & Palkovits, M. (2004). Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience* 127: 385–397.
277. Szymusiak, R., DeMory, A., Kittrell, E. M. W. & Satinoff, E. (1985). Diurnal changes in thermoregulatory behavior in rats with medial preoptic lesions. *American Journal of Physiology* 249: R219–R227.
278. Satinoff, E. & Shan, S. Y. Y. (1971). Loss of behavioral thermoregulation after lateral hypothalamic lesions in rats. *Journal of Comparative and Physiological Psychology* 77: 302–312.
279. Refinetti, R. & Carlisle, H. J. (1986). Effects of lateral hypothalamic lesions on thermoregulation in the rat. *Physiology and Behavior* 38: 219–228.
280. Refinetti, R., Kaufman, C. M. & 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.
281. Refinetti, R. (1995). Effects of suprachiasmatic lesions on temperature regulation in the golden hamster. *Brain Research Bulletin* 36: 81–84.
282. Osborne, A. R. & Refinetti, R. (1995). Effects of hypothalamic lesions on the body temperature rhythm of the golden hamster. *NeuroReport* 6: 2187–2192.
283. Ruby, N. F., Ibuka, N., Barnes, B. M. & Zucker, I. (1989). Suprachiasmatic nuclei influence torpor and circadian temperature rhythms in hamsters. *American Journal of Physiology* 257: R210–R215.
284. Ruby, N. F. & Zucker, I. (1992). Daily torpor in the absence of the suprachiasmatic nucleus in Siberian hamsters. *American Journal of Physiology* 263: R353–R362.
285. Paul, M. J., Kauffman, A. S. & Zucker, I. (2004). Feeding schedule controls circadian timing of daily torpor in SCN-ablated Siberian hamsters. *Journal of Biological Rhythms* 19: 226–237.
286. Ruis, J. F., Rietveld, W. J. & 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.
287. Eastman, C. I., Mistlberger, R. E. & Rechtschaffen, A. (1984). Suprachiasmatic nuclei lesions eliminate circadian temperature and sleep rhythms in the rat. *Physiology and Behavior* 32: 357–368.
288. Honma, S., Honma, K., Shirakawa, T. & Hiroshige, T. (1988). Rhythms in behavior, body temperature and plasma corticosterone in SCN lesioned rats given methamphetamine. *Physiology and Behavior* 44: 247–255.
289. Warren, W. S., Champney, T. H. & 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.
290. Wachulec, M., Li, H., Tanaka, H., Peloso, E. & Satinoff, E. (1997). Suprachiasmatic nuclei lesions do not eliminate homeostatic thermoregulatory responses in rats. *Journal of Biological Rhythms* 12: 226–234.
291. Liu, S., Chen, X. M., Yoda, T. Nagashima, K., Fukuda, Y. & Kanosue, K. (2002). Involvement of the suprachiasmatic nucleus in body temperature modulation by food deprivation in rats. *Brain Research* 929: 26–36.
292. Ruby, N. F., Dark, J., Burns, D. E., Heller, H. C. & Zucker, I. (2002). The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *Journal of Neuroscience* 22: 357–364.
293. Satinoff, E., Liran, J. & Clapman, R. (1982). Aberrations of circadian body temperature rhythms in rats with medial preoptic lesions. *American Journal of Physiology* 242: R352–R357.
294. Briese, E. (1989). Do medial preoptic lesions interfere with the set-point of temperature regulation? *Brain Research Bulletin* 23: 137–144.
295. Yamazaki, S., Kerbeshian, M. C., Hocker, C. G., Block, G. D. & Menaker, M. (1998). Rhythmic properties of the hamster suprachiasmatic nucleus *in vivo*. *Journal of Neuroscience* 18: 10709–10723.
296. Ralph, M. R., Foster, R. G., Davis, F. C. & Menaker, M. (1990). Transplanted suprachiasmatic nucleus determines circadian period. *Science* 247: 975–978.
297. Saitoh, Y., Matsui, Y., Nihonmatsu, I. & 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.

298. Romero, M. T., Lehman, M. N. & Silver, R. (1993). Age of donor influences ability of suprachiasmatic nucleus grafts to restore circadian rhythmicity. *Developmental Brain Research* 71: 45–52.
299. LeSauter, J. & Silver, R. (1994). Suprachiasmatic nucleus lesions abolish and fetal grafts restore circadian gnawing rhythms in hamsters. *Restorative Neurology and Neuroscience* 6: 135–143.
300. Viswanathan, N. & Davis, F. C. (1995). Suprachiasmatic nucleus grafts restore circadian function in aged hamsters. *Brain Research* 686: 10–16.
301. Sollars, P. J., Kimble, D. P. & Pickard, G. E. (1995). Restoration of circadian behavior by anterior hypothalamic heterografts. *Journal of Neuroscience* 15: 2109–2122.
302. Grosse, J. & 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.
303. Boer, G. J., van Esseveldt, K. E., van der Geest, B. A. M., Duindam, H. & Rietveld, W. J. (1999). Vasopressin-deficient suprachiasmatic nucleus grafts re-instate circadian rhythmicity in suprachiasmatic nucleus lesioned arrhythmic rats. *Neuroscience* 89: 375–385.
304. Earnest, D. J., Liang, F. Q., Ratcliff, M. & Cassone, V. M. (1999). Immortal time: circadian clock properties of rat suprachiasmatic cell lines. *Science* 283: 693–695.
305. Zlomanczuk, P., Mrugala, M., de la Iglesia, H. O., Ourednik, V., Quesenberry, P. J., Snyder, E. Y. & 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.
306. Tousson, E. & Meissl, H. (2004). Suprachiasmatic nuclei grafts restore the circadian rhythm in the paraventricular nucleus of the hypothalamus. *Journal of Neuroscience* 24: 2983–2988.
307. Lehman. M. N., LeSauter, J. & Silver, R. (1998). Fiber outgrowth from anterior hypothalamic and cortical xenografts in the third ventricle. *Journal of Comparative Neurology* 391: 133–145.
308. Meyer-Bernstein, E. L., Jetton, A. E., Matsumoto, S., Markuns, J. F. Lehman, M. N. & Bittman, E. L. (1999). Effects of suprachiasmatic transplants on circadian rhythms of neuroendocrine function in golden hamsters. *Endocrinology* 140: 207–218.
309. Silver, R., LeSauter, J., Tresco, P. A. & Lehman, M. N. (1996). A diffusible coupling signal from the transplanted suprachiasmatic nucleus controlling circadian locomotor rhythms. *Nature* 382: 810–813.
310. Allen, G., Rappe, J., Earnest, D. J. & 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.
311. McDonald, M. J. & Rosbash, M. (2001). Microarray analysis and organization of circadian gene expression in *Drosophila*. *Cell* 107: 567–578.
312. Claridge-Chang, A., Wijnen, H., Naef, F., Boothroyd, C., Rajewsky, N. & Young, M. W. (2001). Circadian regulation of gene expression systems in the *Drosophila* head. *Neuron* 32: 657–671.
313. Ceriani, M. F., Hogenesch, J. B., Yanovsky, M., Panda, S., Straume, M. & Kay, S. A. (2002). Genome-wide expression analysis in *Drosophila* reveals genes controlling circadian behavior. *Journal of Neuroscience* 22: 9305–9319.
314. Lin, Y., Han, M., Shimada, B., Wang, L., Gibler, T. M., Amarakone, A., Awad, T. A., Stormo, G. D., van Gelder, R. N. & 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 U.S.A.* 99: 9562–9567.
315. Kita, Y., Shiozawa, M., Jin, W., Majewski, R. R., Besharse, J. C., Greene, A. S. & Jacob, H. J. (2002). Implications of circadian gene expression in kidney, liver and the effects of fasting on pharmacogenomic studies. *Pharmacogenetics* 12: 55–65.
316. Akhtar, R. A., Reddy, A. B., Maywood, E. S., Clayton, J. D., King, V. M., Smith, A. G., Gant, T. W., Hastings, M. H. & 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.
317. Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H. & Weitz, C. J. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83.
318. Duffield, G. E., Best, J. D., Meurers, B. H., Bittner, A., Loros, J. J. & 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.
319. Oishi, K., Miyazaki, K., Kadota, K., Kikuno, R., Nagase, T., Atsumi, G., Ohkura, N., Azama, T., Mesaki, M., Yukimasa, S., Kobayashi, H., Iitaka, C., Umehara, T., Horikoshi, M., Kudo, T., Shimizu, Y., Yano, M. & Monden, M. (2003). Genome-wide expression analysis of mouse liver reveals CLOCK-regulated circadian output genes. *Journal of Biological Chemistry* 278: 41519–41527.
320. Desai, V. G., Moland, C. L., Branham, W. S., Delongchamp, R. R., Fang, H., Duffy, P. H., Peterson, C. A., Beggs, M. L. & 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.
321. Marshall, E. (2004). Getting the noise out of gene arrays. *Science* 306: 630–631.

Part V

Applications



A patient undergoes surgical biopsy for breast cancer. (Photograph courtesy of the U.S. National Cancer Institute.)

14 Optimal Timing on Earth and in Space

CHAPTER OUTLINE

14.1 Best Time for Sports and Intellectual Activities
14.2 Space Exploration

14.1 BEST TIME FOR SPORTS AND INTELLECTUAL ACTIVITIES

Part I of this book discussed historical and methodological aspects of research in circadian physiology, while Part II examined the phenomenology of circadian rhythms. Endogenous and environmental mechanisms that control circadian rhythms were discussed in Part III. Part IV addressed the cellular and molecular substrates of circadian rhythms. It is now time to try to relate these fundamental principles to the complexity of human life. The four chapters in Part V discuss 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 this chapter, the discussion of optimal timing for human performance starts here on Earth (Section 14.1) and later considers the issue of space exploration (Section 14.2).

14.1.1 Physical Activities

I discuss first the best time of day for physical activities, such as sports (Figure 14.1). Previous chapters showed repeatedly that practically every variable in the body exhibits daily or circadian rhythmicity. Therefore, it is reasonable to expect that the ability to perform physical activities will exhibit daily rhythmicity. Previous chapters, however, also showed that quite a few "obvious" expectations are sometimes wrong. Rather than trust intuition, one must examine the facts.

Laboratory studies on human subjects performing various forms of physical exercise have confirmed researchers' expectation of daily rhythmicity. Whether one considers maximal isometric lifting strength,[1] endurance in concentric leg movements,[2] spontaneous variation in bicycle pedaling rate,[3,4] anaerobic (strenuous) capacity,[5] or the subjective feeling of exertion,[6] 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 with the early morning. As an example, Figure 14.2 shows the daily variation in knee-extension torque ("force") of trained bicyclists tested in the laboratory. Note that torque is maximal at 19:00 hours (7 P.M.) and minimal in the early morning.[7]

FIGURE 14.1 On the ball. Because many physiological variables exhibit circadian rhythmicity, performance in sports and other physical activities may depend on time of day. (Source: © ArtToday, Tucson, AZ.)

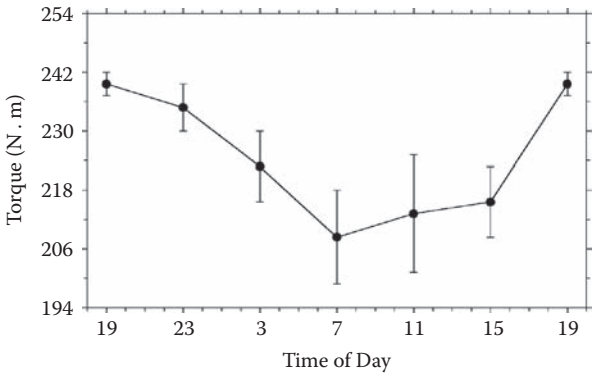


FIGURE 14.2 Daily variation in muscle torque. The graph shows the daily variation in torque of trained bicyclists. Torque was measured in the laboratory, using the isometric contraction of the right knee extensor muscles at 65° knee flexion. The data points represent the means (± SE) of six subjects. Note that torque is lowest in the morning and early afternoon. (Source: Callard, D., Davenne, D., Gauthier, A., Lagarde, D. & Van Hoecke, J. (2000). Circadian rhythms in human muscular efficiency: continuous physical exercise versus continuous rest, a crossover study. *Chronobiology International* 17: 693–704.)

Conditions in the laboratory are much more stable than in the real world. Variables studied in the laboratory

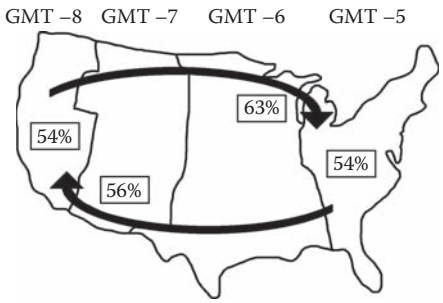


FIGURE 14.3 Home team advantage. The number of wins by American baseball teams depends on whether the visiting team travels west or east. Percentages of wins over a 3-year period are shown for the home team according to the travel status of the visiting team (local, traveling east, or traveling west). (Source: Recht, L. D., Lew, R. A. & Schwartz, W. J. (1995). Baseball teams beaten by jet lag. *Nature* 377: 583.)

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. To gain a better understanding of the issue, circadian physiologists must examine the performance records of professional athletes. One group of researchers analyzed the performance of major league baseball teams from 1991 to 1993.[8] Scientists cannot tell multimillion-dollar baseball players when to play, of course, but the regular schedule of games often involves travel from one coast of the Unites States to the other. Because games are played at the same local time on both coasts, the visiting team from one coast is forced to play 3 hours earlier (or later) than the team usually plays. This schedule provides researchers with the opportunity to compare the performance of the teams at different times of the biological day. Figure 14.3 shows the percentages of wins by 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. Westward travel, however, 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 comparable 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 east, may be due to jet lag (which is discussed in detail in Chapter 15) or it simply may be an incidental occurrence in the 1991 to 1993 seasons. A separate group of researchers analyzed the performance of professional basketball teams (NBA) from 1987 to 1995.[9] Although there was a small difference in the percentages of games won by home teams on the two Coasts, the difference was not statistically significant.

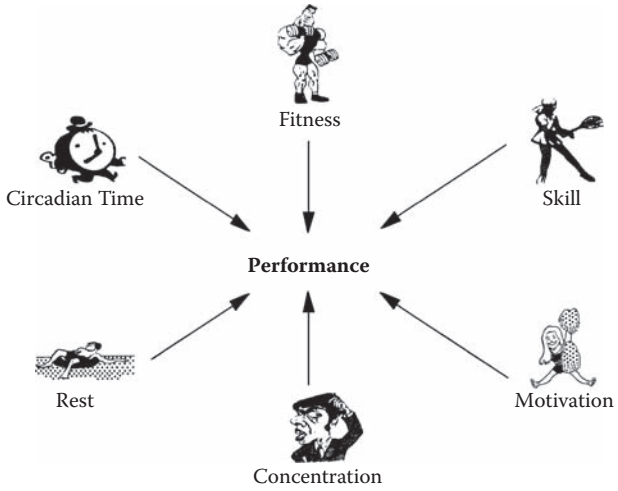


FIGURE 14.4 More than just hard work. This diagram illustrates factors that affect athletic performance.

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 14.4). It makes sense then that *not* playing at one's best time of day does *not* have a major effect on one's overall performance. Evaluation of the existing literature reveals no consistent evidence of daily rhythmicity in sport-specific performance.[10,11] Even in physically fit but nonprofessional athletes tested under seminatural conditions, time of day does not seem to affect overall performance.[12–14] An example is shown in Figure 14.5. Assiduous members of a fitness club (who work out an average of 4 days per week) were asked to exercise at different times of the day. Body temperature, measured immediately before the workout, exhibited the expected daily variation with a peak in the mid- 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 lunchtime.

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 remember, however, that circadian variation in physical prowess is only one of many variables that determine your overall performance. Depending on your level of fitness, your motivation, and the amount of rest you have had, the effect of circadian time on performance may be negligible.

14.1.2 Intellectual Activities

Chapter 5 briefly mentioned the existence of daily and circadian rhythmicity in cognitive processes. Such rhythmicity may or may not have significant implications for a variety of intellectual activities, including, for example,

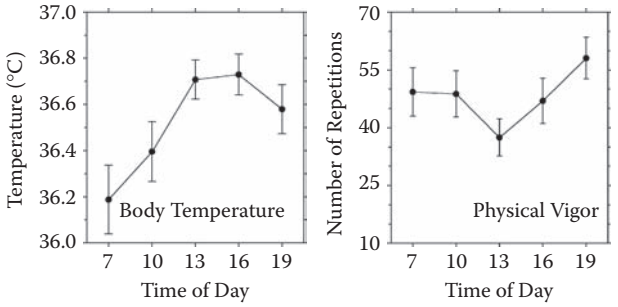


FIGURE 14.5 Best time to work out? The graphs show the daily variation in body core temperature and physical vigor of assiduous members of a fitness club (who work out an average 3.8 days per week). The data points correspond to the means (± SE) of 25 subjects. Physical vigor was measured as the number 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"). Note that body temperature shows the expected peak in the mid- to late afternoon, but physical vigor shows only a nonstatistically significant dip after lunch. (Source: Refinetti, R. & 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.)

FIGURE 14.6 Exam time! Because many psychological variables exhibit circadian rhythmicity, performance in exams may depend on time of day. (Source: © ArtToday, Tucson, AZ.)

learning and exam-taking (Figure 14.6). I have taught Introduction to Psychology to undergraduate students for many years. I have presented 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

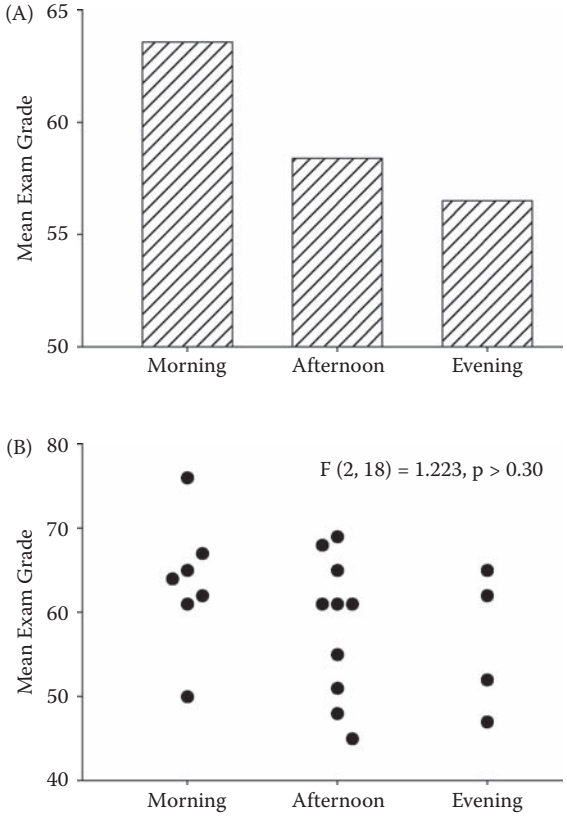


FIGURE 14.7 Comparing exam grades. The top graph (A) shows the mean grades attained by students in the first midterm exam in introductory psychology classes taught by the same professor — at the same university — in the morning, in the afternoon, or in the evening, between 1999 and 2003. Although the 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. (Source: R. Refinetti's archives of Psychology 101 grades, University of South Carolina, Salkehatchie.)

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 14.7, 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 to 100), but grades are a topic 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, 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 explained easily by random variability ($p > 0.30$). I showed you the data in Figure 14.7 because I wanted to emphasize the importance of proper data analysis procedures in the interpretation of real-world data. Too often, educators and some members of the media publicize differences in mean performance without accompanying indices of intragroup variability. This practice is dangerous, as it can be intentionally deceiving. You should be careful when interpreting these data.

Numerous laboratory studies have demonstrated daily (and, occasionally, circadian) rhythmicity in cognitive performance. Whether one considers general alertness,[15–19] reaction time,[16,18,20] memory,[16,18,21] the accuracy of estimates of time,[22,23] or visual-search speed,[24] performance is worst in the early morning (around wake-up time) and best in the late afternoon or early evening (around the time the body temperature rhythm peaks), with a variation in performance of 20 to 100% between peak and trough. Some evidence even suggests that people are more likely to a buy a new brand of shampoo when they watch the advertisement in the evening than when they watch it in the morning or afternoon.[25] However, I must add 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.[26–31] Two alternative explanations for the disagreement in findings 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.

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, while performance in other tasks is better later in the day.[32] 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 well-controlled study in which subjects were kept in bed rest (but awake) for 60 consecutive hours. Body temperature and several cognitive variables were recorded continuously.[33] Figure 14.8 shows some of the results. In the bottom panel, 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.

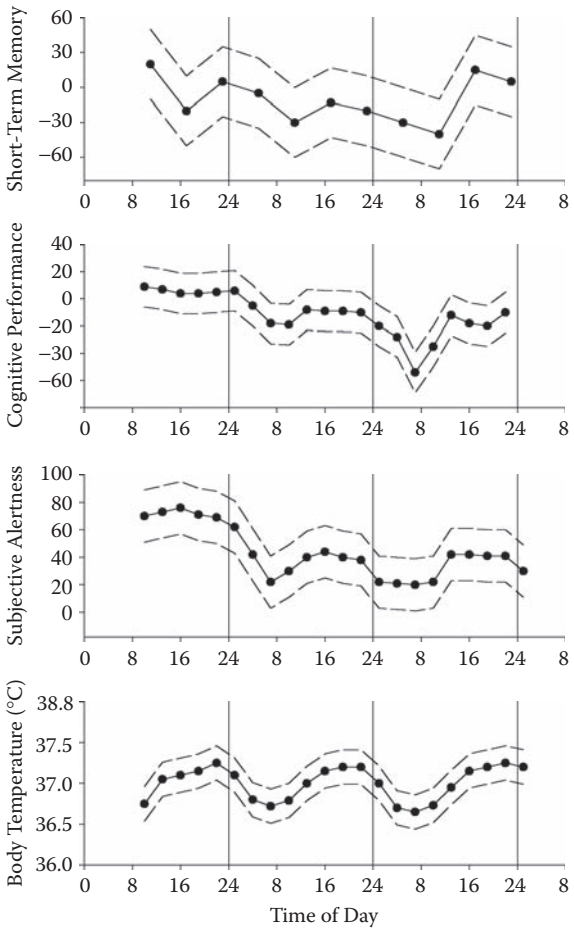


FIGURE 14.8 Daily variation in psychological functions. The graphs show the daily variation in three psychological functions (as well as body temperature) of human subjects studied under a constant routine protocol (that is, in continuous bed rest, receiving equally spaced meals, and not allowed to sleep) for 60 consecutive hours. The data points correspond to the means (± SE) of five subjects. Note that the rhythm of body temperature is very robust, but the rhythms of the psychological functions (especially short-term memory) are rather sloppy. (Source: Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M. & 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.)

The temporary elevation in values 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 clear, but rhythmicity in cognitive performance and subjective alertness is quite robust. Note that the cognitive rhythms peak in the early evening, along with body temperature. Because the subjects were not allowed to sleep during the experiment, it is clear that the circadian variation in cognitive performance and subjective alertness is not contingent on the increase of sleepiness during wake hours (as discussed previously in Chapter 10). In a more recent

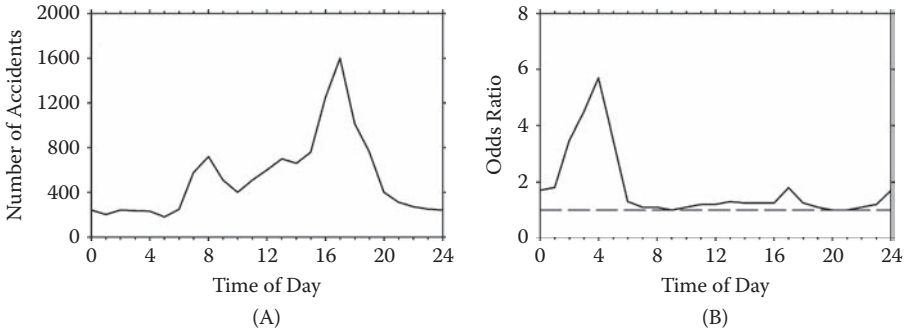


FIGURE 14.9 Daily variation in highway traffic accidents. The left graph (A) shows the hourly distribution of all highway traffic accidents involving personal injury or death in Sweden from 1987 to 1991. More accidents clearly occur around 8 A.M. and 5 P.M. Traffic also is heavier at these times of day, so the graph on the right (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. (Source: Åkerstedt, T., Kecklund, G. & Hörte, L. G. (2001). Night driving, season, and the risk of highway accidents. *Sleep* 24: 401–406.)

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.[34]

It seems appropriate to conclude that the studies that found the rhythms of cognitive performance to peak at times other than those associated with the peak of the body temperature rhythm[26–31] 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 differ from phasing observed in the laboratory. For example, 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.[35] This finding 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 an interesting analysis of data on highway traffic accidents involving personal injury or death.[36] The left panel in Figure 14.9 illustrates the well-known fact that more traffic accidents occur during rush hour (8 A.M. and 5 P.M.) than at other times of the day. However, because rush hour is the time of day when the largest number of vehicles are 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 calculated easily as the quotient of the number of accidents and the number of vehicles on the road at each time of day. As shown in the right panel, the odds ratio peaks at 4 A.M. 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 must drive late at night, you should feel lucky that few other people are driving at that time. However, you are likely to get into an accident at that time even if you are the only driver on the road!

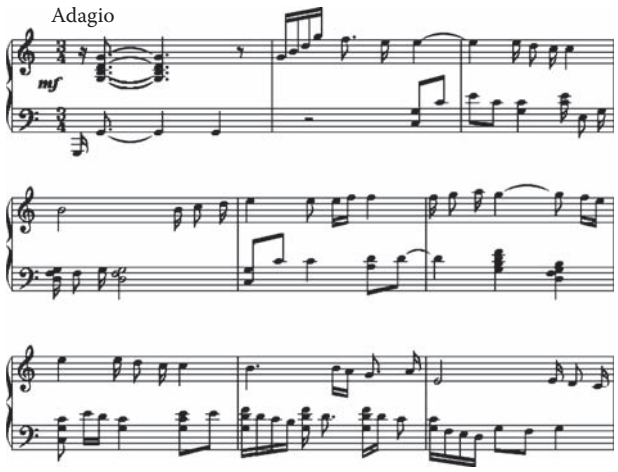


FIGURE 14.10 A musical score for the piano. Music playing requires accurate temporal discrimination, detailed memory, precise coordination of muscle movements, and many other abilities. Some or all of these abilities may vary during the day. (Source: Excerpt from *Anita* by R. Refinetti. © 1983–2001 R. Refinetti.)

As was the case for physical activities, it is possible that performance in most intellectual activities — such as playing the piano (Figure 14.10) — is affected by so many variables that the effect of circadian variation is negligible. This hypothesis does not seem to apply to traffic accidents, but no thorough study has been conducted to prove or disprove 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 — not because cognitive processes are at their circadian low point. The case of tiredness in the workplace mentioned earlier also suggested that the circadian signal may be overridden by other signals. More research clearly is needed on this issue. A pianist should perform better in the early evening; but talent, practice, fatigue, mood, behavior of the audience, and numerous other variables contribute to the quality of the performance to such an extent that one cannot say confidently that circadian variation in cognitive processes plays a significant role in it. The importance of the circadian system in the determination of intellectual performance in real-world activities has yet to be demonstrated adequately.

14.1.3 The Morningness–Eveningness Typology

Physical and intellectual capacities are higher in the evening for an "average" human. However, many human beings are not *average*. To advise people about "when to work and when to play,"[37] circadian physiologists need to consider individual differences. Chapters 7 and 8 discussed the mechanisms that determine the phase relationship between the rhythms of an organism and the rhythms of environmental factors. The environmental alternation of light and darkness (the light–dark cycle) usually is the most important *zeitgeber* for circadian rhythms, and the timing of circadian rhythms is determined primarily by the mechanism of *entrainment* — which is predicated on the free-running period of the organism, the period of the zeitgeber, and the properties of the *phase-response curve* (PRC). 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 PRC. Figure 14.11 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 to 60 minutes before lights-on each day, but some start only 20 minutes before lights-on, while others start as early as 80 minutes before lights-on. Thus, some grass rats are "late risers" and some are "early risers."

As mentioned in Chapter 2, a *morningness–eveningness inventory* for humans was developed by Horne and Östberg in 1976,[38] and similar inventories have been developed since then.[39–42] 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

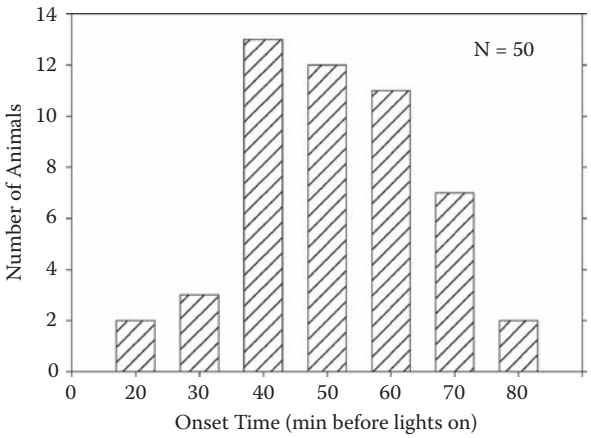


FIGURE 14.11 Inter-individual variability in the daily start of activity. The graph shows the distribution of activity onset times of 50 Nile grass rats (*Arvicanthis niloticus*) housed individually with running wheels under a light–dark cycle (LD 12:12). Most animals start running on the wheels 40 to 60 minutes before the lights come on each day. However, a full hour separates early risers from late risers. (Source: 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.)

the understanding of fundamental principles of circadian physiology, but it has a great appeal to people who must live in a world structured around the 24-hour day (Figure 14.12). Many visitors to my web site (www.circadian.org) write to me lamenting that as extreme evening types they are frequently chastised for being "lazy" or "disorganized." I sympathize with their sort. Not being "normal" certainly can 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 explain these two arguments in detail in the following paragraphs.

Figure 14.13 shows the distribution of morningness–eveningness scores for 2135 university students from Italy and Spain. The distribution is unremarkable for distributions of biological and psychological functions. It is bell-shaped with a mean of 48 and a standard deviation of 10.[43] Lower scores indicate eveningness (*evening chronotype*), while higher scores indicate morningness (*morning chronotype*). Of course, the cut-off points for the typology are arbitrary. Horne and Östberg[38] 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,[44] Brazil,[45,46] Canada,[47] Finland,[48] France,[49,50] Germany,[51] Italy,[31,43,52,53] Japan,[54,55] Korea,[54]

FIGURE 14.12 Sunrise at the beach. Some people do not like to wake up early; some do. (Source: © ArtToday, Tucson, AZ.)

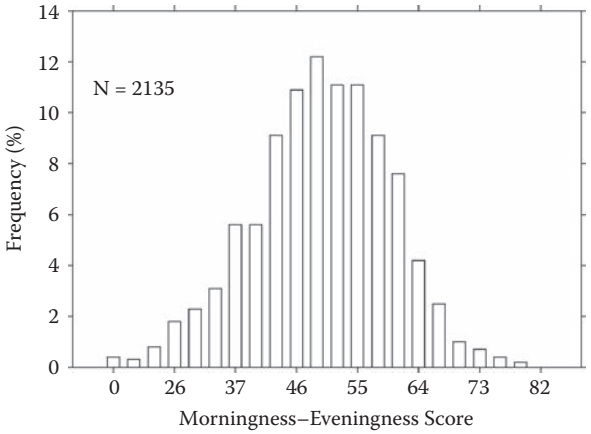


FIGURE 14.13 Morning types, evening types, and everyone in between. The graph shows the distribution of scores in the Morningness–Eveningness Questionnaire answered by 2135 university students from Italy and Spain. The distribution is bell-shaped with a mean of 48.2. Lower scores characterize evening types; higher scores characterize morning types. (Source: Adan, A. & Natale, V. (2002). Gender differences in morningness–eveningness preference. *Chronobiology International* 19: 709–720.)

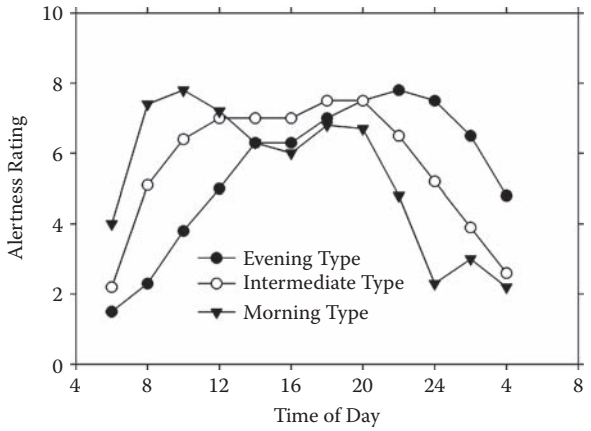


FIGURE 14.14 Morningness–eveningness typology and alertness. The graph shows the daily variation in alertness of morning types, intermediate types, and evening types. Subjective self-evaluation of alertness was conducted by 1800 university students from six countries. Mean values for each of the three types are shown. Note 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. (Source: Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M. & Tisak, J. (2002). Investigation of morning–evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.)

Spain,[43] and the United States.[21,56–59] 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).[60] The daily rhythms of body temperature,[38,57] heart rate,[49,61] and melatonin secretion[47,51,59] consistently peak earlier in the day in morning types than in evening types. Morning types are more alert at wake-up time,[31,40] are better at recognition of sentences presented in the morning than in the afternoon,[52] and are less stressed out by morning commute driving than are evening types.[44] A follow-up study in Croatia revealed a stable distribution of scores for two generations of university students tested 21 years apart.[39]

Figure 14.14 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 rhythm peaks much earlier in the day, while in evening types, the rhythm peaks much later in the day.[40]

Although some researchers have reported that women tend to be slightly more morning-oriented than men,[41,43] others have found no sexual difference.[62,63] Studies have been consistent, however, in finding young children to be more morning-oriented than young adults[55,62,63] and young adults to be more evening-oriented than older

adults.[21,50,56,64] Thus, morningness seems to decrease (by about 20%) from childhood to adult years and to return to the childhood level in old age.

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.[58] This correlation 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). One study found an association between the morningness–eveningness typology and a polymorphism in the human *clock* gene.[65] However, a follow-up study by a different research team found no such association.[64]

My argument that "the norms about official work times are most likely wrong" 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 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."[41] 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.

My argument that "being abnormal may many times be a good thing" is based on two principles. The first principle is that abnormality by itself does not have a negative connotation. Bill Gates, cofounder and chairman of Microsoft Corporation, is the richest man in the world.[66] He is, unquestionably, *abnormally rich*. Although some people may think of his wealth in a negative way, I am sure that most people would be very happy to be 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.[67] So, it is possible that morning types are slightly more diligent and goal-oriented than evening types. If true, the difference might be because 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* simply means 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 A.M. Such a person may often arrive late for work and be unproductive for most of the work day. 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 A.M. In 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. The issue of normality clearly 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.

14.2 SPACE EXPLORATION

Cosmonaut Yuri Gagarin's orbital flight in 1961 and astronaut Neil Armstrong's landing on the moon in 1969 were rightfully heralded as proof of the possibility of space exploration. Later in the 20th century, the Russian space station and the American space shuttle programs added support to the idea.[68] The International Space Station, whose on-orbit assembly started in 1998 and is ongoing (Figure 14.15), promises to spearhead space-science research in the 21st century. The largest scientific and technological endeavor ever undertaken by humankind, the station is being 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. The program currently involves more than 100,000 people at space agencies and contractor companies around the world. The station will provide a permanent laboratory for research and development in multiple fields, from materials science to human physiology.

The Web site of the National Aeronautics and Space Administration (www.nasa.gov) proudly states that the goals of its Human Exploration and Development of Space enterprise are to "increase human knowledge of nature's processes using the space environment, explore the solar system, achieve routine space travel, and enrich life on Earth through people living and working in space." Colonization of other planets is a more remote but not unlikely possibility (Figure 14.16). In addition to complex new developments in spacecraft design and operation — such as new propulsion systems[69] and on-board computers capable of assuming complete control of the

FIGURE 14.15 The International Space Station. The International Space Station, still under construction in Earth's orbit, is the largest scientific and technological endeavor ever undertaken by humankind. (Source: Image courtesy of NASA.)

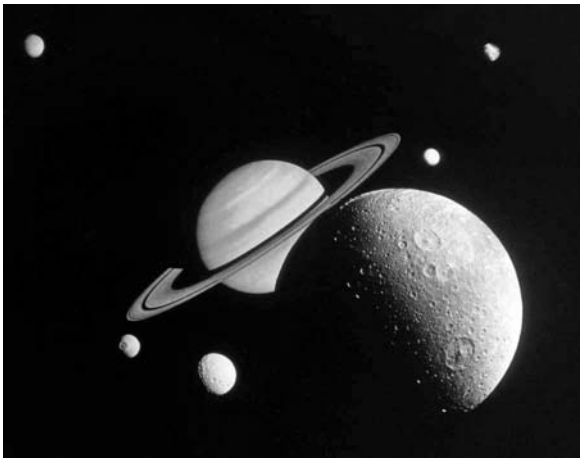


FIGURE 14.16 The solar system. Seven of the nine planets of the solar system are seen in this artistic representation that shortens the interplanetary distances by several orders of magnitude. The largest planet is Jupiter. (Source: © ArtToday, Tucson, AZ.)

spacecraft[70] — what type of challenges can be expected? How will humans adapt to life away from Earth?

The bulk of research on human physiology in space has focused on the effects of reduced gravity. It is well established that microgravity leads to bone loss,[71] which is a major problem, but little is known about the effects of microgravity on circadian rhythms. Although reductions in sleep time and changes in sleep pattern (as compared with preflight baseline measurements) have been observed in orbiting astronauts,[72] fundamental parameters of circadian rhythmicity have not been properly investigated.[73] 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.[74] Rhythms of heart rate, activity, and axillary temperature of monkeys aboard Cosmos missions exhibited changes in amplitude and phase,[75] 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 one study aboard Mir, only a 2-hour delay (and no change in other parameters) was noticed in the body temperature rhythm,[76] and the delay 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.[77] Neither study (which lasted 30 and 17 days, respectively) found that the rhythms freeran. 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. The illumination was less bright than that experienced during the baseline period on Earth, but it was evidently sufficient to maintain entrainment. In another study of astronauts aboard space shuttle missions, the robustness of the body temperature rhythm was reduced during the flight,[78] but the awkward sleep schedule that was forced on the astronauts most likely masked the body temperature rhythm. To coordinate the times of launching and landing, NASA must advance the time of landing by 4 to 5 hours in relation to launching. Rather than conduct the phase shift on the day of landing, NASA spreads it out over the duration of the mission, so that the astronauts

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	1,410	88
Venus	108	12.1	5,830	225
Earth	150	12.8	24	365
Mars	228	6.8	24	687
Jupiter	778	142.8	10	4,380
Saturn	1,427	120.7	11	10,750
Uranus	2,870	51.8	17	30,660
Neptune	4,497	49.5	16	59,400
Pluto	5,900	2.3	144	90,520

Source: TIME Almanac 2004. (2003). Des Moines, IA: TIME Books.

are placed on a sleep schedule with 23.7- or 23.4-hour days. I doubt that 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. It is not surprising that the body temperature rhythm is 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 in later months.[79]

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.[80] The artificial living conditions maintained in spacecraft are sufficient to preserve entrainment. However, artificial conditions are not viable in large-scale enterprises. If a full light–dark cycle had to be maintained artificially on an entire planet, the costs would be exorbitant. To colonize a planet whose day is longer or shorter than 24 hours, humans will have to learn how to adapt their circadian systems to non-24-hour days. The human earthly biological clock can be entrained to light–dark cycles that do not differ much from 24 hours. However, even within the solar system of Earth, few planets have day lengths similar to those on Earth (Table 14.1). How can humans adjust to life on a planet that has, for example, a 35-hour day? In some cases, frequency demultiplication (discussed in Chapter 7) might help humans deal with unusual day lengths (say, 48-hour days), but this technique will not help people adjust to life on planets with 15-hour days, 35-hour days — or 5830-hour days, such as on Venus!

A better understanding of entrainment mechanisms might lead to the development of potent and inexpensive zeitgebers capable of entraining people to the unusual day length prevailing in other planets, although the problem of wanting to be awake for much of the long alien night would still exist. As circadian physiologists learn more about the mechanisms of action of circadian genes, they might use this knowledge to biologically engineer a circadian mutant gene for humans that could set the human circadian period to that of the destination planet. The creation of this gene might provide the optimal solution for adapting to life on planets whose day lengths differ from that of Earth by several hours.

To colonize an alien planet, however, humans first must get there. People have already been to the Moon (Figure 14.17), and there are elaborate plans to return there.[81–83] Planets in far-away galaxies present a much greater challenge. Spacecraft speed may be increased, but travel is still 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 be negligible compared with the costs of generating breathable air and providing food, neutral ambient temperature, and health care for all the travelers. However, accurate knowledge of human circadian rhythms and the zeitgebers that affect them will be essential to provide the sojourners with an environment that relieves rather than accentuates the stress of the journey.

In the immediate future, colonization of the Moon is the most realistic goal. Even this short-distance migration, however, requires a monumental enterprise.[84,85] For example, occupational health issues that must be considered include adequate food supply, medical care, mental health promotion, environmental hazard control, occupational injury prevention, preflight training, and crew selection. Environmental health issues include air quality, water, sanitation, and waste management.[85] A closed ecosystem would need to be developed and tested on Earth as a rehearsal for "the real thing." Developing a closed

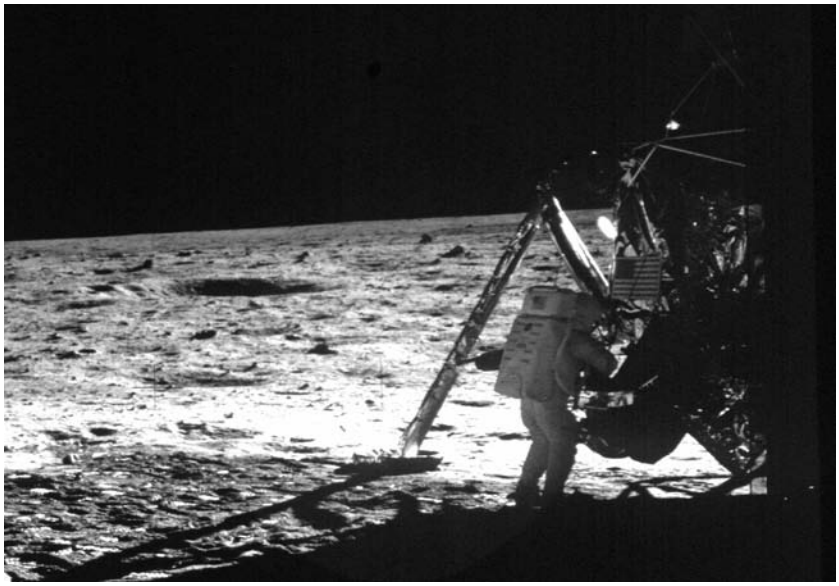


FIGURE 14.17 Man on the Moon. On July 20, 1969, Neil Armstrong became the first man to set foot on the Moon. (Source: Image courtesy of NASA.)

FIGURE 14.18 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. (Source: Biosphere 2 Center, Columbia University. Used under license.)

ecosystem was the main goal of the Biosphere 2 Center (Figure 14.18), which was privately constructed in the late 1980s and entrusted to Columbia University in 1996. The project was not successful, however, and the university ended its involvement in 2003.[86]

Although Russia and the United States were the only space-faring nations until a few decades ago, numerous countries now have organized space programs (Figure 14.19). At the beginning of the U.S. space program in the early 1960s, Pittendrigh pointed out that biologists had great interest in the exploration of Mars.[87] Several unmanned missions to Mars have been conducted in recent years, and many more are planned for the near future[88] (Table 14.2). Manned missions to Mars have been proposed,[89,90] and U.S. President George W. Bush presented his own view on human exploration of Mars in 2004.[83,91,92] The prospect is an exciting one, and circadian physiologists certainly will be involved to provide their expertise to the advancement and ultimate success of the enterprise.

SUMMARY

1. 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 peak time of the body temperature rhythm, which usually occurs 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.
2. Microgravity in space does not seem to have major effects on fundamental properties of circadian rhythms. Circadian physiology, however, can provide significant contributions to the space-exploration enterprise by assisting in the design of work and living schedules that optimize physical and cognitive performance in environments that lack the 24-hour cycle of light and dark prevailing on Earth.

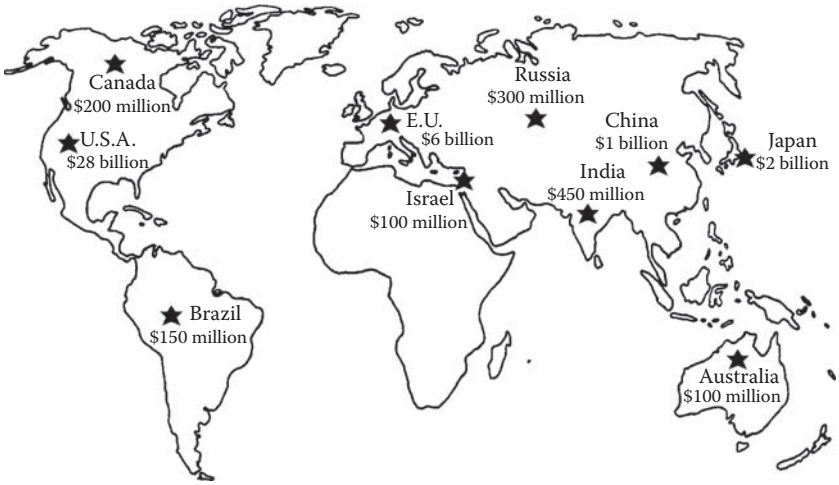


FIGURE 14.19 The space club. Ten nations currently have active space programs. The United States invests in space science and technology more than all other nations combined ($28 billion per year). (Source: Verger, F., Sourbès-Verger, I. & Ghirardi, R. (2003). *The Cambridge Encyclopedia of Space* (Trans. by S. Lyle & P. Reilly). Cambridge, UK: Cambridge University Press.)

TABLE 14.2
Recent and Planned Expeditions to Mars

Year	Mission	Space Agency
1996	Mars Global Surveyor	NASA
1998	Nozomi	Japan
2001	Mars Odyssey	NASA
2003	Mars Exploration Rovers	NASA
2003	Mars Express	European Space Agency
2005	Mars Reconnaisance Orbiter	NASA
2007	Scout 1	NASA
2007	NetLander	France/NASA
2009	Smart Lander	NASA
2011	Scout 2	NASA
2014	Sample Return 1	NASA/Italy/France
2016	Sample Return 2	NASA/Italy/France

Source: Kunzig, R. (2003). Mars express. *Discover* 24(5): 34–43.

EXERCISES

Exercise 14.1 Calculating your best time of day

Section 14.1 discussed how the knowledge of daily rhythmicity in the human body can help people 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.

Exercise 14.2 Daily variation in memory/attention

This exercise examines 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 influence it.

1. Ideally, you should use a hand-held electronic game that requires you to repeat a random sequence of stimuli. Two versions of this toy are shown in Figure 14.20. If you cannot obtain an equivalent game, use the computer program included on the CD-ROM 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 Game.exe in the folder where the other programs are located (\Program Files\Circadian). Game.exe works in a similar way to the hand-held game.
2. You must use the game several times before starting the study. As you become familiar with the game, your skills will improve.
3. When you are ready to start the exercise, use a pencil and a sheet of paper to record the data. You will need 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 is ideal.

FIGURE 14.20 Simon says. This figure shows two commercially available hand-held electronic games that may be used for the memory/attention task in Exercise 14.2.

Occasionally, you may also use an alarm clock to wake yourself 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, recruit a friend to count the number of repetitions; otherwise, you may get distracted by counting and make errors.
5. 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 five consecutive times with the same color followed by five consecutive times with another color). You might discard patterns that are too easy and redo the trial, but you will find it difficult to objectively define "too easy." The best solution is to conduct two or three trials, one after the other, and then average the results.
6. When you finish collecting the data, draw a graphic 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 1.3 and 5.1. If you find no daily rhythm in the raw data, try to average all days into a single day. This average will limit your analysis to a single 24-hour cycle, but it will also eliminate random variations in performance, which may unmask the daily oscillation.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general 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 book is helpful, with the caveats presented in this chapter.

Gardner, H., Kornhaber, M. L. & 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.

Singer, R. N., Hausenblas, H. A. & Janelle, C. (Eds.). (2001). ***Handbook of Sport Psychology*****, 2nd Edition. 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 ten planetary scientists describe their favorite planets in the solar system.

Verger, F., Sourbès-Verger, I. & 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 mid-20th century.

WEB SITES TO EXPLORE

China National Space Administration:
http://www.cnsa.gov.cn/main_e.asp
College Rankings (U.S. News & World Report):
http://www.usnews.com/usnews/edu/college/cohome.htm
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

LITERATURE CITED

1. Birch, K. & Reilly, T. (2002). The diurnal rhythm in isometric muscular performance differs with eumenorrheic menstrual cycle phase. *Chronobiology International* 19: 731–742.
2. Deschenes, M. R., Kraemer, W. J., Bush, J. A., Doughty, T. A., Kim, D., Mullen, K. M. & Ramsey, K. (1998). Biorhythmic influences on functional capacity of human muscle and physiological responses. *Medicine and Science in Sports and Exercise* 30: 1399–1407.

3. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C. & 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.
4. Moussay, S., Dosseville, F., Gauthier, A., Larue, J., Sesboüe, B. & Davenne, D. (2002). Circadian rhythms during cycling exercise and finger-tapping task. *Chronobiology International* 19: 1137–1149.
5. Hill, D. W. & Smith, J. C. (1991). Circadian rhythm in anaerobic power and capacity. *Canadian Journal of Sport Science* 16: 30–32.
6. Waterhouse, J., Edwards, B., Bedford, P., Hughes, A., Robinson, K., Nevill, A., Weinert, D. & Reilly, T. (2004). Thermoregulation during mild exercise at different circadian times. *Chronobiology International* 21: 253–275.
7. Callard, D., Davenne, D., Gauthier, A., Lagarde, D. & Van Hoecke, J. (2000). Circadian rhythms in human muscular efficiency: continuous physical exercise versus continuous rest, a crossover study. *Chronobiology International* 17: 693–704.
8. Recht, L. D., Lew, R. A. & Schwartz, W. J. (1995). Baseball teams beaten by jet lag. *Nature* 377: 583.
9. Steenland, K. & Deddens, J. A. (1997). Effect of travel and rest on performance of professional basketball players. *Sleep* 20: 366–369.
10. Youngstedt, S. D. & O'Connor, P. J. (1999). The influence of air travel on athletic performance. *Sports Medicine* 28: 197–207.
11. Youngstedt, S. D. & Buxton, O. M. (2003). Jet lag and athletic performance. *American Journal of Medicine in Sports* 5: 219–226.
12. O'Connor, P. J., Morgan, W. P., Koltyn, K. F., Raglin, J. S., Turner, J. G. & Kalin, N. H. (1991). Air travel across four time zones in college swimmers. *Journal of Applied Physiology* 70: 756–763.
13. Refinetti, R. & 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.
14. Racinais, S., Hue, O., Hertogh, C., Damiani, M. & 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.
15. Van Dongen, H. P. A., Kerkhof, G. A. & Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
16. Wright, K. P., Hull, J. T. & Czeisler, C. A. (2002). Relationship between alertness, performance, and body temperature in humans. *American Journal of Physiology* 283: R1370–R1377.
17. Hull, J. T., Wright, K. P. & 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.
18. Varkevisser, M. & Kerkhof, G. A. (2003). 24-Hour assessment of performance on a palmtop computer: validating a self-constructed test battery. *Chronobiology International* 20: 109–121.
19. Horowitz, T. S., Cade, B. E., Wolfe, J. M. & 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.
20. Graw, P., Kräuchi, K., Knoblauch, V., Wirz-Justice, A. & Cajochen, C. (2004). Circadian and wake-dependent modulation of fastest and slowest reaction times during psychomotor vigilance task. *Physiology and Behavior* 80: 695–701.
21. May, C. P., Hasher, L. & Stoltzfus, E. R. (1993). Optimal time of day and the magnitude of age differences in memory. *Psychological Science* 4: 326–330.
22. Campbell, S. S., Murphy, P. J. & Boothroyd, C. E. (2001). Long-term time estimation is influenced by circadian phase. *Physiology and Behavior* 72: 589–593.
23. Kuriyama, K., Uchiyama, M., Suziki, H., Tagaya, H., Ozaki, A., Aritake, S., Kamei, Y., Nishikawa, T. & Takahashi, K. (2003). Circadian fluctuation of time perception in healthy human subjects. *Neuroscience Research* 46: 23–31.
24. De Gennaro, L., Ferrara, M., Curcio, G. & 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.
25. Pornpitakpan, C. (2004). The persuasive effect of circadian arousal, endorser expertise, and argument strength in advertising. *Journal of Global Marketing* 17: 141–172.
26. Gates, A. I. (1916). Diurnal variations in memory and association. *University of California Publications in Psychology* 1: 323–344.
27. De Castro, J. M. (1987). Circadian rhythms of the spontaneous meal pattern, macronutrient intake, and mood of humans. *Physiology and Behavior* 40: 437–446.
28. Watson, D., Wiese, D., Vaidya, J. & 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.
29. Kraemer, S., Danker-Hopfe, H., Dorn, H., Schmidt, A., Ehlert, I. & 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.
30. Murray, G., Allen, N. B. & Trinder, J. (2002). Mood and the circadian system: investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
31. Natale, V., Alzani, A. & Cicogna, P. (2003). Cognitive efficiency and circadian typologies: a diurnal study. *Personality and Individual Differences* 35: 1089–1105.

32. Folkard, S. (1990). Circadian performance rhythms: some practical and theoretical implications. *Philosophical Transactions of the Royal Society of London B* 327: 543–553.
33. Johnson, M. P., Duffy, J. F., Dijk, D. J., Ronda, J. M., Dyal, C. M. & 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.
34. Van Eekelen, A. P. J. & Kerkhof, G. A. (2003). No interference of task complexity with circadian rhythmicity in a constant routine protocol. *Ergonomics* 46: 1578–1593.
35. Kahneman, D., Krueger, A. B., Schkade, D. A., Schwarz, N. & Stone, A. A. (2004). A survey method for characterizing daily life experience: the day reconstruction method. *Science* 306: 1776–1780.
36. Åkerstedt, T., Kecklund, G. & Hörte, L. G. (2001). Night driving, season, and the risk of highway accidents. *Sleep* 24: 401–406.
37. Brody, J. E. (1981). Body's many rhythms send messages on when to work and when to play. *New York Times*, 11 August 1981, pp. C1–C2.
38. Horne, J. A. & Östberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology* 4: 97–110.
39. Koscec, A., Radosevic-Vidacek, B. & Kostovic, M. (2001). Morningness-eveningness across two student generations: would two decades make a difference? *Personality and Individual Differences* 31: 627–638.
40. Smith, C. S., Folkard, S., Schmieder, R. A., Parra, L. F., Spelten, E., Almiral, H., Sen, R. N., Sahu, S., Perez, L. M. & Tisak, J. (2002). Investigation of morning-evening orientation in six countries using the preference scale. *Personality and Individual Differences* 32: 949–968.
41. Roenneberg, T., Wirz-Justice, A. & Merrow, M. (2003). Life between clocks: daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
42. Di Milia, L., Smith, P. A. & Folkard, S. (2004). Refining the psychometric properties of the circadian type inventory. *Personality and Individual Differences* 36: 1953–1964.
43. Adan, A. & Natale, V. (2002). Gender differences in morningness-eveningness preference. *Chronobiology International* 19: 709–720.
44. Langford, C. & Glendon, A. I. (2002). Effects of neuroticism, extraversion, circadian type and age on reported driver stress. *Work and Stress* 16: 316–334.
45. Benedito-Silva, A. A., Menna-Barreto, L., Alam, M. F., Rotenberg, L., Moreira, L. F. S., Menezes, A. A. L., Pereira da Silva, H. & Marques, N. (1998). Latitude and social habits as determinats of the distribution of morning and evening types in Brazil. *Biological Rhythm Research* 29: 591–597.
46. Medeiros, A. L. D., Mendes, D. B. F., Lima, P. F. & Araujo, J. F. (2001). The relationships between sleep-wake cycle and academic performance in medical students. *Biological Rhythm Research* 32: 263–270.
47. Laberge, L., Carrier, J., Lespérance, P., Lambert, C., Vitaro, F., Tremblay, R. E. & Montplaisir, J. (2000). Sleep and circadian phase characteristics of adolescent and young adult males in a naturalistic summertime condition. *Chronobiology International* 17: 489–501.
48. Johansson, C., Willeit, M., Smedh, C., Ekholm, J., Paunio, T., Kieseppä, T., Lichtermann, D., Neumeister, A., Nilsson, L. G., Kasper, S., Peltonen, L., Adolfsson, R., Schalling, M. & Partonen, T. (2003). Circadian clock-related polymorphisms in seasonal affective disorder and their relevance to diurnal preference. *Neuropsychopharmacology* 28: 734–739.
49. Taillard, J., Sanchez, P., Lemoine, P. & 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.
50. Taillard, J., Philip, P., Chastang, J. F. & 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.
51. Roemer, H. C., Griefahn, B., Kuenemund, C., Blaszkewicz, M. & Gerngroß, H. (2003). The reliability of melatonin synthesis as an indicator of the individual circadian phase position. *Military Medicine* 168: 674–678.
52. Natale, V. & Lorenzetti, R. (1997). Influences of morningness-eveningness and time of day on narrative comprehension. *Personality and Individual Differences* 23: 685–690.
53. Mecacci, L., Righi, S. & Rocchetti, G. (2004). Cognitive failures and circadian typology. *Personality and Individual Differences* 37: 107–113.
54. Park, Y. M., Matsumoto, K., Seo, Y. J. & 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.
55. Shinkoda, H., Matsumoto, K., Park, Y. M. & Nagashima, H. (2000). Sleep-wake habits of schoolchildren according to grade. *Psychiatry and Clinical Neurosciences* 54: 287–289.
56. May, C. P. & Hasher, L. (1998). Synchrony effects in inhibitory control over thought and action. *Journal of Experimental Psychology: Human Perception and Performance* 24: 363–379.
57. Baehr, E. K., Revelle, W. & 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.
58. Duffy, J. F., Rimmer, D. W. & Czeisler, C. A. (2001). Association of intrinsic circadian period with morningness-eveningness, usual wake time, and circadian phase. *Behavioral Neuroscience* 115: 895–899.
59. Martin, S. K. & 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.

60. Horne, J. A. & Östberg, O. (1977). Individual differences in human circadian rhythms. *Biological Psychology* 5: 179–190.
61. Kerkhof, G. A., Van Dongen, H. P. A. & Bobbert, A. C. (1998). Absence of endogenous circadian rhythmicity in blood pressure? *American Journal of Hypertension* 11: 373–377.
62. Kim, S., Dueker, G. L., Hasher, L. & Goldstein, D. (2002). Children's time of day preference: age, gender and ethnic differences. *Personality and Individual Differences* 33: 1083–1090.
63. Giannotti, F., Cortesi, F., Sebastiani, T. & Ottaviano, S. (2002). Circadian preference, sleep and daytime behaviour in adolescence. *Journal of Sleep Research* 11: 191–199.
64. Robilliard, D. L., Archer, S. N., Arendt, J., Lockley, S. W., Hack, L. M., English, J., Leger, D., Smits, M. G., Williams, A., Skene, D. J. & von Schantz, M. (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.
65. Katzenberg, D., Young, T., Finn, L., Lin, L., King, D. P., Takahashi, J. S. & Mignot, E. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep* 21: 569–576.
66. Grossman, L. (2004). 10 Questions for Bill Gates. *Time* 163(10): 8.
67. Watts, B. L. (1982). Individual differences in circadian activity rhythms and their effects on roommate relationships. *Journal of Personality* 50: 374–384.
68. Verger, F., Sourbès-Verger, I. & Ghirardi, R. (2003). *The Cambridge Encyclopedia of Space* (Trans. by S. Lyle & P. Reilly). Cambridge, UK: Cambridge University Press.
69. Weed, W. S. (2003). Star trek. *Discover* 24(8): 34–41.
70. Normile, D. (1998). NASA craft to take the controls in flight. *Science* 282: 604–605.
71. Lang, T., LebLanc, A., Evans, H., Lu, Y., Genant, H. & 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.
72. Izumi, R., Ishioka, N., Mizuno, K. & Goka, T. (2001). Space environment, electromagnetic fields, and circadian rhythm. *Biomedicine and Pharmacotherapy* 55: 25–31.
73. Cassone, V. M. & 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.
74. Hoban-Higgins, T. M., Alpatov, A. M., Wassmer, G. T., Rietveld, W. J. & 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.
75. Fuller, C. A., Hoban-Higgins, T. M., Klimovitsky, V. Y., Griffin, D. W. & Alpatov, A. M. (1996). Primate circadian rhythms during spaceflight: results from Cosmos 2044 and 2229. *Journal of Applied Physiology* 81: 188–193.
76. Gundel, A., Polyakov, V. V. & Zulley, J. (1997). The alteration of human sleep and circadian rhythms during spaceflight. *Journal of Sleep Research* 6: 1–8.
77. Monk, T. H., Buysse, D. J., Billy, B. D., Kennedy, K. S. & Willrich, L. M. (1998). Sleep and circadian rhythms in four orbiting astronauts. *Journal of Biological Rhythms* 13: 188–201.
78. Dijk, D. J., Neri, D. F., Wyatt, J. K., Ronda, J. M., Riel, E., de Cecco, A. R., Hughes, R. J., Elliott, A. R., Prisk, G. K., West, J. B. & Czeisler, C. A. (2001). Sleep, performance, circadian rhythms, and light-dark cycles during two space shuttle flights. *American Journal of Physiology* 281: R1647–R1664.
79. Monk, T. H., Kennedy, K. S., Rose, L. R. & Linenger, J. M. (2001). Decreased human circadian pacemaker influence after 100 days in space: a case study. *Psychosomatic Medicine* 63: 881–885.
80. Pittendrigh, C. S. (1967). Circadian rhythms, space research and manned space flight. *Life Sciences and Space Research* 5: 122–134.
81. Lawler, A. (2003). The new race to the Moon. *Science* 300: 724–727.
82. Spudis, P. D. (2003). The new moon. *Scientific American* 289(6): 86–93.
83. Kluger, J. (2004). Mission to Mars. *Time* 163(4): 42–50.
84. Bluth, B. J. (1988). Lunar settlements: a socio-economic outlook. *Acta Astronautica* 17: 659–667.
85. Litton, C. E. (1997). Lessons learned studying design issues for lunar and Mars settlements. *Life Support and Biosphere Science* 4: 127–144.
86. Mervis, J. (2003). Bye, bye Biosphere 2. *Science* 302: 2053.
87. 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.
88. Kunzig, R. (2003). Mars Express. *Discover* 24(5): 34–43.
89. Jaffe, S. (2003). Shuttle squeezes science in space program. *Scientist* 17(20): 46–47.
90. Stone, R. (2003). One nuclear leap to Mars? *Science* 301: 906–909.
91. Lawler, A. (2004). Scientists add up gains, losses in Bush's new vision for NASA. *Science* 303: 444–445.
92. Lawler, A. (2004). How much space for science? *Science* 303: 610–612.

15 Jet Lag and Shift Work

CHAPTER OUTLINE

15.1 The Jet-Lag Syndrome
15.2 The Shift-Work Malaise

15.1 THE JET-LAG SYNDROME

15.1.1 Diagnosis

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 example, from New York to London) probably has experienced jet lag. Some of the symptoms often reported are fatigue, irritability, and inability to concentrate during the day; difficulty 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.

Three main elements are involved in transmeridian flights: the actual traveling, the crossing of time zones, and the arrival at a different geographical area. The actual traveling — 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. If you walk across several time zones (which is far more exhausting than sitting in an airplane but allows you to adapt slowly to the successive zones), then you do not experience jet lag. Even better, if you fly from New York in North America to Lima in South America (which is a long trip but involves no crossing of time zones), you do not experience jet lag.

FIGURE 15.1 Tourist attractions. Places such as New York, London, Rio de Janeiro, and Cairo attract visitors from all over the world. (Source: © ArtToday, Tucson, AZ.)

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. Depending on your predisposition, even everyday problems and the minor nuisances of life can be important forms of stress with significant impact on 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 preference 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.

The element of jet lag that causes the most distress, therefore, seems to be the rapid crossing of time zones. The cause of jet lag is travel by *jet* (Figure 15.2) across time zones. In terms of circadian rhythms, what does this mean? 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.

Consider an eastbound trip across eight 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 (plus or minus 1 hour, depending on daylight-saving schedules). If you managed to sleep on the plane, you will be quite awake and ready to do business at a time when everyone is getting ready to go

FIGURE 15.2 Flying overseas. As the best-selling jetliner in the world, the Boeing 737 transports millions of travelers on trans-meridian flights. (Source: Boeing™ & ©. Used under license.)

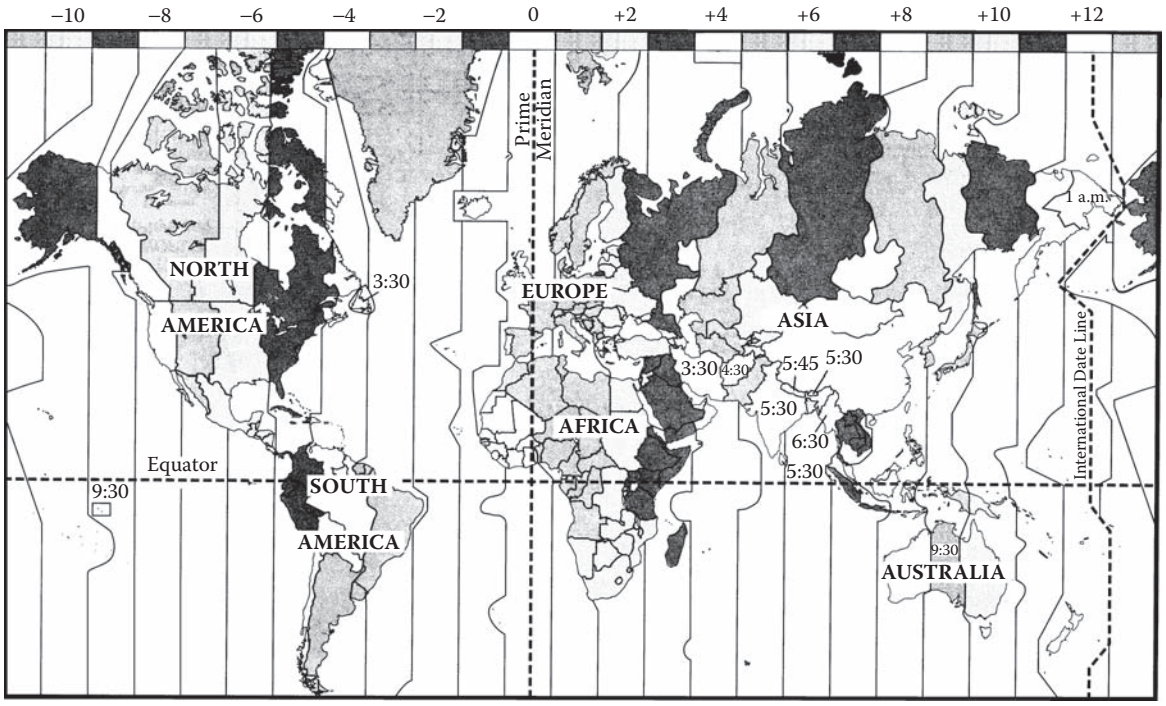


FIGURE 15.3 Time zones. By convention, the world is divided into 24 time zones, starting at the Greenwich meridian (England). When it is noon in London, it is still 4 A.M. in California (GMT –8) and it is already 9 P.M. in Japan (GMT +9). (Note: GMT = Greenwich Mean Time.) (Source: Adapted from *TIME Almanac 2006* (2005). Des Moines, IA: TIME Books.)

home. Worse, when the Moscow clock shows 12 midnight (time to go to sleep), your clock says it is only 4 P.M. You obviously do not feel sleepy. By the time you are tired (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! Note 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. You also will adapt, 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 ability to adapt to a new time zone explains 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).

In Figure 15.4, note that a flight from New York to Santiago (in Chile) is just as long as the flight to Moscow, but it involves a change of only one 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

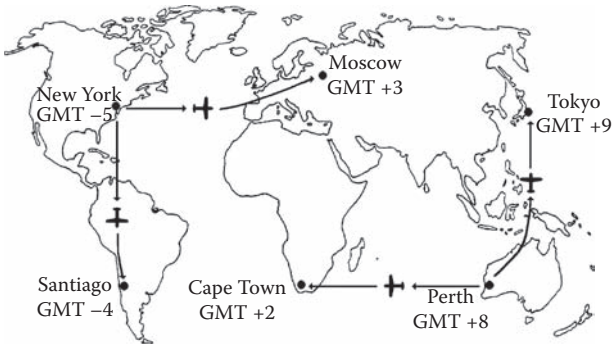


FIGURE 15.4 Traveling east–westward or north–southward. Although a flight from New York to Moscow (Russia) is about as long as a flight to Santiago (Chile), the trip to Moscow requires the crossing of eight time zones, while the trip to Santiago requires the crossing of only one time zone. Similarly, a trip from Perth (Australia) to Cape Town (South Africa) requires the crossing of six time zones, while a trip to Tokyo (Japan) requires the crossing of only one time zone.

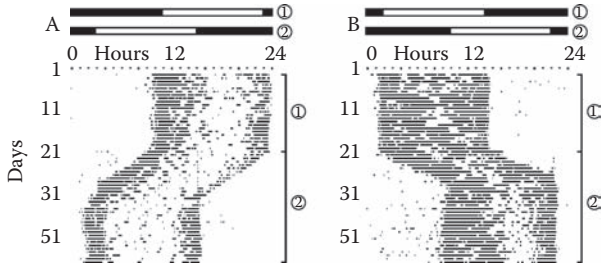


FIGURE 15.5 Simulating jet lag. The actograms show the rhythms of running-wheel activity of two Nile grass rats (*Arvicanthis niloticus*) subjected to phase shifts of the light–dark cycle as simulations of transmeridian flights. As indicated by the dark and white horizontal bars above the actograms, the condition depicted in Panel A is an 8-hour phase advance, similar to that experienced after a flight from New York to Moscow. The condition depicted in Panel B is an 8-hour phase delay, similar to that experienced after a flight from Tokyo to Rome. Re-entrainment 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). (Source: Archives of the Refinetti lab.)

flight to Tokyo (in Japan), but the flight to Cape Town involves a change of six time zones, while the flight to Tokyo involves a change of only one 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.

The trouble with jet lag is that you are suddenly expected to be awake during your night and asleep during your day. Because you cannot sleep during the day (local night), you are tired, irritable, and unable to concentrate at night (local day). This reasoning 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, so that the day starts earlier as one moves eastward), 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 the left panel was subjected to an 8-hour phase advance of the zeitgeber, which corresponds to the flight from New York to Moscow. The animal whose records are shown on the right panel was subjected to an 8-hour phase delay of the zeitgeber, which corresponds to a flight from Tokyo (Japan) to Rome (Italy). Note that re-entrainment takes several days in both cases but that the phase delay (right panel) is accomplished in 10 days, while the phase advance (left panel) requires 16 days. The principles discussed in Chapter 7 can help you undersand that the rate of re-entrainment depends on the circadian period of the organism, on the amplitude of the phase-response curve (PRC), and on various properties of the zeitgeber (such as the proportion of light and darkness and the magnitude of the shift).

Simulations of jet lag have been conducted in numerous species in numerous laboratories.[6–16] Although cases of instantaneous re-entrainment have been observed,[11,12] 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, re-entrainment was found to require approximately 1 day for each hour of the shift in the zeitgeber. For example, 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 re-entrainment was attained in 6 days (that is, 1 day for each hour of the full shift).[10] In a similar study in a different laboratory, it took rats 6 to 7 days to re-entrain after an 8-hour advance in the light–dark cycle.[9]

Studies in which many physiological variables were simultaneously recorded indicated that different variables may re-entrain at different rates. For example, in rats subjected to a 12-hour shift of the light–dark cycle, the body temperature rhythm re-entrained in 9 days, while the rhythms of drinking and feeding re-entrained in 7 days.[17] In rhesus monkeys subjected to a 6-hour phase advance, the activity rhythm re-entrained in 4 days, while the body temperature rhythm re-entrained in 7 days.[18] In squirrel monkeys subjected to an 8-hour phase delay, the rhythms of drinking, feeding, activity, urination, and body temperature all re-entrained at different rates.[19] 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.[20] As you remember from Chapter 12, the ventrolateral SCN receives most of the photic afferent input, while the dorsomedial SCN contains most cells that are intrinsically rhythmic. Note 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 to 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.[21] This finding is interesting because, as shown 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.[22–29] The time needed for re-entrainment was found to be a little less than 1 day per time zone — say, about 6 days for an 8-hour shift — and differences existed 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,[30,31] 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 six time zones and eastward flights across eight time zones, but it is not clear that athletic performance was actually impaired.[32] Whether researchers study athletes or sedentary subjects, subjective ratings of jet lag are very closely related to subjective ratings of fatigue,[33] 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.

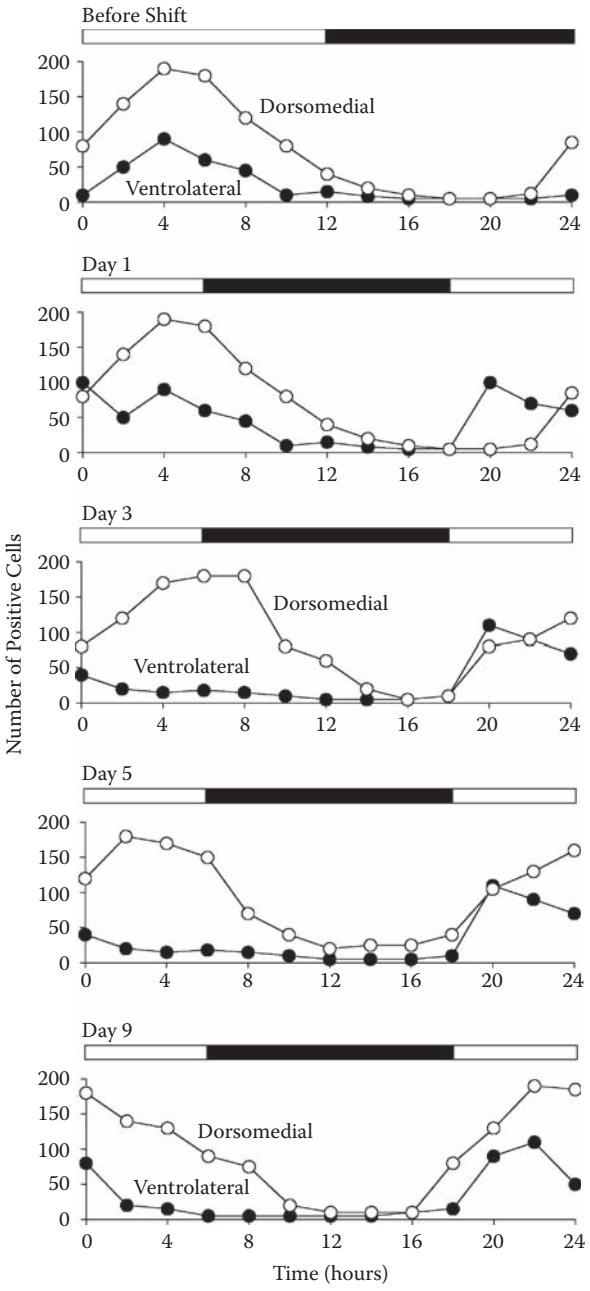


FIGURE 15.6 Looking for the molecular substrate of jet lag. The graphs show the daily variation in the expression of the *per1* gene in the suprachiasmatic nucleus (SCN) of rats (*Rattus norvegicus*) subjected to a 6-hour advance of the light–dark cycle. Note that the *per1*-expression rhythm in the ventrolateral section of the SCN shifts almost immediately after the shift of the light–dark cycle, while 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. (Source: Nagano, M. et al. (2003). An abrupt shift in the day-night cycle causes desynchrony in the mammalian circadian center. *Journal of Neuroscience* 23: 6141–6151.)

15.1.2 Treatment

To avoid or reduce jet lag, consider the information about circadian rhythms presented 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. This type of travel is often impractical, however, so the next possible solution is to conduct business on the west leg of the trip, rather than on the east leg. Many frequent travelers have discovered 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 they will wake up ready for business the following morning. After an eastbound flight, however, travelers must 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).

For example, suppose 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. Since you crossed eight 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 at all. Of course, if it will take you 6 to 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 fact 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 return home without ever experiencing jet lag.

In some cases, 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 is discussed in greater detail later in this chapter). 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. The standard time difference between Chicago and Rio is 3 hours, but in July Chicagoans have their clocks advanced by 1 hour (their summer), while *cariocas* are back on standard time (their winter) — thus, 3 – 1 = 2 hours 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 plan your trip to reduce jet lag (see Exercise 15.2).

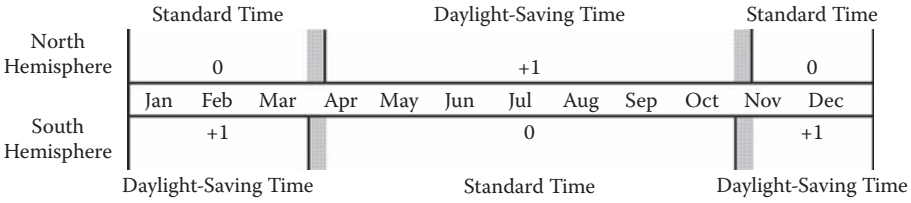


FIGURE 15.7 Hemispheric differences in daylight-saving time. When traveling between two countries that are on different time zones, but that are located on opposite sides of the Equator and adopt daylight-saving time, one rarely encounters the standard difference in time zones. Because the 1-hour delay 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.

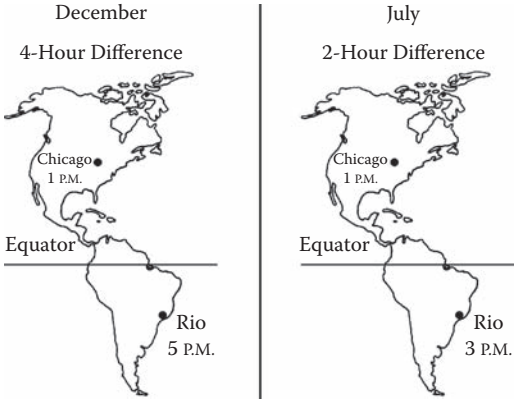


FIGURE 15.8 Two-, three-, or four-hour difference? The standard time difference between Chicago and Rio is 3 hours. However, during most of the year, daylight-saving time causes the difference to be either 4 hours (northern winter) or 2 hours (northern summer).

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 a faster shift, 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 tested thoroughly in real-life jet-lag situations, but many studies have produced encouraging results. Before these studies are discussed, however, I will review the rationale, using Figure 15.9 as a guide. In Panel A, 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 four 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. Chapter 7 showed that the human circadian system can be phase shifted by discrete pulses of light presented at the appropriate circadian time.[34–49] Panel B provides 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. Assume that sunlight at the origin lasted from 5 A.M. to 7 P.M. ("Previous day"). Sunlight at the destination that is four time zones ahead will last (according to the internal clock) from 1 A.M. to 3 P.M. ("Next day"). To avoid jet lag, the internal clock must be advanced by 4 hours. According to the human PRC for photic stimuli (Panel C), a 4-hour phase advance can be obtained by a light pulse presented at circadian time two (CT 2).Thus, on the travel day (middle diagram in Panel B), the traveler must spend the entire day in darkness except for a few hours

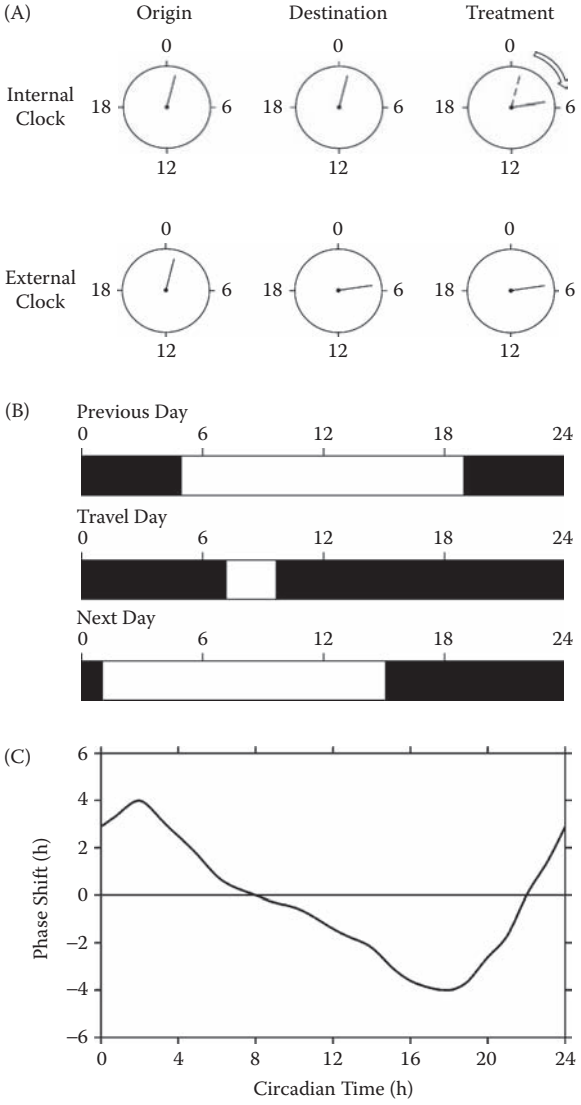


FIGURE 15.9 Treatment of jet lag: theory. The diagrams in Panel A illustrate the fact that, after a flight eastward across four time zones, the internal clock is 4 hours behind the external clock at the destination, and that, therefore, treatment must produce a 4-hour advance of the internal clock. The 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 (PRC) (values averaged from four independently determined PRCs). (Sources: Honma, K. & Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167-168; Dawson, D., Lack, L. & Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102; Jewett, M. E. et al. (1994). Phase-amplitude resetting of the human circadian pacemaker via bright light: a further analysis. *Journal of Biological Rhythms* 9: 295–314; Khalsa, S. B. S., Jewett, M. E., Cajochen, C. & Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.)

— say, 3 hours — starting at (or centered around) CT 2. Because CT 2 is 2 hours after the traveler's wake 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 move around safely but not enough to stimulate the circadian system (which, as shown in Chapter 7, is less sensitive to light than the visual system). Wearing the goggles 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 PRC is relatively modest. A phase shift of 4-hours is about the largest phase shift that can be attained. Still, 4 hours is better than nothing (and would be more than enough for a trip that involves the crossing of only four time zones). Several researchers put this protocol to the test.[42,50–53] 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.[42] 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. Figure 15.10 shows the results from a study in which the subjects traveled from Zurich (Switzerland) to New York. New York is 6 hours behind Zurich, so 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 PRC 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. 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 increased the speed of re-entrainment. Of course, the treatment added only 1 hour to the shift, while 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 re-entrainment of the melatonin rhythm was *not* accompanied by improvement in sleep, performance, or subjective assessment of jet lag symptoms.[51] This study clearly will not cause newspapers to print the headline "Cure for Jet Lag Found!"

A popular remedy for jet lag these days is the hormone melatonin, which — as discussed in Chapter 13 — has

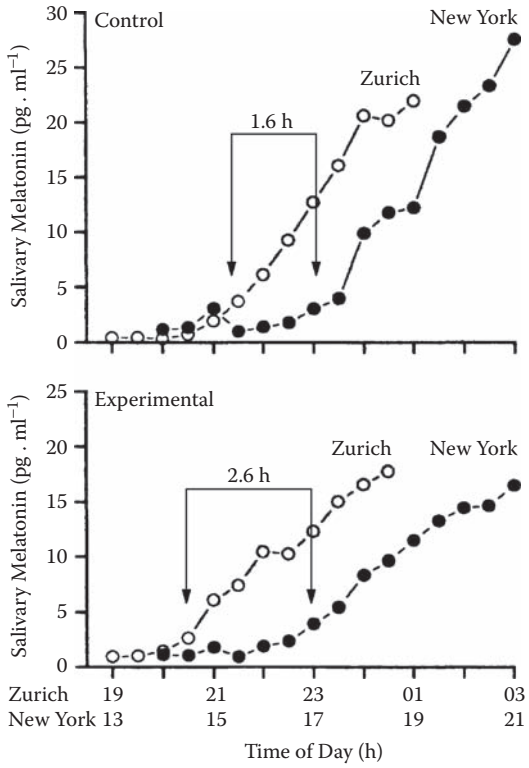


FIGURE 15.10 Treatment of jet lag: real case. The graphs show the results of a study that investigated the use of light visors as a remedy for jet lag after a flight from Zurich (Switzerland) to New York. As indicated in the abscissa, New York time is 6 hours behind Zurich time, which means that the 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 represent the means of eight experimental subjects (who wore a visor emitting white light at 3000 lux for 3 hours starting at CT 18 after arrival in New York) and eight control subjects (who wore a visor emitting dim red light at 10 lux). Note that the subjects of the experimental group exhibited a significantly greater delay than the members of the control group. (Source: Boulos, Z., Macchi, M. M., Stürchler, M. P., Stewart, K. T., Brainard, G. C., Suhner, A., Wallace, G. & Steffen, R. (2002). Light visor treatment for jet lag after westward travel across six time zones. *Aviation, Space, and Environmental Medicine* 73: 953–963.)

the ability to shift the mammalian clock. Because melatonin is not a regulated drug, it can be purchased at natural-food stores and used by anyone for self-medication. *Theoretically*, melatonin can be almost as effective as light pulses,[54–58] 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.[59,60] Articles in the medical literature have claimed both that melatonin can relieve symptoms of jet lag[61] and that it cannot.[62] Other

potential treatments include the use of exercise "pulses" to shift the clock after transmeridian flight[63–65] and the administration of drugs known to have phase-shifting properties,[66–68] although additional studies are needed to verify their safety and effectiveness. A recent animal study showed that inhibition of cortisol synthesis can accelerate re-entrainment after a 6-hour shift of the light–dark cycle.[69] 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 re-entrainment times and, consequently, to faster recovery from jet lag.

When inability to sleep at night is the major complaint, sleeping pills (benzodiazepines) can be taken as treatment for jet lag.[70] As a matter of fact, melatonin has soporific effects,[71] 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.[72] Although chemically induced sleep takes care of only 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. 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 so 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 caused the problem!

15.2 THE SHIFT-WORK MALAISE

15.2.1 Diagnosis

Shift work refers to work performed in shifts (i.e., changes in configuration), although the term is used most often in connection with *night* shifts or *rotating* shifts. Truck drivers (Figure 15.11) and emergency medical personnel (Figure 15.12) are two examples of workers who often must labor in nonstandard shifts. Shift work often produces a malaise characterized by both sleepiness and inability to concentrate during work hours and by difficulty sleeping at the scheduled sleep time.[73] Complaints of gastrointestinal disturbances are also common.[74] Considering that two out of five American workers are employed at jobs with nonstandard shifts,[75] the malaise associated with shift work has the potential to be a major public health issue. The impairment in performance during nocturnal work hours also can create additional health problems. For example, 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.[76]

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. (Source: Photograph 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. (Source: Photograph by R. Refinetti.)

The previous section showed 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. Shift workers face the same problem. A major difference exists between the two situations, however, as diagrammed in Figure 15.13. 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 the 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

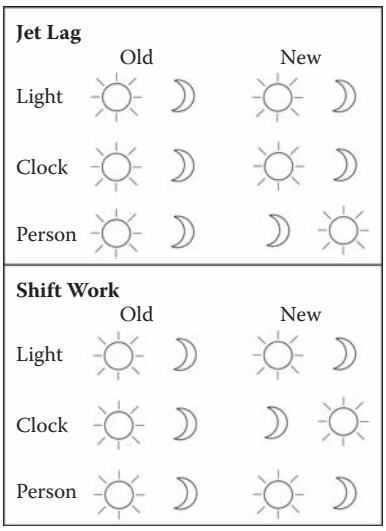


FIGURE 15.13 Comparing jet lag and shift work. The diagram illustrates the fact that although both jet lag and shift work involve a mismatch between the timing of a person's internal clock and the timing of the external clock, shift work is complicated by the mismatch between sunlight and the external clock. See text for details.

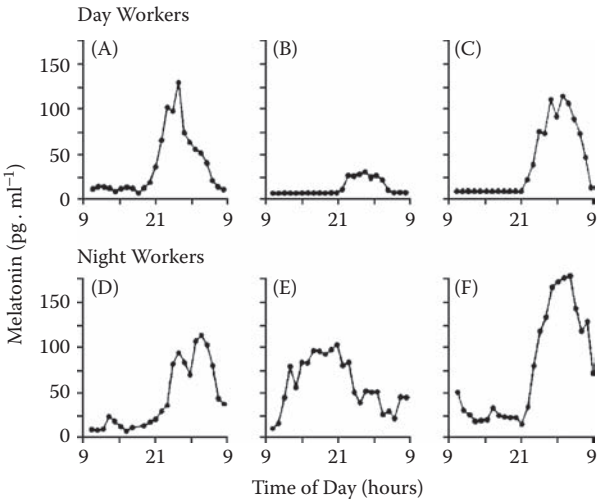


FIGURE 15.14 Shift workers do not shift. The graphs show the daily rhythm of melatonin secretion (as determined by serum melatonin concentration) of three day workers (A–C) and three night workers (D–F). Day workers were active from 09:00 to 18:00 daily, while night workers worked five to six night shifts per week (19:00 to 07:00) for at least a year. Note 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 six day workers and nine night workers, subject E was the only worker not to exhibit a peak around 04:00. (Source: Roden, M., Koller, M., Pirich, K., Vierhapper, H. & Waldhauser, F. (1993). The circadian melatonin and cortisol secretion pattern in permanent night shift workers. *American Journal of Physiology* 265: R261–R267.)

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 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 days off from the night shift so that, for example, 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. Are shift workers doomed to lead a miserable existence? The next paragraph shows what research in this field has revealed.

Many businesses schedule three basic work shifts, which vary with location and number of hours in the shifts: 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.[77] 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.[78] He was dissatisfied with the use of mercury thermometers in long-term studies, so he developed 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 discovered 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 do not adapt properly to the reversed work schedule.[79–83] Figure 15.14 shows data from one of these studies. Serum melatonin concentration was used as a marker of the phase of the circadian pacemaker and 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.[79] Subject E exhibits an earlier peak, but it is not early enough to characterize entrainment to the nocturnal work schedule. The study involved six day workers and nine night workers,

and subject E was the only worker of the 15 subjects who did not exhibit a peak in the middle of the night.

Alain Reinberg's team, in France, examined 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).[84] They also observed that rhythms with smaller amplitude are more easily shifted, as assessed on the first night of shift work.[85] Consequently, people who are less tolerant to shift work synchronize to the new schedule more rapidly.[86] This finding 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. 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 is not that one has to work during the night but that one has to make the transition from day to night work. In this case, shift work is not different from jet lag after all. However, Reinberg's team did not follow the workers' rhythms long enough to ascertain that they actually synchronized to the night schedule. In 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. It may have resulted, therefore, from individual characteristics developed during the course of shift work.

Stjepan Vidacek's team, in Croatia, conducted a longitudinal study (that is, they examined the body temperature rhythms before individuals started shift work and then compared the rhythms with health-related complaints after the individuals started shift work). This research team found 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[87] (that is, the opposite of Reinberg's finding). Therefore, further studies — especially by different researchers — are necessary to elucidate the phenomenon.

15.2.2 Treatment

The cure for the shift-work malaise is as nebulous as that for jet lag. The best way to avoid the malaise is to avoid shift work altogether. This option is not feasible for many workers. In some cases, the schedule of work hours is so bad that nothing can be done about it. For example, some health-care workers are scheduled for 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.[88] Consider the work schedule of an American submarine crew. Sailors are required to live

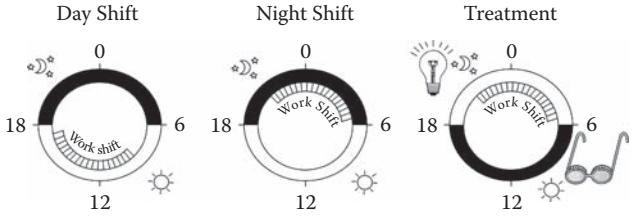


FIGURE 15.15 Treatment of the shift-work malaise. The ideal treatment for the malaise associated with shift work is to simulate a shift in the daily cycle of light and darkness that matches the shift in work schedule. This simulation can be achieved by the use of artificial light during the night and dark goggles during the day. The night shift must be permanent (that is, nonrotating).

under an 18-hour schedule (6 hours on-duty and 12 hours off-duty), which is incompatible with entrainment of human circadian rhythms.[89] Deleterious effects of shift work on workers' long-term health are not well known, but, at least in golden hamsters, rotating shifts can be deadly. Cardiomyopathic hamsters subjected to 12-hour phase shifts of the light–dark cycle on a weekly basis experienced an 11% reduction in life span compared with animals maintained under a constant light–dark cycle.[90] In humans, this percentage would equate to an 8-year reduction in life span (from 76 to 68 years).

For those who must work nonstandard but reasonable shifts, one option is to reduce the number of shift changes and allow the internal clock to adapt to whatever schedule 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).[91] However, recent studies revealed no benefit of clockwise rotation as compared with counterclockwise rotation.[92,93] Theoretically, the best approach to avoid the malaise associated with shift work is to not 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 15.15. 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

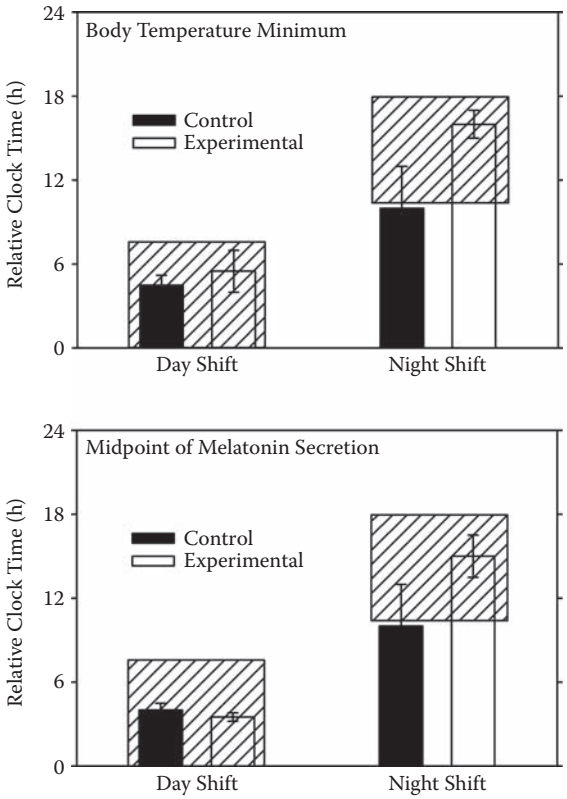


FIGURE 15.16 Testing the treatment. The graphs show the mean times of the trough of the body temperature rhythm (top panel) and the midpoint of the rhythm of melatonin secretion (bottom panel) of workers on day and night shifts under normal conditions (Control) or after treatment with bright light during 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. Note 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. (Source: Boivin, D. B. & James, F. O. (2002). Circadian adaptation to night-shift work by judicious light and darkness exposure. *Journal of Biological Rhythms* 17: 556–567.)

cycle will allow re-entrainment of the circadian clock after a week or so.

In studies with shift workers, treatment with light exposure at night and use of dark goggles during the day produced encouraging results.[94–96] An example is shown in Figure 15.16. 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, while 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. Note 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.[95] In principle, control subjects should not have exhibited any shift, but the experimental subjects clearly exhibited much greater shifts, thus indicating that the treatment was effective. Some researchers used light pulses at night without control of photic exposure during the day,[97–100] and the results were not encouraging. Other researchers used physical exercise,[101,102] melatonin,[103,104] or mere enforcement of diurnal sleep time,[105–107] without great success.

You may wonder why shift work is used at all if it causes such negative effects on an individual's well-being. 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, to be profitable.[91] Thus, as long as the night worker's performance is not poor enough to compromise the quality of the product, the reduction in productivity is not a major issue. 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.[75]

15.2.3 Daylight-Saving Time

The regimen of daylight-saving time ("summer time") resembles the condition of shift work albeit on a very small scale. The regimen consists of advancing the clocks by 1 hour in the spring and then turning them back 1 hour in the fall (Figure 15.17). 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.[108] The rationale for daylight-saving time was mainly the conservation of fuel in a time of war (World War I). As shown in Chapter 4, there are many more hours of sunlight in a day in the summer than in the winter. Because more 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.

In terms of entrainment of circadian rhythms, daylight-saving time has a small effect that is inconsequential

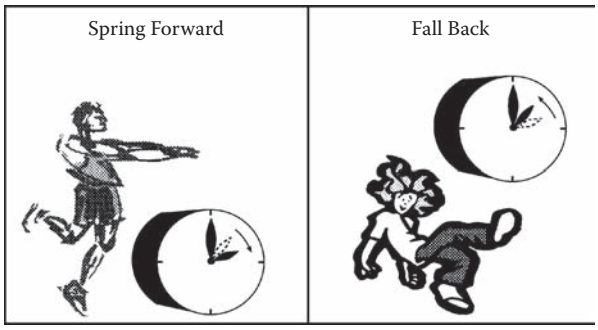


FIGURE 15.17 Daylight-saving time. In regions that observe daylight-saving time, the clocks are advanced by 1 hour in the spring and delayed by 1 hour in the fall.

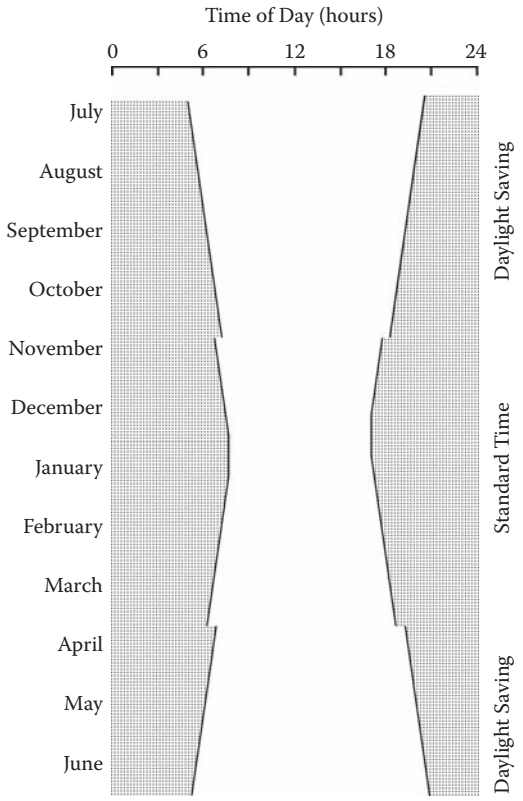


FIGURE 15.18 Photoperiod and daylight-saving time. The graph shows the annual variation in photoperiod at a latitude slightly north of the tropic of Cancer with the adjustment for daylight-saving time. Because of the large seasonal effect, the 1-hour change of daylight-saving time is barely noticeable.

for most people. Consider Figure 15.18. The figure shows the annual variation in day length. The magnitude of the variation depends on 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 with 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 are, however, substantial. Thus, another act of Congress in 1966 instituted daylight-saving time as a permanent fixture in our lives.[108]

Although daylight-saving time is not a major nuisance for the human circadian system, some people do not like it for other reasons. 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. One complaint about daylight-saving time from early risers 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 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 which individuals you ask. Seventy countries in the world observe daylight-saving time in some form.[109]

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 sleeping at night; and gastrointestinal discomfort. If untreated, the condition undergoes spontaneous remission in 1 to 10 days, depending on the number of time zones crossed and the direction of travel. Jet lag sometimes can 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.

EXERCISES

Exercise 15.1: Dealing with shift work and jet lag

This chapter 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 contains two sections that deal with this issue.

1. Double-click on the Circadian icon to open the program banner, then click on Health (the third icon from the right).
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 the program Health that is pertinent to this chapter is the fourth option from the top ("I traveled recently and can't get over my jet lag"). This section is very brief and is intended mainly to remind you that jet lag is, by nature, a transient illness. Exercise 15.2 deals with the prevention of jet lag.

Exercise 15.2 Planning a transcontinental trip

This exercise is meant for entertainment as well as 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. Double-click on the Circadian icon to open the program banner, then click on Jet-lag (the fourth icon from the right).
2. Click on the button under Origin, then click on Florida in the map (Florida should appear in bright blue on most monitors).
3. Click on the button under Destination, then click on Western Europe in the map (Western Europe should appear in dark blue on most monitors).
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 off by an hour. Click on the button under Travel.
5. A message is displayed indicating that you will cross six 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 schedule will help you sleep on your first night in Europe and not be sleepy the following day. Essentially, you will behave as if the external clock had not been phase-advanced by 6 hours.
6. Now, change the origin of the trip. Click on the button under Origin, then click on California (purple), and then click on the button under Travel.
7. A message is displayed that indicates you will cross nine time zones to travel from California to Western Europe. This change in time zones will require a large shift of your internal clock. Although you could try the same approach as in the previous trip (item 5 above), most airlines do not offer flights at times that help you attain synchronization. Flights are scheduled so that 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 able to sleep reasonably well even though your internal clock says that it is not time to sleep.
8. Repeat the procedure with other origins and destinations of your choice. If your travel requires you to cross the Equator, a message will be displayed to remind you that daylight-saving time, if applicable, is 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 contact 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

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Ehret, C. F. & Scanlon, L W. (1993). *Overcoming Jet Lag* (Reissue Edition). New York: Berkley. A "cookbook" for preventing jet lag after transmeridian travel. As is the case for any book aimed at popular audiences, this text 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 with 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.

WEB SITES TO EXPLORE

Circadian Technologies Inc.:
http://www.circadian.com

Jet Lag Help Kit:
http://www.outsidein.co.uk/jetlag.htm

Shiftwork Solutions LLC:
http://www.shift-work.com

Travel Online:
http://www.travel.com

Traveler's Health (U.S. Centers for Disease Control):
http://www.cdc.gov/travel/

LITERATURE CITED

1. Siegel, P. V., Gerathewohl, S. J. & 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. & 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. & 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. & 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. Johnson, R. F. & Randall, W. (1985). Freerunning and entrained circadian rhythms in body temperature in the domestic cat. *Journal of Interdisciplinary Cycle Research* 16: 49–61.
8. Honrado, G. I. & 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.
9. Takamure, M., Murakami, N., Takahashi, K., Huroda, H. & 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.
10. Refinetti, R. & Menaker, M. (1993). Effects of imipramine on circadian rhythms in the golden hamster. *Pharmacology, Biochemistry and Behavior* 45: 27–33.
11. Marumoto, N., Murakami, N., Kuroda, H. & 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.
12. Kilduff, T. S., Vugrinic, C., Lee, S. L., Milbrandt, J. D., Mikkelsen, J. D., O'Hara, B. F. & 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.
13. Schilling, A., Richard, J. P. & 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.
14. Perret, M., Aujard, F., Séguy, M. & 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.
15. Sage, D., Ganem, J., Guillaumond, F., Laforge-Anglade, G., François-Bellan, A. M., Bosler, O. & Becquet, D. (2004). Influence of the corticosterone rhythm on photic entrainment of locomotor activity in rats. *Journal of Biological Rhythms* 19: 144–156.
16. Colwell, C. S., Michel, S., Itri, J., Rodriguez, W., Lelièvre, V., Hu, Z. & Waschek, J. A. (2004). Selective deficits in the circadian light response in mice lacking PACAP. *American Journal of Physiology* 287: R1194–R1201.
17. Zerath, E., Holy, X., Lagarde, D., Fernandes, T., Rousselet, D. & 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.
18. Tapp, W. N. & Natelson, B. H. (1989). Circadian rhythms and patterns of performance before and after simulated jet lag. *American Journal of Physiology* 257: R796–R803.
19. Moore-Ede, M. C., Kass, D. A. & Herd, J. A. (1977). Transient circadian internal desynchronization after light-dark phase shift in monkeys. *American Journal of Physiology* 232: R31–R37.
20. Nagano, M., Adachi, A., Nakahama, K., Nakamura, T., Tamada, M., Meyer-Bernstein, E., Sehgal, A. & Shigeyoshi, Y. (2003). An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *Journal of Neuroscience* 23: 6141–6151.
21. Reddy, A. B., Field, M. D., Maywood, E. S. & 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.
22. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.

23. Elliott, A. L., Mills, J. N., Minors, D. S. & 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.
24. Mills, J. N., Minors, D. S. & Waterhouse, J. M. (1978). Adaptation to abrupt time shifts of the oscillator(s) controlling human circadian rhythms. *Journal of Physiology* 285: 455–470.
25. Wever, R. A. (1980). Phase shifts of human circadian rhythms due to shifts of artificial zeitgebers. *Chronobiologia* 7: 303–327.
26. Monk, T. H., Moline, M. L. & 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.
27. Folkard, S., Minors, D. S. & Waterhouse, J. M. (1991). "Demasking" the temperature rhythm after simulated time zone transitions. *Journal of Biological Rhythms* 6: 81–91.
28. Lowden, A. & 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.
29. Waterhouse, J., Edwards, B., Nevill, A., Carvalho, S., Atkinson, G., Buckley, P., Reilly, T., Godfrey, R. & 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.
30. Youngstedt, S. D. & O'Connor, P. J. (1999). The influence of air travel on athletic performance. *Sports Medicine* 28: 197–207.
31. Youngstedt, S. D. & Buxton, O. M. (2003). Jet lag and athletic performance. *American Journal of Medicine in Sports* 5: 219–226.
32. Lemmer, B., Kern, R. I., Nold, G. & Lohrer, H. (2002). Jet lag in athletes after eastward and westward time-zone transition. *Chronobiology International* 19: 743–764.
33. Waterhouse, J., Nevill, A., Edwards, B., Godfrey, R. & Reilly, T. (2003). The relationship between assessments of jet lag and some of its symptoms. *Chronobiology International* 20: 1061–1073.
34. Czeisler, C. A., Allan, J. S., Strogatz, S. H., Ronda, J. M., Sánchez, R., Ríos, C. D., Freitag, W. O., Richardson, G. S. & Kronauer, R. E. (1986). Bright light resets the human circadian pacemaker independent of the timing of the sleep-wake cycle. *Science* 233: 667–671.
35. Honma, K., Honma, S. & Wada, T. (1987). Phase-dependent shift of free-running human circadian rhythms in response to a single bright light pulse. *Experientia* 43: 1205–1207.
36. Honma, K. & Honma, S. (1988). A human phase response curve for bright light pulses. *Japanese Journal of Psychiatry and Neurology* 42: 167–168.
37. Dijk, D. J., Beersma, G. M., Daan, S. & Lewy, A. J. (1989). Bright morning light advances the human circadian system without affecting NREM sleep homeostasis. *American Journal of Physiology* 256: R106–R111.
38. Frennan, M., Kripke, D. F. & Gillin, J. C. (1989). Bright light can delay human temperature rhythm independent of sleep. *American Journal of Physiology* 257: R136–R141.
39. Shanahan, T. L. & 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.
40. Buresová, M., Dvoráková, M., Zvolsky, P. & Illnerová, H. (1991). Early morning bright light phase advances the human circadian pacemaker within one day. *Neuroscience Letters* 121: 47–50.
41. Dawson, D., Lack, L. & Morris, M. (1993). Phase resetting of the human circadian pacemaker with use of a single pulse of bright light. *Chronobiology International* 10: 94–102.
42. Honma, K., Honma, S., Nakamura, K., Sasaki, M., Endo, T. & Takahashi, T. (1995). Differential effects of bright light and social cues on reentrainment of human circadian rhythms. *American Journal of Physiology* 268: R528–R535.
43. Tzischinsky, O. & Lavie, P. (1997). The effects of evening bright light on next-day sleep propensity. *Journal of Biological Rhythms* 12: 259–265.
44. Shanahan, T. L., Kronauer, R. E., Duffy, J. F., Williams, G. H. & 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.
45. Zeitzer, J. M., Dijk, D. J., Kronauer, R. E., Brown, E. N. & Czeisler, C. A. (2000). Sensitivity of the human circadian pacemaker to nocturnal light: melatonin phase resetting and suppression. *Journal of Physiology* 526: 695–702.
46. Khalsa, S. B. S., Jewett, M. E., Cajochen, C. & Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *Journal of Physiology* 549: 945–952.
47. Lockley, S. W., Brainard, G. C. & 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.
48. Warman, V. L., Dijk, D. J., Warman, G. R., Arendt, J. & Skene, D. J. (2003). Phase advancing human circadian rhythms with short wavelength light. *Neuroscience Letters* 342: 37–40.
49. Burgess, H. J., Fogg, L. F., Young, M. A. & Eastman, C. I. (2004). Bright light therapy for winter depression: is phase advancing beneficial? *Chronobiology International* 21: 759–775.
50. Whitson, P. A., Putcha, L., Chen, Y. M. & Baker, E. (1995). Melatonin and cortisol assessment of circadian shifts in astronauts before flight. *Journal of Pineal Research* 18: 141–147.

51. Boulos, Z., Macchi, M. M., Stürchler, M. P., Stewart, K. T., Brainard, G. C., Suhner, A., Wallace, G. & Steffen, R. (2002). Light visor treatment for jet lag after westward travel across six time zones. *Aviation, Space, and Environmental Medicine* 73: 953–963.
52. Boivin, D. B. & 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.
53. Burgess, H. J., Crowley, S. J., Gazda, C. J., Fogg, L. F. & 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.
54. Lewy, A. J., Ahmed, S., Jackson, J. M. L. & Sack, R. L. (1992). Melatonin shifts human circadian rhythms according to a phase-response curve. *Chronobiology International* 9: 380–392.
55. Lewy, A. J., Ahmed, S. & Sack, R. L. (1996). Phase shifting the human circadian clock using melatonin. *Behavioural Brain Research* 73: 131–134.
56. Matsumoto, M., Sack, R. L., Blood, M. L. & 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.
57. Rajaratnam, S. M. W., Dijk, D. J., Middleton, B., Stone, B. M. & 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.
58. Wirz-Justice, A., Kräuchi, K., Cajochen, C., Danilenko, K. V., Renz, C. & 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.
59. Reiter, R. J. & Robinson, J. (1996). *Melatonin: Breakthrough Discoveries That Can Help You*. New York: Bantam Books.
60. Pierpaoli, W., Davies, O. & Regelson, W. (1995). *The Melatonin Miracle: Nature's Age-Reversing, Disease-Fighting, Sex-Enhancing Hormone*. New York: Simon & Schuster.
61. Sack, R. L., Lewy, A. J. & Hughes, R. J. (1998). Use of melatonin for sleep and circadian rhythm disorder. *Annals of Medicine* 30: 115–121.
62. Edwards, B. J., Atkinson, G., Waterhouse, J., Reilly, T., Godfrey, R. & Budgett, R. (2000). Use of melatonin in recovery from jet-lag following an eastward flight across 10 time-zones. *Ergonomics* 43: 1501–1513.
63. Shiota, M., Sudou, M. & Ohshima, M. (1996). Using outdoor exercise to decrease jet lag in airline crewmembers. *Aviation, Space, and Environmental Medicine* 67: 1155–1160.
64. Buxton, O. M., Lee, C. W., L'Hermite-Balériaux, M., Turek, F. W. & 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.
65. Barger, L. K., Wright, K. P., Hughes, R. J. & Czeisler, C. A. (2004). Daily exercise facilitates phase delays of circadian melatonin rhythm in very dim light. *American Journal of Physiology* 286: R1077–R1084.
66. Van Reeth, O. & 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.
67. Van Reeth, O. & 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.
68. Buxton, O. M., Copinschi, G., van Onderbergen, A., Karrison, T. G. & 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.
69. Mohawk, J. A., Cashen, K. & Lee, T. M. (2005). Inhibiting cortisol response accelerates recovery from a photic phase shift. *American Journal of Physiology* 288: R221–R228.
70. Nicholson, A. N., Spencer, M. B., Pascoe, P. A., Stone, B. M., Roehrs, T. & Roth, T. (1986). Sleep after transmeridian flights. *Lancet* 8517: 1205–1208.
71. Zhdanova, I. V. & Wurtman, R. J. (1997). Efficacy of melatonin as a sleep-promoting agent. *Journal of Biological Rhythms* 12: 644–650.
72. Paul, M. A., Gray, G., Sardana, T. M. & 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.
73. Rajaratnam, S. M. W. & Arendt, J. (2001). Health in a 24-h society. *Lancet* 358: 999–1005.
74. Caruso, C. C., Lusk, S. L. & 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.
75. Presser, H. B. (1999). Toward a 24-hour economy. *Science* 284: 1778–1779.
76. Parks, D. K., Yetman, R. J., McNeese, M. C., Burau, K. & Smolensky, M. H. (2000). Day-night pattern in accidental exposures to blood-borne pathogens among medical students and residents. *Chronobiology International* 17: 61–70.
77. Singer, B., Terborg, J. & Mayer, S. (1994). Attitudinal, circadian, circumstantial, and subject selection explanations of shiftwork effects on health. *Journal of Occupational Medicine* 36: 66–69.
78. 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.
79. Roden, M., Koller, M., Pirich, K., Vierhapper, H. & Waldhauser, F. (1993). The circadian melatonin and cortisol secretion pattern in permanent night shift workers. *American Journal of Physiology* 265: R261–R267.

80. Pati, A. K. & Gupta, S. (1994). Time estimation circadian rhythm in shift workers and diurnally active humans. *Journal of Biosciences* 19: 325–330.
81. Weibel, L. & Brandenberger, G. (1998). Disturbances in hormonal profiles of night workers during their usual sleep and work times. *Journal of Biological Rhythms* 13: 202–208.
82. Dumont, M., Benhaberou-Brun, D. & 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.
83. Waterhouse, J., Buckley, P., Edwards, B. & Reilly, T. (2003). Measurement of, and some reasons for, differences in eating habits between night and day workers. *Chronobiology International* 20: 1075–1092.
84. Andlauer, P., Reinberg, A., Fourré, L., Battle, W. & Duverneuil, G. (1979). Amplitude of the oral temperature circadian rhythm and the tolerance to shift-work. *Journal de Physiologie* 75: 507–512.
85. Reinberg, A., Vieux, N., Ghata, J., Chaumont, A. J. & 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.
86. Reinberg, A., Andlauer, P., Guillet, P., Nicolai, A., Vieux, N. & Laporte, A. (1980). Oral temperature, circadian rhythm amplitude, ageing and tolerance to shift-work. *Ergonomics* 23: 55–64.
87. Vidacek, S., Radosevic-Vidacek, B., Kaliterna, L. & Prizmic, Z. (1993). Individual differences in circadian rhythm parameters and short-term tolerance to shift-work: a follow-up study. *Ergonomics* 36: 117–123.
88. Borges, F. N. S. & Fischer, F. M. (2003). Twelve-hour night shifts of healthcare workers: a risk to the patients? *Chronobiology International* 20: 351–360.
89. Kelly, T. L., Neri, D. F., Grill, J. T., Ryman, D., Hunt, P. D., Dijk, D. J., Shanahan, T. L. & Czeisler, C. A. (1999). Nonentrained circadian rhythms of melatonin in submariners scheduled to an 18-hour day. *Journal of Biological Rhythms* 14: 190–196.
90. Penev, P. D., Kolker, D. E., Zee, P. C. & Turek, F. W. (1998). Chronic circadian desynchronization decreases the survival of animals with cardiomyopathic heart disease. *American Journal of Physiology* 275: H2334–H2337.
91. Monk, T. H. (2000). What can the chronobiologist do to help the shift worker? *Journal of Biological Rhythms* 15: 86–94.
92. Cruz, C., Detwiler, C., Nesthus, T. & Boquet, A. (2003). Clockwise and counterclockwise rotating shifts: effects on sleep duration, timing, and quality. *Aviation, Space, and Environmental Medicine* 74: 597–605.
93. Cruz, C., Boquet, A., Detwiller, C. & Nesthus, T. (2003). Clockwise and counterclockwise rotating shifts: effects on vigilance and performance. *Aviation, Space, and Environmental Medicine* 74: 606–614.
94. Baehr, E. K., Fogg, L. F. & Eastman, C. I. (1999). Intermittent bright light and exercise to entrain human circadian rhythms to night work. *American Journal of Physiology* 277: R1598–R1604.
95. Boivin, D. B. & James, F. O. (2002). Circadian adaptation to night-shift work by judicious light and darkness exposure. *Journal of Biological Rhythms* 17: 556–567.
96. Crowley, S. J., Lee, C., Tseng, C. Y., Fogg, L. F. & 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.
97. Gallo, L. C. & Eastman, C. I. (1993). Circadian rhythms during gradually delaying and advancing sleep and light schedules. *Physiology and Behavior* 53: 119–126.
98. Eastman, C. I., Liu, L. & Fogg, L. F. (1995). Circadian rhythm adaptation to simulated night shift work: effect of nocturnal bright-light duration. *Sleep* 18: 399–407.
99. Leppämäki, S., Partonen, T., Piiroinen, P., Haukka, J. & 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.
100. Lowden, A., Åkerstedt, T. & Wibom, R. (2004). Suppression of sleepiness and melatonin by bright light exposure during breaks in night work. *Journal of Sleep Research* 13: 37–43.
101. Härmä, M. I., Ilmarinen, J., Knauth, P., Rutenfranz, J. & 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.
102. Eastman, C. I., Hoese, E. K., Youngstedt, S. D. & Liu, L. (1995). Phase-shifting human circadian rhythms with exercise during the night shift. *Physiology and Behavior* 58: 1287–1291.
103. James, M., Tremea, M. O., Jones, J. S. & Krohmer, J. R. (1998). Can melatonin improve adaptation to night shift? *American Journal of Emergency Medicine* 16: 367–370.
104. Sharkey, K. M., Fogg, L. F. & Eastman, C. I. (2001). Effects of melatonin administration on daytime sleep after simulated night shift work. *Journal of Sleep Research* 10: 181–192.
105. Jelínková-Vondrasová, D., Hájek, I. & 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.
106. Horowitz, T. S., Cade, B. E., Wolfe, J. M. & 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.
107. Burgess, H. J. & 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.
108. Jespersen, J. & Fitz-Randolph, J. (1999). *From Sundials to Atomic Clocks: Understanding Time and Frequency,* 2nd Edition. Mineola, NY: Dover.
109. Brunner, B. (Ed.) (2005). *TIME Almanac 2006*. Des Moines, IA: TIME Books.

16 Human Medicine

CHAPTER OUTLINE

16.1 Chronotherapeutics
16.2 Sleep Disorders
16.3 Depression

16.1 CHRONOTHERAPEUTICS

Chronotherapeutics is the prevention and treatment of disease informed by knowledge of circadian rhythms. In Chapter 5, and throughout this book, circadian rhythmicity was shown to affect numerous physiological and behavioral processes. Therefore, it is not surprising to discover 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 suggested by Arthur Jores in 1936[2] and Franz Halberg in 1958.[3]

The relationship of circadian rhythmicity to human medicine starts at the most basic level of physiological processes. For example, previous chapters frequently discussed the rhythm of body temperature. Like other animals, humans exhibit robust rhythmicity of body temperature.[4–33] It is not surprising, then, 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. Note 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) because the measurements were taken under the tongue rather than rectally. Note 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. The "normal" threshold for a fever clearly 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.[34] As shown in Figure 16.2,

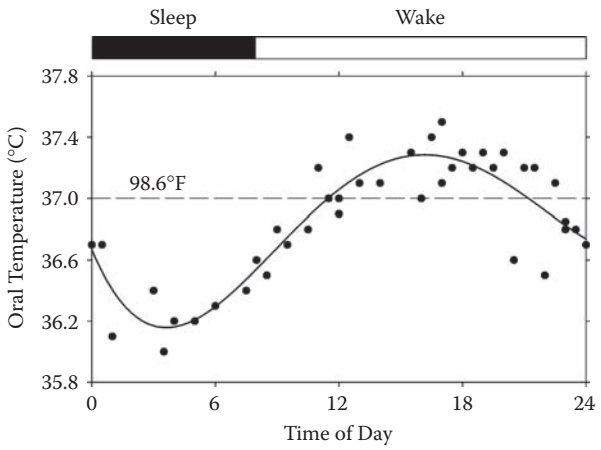


FIGURE 16.1 Do you have a fever? The graph shows the daily rhythm of oral temperature of a normal healthy human subject. Because the temperature oscillates from 36.0 to 37.5°C, the traditional value of 37.0°C (98.6°F) should not be used as a criterion for ascertaining the presence of a fever. (Source: Refinetti, R. (1992). Analysis of the circadian rhythm of body temperature. *Behavior Research Methods, Instruments, & Computers* 24: 28–36.)

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.[35] 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.

Body temperature, heart rate, and blood pressure are routinely monitored in medical visits. Because all three of these variables exhibit daily rhythmicity, disregard for the rhythmicity may lead to erroneous interpretation of the measurements — as demonstrated 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. Note that the ranges for blood pressure and heart rate are the same in the morning and afternoon. This finding 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

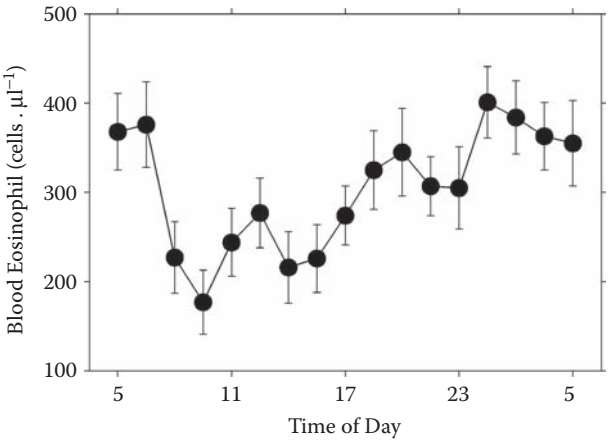


FIGURE 16.2 Daily rhythmicity in white blood cell count. The graph shows the daily oscillation of blood eosinophils (leukocytes with distinctive antiparasitic functions) in healthy human subjects. The data points correspond to the means (± SE) of 17 young men. Note that cell count is higher during sleep time (between midnight and 7 A.M.). (Source: Halberg, F., Visscher, M. B., Flink, E. B., Berge, K. & Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *The Journal-Lancet* 71: 312–319.)

TABLE 16.1
Normative Ranges for Human Vital Signs

	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

Note: 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 is 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 inter-individual variability for healthy individuals.

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. Note 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 inter-individual variability observed among healthy individuals. For long-term health care, normative ranges should be determined empirically 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 at a later time. Devices for ambulatory monitoring of body temperature, heart rate, and blood pressure are now easily available, so that patients may live 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 Harrell Medical Inc. (Canby, Oregon), Mini-Mitter Co. (Bend, Oregon), SpaceLabs Inc. (Issaquah, Washington), Tiba Medical Inc. (Plano, Texas), and VivoMetrics Inc. (Ventura, California). 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.[36] In the case of blood pressure, ambulatory monitoring can provide the normative baseline data and prevent the occurrence of "white-coat hypertension" (i.e., the spurious elevation of blood pressure caused by patient anxiety in the physician's office).[37–39]

Chronotherapeutics may be relevant to every human disease, but space limitations allow me to discuss in detail only a few selected conditions. Two diseases — heart

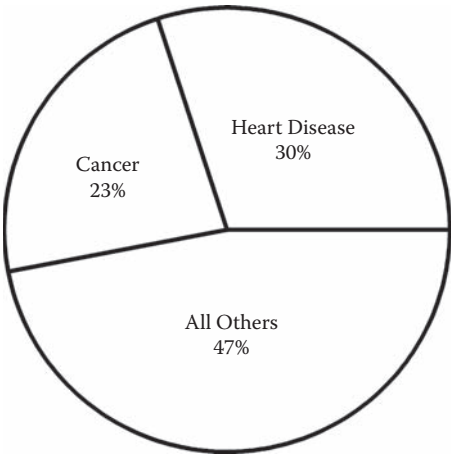


FIGURE 16.3 Leading causes of death in the United States. Two illnesses — heart disease and cancer — are responsible for more than half of all deaths. (Source: *TIME Almanac 2003*. (2002). Des Moines, IA: TIME Books.)

disease (or, more generally, cardiovascular disease) and cancer — are responsible for more than half of all deaths in the United States (Figure 16.3). Of course, every human being will die one day, so that reductions in mortality by one disease will lead to increases in mortality by other diseases. However, improvements in the treatment of cardiovascular disease and cancer can improve the patient's quality of life, even if it only postpones his or her death by other causes. Having money to spend on essential (or frivolous) things is one determinant of quality of life. Yet, Figure 16.4 shows that the cost of medical care has become a disproportionately large expense for American consumers in the last two decades. To the extent that improvements in medical treatments can eventually reduce health care costs, advances in the treatment of cardiovascular disease and cancer can effectively lead to improvements in quality of life.

16.1.1 Cardiovascular Disease

One of the leading causes of cardiovascular disease is hypertension (high blood pressure). Regular monitoring of blood pressure (Figure 16.5) is a simple, noninvasive way to ensure early diagnosis of hypertension. However, humans — like other animals — exhibit robust daily rhythmicity in cardiovascular parameters.[7,15,24,26,29,30,37–49] As mentioned earlier, this finding 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 4 A.M. to noon. Consequently, antihypertensive control should be concentrated in the early morning. To attain peak plasma level

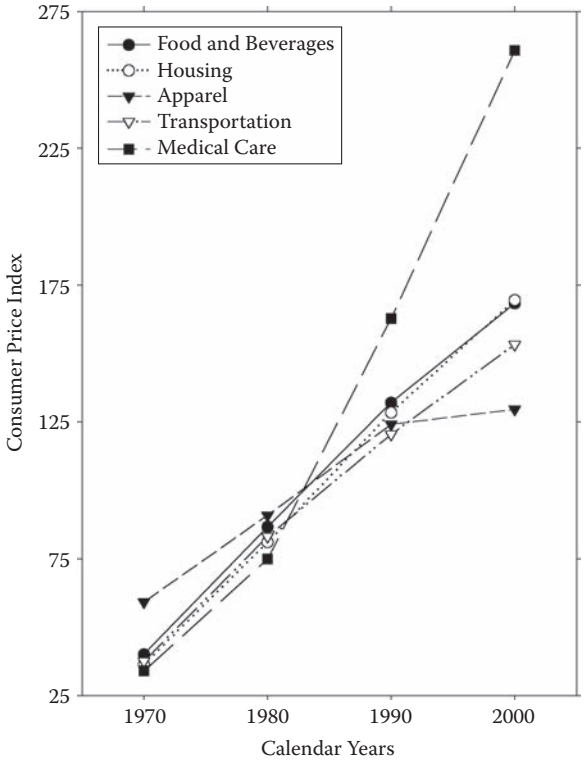


FIGURE 16.4 Escalating medical costs. Inflation has raised the costs of most goods in the United States during the last few decades, but the costs of medical care have risen disproportionally faster since 1980. (Source: *TIME Almanac 2003*. (2002). Des Moines, IA: TIME Books.)

FIGURE 16.5 Monitoring blood pressure. Frequent monitoring of blood pressure can facilitate the early detection of elevated blood pressure and the prevention of deadly cardiovascular diseases. (Source: © ArtToday, Tucson, AZ.)

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,

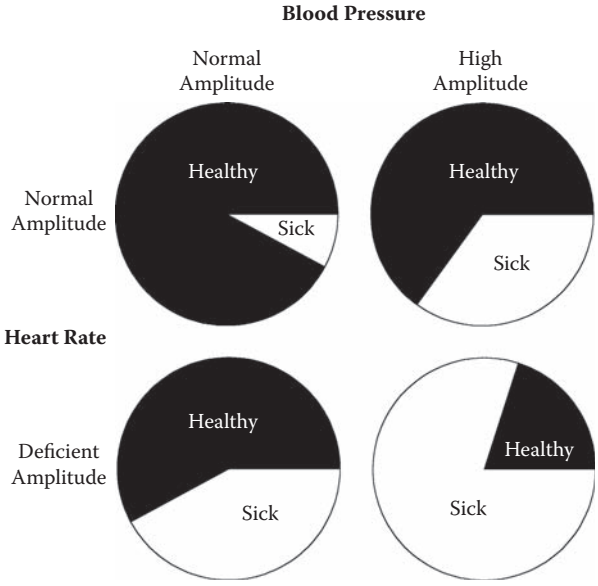


FIGURE 16.6 Prediction of morbidity by rhythm amplitude. Franz Halberg conducted ambulatory measurements of blood pressure and heart rate in a number of individuals and then counted how many of them developed cardiovascular morbidity during the following 6 years. The graphs show that large amplitude of the rhythm of blood pressure and small amplitude of the rhythm of heart rate (without abnormal mean levels) are strong predictors of morbidity. (Source: Data courtesy of Franz Halberg, University of Minnesota.)

thus ensuring adequate dosage at the time when the drug is most needed and avoiding toxic overdosage at other times.[49]

Halberg has 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 alone is not a morbid state and does not imply current hypertension) eventually developed vascular morbidity.[42,50] 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.[51] 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. The sample sizes in Halberg's exploratory studies were rather small, however, 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. A full-fledged clinical trial is urgently needed to evaluate the merits of the preliminary observations.

Chapter 5 showed that heart attacks tend to occur in the morning or early afternoon.[52–54] This rhythm is populational, but it would not exist if individuals were not more likely to have heart attacks 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 obtain early diagnosis of cardiac arrhythmia.[55]

16.1.2 Cancer

Recognizing that circadian rhythms exist is important not only for diagnostic purposes but also for therapeutic reasons. If physiological functions oscillate daily, then sensitivity of the patient to therapeutic agents also should oscillate daily. Studies in laboratory animals have identified daily rhythmicity in the body's response to numerous drugs, including anesthetics,[56,57] immunosuppressants,[58] antibiotics,[59] and alcohol.[60] In a similar fashion, clinical research on human patients has demonstrated that the pharmacodynamics and pharmacokinetics of drugs such as H2-blockers, antiasthmatics (theophylline, β agonists, anticholinergics, glucorticoids), cardiovascular active drugs (β blockers, organic nitrates, calcium channel blockers), and anticancer drugs display daily rhythmicity.[61,62] In fact, 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 the fewest side effects). Consider Figure 16.7. It shows daily variation in plasma concentration of fluorouracil (one of the oldest anticancer drugs, still used widely today) in patients with bladder carcinoma. The drug, which has a half life in the blood of 10 to 20 minutes, was infused intravenously at a constant rate. Note the clear daily variation in plasma concentration despite the constant rate of infusion.[63] Breakdown clearly is much faster during the day than at night, which results in higher plasma concentration at night. This finding means that lower doses (with potentially lower toxicity) could be used at night.

In contrast, parallel research conducted by Francis Lévi's team (in France) has shown that fluorouracil is more effective and less toxic at night than during the day.[64] This finding means that the variation in drug breakdown can be ignored, and *higher* doses should be used at night. Many oncologists administer fluorouracil only at night

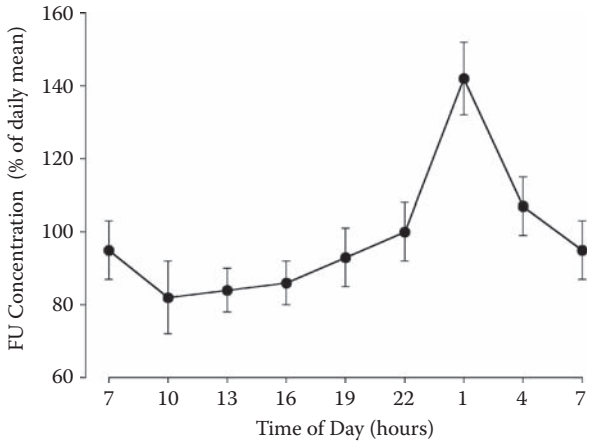


FIGURE 16.7 Daily rhythmicity in chemical breakdown of anticancer drug. The graph shows the daily oscillation in plasma concentration of fluorouracil (FU, a drug used in cancer treatment) administered by continuous intravenous infusion at constant rate in human subjects. The data points correspond to the means (± SE) of seven patients with bladder carcinoma. Note 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. (Source: Milano, G. & Chamorey, A. L. (2002). Clinical pharmacokinetics of 5-fluorouracil with consideration of chronopharmacokinetics. *Chronobiology International* 19: 177–189.)

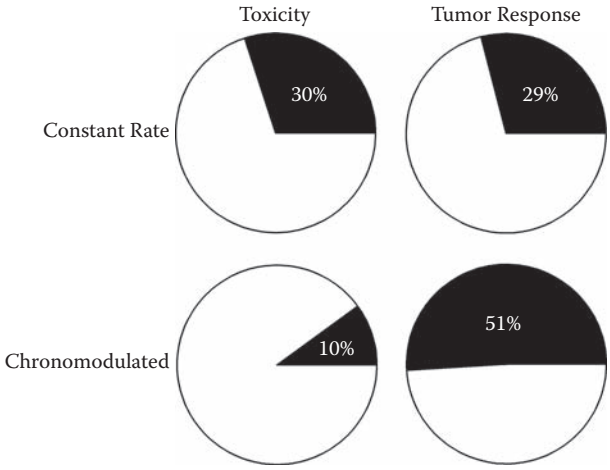


FIGURE 16.8 Chemotherapy of colorectal cancer. The graphs show the percentages of patients hospitalized for toxicity (Toxicity) and the percentages of patients exhibiting greater than 50% reduction of tumor size (Tumor Response) associated with constant-rate or chronomodulated chemotherapy (fluorouracil, leucovorin, and oxaliplatin). The percentages are derived from two multicenter randomized trials involving a total of 278 patients with metastatic colorectal cancer. Note that chronomodulated chemotherapy is less toxic and more effective than traditional chemotherapy. (Source: Lévi, F., Giacchetti, S., Zidani, R., Brezault-Bonnet, C., Tigaud, J. M., Goldwasser, F. & Misset, J. L. (2001). Chronotherapy of colorectal cancer metastases. *Hepato-Gastroenterology* 48: 320–322.)

(with the assistance of time-controlled infusion pumps).[65] Figure 16.8 shows a comparison of the results of chemotherapy of colorectal cancer by constant infusion ("Constant Rate") and by time-controlled infusion ("Chronomodulated"). 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. Note that chronomodulated infusion resulted in both lower toxicity and higher tumor response.[65] As early as 1985, William Hrushesky (now at the University of South Carolina) demonstrated greater efficacy and lower toxicity of chronomodulated administration of adriamycin and cisplatin in the treatment of ovarian cancer.[66]

Some researchers have speculated that the development of breast cancer may be more likely in individuals who lack robust circadian rhythms.[67] 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.[68] However, it is not clear whether weak rhythmicity is a contributing factor to cancer growth or a consequence of the illness. As discussed in Chapter 6, illness is known to weaken circadian rhythms.

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 States alone.[69] It is particularly worthy of mention here because its symptoms exhibit clear daily rhythmicity.[70,71] 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 primary symptom is difficulty in breathing.

The first large-scale investigation of daily rhythmicity in asthma was conducted by Margaret Turner-Warwick (in England) and involved 7729 asthmatic outpatients.[72] 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 an organic process, not a consequence of the lack of effective medication during the night.[73] 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. One group of researchers found that the rhythmicity in asthmatic symptoms disappears if

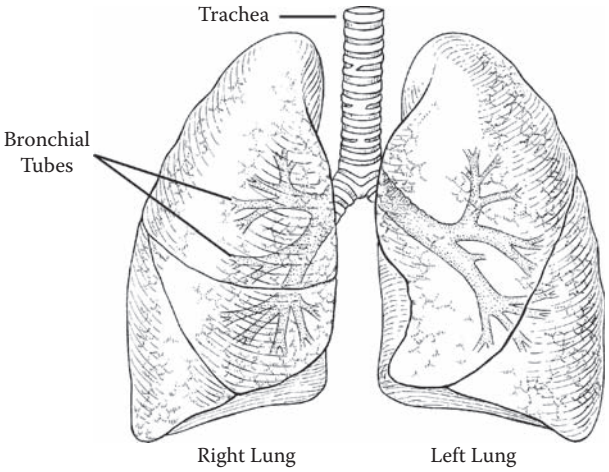


FIGURE 16.9 In the lungs. Asthma is a pulmonary disorder resulting from spasmodic contraction of the muscles of the bronchioles, a swelling of the tissues of the bronchial tubes, and the retention of the secretions of the bronchial glands. (Source: Adapted from *Medical Illustration Library.* (1994). Baltimore, MD: Williams & Wilkins.)

the data are corrected for daily changes in psychosocial factors that contribute to asthma, such as activities, locations, social contacts, mood, and stressors.[74]

Two indices of expiratory flow have been used as objective measures to facilitate the management of asthma: peak expiratory flow rate (PEF)[75–77] and forced expiratory volume in 1 second (FEV1).[78–80] The two measures correlate well with each other and with the caliber of the airway.[81] Measurements conducted at several time points during a day revealed clear rhythmicity in asthmatic patients as well as in normal subjects. For both groups, peak lung function occurs at approximately 4 P.M. and lowest lung function is seen 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.[82,83] 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 the strict sense, but one should not infer that daily rhythmicity in asthma severity is not clinically relevant in general. Because a large number of uncontrolled variables determines 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 also makes use of chronobiological information to maximize pharmacological effects and minimize side effects.[70,71] Because 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,[84] theophylline,[85] anticholinergics,[86] and β-adrenergic agonists.[87] 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.

16.1.4 Summer Course on Chronopharmacology

For readers interested in practical training in chronopharmacology and, more generally, in chronotherapeutics, an International Course on Chronopharmacology has been offered at the University of Heidelberg (in Germany) every summer since 2000. The course, which lasts about a week in July, is organized by Björn Lemmer and includes lecturers from the United States, Canada, United Kingdom, France, Switzerland, Italy, and Germany. Tuition often is waived for graduate students and postdoctoral fellows. Details can be found at the course's web site, whose address is: www.chronopharmacology.de.

16.2 SLEEP DISORDERS

Chapter 10 discussed the regulation of sleep. Sleep is a restorative process gated by the circadian system (Figure 16.10).[88] The longer you stay awake, the greater 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 associated with sleep 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.[89–93] If a causal link exists 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.[23,94] Also, during the deeper stages of sleep, the homeostatic control of body temperature is relaxed,[89,93,95–106] 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 as long as they want sleep approximately 8 hours each day. (Source: © ArtToday, Tucson, AZ.)

Because most humans are awake during the day and asleep at night, day length (photoperiod) can affect sleep. Also, as shown 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 moment (Figure 16.11). I mentioned this phenomenon in Chapter 15 when discussing 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 July, locations above the Arctic Circle experience 24 hours of sunlight each day, while 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. Chapter 7 and Chapter 9 showed that animals expand and contract their daily intervals of activity in response to changes in photoperiod. Therefore, the activity interval of a given species can be expected to vary with latitude. This variance does not occur in 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 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., and most of them obtain 7 to 8 hours of sleep each night. A survey of college students conducted in California in 1916 indicated that average bedtime and wake time were 11 P.M. and 7 A.M., respectively,[107] while a survey conducted in Virginia in 1995 indicated that average bedtime and wake time were 1:30 A.M. and 9 A.M.[108] 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, wake times are strongly determined by class schedules, so that a weekly pattern of wake times (with delayed wake times on weekends) can be observed in the records of many students.[108,109]

Most humans need 8 hours of sleep each day,[110,111] but most people sleep fewer than 8 hours a day — indicating that most people are chronically sleep deprived. A non-scientifically rigorous survey reported in *Time Magazine* indicated that 75% of American adults do not feel fully refreshed when waking up in the morning (Figure 16.13). It also indicated that 64% of the respondents hit the circadian gate on weekend mornings. Most people, however, can get a good night of sleep if their work schedules allow it. Some people 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 wake time), somnambulism (sleepwalking), and night terrors.[111] The prevalence of insomnia in the general population is reported to be quite high (10 to 35% of all adults),[111] although it has been noted that many people who complain of inability to sleep actually misjudge their real needs and stay in bed longer than required.[112,113] In a study of 121 people who complained of insomnia and 56 who did not, electroencephalographic records over 2 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.[114]

Some cases of insomnia, such as those caused by sleep apnea, have no obvious relationship with circadian rhythms. Other cases, however, potentially could be due to disorders of the circadian system; these disorders include 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.[115] 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 1,000.[116] Both conditions have an inherited component, and familial ASPS has been traced to an abnormality in the *per2* gene.[117] Neither syndrome involves actual lack of sleep if the patients are able to set their work schedules according to their sleep habits, and studies of DSPS patients using

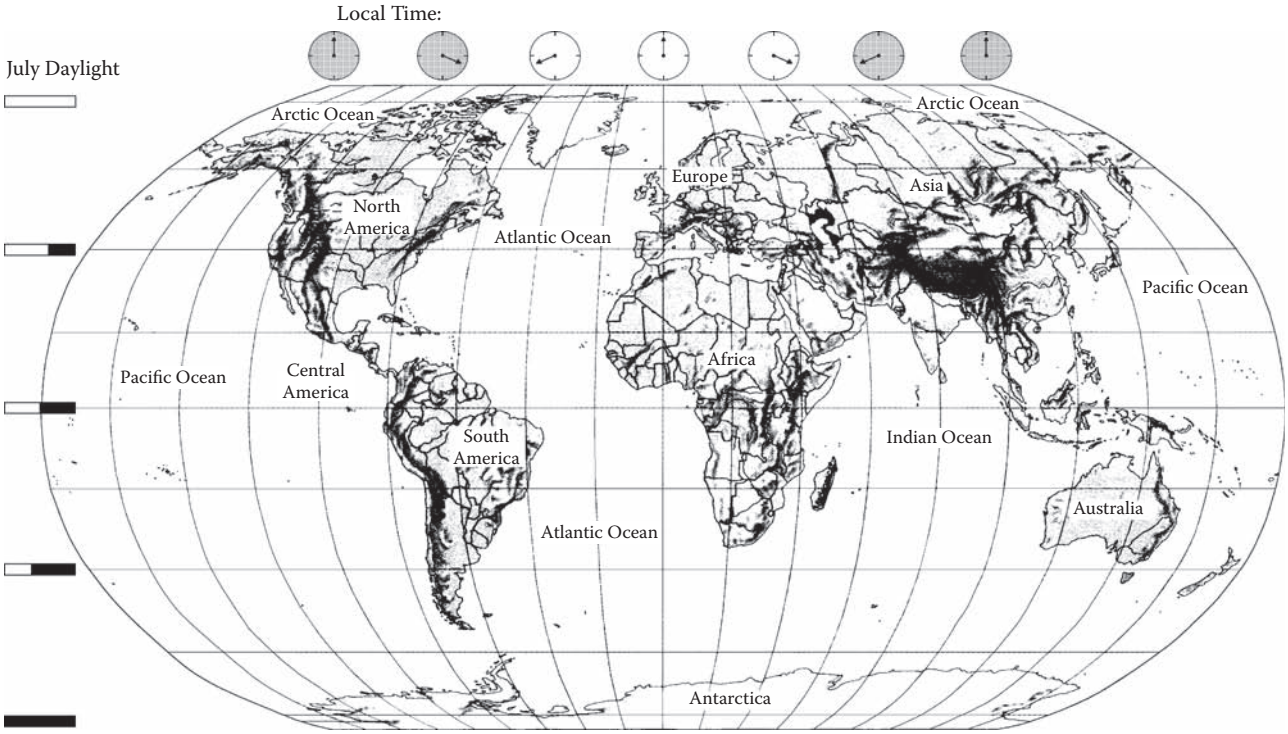


FIGURE 16.11 Latitude and longitude. Latitude and longitude affect the time of day differently. Longitude affects time of day according to the time zones, but the days are similar regarding local time. Latitude does not affect time of day, but it has a drastic effect on day length. As indicated by the 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. (Source: *TIME Almanac 2004*. (2003). Des Moines, IA: TIME Books.)

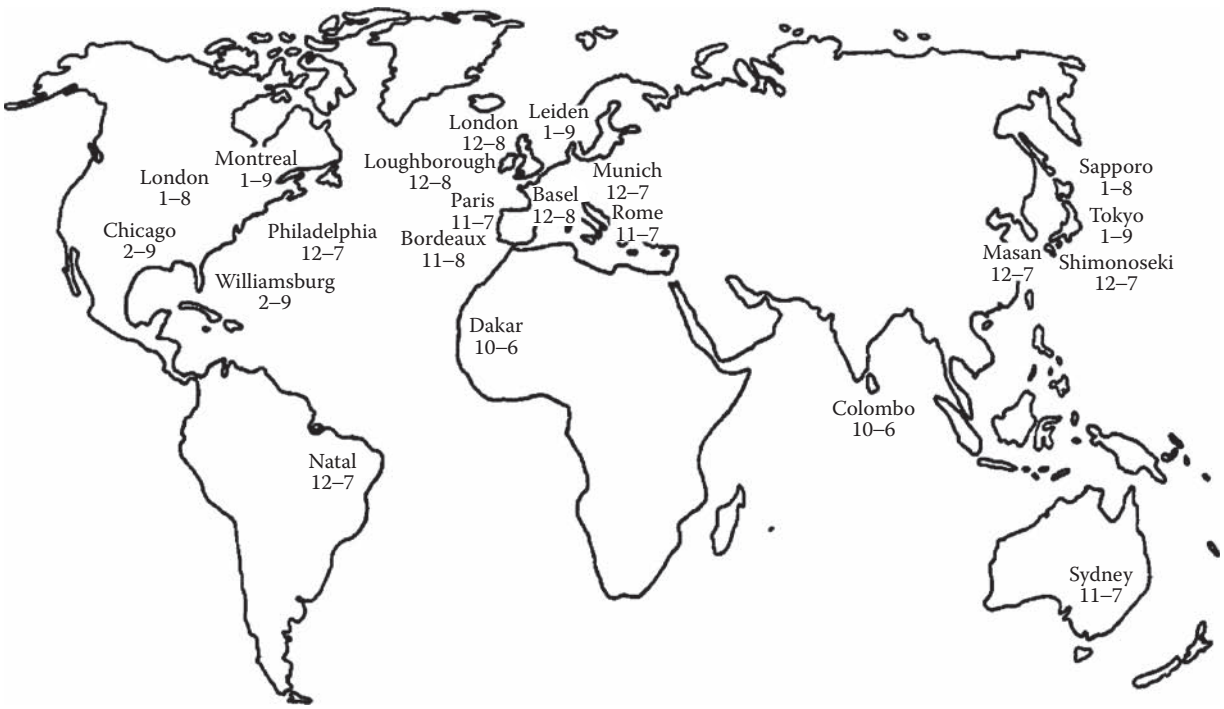


FIGURE 16.12 Sleep time. This diagram of the world indicates the habitual bedtimes and wake times in various locations. To improve readability, values were rounded to full hours. All times are A.M. local time, except bedtimes 10 and 11, which are P.M. (Sources: See references 19, 22, 26, 32, 108, 215–228 in the *Literature Cited* section.)

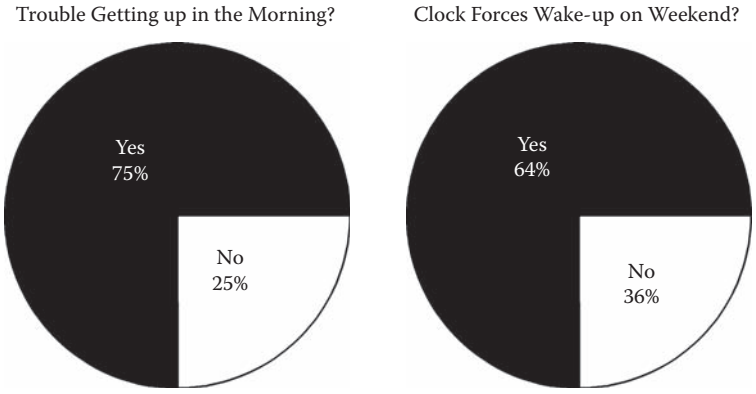


FIGURE 16.13 Common complaints about sleep. In a large — though not strictly scientific — survey conducted in the United States, randomly selected members of the general population were asked whether they regularly had difficulty getting up in the morning during weekdays and whether their body clocks forced them to wake up earlier than desired on weekends. (Source: *TIME* 161(21): 24, 2003.)

electroencephalographic recording revealed no abnormalities in sleep structure.[118,119] 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.

Figure 16.14 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 bed time. In a person with ASPS, the clock is advanced by several hours. In Figure 16.14, 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 usually can stay awake past the expected bed time (probably watching television) and not be troubled by the advanced rhythm. However, because the rhythm is advanced, his or her expected wake 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 awaken the individual 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 the knowledge of circadian rhythms should allow circadian physiologists to understand the etiology

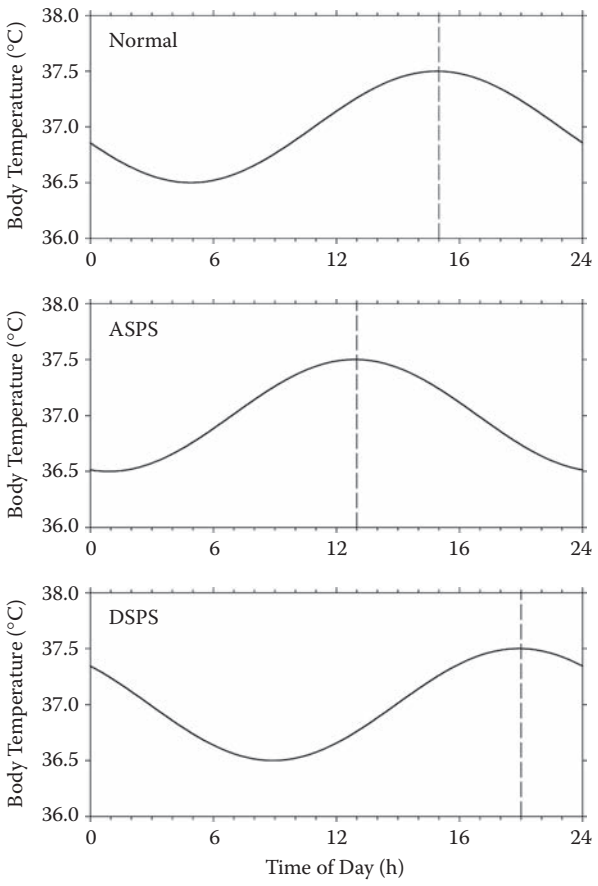


FIGURE 16.14 Sleep phase syndromes. These diagrams indicate that the phase of the circadian clock, as inferred from the phase of the human body temperature rhythm, is advanced in patients suffering from Advanced Sleep Phase Syndrome (ASPS) and is delayed in patients suffering from Delayed Sleep Phase Syndrome (DSPS). The vertical dashed lines indicate the acrophase (time of peak) of the temperature rhythm.

of the disease. Chapter 7 showed that entrainment depends on three variables: the period of the pacemaker (τ), the period of the zeitgeber (T), and the phase-response curve (PRC) of the pacemaker. The period of the zeitgeber presumably is 24 hours for all patients living in the real world, so the other two variables should be examined. 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, which is the case for most people (because the human τ is usually longer than 24 hours), but it could be exaggerated in DSPS patients (say, τ = 28 hours). Just as the activity of the nocturnal mouse with a short τ was displaced into the daylight (Figure 7.24 in Chapter 7), so will the activity of the diurnal human with a long τ be displaced into the nighttime. Alternatively (or in combination with an abnormal τ), the PRC could be affected. Thus, the relative phase delay of DSPS patients could be due to reduced amplitude of the phase-advance region of the PRC. A reduced amplitude would require that a wider section of the phase-advance region be photically stimulated, which could be accomplished only by starting the stimulation earlier during subjective night and terminating the stimulation earlier during subjective day, thus extending the duration between lights-off and bedtime. The combination of a small reduction in the amplitude of the PRC and a small lengthening of τ would produce equivalent results.

Of course, this discussion 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, Chapter 6 showed that the wake-sleep rhythm in humans can be 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.[120–122] Have the circadian period and the amplitude of the PRC been determined in patients with sleep phase disorders? Not really.[123] Despite this scarcity of data about the etiology of sleep phase disorders, several forms of treatment have been employed. These include chronotherapy, treatment with bright light exposure, and treatment with melatonin administration. All three treatments do not involve controlled drugs and, therefore, do not require a prescribing physician. Sleeping pills, which traditionally are used in the treatment of sleep disorders, require a prescription from a physician. Sleeping pills (sedatives and hypnotics) include barbiturates, benzodiazepines, and other substances (such as zaleplon).[124] The most common sleeping pills today are benzodiazepines, such as diazepam (Valium®) and estazolam (Prosom®).[111] The structural formulas of diazepam and estazolam, which are very similar, are shown in Figure 16.15. Although

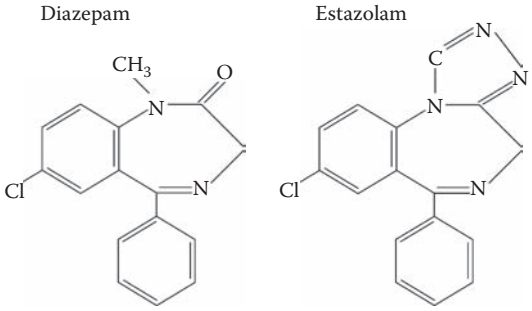


FIGURE 16.15 Sleeping pills. Benzodiazepines are often prescribed for the treatment of insomnia. The structural formulas of two benzodiazepines are shown: diazepam (Valium) and estazolam (Prosom). Diazepam also is prescribed widely for the treatment of anxiety. (Source: *Physician's Desk Reference*, 57th Edition. (2003). Montvale, NJ: Thomson PDR.)

sleeping pills are effective in inducing sleep, they may not affect the circadian system (even if they did, one would have to administer them in accordance with the PRC for each specific drug, rather than at bedtime). Therefore, sleeping pills correct only the homeostatic component of sleep, leaving the circadian component unaffected. Sleeping pills also can induce tolerance and dependence.

Chronotherapy is more like a business time-management initiative than a medical treatment. It involves the realization that the problem 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 of going to bed earlier. Of course, the person cannot arrive at a solution without help from others — 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.[125] The new phase angle of entrainment is unlikely to persist if the old phase angle was determined by abnormalities in τ or the PRC, 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 not disruptive and can be performed by the patient without the consultation of a physician or any other person. Therefore, it provides the convenience of a self-help method. The therapeutic action of chronotherapy may result simply from the establishment of a structured lifestyle, as a high level of regularity in lifestyle is associated with a high level of subjective sleep quality.[126] An attempt to obtain a 6-hour phase-advance of the sleep-wake schedule of healthy subjects by successive 2-hour delays was unsuccessful; it was plagued by disruptions in sleep, alertness, and circadian rhythms.[127]

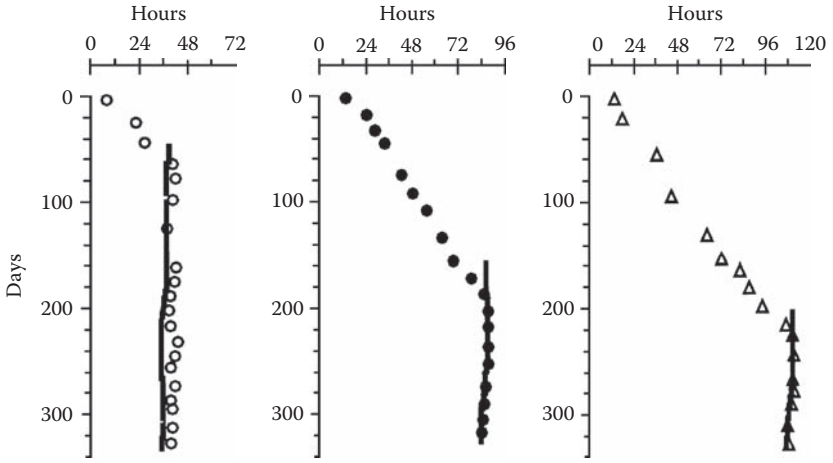


FIGURE 16.16 Entraining the blind. These pseudo-actograms (single plots on multiple-day axes) show the onset times of the circadian rhythms of melatonin secretion of totally blind human subjects before and during daily administration of exogenous melatonin as a zeitgeber (0.5 mg orally). Before treatment, 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. (Source: Adapted from Lewy, A. J., Emens, J. S., Bernert, R. A. & 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.)

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 PRC should cause a corrective phase advance. Successful treatment of DSPS patients with phototherapy has been reported.[128–131] 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). Of course, 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 were 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.[132,133] 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.[134,135] As shown in Figure 16.16, 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.[136] 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 relevant, however, that melatonin cannot be used simply as a corrective measure. To maintain entrainment (or masking), the patient would need to remain under melatonin treatment for the rest of his or her life.

The fact that physicians employ some form of treatment (and seem to obtain positive results) does not imply that the treatment is effective. As discussed 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, clinicians are offering potential solutions to patients' predicaments, which may be 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 16.17, 35% of the United States population suffers from and/or is treated for mental illness. Note 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. A full 20% (or one fifth of the population) is diagnosed but not treated.[137] Although biomedical researchers have identified many genes and gene polymorphisms associated with mental disorders, the causes of mental illness remain mostly unknown.[138]

Affective disorders (which include depression, mania, and bipolar disorders) are a major component of mental illness. Figure 16.18 shows the percentages of U.S.

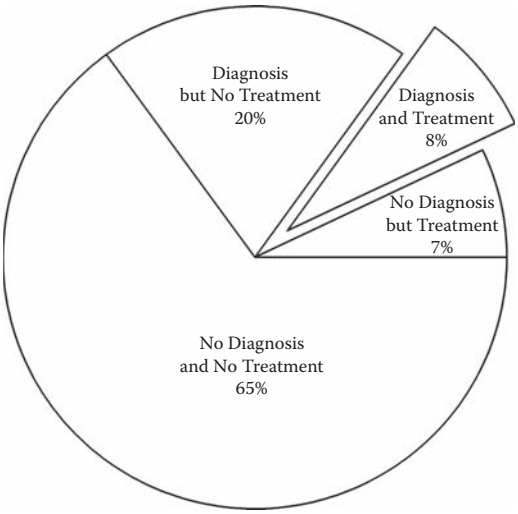


FIGURE 16.17 Prevalence of mental disorders and treatment. The graph shows the percentages of the United States population that are diagnosed and treated for mental disorders each year. Note that 8% of the population is diagnosed and treated annually and that 7% of the population is treated even though not diagnosed with a mental disorder. Thus, 15% of the population (or about 43 million people) receive treatment for a mental disorder each year. (Source: *Mental Health — A Report of the Surgeon General*. (1999). Rockville, MD: U.S. Department of Health and Human Services.)

residents who are diagnosed with an affective disorder (whether treatment is rendered or not) each year or over their lifetime. Each year, 14% of all women and 9% of all men are diagnosed with an affective disorder. Over their lifetime, the percentages rise to 24% for women and 15% for men.[139]

Although affective disorders usually do not involve the breakdown in logical thinking that characterizes other forms of mental illness, they can be incapacitating and may lead to suicide. Everyone is subject to occasional alterations in mood elicited by both internal and external factors (losing a job, going through a divorce, and so on). Overall happiness seems to be related more to one's constitution than to life events,[140] but small mood alterations are common. Alterations in mood, however, are sometimes strong enough to be diagnosed as depression or mania and, therefore, require medical attention. Depression is the most common affective disorder.[138] Table 16.2 shows the diagnostic criteria for depression (Major Depressive Disorder) according to the American Psychiatric Association.[141] Six conditions must be met to justify the diagnosis of major depressive disorder, although the main defining feature is a state of profound sadness and lack of pleasure that persists for at least 2 weeks. The program Health (see Exercise 16.1) can help you conduct a brief self-diagnosis if you think you may be suffering from depression. The program is not a licensed therapist, however, and you should consult a psychiatrist or a psychologist if you believe that you need a professional diagnosis.

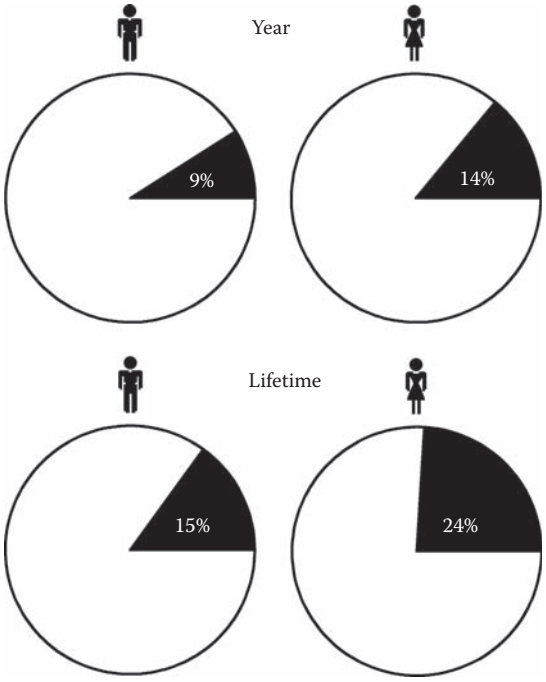


FIGURE 16.18 Prevalence of affective disorders. The diagrams show the prevalence of affective disorders in the U.S. population. The most common affective disorder is depression. The values shown are the percentages of the population that were diagnosed with the disorder during the past 12 months ("Year") or during their lifetime ("Lifetime"). (Source: Kessler, R. C. & Zhao, S. (1999). The prevalence of mental illness. In: Horvitz, A. V. & 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.)

16.3.1 Traditional Treatment of Affective Disorders

Professionals involved in the treatment of mental disorders generally are either psychiatrists or psychologists. As medical doctors, psychiatrists can prescribe *psychotropic drugs*, while psychologists must rely on *psychotherapy* (the "talking cure"). Both psychiatrists and psychologists use scientific knowledge to guide their diagnostic and therapeutic procedures. However, medical and psychological practice involves more than just the application of scientific knowledge to "real world" situations. Graduate school programs in clinical psychology often express the goal of training "scientist practitioners."[142] The central mission of medicine (or psychological practice), however, 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 16.19). When no scientific knowledge about a given condition is available, the

TABLE 16.2
Diagnostic Criteria for Depression (Major Depressive Disorder)

A. At least one of the following three abnormal moods has significantly interfered with the person's life for at least two weeks:
   1. Abnormal depressed mood most of the day, nearly every day
   2. Abnormal loss of all interest and pleasure most of the day, nearly every day
   3. If 18 years of age or younger, abnormal irritable mood most of the day, nearly every day

B. At least five of the following nine symptoms have been present during the same 2 weeks as in criterion A:
   1. Abnormal depressed mood (or irritable mood if under 18 years of age)
   2. Abnormal loss of all interest and pleasure
   3. Abnormal disturbance in body weight and/or appetite (abnormal loss or gain)
   4. Sleep disturbance (abnormal insomnia or abnormal hypersomnia)
   5. Activity disturbance (abnormal agitation or abnormal slowing observable by others)
   6. Abnormal fatigue or loss of energy
   7. Abnormal self-reproach or inappropriate guilt
   8. Abnormal poor concentration or indecisiveness
   9. Abnormal morbid thoughts of death or suicide

C. The symptoms are not due to psychosis (mood-incongruent psychosis)

D. The person has not previously experienced a manic episode, a mixed episode, or a hypomanic episode

E. The symptoms are not due to physical illness or to the use of alcohol, medication, or street drugs

F. The symptoms are not due to normal bereavement

Source: Adapted from *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition. (1994). Washington, DC: American Psychiatric Association.

practitioner is expected to do his or her best to alleviate the patient's ailment, while 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 one appreciate their efforts. Lack of this understanding was likely the main cause of the division of circadian physiology into two antagonistic factions during most of its recent history, as shown 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 proceed. From the perspective of the practitioner, hard-headed adherence to fundamental principles deters applications that might relieve a patient's suffering. The application of circadian physiology to psychiatric practice should be seen 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 on psychotropic drugs or on psychotherapy (or both). Antidepressant drugs are effective in the treatment of depression for some people.[143] Scientists know very little about how they work, however. A major roadblock in the understanding of the action of antidepressants (and other psychotropic drugs) is that, despite great progress in recent years, scientists know very little about how the brain processes mental activity. Because the antidepressant imipramine (Tofranil®) — the first widely used antidepressant — increases activity at synapses that utilize norepinephrine and dopamine as neurotransmitters, it was initially

FIGURE 16.19 Physicians and researchers. This cartoon poignantly identifies the fundamental difference in the problem-solving approaches of physicians and researchers. The two approaches are antithetical and cannot be simultaneously applied to the same problem. Understanding the difference between the two approaches is a prerequisite for the appreciation of their merits. (Source: © 1991 Sidney Harris. Reprinted with permission.)

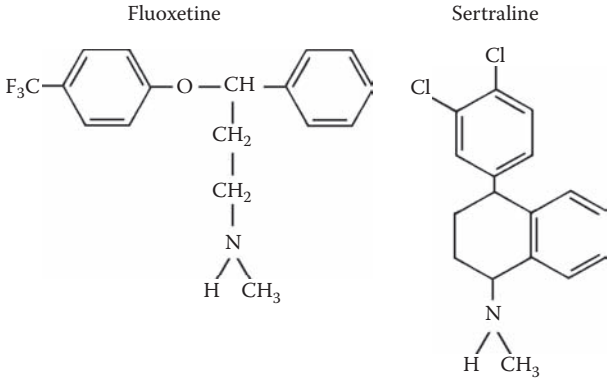


FIGURE 16.20 Antidepressant drugs. Selective serotonin reuptake inhibitors (SSRIs) are often prescribed for the treatment of depression. The structural formulas of two SSRIs are shown: fluoxetine (Prozac) and sertraline (Zoloft). (Source: *Physician's Desk Reference, 57th Edition*. (2003). Montvale, NJ: Thomson PDR.)

conjectured that depression might be the result of a deficiency in noradrenergic and dopaminergic neurotransmission.[144] Fluoxetine (Prozac) and other *selective serotonin reuptake inhibitors* (Figure 16.20) were introduced later. Because these potent antidepressants inhibit the synaptic re-uptake of serotonin (thus increasing activity in serotonergic synapses), it was conjectured that depression might be the result of a deficiency in serotonergic neurotransmission.[145,146] In the late 1990s, Merck & Co. developed a new antidepressant (MK-869) that blocks synaptic receptors for the neurotransmitter *substance P* and that does not seem to affect receptors for either serotonin or norepinephrine.[147] MK-869 turned out not to be better than placebo,[148] but researchers clearly can produce drugs to treat depression — and at least some of them work well. However, scientists cannot say how they really work. Even if researchers knew exactly which neurotransmitter systems are involved in depression, they still would need to identify the areas of the brain where these systems are located. A recent study in mice raised the possibility that selective serotonin reuptake inhibitors act by stimulating neurogenesis in the hippocampus,[149] but further studies on humans are necessary to determine the significance of this finding.

Psychotherapy is the other traditional treatment of depression. From a pharmacological perspective, all psychotherapy is placebo medication — because no drug is actually administered. However, psychotherapy must have a pharmacological effect on the brain that accounts for the changes in the patient's behavior. Evaluation of the efficacy of psychotherapy is complicated by the fact that traditionally only a small fraction of people affected by mental disorders is treated with psychotherapy[137,139,150] (Figure 16.21). Thus, most cases of "antisocial personality" (i.e., psychotics, criminals, and so on) are isolated in special institutions and do not undergo psychotherapy (i.e., they receive other types of treatment or no treatment

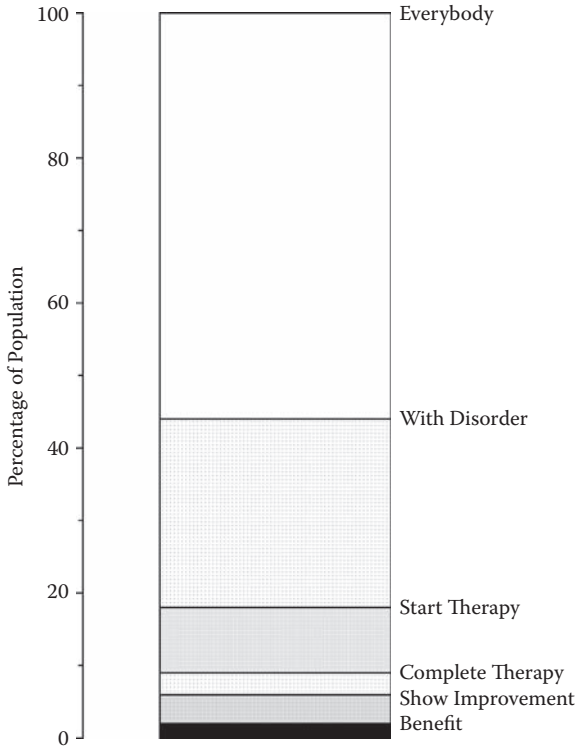


FIGURE 16.21 The benefit of psychotherapy. Less than half of the general population has a mental disorder. Less than half of those with a mental disorder undergo psychotherapy. Less than half of those who undergo psychotherapy show improvement. Less than half of those who show improvement actually benefit from psychotherapy. Still, about 3 million people in the United States benefit from psychotherapy each year. (Sources: Bandura, A. (1969). *Principles of Behavior Modification*. New York: Holt, Rinehart and Winston; Shapiro, S. et al. (1984). Utilization of health and mental health services. *Archives of General Psychiatry* 41: 971–978; Kessler, R. C. & Zhao, S. (1999). The prevalence of mental illness. In: Horvitz, A. V. & 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.)

at all). Thirty to sixty percent of those who consult a psychotherapist and are accepted for treatment stop coming back before their treatments are finished. Of those who stay, about two thirds show improvement. Thus, out of 100 people with mental disorders, only 40 undergo psychotherapy, 20 remain under treatment for the prescribed period, and 14 show improvement. Most people would not call a 14/100 ratio a success. But they would not call it a failure either. After all, it is not the therapist's fault that patients are not sent for treatment or do not wish to stay. What about the 2/3 improvement ratio? This ratio may not be a success either. Because 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 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 whether or not they undergo psychotherapy.[151] A few years later, a similar study on children reached a similar conclusion.[152]

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. These findings mean that psychotherapy provides no benefit at all! By the 1980s, a consensus developed that psychotherapy does have a small but real benefit.[153,154] A major problem, however, was that — although there are many different types of psychotherapy, many of them based on principles totally different from, and often antithetical to, the other types — all therapies were declared to be equally effective.[154,155] 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." 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 was at the heart of my own consulting practice.[156] It is consistent with the idea of "social construction" of the concept of mental illness.[157,158] Recently, it has been shown that even the participation of a therapist or consultant may not be necessary. Several health benefits apparently can be obtained simply by writing about one's own deep thoughts and feelings without any feedback from a therapist.[159]

Figure 16.22 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 note that the rate for pill-placebo treatment is only 30%, which means that 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).[143]

16.3.2 Circadian Rhythmicity and Affective Disorders

Clinically depressed patients exhibit various abnormalities in circadian rhythms.[41,160–170] The most common

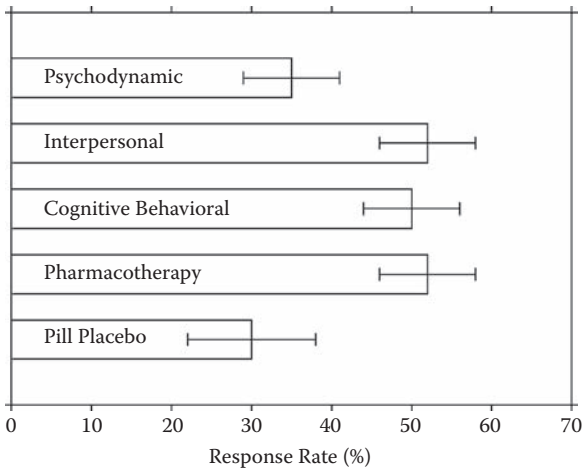


FIGURE 16.22 The efficacy of psychotherapy. The graph shows the mean (± SD) response rate of depressed patients who undergo different forms of psychotherapy compared with the mean response rate of patients receiving psychiatric medication or a pill placebo. Even for the best treatments, response rate is only 50%, while the response rate for placebo is up to 30%. (Source: Means and approximate SDs computed from data provided by Hollon, S. D., Thase, M. E. & Markowitz, J. C. (2002). Treatment and prevention of depression. *Psychological Science in the Public Interest* 3: 39–77.)

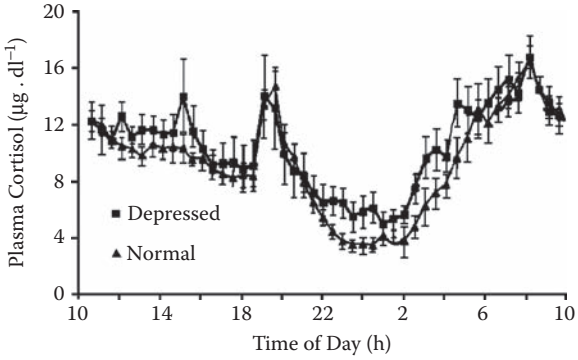


FIGURE 16.23 Depression and circadian rhythms. The graph shows the mean daily oscillation in plasma cortisol concentration (± SD) of 15 normal human subjects and 14 patients with major depression. Note that the nocturnal trough of the rhythm is less accentuated in depressed patients than in healthy controls. (Source: Adapted from Yehuda, R., Teicher, M. H., Trestman, R. L., Levengood, R. A. & Siever, L. J. (1996). Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. *Biological Psychiatry* 40: 79–88.)

abnormality reported in the literature is a small reduction in the amplitude of various daily rhythms, as exemplified in Figure 16.23. 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

7 P.M.) with a transient elevation in cortisol secretion. Note, however, that the nocturnal fall in cortisol concentration is slightly smaller in depressed patients than in normal subjects,[166] 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 example, in two recent studies, depressed patients were reported to have both delayed[168] and advanced[170] rhythms. It is possible that different rhythms behave differently (one group studied the melatonin rhythm while the other studied the cortisol rhythm), but if this possibility is true, the phase change evidently is not a characteristic of the central pacemaker. More important, 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. However, 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.[171–175] One suggestion was that bipolar (manic depressive) disorder results from an abnormally phase-advanced clock.[162] 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 treatment would eliminate the undesirable side effects that often accompany psychotropic medication, provide a "natural" alternative to pharmaceutical products, and greatly reduce the financial burden of drug treatment. (When this book was written, a year's supply of Prozac, for example, cost $1800). The data on which the suggestion was based, however, were very noisy and unconvincing. I averaged the various data sets and plotted the means in Figure 16.24. 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 delay of the circadian clock. The team's data are shown in Figure 16.25. The researchers 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).[168] However, the

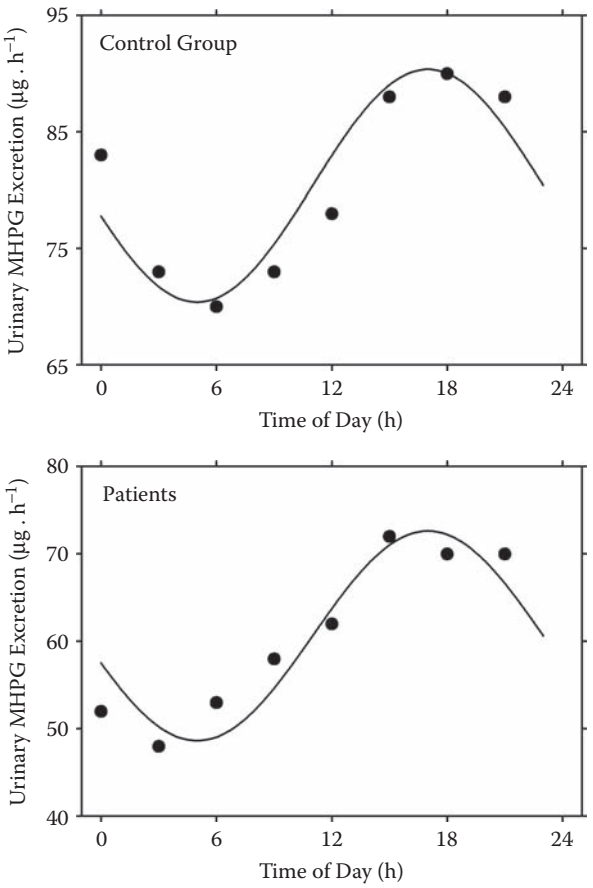


FIGURE 16.24 Are depressed patients phase-advanced? It has been suggested that the circadian clock of bipolar patients (manic depressives) is 2 to 4 hours advanced in relation to that of healthy controls. The graphs show the data purported to support the phase-advance hypothesis. The data points are the means of two separate studies, one with 10 patients and 14 controls, the other with 8 patients and 15 controls. MHPG (3-methoxy, 4-hydroxy-phenyl-glycol) 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 no difference between the acrophases of the control group and of the patients. (Source: Wehr, T. A. and Wirz-Justice, A. (1982). Circadian rhythm mechanisms in affective illness and in antidepressant drug action. *Pharmacopsychiatry* 15: 31–39.)

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.

Because of the claims of correlation between circadian phase and depression, many basic researchers conducted experimental research in laboratory animals to determine

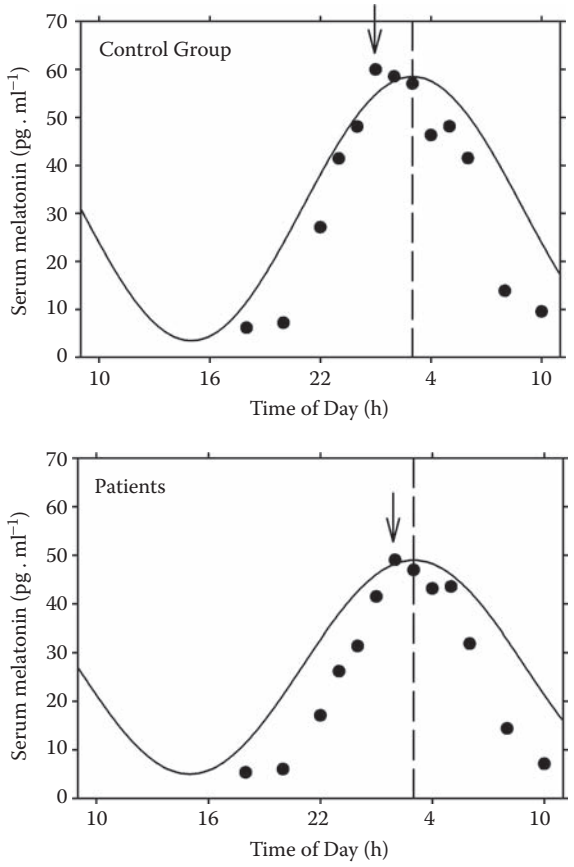


FIGURE 16.25 Are depressed patients phase-delayed? The graphs show the mean concentrations of serum melatonin of 14 patients with major depression and 14 healthy controls, as measured in bed rest from 6 P.M. to 10 A.M. Because the highest mean value in patients occurred over an hour after the mean value 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). (Source: Crasson, M., Kjiri, S., Colin, A., Kjiri, K., L'Hermite-Baleriaux, M., Ansseau, M. & Legros, J. J. (2004). Serum melatonin and urinary 6-sulfatoxymelatonin in major depression. *Psychoneuroendocrinology* 29: 1–12.)

whether the correlation implied causation. "Animal models" of depression admittedly have several limitations.[176] 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,[177–180] other drugs such as lithium (Eskalith®, Lithobid®),[181–184] fluoxetine (Prozac®),[185] clorgyline (an experimental antidepressant),[186,187] and rubidium (also an experimental antidepressant)[188] have been found to have small but significant effects. Thus, the hypothesis that circadian disruption

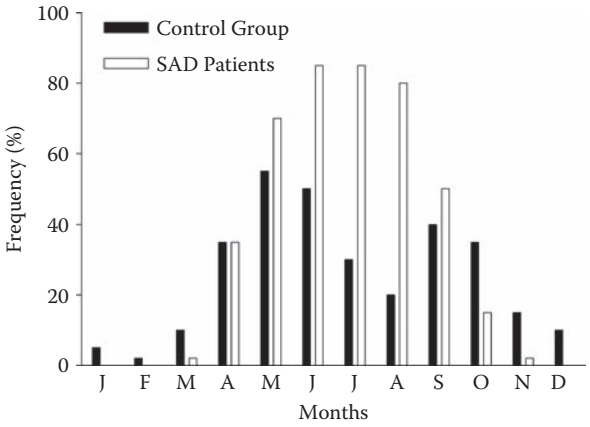


FIGURE 16.26 Seasonal depression. The graph shows the frequency distribution of answers to the question "When do you feel best?" asked to patients diagnosed with Seasonal Affective Disorder (SAD) and to a random sample of the general population (Control group). Although people in the control group feel best mostly in the spring and fall, SAD patients show a much sharper seasonal variation in mood. (Source: 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.)

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. 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 they could provide solid evidence to support the correlational association.

16.3.3 Seasonal Affective Disorder

One 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.[189,190] Chapter 4 showed that many organismal variables, including mood, exhibit annual rhythmicity. As shown in Figure 16.26, normal people (dark bars) feel best mostly in the spring and fall.[191,192] However, the distribution for SAD patients (white bars) is much sharper: no patient feels best during the winter months (December to February), and most of them feel best during the late spring and summer months (May to August).[192] 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 are many annual rhythms in animals (see Chapter 4). Although weather conditions were found to affect patients' self-rating of energy level, this effect was

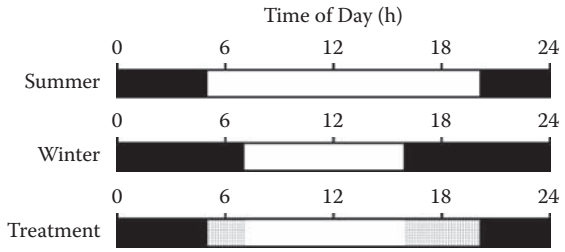


FIGURE 16.27 The rationale for phototherapy. Because seasonal affective disorder (SAD) is more common in the winter than in the summer, phototherapy was designed to simulate a summer photoperiod during the winter. Artificial light (hatched area) was considered to be necessary in the early morning and in the evening. Later studies revealed that artificial light only once a day may be sufficient.

FIGURE 16.28 Phototherapy. Timed exposure to artificial light is a common treatment for seasonal affective disorder (SAD). (Source: Image courtesy of BioLight Group, Santa Barbara, California.)

smaller than those of seasonal changes.[193] As a result, researchers attempted 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 16.27. During the summer, the days are long (sunlight from 5 A.M. to 8 P.M.), while the days are short in the winter (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 A.M. to 7 A.M. and from 4 P.M. to 8 P.M.

Various authors reported mood improvements in SAD patients subjected to this form of treatment,[194–197] and light boxes like the one pictured in Figure 16.28 gained popularity. However, it soon became clear that the effects of light therapy (or *phototherapy*) were not due to the extension of day length.[198–205] For example, it was found that a single interval of light stimulation in the morning was just as effective as the morning–evening combination and that

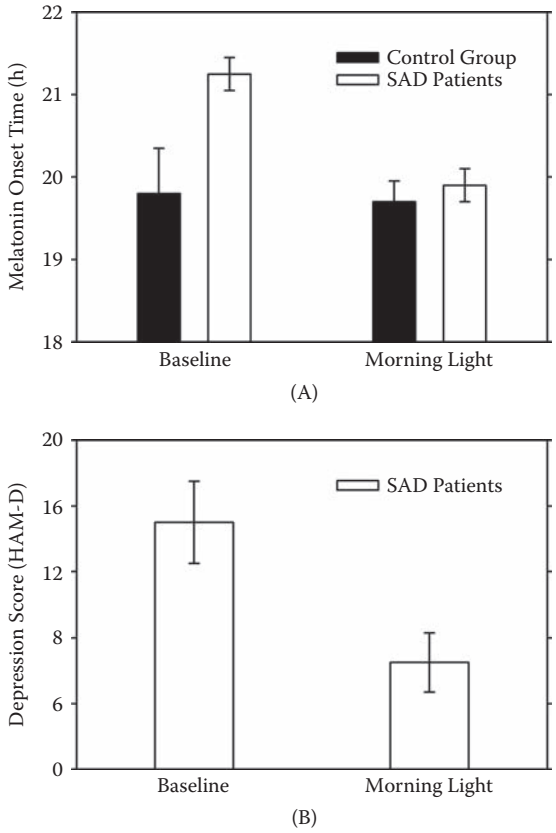


FIGURE 16.29 SAD and phase delay. The graphs show the onset time of melatonin secretion (as a phase marker of the circadian clock) and the depression score (in the Hamilton depression scale, HAM-D) of seasonal affective disorder (SAD) patients and healthy controls before and after phototherapy. Phototherapy 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 eight SAD patients and seven controls. Note 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). (Source: Lewy, A. J., Sack, R. L., Miller, K. S. & Hoban, T. M. (1987). Antidepressant and circadian phase-shifting effects of light. *Science* 235: 352–354.)

a single interval of stimulation in the early afternoon (which does not extend the day length) was also effective.[199] Attention was then focused on the potential phase-shifting effects of phototherapy. As discussed in Chapter 7, appropriately timed light pulses can phase shift circadian rhythms in humans and other animals. If, for example, SAD were associated with an intrinsic phase delay of the circadian clock, then light pulses presented in the early morning should evoke a compensatory phase advance. Figure 16.29 summarizes the results of a study that tested this possibility. The circadian phase marker used in the study was the onset time of nocturnal melatonin secretion. Clearly, SAD patients had delayed rhythms, as compared with 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. The depression scores of SAD patients also were reduced by the treatment (Panel B).[200]

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,[204,205] others did not.[206,207] In addition, while some found untreated SAD patients to have phase-delayed rhythms,[204,205] others found them to have normally phased rhythms.[196,208–210] The results of a careful study conducted on SAD patients and healthy individuals under controlled laboratory conditions are summarized in Figure 16.30. Patients were tested both when they were depressed and when they were not. Mean values of circadian 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.[209] As in patients with major depressive disorder, SAD patients had rhythms with smaller amplitude. Note, however, that the amplitude is smaller than that of controls both when the patients are depressed and when they have remitted. This finding implies that reduced amplitude is a constitutive characteristic of people predisposed to depression and not a functional property of the depressive state.

It would seem justified to conclude that the mechanism by which phototherapy relieves SAD symptoms is not yet known. However, whether or not the therapeutic action of bright light stimulation is understood, the treatment itself may be effective. A major problem involving efficacy evaluation in the treatment of SAD 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.[211] 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 to 40%).[192] 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).[212] Clearly, the outcome of treatment for SAD is not impressive. Attempts to conduct placebo-controlled studies by using sounds and dim lights as the placebo condition produced minimal or null evidence that phototherapy is more effective than placebo alone.[213,214] Much more research is 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."

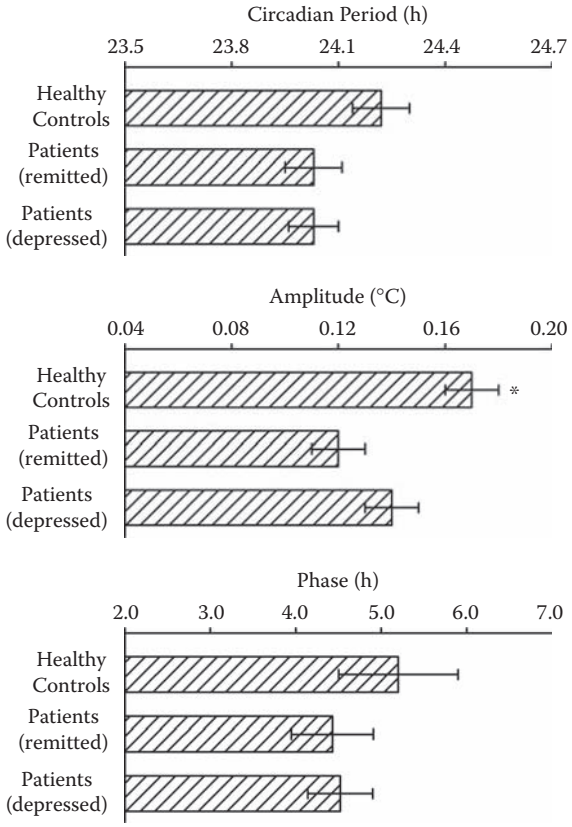


FIGURE 16.30 SAD and circadian rhythms. The graphs compare healthy controls with depressed and remitted seasonal affective disorder (SAD) patients in terms of circadian period, amplitude, and phase of the rhythm of melatonin secretion. The bars correspond to the means (± SE) of seven patients and seven controls. Healthy controls have slightly higher means in the three variables, but the values are significantly higher only in amplitude (asterisk). Note that depressed and remitted patients do not differ in any of the three variables. (Source: Koorengevel, K. M., Beersma, D. G. M., den Boer, J. A. & 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.)

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 cases of insomnia may be due to disorders of the circadian system. People suffering from Delayed Sleep Phase Syndrome (DSPS) experience sleep-onset insomnia, while people suffering from Advanced Sleep Phase Syndrome (ASPS) experience sleep-termination insomnia. These sleep disorders often are 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 evidence suggests 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 SAD.

EXERCISES

Exercise 16.1 Are you depressed?

Section 16.3 showed 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. The diagnosis will be unreliable if you are not honest and accurate in your answers. Also, although the questions are based on valid questionnaires developed by practicing psychiatrists, *the program 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.

Exercise 16.2 Can you sleep well at night?

Section 16.2 discussed sleep disorders, particularly those due to circadian dysfunction. The program Health used in the previous exercise contains a section on sleep disorders. 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. The program assumes 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, consult a sleep therapist. Most hospitals have a sleep clinic or are associated with one.

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Halberg, F. & Watanabe, H. (Eds.). (1992). *Chronobiology and Chronomedicine*. Tokyo: 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.

Smolensky, M. & Lamberg, L. (2001). *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. 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. & Van Cauter, E. (2002). Sleep and biological clocks. In: Weiner, I. B. (Ed.). *Biological Psychology* (Volume 3 of *Handbook of Psychology*). 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.). (1999). *Sleep Disorders Medicine: Basic Science, Technical Considerations, and Clinical Aspects* (2nd Edition). New York: Butterworth–Heinemann Medical. A handbook of sleep medicine for medical practitioners.

Baum, A., Newman, S., Weinman, J., West, R. & McManus, C. (Eds.). (1997). *Cambridge Handbook of Psychology, Health and Medicine*. Cambridge, UK: 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. (1998). *Winter Blues: Seasonal Affective Disorder* (Updated Edition). New York: Guilford Press. A book on seasonal affective disorder written for general audiences by a renowned medical researcher from the National Institute of Mental Health.

WEB SITES TO EXPLORE

American Psychiatric Association:
http://www.psych.org
American Psychological Association:
http://www.apa.org
Circadian Lighting Association:
http://www.claorg.org
Feeling Good (David D. Burns, MD):
http://www.FeelingGood.com
Lighting Research Center at Rensselaer Polytechnic Institute:
http://www.lrc.rpi.edu/programs/lighthealth/
Sleep Network:
http://www.sleepnet.com
U.S. National Institute of Mental Health:
http://www.nimh.nih.gov

LITERATURE CITED

1. Smolensky, M. H. & 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. & 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. & Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
8. Mellette, H. C., Hutt, B. K., Askovitz, S. I. & 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. & 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. & Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
13. Marotte, H. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & 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. & Morris, M. (1993). The sleep-evoked decrease of body temperature. *Sleep* 16: 93–99.
24. Lee, Y. H. & 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. & 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. & 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. & 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. & 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. & 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. & Davenne, D. (2002). Circadian rhythms during cycling exercise and finger-tapping task. *Chronobiology International* 19: 1137–1149.
31. Murray, G., Allen, N. B. & Trinder, J. (2002). Mood and the circadian system: investigation of a circadian component in positive affect. *Chronobiology International* 19: 1151–1169.
32. Varkevisser, M. & 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. & 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. Halberg, F., Visscher, M. B., Flink, E. B., Berge, K. & Bock, F. (1951). Diurnal rhythmic changes in blood eosinophil levels in health and in certain diseases. *Journal-Lancet* 71: 312–319.
35. Ganong, W. F. (2001). *Review of Medical Physiology,* 20th Edition. New York: McGraw-Hill.
36. Williams, R. S., Willard, H. F. & Snyderman, R. (2003). Personalized health planning. *Science* 300: 549.
37. Hermida, R. C., Ayala, D. E., Fernández, J. R., Mojón, A., Alonso, I. & Calvo, C. (2002). Modeling the circadian variability of ambulatorily monitored blood pressure by multiple-component analysis. *Chronobiology International* 19: 461–481.
38. Leary, A. C., Struthers, A. D., Donnan, P. T., MacDonald, T. M. & 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.
39. Hermida, R. C., Fernández, J. R., Ayala, D. E., Mojón, A., Alonso, I. & Calvo, C. (2004). Circadian time-qualified tolerance intervals for ambulatory blood pressure monitoring in the diagnosis of hypertension. *Chronobiology International* 21: 147–160.
40. Kriebel, J. (1974). Changes in internal phase relationships during isolation. In: Scheving, L. E., Halberg, F. & Pauly, J. E. (Eds.). *Chronobiology.* Tokyo: Igaku Shoin, pp. 451–459.
41. Taillard, J., Sanchez, P., Lemoine, P. & 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.
42. Kumagai, Y., Shiga, T., Sunaga, K., Cornélissen, G., Ebihara, A. & Halberg, F. (1992). Usefulness of circadian amplitude of blood pressure in predicting hypertensive cardiac involvement. *Chronobiologia* 19: 43–58.
43. Scheer, F. A. J. L., van Doornen, L. J. P. & 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.
44. Viola, A. U., Simon, C., Ehrhart, J., Geny, B., Piquard, F., Muzet, A. & 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.
45. Lee, M. S., Lee, J. S., Lee, J. Y., Cornélissen, G., Otsuka, K. & Halberg, F. (2003). About 7-day (circaseptan) and circadian changes in cold pressor test (CPT). *Biomedicine and Pharmacotherapy* 57: 39s–44s.
46. O'Sullivan, C., Duggan, J., Atkins, N. & 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.
47. Van Eekelen, A. P. J., Houtveen, J. H. & Kerkhof, G. A. (2004). Circadian variation in cardiac autonomic activity: reactivity measurements to different types of stressors. *Chronobiology International* 21: 107–129.
48. Hadtstein, C., Wühl, E., Soergel, M., Witte, K. & Schaefer, F. (2004). Normative values for circadian and ultradian cardiovascular rhythms in childhood. *Hypertension* 43: 547–554.
49. Hermida, R. C., Calvo, C., Ayala, D. E., Domínguez, M. J., Covelo, M., Fernández, J. R., Fontao, M. J. & López, J. E. (2004). Administration-time-dependent effects of doxazosin GITS on ambulatory blood pressure of hypertensive subjects. *Chronobiology International* 21: 277–296.
50. Watanabe, Y., Cornélissen, G., Halberg, F., Bingham, C., Siegelová, J., Otsuka, K. & Kikuchi, T. (1997). Incidence pattern and treatment of a clinical entity: overswinging or circadian hyperamplitudetension (CHAT). *Scripta Medica* 70: 245–261.
51. Halberg, F., Cornélissen, G., Katinas, G., Syutkina, E.V., Sothern, R.B., Zaslavskaya, R., Halberg, F., Watanabe, Y., Schwartzkopff, O., Otsuka, K., Tarquini, R., Frederico, P. & Siggelova, J. (2003). Transdisciplinary unifying implications of circadian findings in the 1950s. *Journal of Circadian Rhythms* 1: art. 2.
52. Fromm, R. E., Levine, R. L. & Pepe, P. E. (1992). Circadian variation in the time of request for helicopter transport of cardiac patients. *Annals of Emergency Medicine* 21: 1196–1199.
53. Spielberg, C., Falkenhahn, D., Willich, S. N., Wegscheider, K. & 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.

54. López-Messa, J. B., Garmendia-Leiza, J. R., Aguilar-García, M. D., Andrés-de-Llano, J. M., Alberola-López, C. & Ardura-Fernández, J. (2004). Factores de riesgo cardiovascular en el ritmo circadiano del infarto agudo de miocardio. *Revista Española de Cardiología* 57: 850–858.
55. Tükek, T., Yildiz, P., Atilgan, D., Tuzcu, V., Eren, M., Erk, O., Demirel, S., Akkaya, V., Dilmener, M. & 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.
56. Scheving, L. E., Vedral, D. F. & Pauly, J. E. (1968). A circadian susceptibility rhythm in rats to pentobarbital sodium. *Anatomical Record* 160: 741–750.
57. Rebuelto, M., Ambros, L., Waxman, S. & Montoya, L. (2004). Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. *Chronobiology International* 21: 591–600.
58. Yamauchi, A., Oishi, R. & 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.
59. Soulban, G., Smolensky, M. H. & Yonovitz, A. (1990). Gentamicin-induced chronotoxicity: use of body temperature as a circadian marker. *Chronobiology International* 7: 393–402.
60. Williams, R. L., Soliman, K. F. A. & Mizinga, K. M. (1993). Circadian variation in tolerance to the hypothermic action of CNS drugs. *Pharmacology, Biochemistry and Behavior* 46: 283–288.
61. 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.
62. Lemmer, B. (2000). Relevance for chronopharmacology in practical medicine. *Seminars in Perinatology* 24: 280–290.
63. Milano, G. & Chamorey, A. L. (2002). Clinical pharmacokinetics of 5-fluorouracil with consideration of chronopharmacokinetics. *Chronobiology International* 19: 177–189.
64. Lévi, F., Zidani, R. & Misset, J. L. (1997). Randomised multicentre trial of chronotherapy with oxaliplatin, fluorouracil, and folinic acid in metastatic colorectal cancer. *Lancet* 350: 681–686.
65. Lévi, F., Giacchetti, S., Zidani, R., Brezault-Bonnet, C., Tigaud, J. M., Goldwasser, F. & Misset, J. L. (2001). Chronotherapy of colorectal cancer metastases. *Hepato-Gastroenterology* 48: 320–322.
66. Hrushesky, W. J. (1985). Circadian timing of cancer chemotherapy. *Science* 228: 73–75.
67. Keith, L. G., Oleszczuk, J. J. & Laguens, M. (2001). Circadian rhythm chaos: a new breast cancer marker. *International Journal of Fertility* 46: 238–247.
68. Mormont, M. C., Waterhouse, J., Bleuzen, P., Giacchetti, S., Jami, A., Bogdan, A., Lellouch, J., Misset, J. L., Touitou, Y. & 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.
69. National Center for Environmental Health (2003). *Behavioral Risk Factor Surveillance System: Asthma*. Atlanta, GA: Centers for Disease Control and Prevention.
70. Pincus, D. J., Beam, W. R. & Martin, R. J. (1995). Chronobiology and chronotherapy of asthma. *Clinics in Chest Medicine* 16: 699–713.
71. Jindal, S. K., Aggarwal, A. N. & Gupta, D. (2002). Diurnal variability of peak expiratory flow. *Journal of Asthma* 39: 363–373.
72. Turner-Warwick, M. (1988). Epidemiology of nocturnal asthma. *American Journal of Medicine* 85(1B): 6–8.
73. Dethlefsen, U. & Repgas, R. (1985). Ein neues Therapieprinzip bei nachtlichen Asthma. *Klinische Medizin* 80: 44–47.
74. Smyth, J. M., Soefer, M. H., Hurewitz, A., Kliment, A. & 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.
75. Lebowitz, M. D., Krzyzanowski, M., Quackenboss, J. J. & O'Rourke, M. K. (1997). Diurnal variation of PEF and its use in epidemiological studies. *European Respiratory Journal* 24: 49S–56S.
76. Meijer, G. G., Postma, D. S., van der Heide, S., de Reus, D. M., Koeter, G. H., Roorda, R. J. & 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.
77. Burioka, N., Sako, T., Tomita, K., Miyata, M., Suyama, H., Igishi, T. & Shimizu, E. (2001). Theophylline chronotherapy of nocturnal asthma using bathyphase of circadian rhythm in peak expiratory flow rate. *Biomedicine and Pharmacotherapy* 55: 142–146.
78. Kondo, S. & Ito, M. (1997). Reevaluation of the relationship between within-day bronchial variability and bronchial responsiveness in asthmatic children. *Chronobiology International* 14: 61–69.
79. Dottorini, M. L., Tantucci, C., Peccini, F., Grassi, V. & Sorbini, C. A. (1996). Diurnal change of bronchial caliber and airway responsiveness in asthmatics during long-term treatment with long-acting β-2 agonist salmeterol. *International Journal of Clinical Pharmacology and Therapeutics* 34: 438–443.
80. Kraft, M., Wenzel, S. E., Bettinger, C. M. & Martin, R. J. (1997). The effect of salmeterol on nocturnal symptoms, airway function, and inflammation in asthma. *Chest* 111: 1249–1254.
81. Troyanov, S., Ghezzo, H., Cartier, A. & Malo, J. L. (1994). Comparison of circadian variations using FEV1 and peak expiratory flow rates among normal and asthmatic subjects. *Thorax* 49: 775–780.

82. Karras, D. J., D'Alonzo, G. E. & Heilpern, K. L. (1995). Is circadian variation in asthma severity relevant in the emergency department? *Annals of Emergency Medicine* 26: 558–562.
83. Brenner, B. E., Chavda, K. K., Karakurum, M. B., Karras, D. J. & Camargo, C. A. (2001). Circadian differences among 4,096 emergency department patients with acute asthma. *Critical Care Medicine* 29: 1124–1129.
84. Reinberg, A., Gervais, P., Chaussade, M., Fraboulet, G. & Duburque, B. (1983). Circadian changes in effectiveness of corticosteroids in eight patients with allergic asthma. *Journal of Allergy and Clinical Immunology* 71: 425–433.
85. Reinberg, A., Pauchet, F., Ruff, F., Gervais, A., Smolensky, M. H., Levi, F., Gervais, P., Chaouat, D., Abella, M. L. & Zidani, R. (1987). Comparison of once-daily evening versus morning sustained release theophylline dosing for nocturnal asthma. *Chronobiology International* 4: 409–419.
86. Morrison, J., Pearson, S. & Dean, H. (1988). Parasympathetic nervous system in nocturnal asthma. *British Medical Journal* 296: 1427–1429.
87. Gaultier, L., Reinberg, A. & Motohashi, Y. (1988). Circadian rhythm in total pulmonary resistance of asthmatic children: effects of a β-agonist agent. *Chronobiology International* 5: 285–290.
88. Daan, S., Beersma, D. G. M. & Borbély, A. A. (1984). Timing of human sleep: recovery process gated by a circadian pacemaker. *American Journal of Physiology* 246: R161–R178.
89. Parmeggiani, P. L. & 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.
90. Schmidek, W. R., Hoshino, K., Schmidek, M. & Timo-Iaria, C. (1972). Influence of environmental temperature on the sleep-wakefulness cycle in the rat. *Physiology and Behavior* 8: 363–371.
91. Szymusiak, R. & Satinoff, E. (1981). Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate. *Physiology and Behavior* 26: 687–690.
92. Roussel, B., Turrillot, P. & Kitahama, K. (1984). Effect of ambient temperature on the sleep-waking cycle in two strains of mice. *Brain Research* 294: 67–73.
93. Muzet, A., Libert, J. P. & Candas, V. (1984). Ambient temperature and human sleep. *Experientia* 40: 425–429.
94. Obál, Jr., F., Rubicsek, G., Alföldi, P., Sáry, G. & 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.
95. Heller, H. C., Graf, R. & Rautenberg, W. (1983). Circadian and arousal state influences on thermoregulation in the pigeon. *American Journal of Physiology* 245: R321–R328.
96. Abrams, R. & Hammel, H. T. (1964). Hypothalamic temperature in unanesthetized albino rats during feeding and sleeping. *American Journal of Physiology* 206: 641–646.
97. Glotzbach, S. F. & Heller, H. C. (1976). Central nervous regulation of body temperature during sleep. *Science* 194: 537–539.
98. Glotzbach, S. F. & Heller, H. C. (1984). Changes in the thermal characteristics of hypothalamic neurons during sleep and wakefulness. *Brain Research* 309: 17–26.
99. Schmidek, W. R., Zachariassen, K. E. & 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.
100. Walker, J. M., Walker, L. E., Harris, D. V. & Berger, R. J. (1983). Cessation of thermoregulation during REM sleep. *American Journal of Physiology* 244: R114–R118.
101. Hendricks, J. C. (1982). Absence of shivering in the cat during paradoxical sleep without atonia. *Experimental Neurology* 75: 700–710.
102. Amoros, C., Sagot, J. C., Libert, J. P. & Candas, V. (1986). Sweat gland response to local heating during sleep in man. *Journal de Physiology* 81: 209–215.
103. Shapiro, C. M., Moore, A. T., Mitchell, D. & Yodaiken, M. L. (1974). How well does man thermoregulate during sleep? *Experientia* 30: 1279–1281.
104. Sagot, J. C., Amoros, C., Candas, V. & Libert, J. P. (1987). Sweating responses and body temperatures during nocturnal sleep in humans. *American Journal of Physiology* 252: R462–R470.
105. Dewasmes, G., Bothorel, B., Candas, V. & 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.
106. Tayefeh, F., Plattnet, O., Sessler, D. I., Ikeda, T. & Marder, D. (1998). Circadian changes in the sweating-to-vasoconstriction interthreshold range. *Pflügers Archiv* 435: 402–406.
107. Gates, A. I. (1916). Diurnal variations in memory and association. *University of California Publications in Psychology* 1: 323–344.
108. 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.
109. Binkley, S., Tome, M. B. & Mosher, K. (1989). Weekly phase shifts of rhythms self-reported by almost feral human students in the U.S.A. and Spain. *Physiology and Behavior* 46: 423–427.
110. Partinen, M. & Hublin, C. (2000). Epidemiology of sleep disorders. In: Kryger, M. H., Roth, T. & Dement, W. C. (Eds.). *Principles and Practice of Sleep Medicine*. Philadelphia: Saunders, pp. 558–579.
111. Buysse, D. J., Carrier, J., Dew, M. A., Hall, M., Monk, T. H., Nowell, P., Reynolds, C. F. & Kupfer, D. J. (2001). Sleep disorders. In: Takahashi, J. S., Turek, F. W. & Moore, R. Y. (Eds.). *Circadian Clocks* (*Handbook of Behavioral Neurobiology, Volume 12*). New York: Kluwer/Plenum, pp. 645–683.
112. Olders, H. (2003). What causes insomnia? *Scientific American* 289(4): 103.

113. Mercer, J. D., Bootzin, R. R. & Lack, L. C. (2002). Insomniacs' perception of wake instead of sleep. *Sleep* 25: 564–571.
114. Rosa, R. R. & Bonnet, M. H. (2000). Reported chronic insomnia is independent of poor sleep as measured by electroencephalography. *Psychosomatic Medicine* 62: 474–482.
115. Campbell, S. S., Murphy, P. J., Van Den Heuvel, C. J., Roberts, M. L. & Stauble, T. N. (1999). Etiology and treatment of intrinsic circadian rhythm sleep disorders. *Sleep Medicine Reviews* 3: 179–200.
116. Vignau, J., Dahlitz, M., Arendt, J., English, J. & 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, UK: Pergamon, pp. 261–271.
117. Toh, K. L., Jones, C. R., He, Y., Eide, E. J., Hinz, W. A., Virshup, D. M., Ptácek, L. J. & Fu, Y. H. (2001). An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* 291: 1040–1043.
118. Weitzman, E. D., Czeisler, C. A., Coleman, R. M., Spielman, A. J., Zimmerman, J. C., Dement, W., Richardson, G. & Pollack, C. P. (1981). Delayed sleep phase syndrome: a chronobiological disorder with sleep-onset insomnia. *Archives of General Psychiatry* 38: 737–746.
119. Alvarez, B., Dahlitz, M. J., Vignau, J. & 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.
120. Morris, M., Lack, L. & Dawson, D. (1990). Sleep-onset insomniacs have delayed temperature rhythms. *Sleep* 13: 1–14.
121. Lack, L. & 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.
122. Lack, L. C., Mercer, J. D. & Wright, H. (1996). Circadian rhythms of early morning awakening insomniacs. *Journal of Sleep Research* 5: 211–219.
123. Terman, M., Lewy, A. J., Dijk, D. J., Boulos, Z., Eastman, C. I. & Campbell, S. S. (1995). Light treatment for sleep disorders: Consensus report. IV. Sleep phase and duration disturbances. *Journal of Biological Rhythms* 10: 135–147.
124. Sifton, D. W. (Ed.) (2003). *Physician's Desk Reference,* 57th Edition. Montvale, N.J.: Thomson PDR.
125. Czeisler, C. A., Richardson, G., Coleman, R., Zimmerman, J., Moore-Ede, M., Dement, W. & Weitzman, E. (1981). Chronotherapy: resetting the circadian clocks of patients with delayed sleep phase insomnia. *Sleep* 4: 1–21.
126. Monk, T. H., Reynolds, C. F., Buysse, D. J., DeGrazia, J. M. & Kupfer, D. J. (2003). The relationship between lifestyle regularity and subjective sleep quality. *Chronobiology International* 20: 97–107.
127. Monk, T. H., Buysse, D. J., Billy, B. D. & 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.
128. Lewy, A. J., Sack, R. L. & Singer, C. M. (1985). Treating phase typed chronobiologic sleep and mood disorders using appropriately timed bright artificial light. *Psychopharmacological Bulletin* 21: 368–372.
129. Rosenthal, N. E., Joseph-Vanderpool, J. R., Levendosky, A. A., Johnston, S. H., Allen, R., Kelly, K. A., Souêtre, E., Schultz, P. M. & Starz, K. (1990). Phase-shifting effects of bright morning light as treatment for delayed sleep phase syndrome. *Sleep* 13: 354–361.
130. Watanabe, T., Kajimura, N., Kato, M., Sekimoto, M. & Takahashi, K. (1999). Effects of phototherapy in patients with delayed sleep phase syndrome. *Psychiatry and Clinical Neurosciences* 53: 231–233.
131. Kirisoglu, C. & 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.
132. Dahlitz, M., Alvarez, B., Vignau, J., English, J., Arendt, J. & Parkes, J. D. (1991). Delayed sleep phase syndrome response to melatonin. *Lancet* 337: 1121–1123.
133. Tzischinsky, O., Dagan, Y. & Lavie, P. (1993). The effects of melatonin on the timing of sleep in patients with delayed sleep phase syndrome. In: Touitou, Y., Arendt, J. & Pévet, P. (Eds.). *Melatonin and the Pineal Gland*. New York: Elsevier, pp. 351–354.
134. Zhdanova, I. V. & Wurtman, R. J. (1997). Efficacy of melatonin as a sleep-promoting agent. *Journal of Biological Rhythms* 12: 644–650.
135. Sack, R. L., Lewy, A. J. & Hughes, R. J. (1998). Use of melatonin for sleep and circadian rhythm disorder. *Annals of Medicine* 30: 115–121.
136. Lewy, A. J., Emens, J. S., Bernert, R. A. & 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.
137. U.S. Department of Health and Human Services (1999). *Mental Health: A Report of the Surgeon General*. Rockville, MD: National Institute of Mental Health.
138. Cowan, W. M., Kopnisky, K. L. & Hyman, S. E. (2002). The human genome project and its impact on psychiatry. *Annual Review of Neuroscience* 25: 1–50.
139. Kessler, R. C. & Zhao, S. (1999). The prevalence of mental illness. In: Horvitz, A. V. & 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.
140. Lykken, D. & Tellegen, A. (1996). Happiness is a stochastic phenomenon. *Psychological Science* 7: 186–189.
141. American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders*, 4th Edition. Washington, DC: American Psychiatric Association.

142. American Psychological Association (2000). *Graduate Study in Psychology*, 33rd Edition. Washington, DC: American Psychological Association.
143. Hollon, S. D., Thase, M. E. & Markowitz, J. C. (2002). Treatment and prevention of depression. *Psychological Science in the Public Interest* 3: 39–77.
144. Schildkraut, J. J. (1965). The catecholamine hypothesis of affective disorders: a review of supporting evidence. *American Journal of Psychiatry* 122: 509–522.
145. Masand, P. S. & Gupta, S. (1999). Selective serotonin-reuptake inhibitors: an update. *Harvard Review of Psychiatry* 7: 69–84.
146. Hiemke, C. & Hartter, S. (2000). Pharmacokinetics of selective serotonin reuptake inhibitors. *Pharmacology and Therapeutics* 85: 11–28.
147. Kramer, M. S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J. J., Reines, S. A., Liu, G., Snavely, D., Wyatt-Knowles, E., Hale, J. J., Mills, S. G., MacCoss, M. et al (1998). Distinct mechanism for antidepressant activity by blockade of central substance P receptors. *Science* 281: 1640–1645.
148. Enserink, M. (1999). Can the placebo be the cure? *Science* 284: 238–240.
149. Santarelli, L., Saxe, M., Gross, C., Surget, A., Battaglia, F., Dulawa, S., Weisstaub, N., Lee, J., Duman, R., Arancio, O., Belzung, C. & Hen, R. (2003). Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* 301: 805–809.
150. Bandura, A. (1969). *Principles of Behavior Modification*. New York: Holt, Rinehart and Winston.
151. Eysenck, H. J. (1952). The effects of psychotherapy: an evaluation. *Journal of Consulting Psychology* 16: 319–324.
152. Levitt, E. E. (1957). The results of psychotherapy with children: an evaluation. *Journal of Consulting Psychology* 21: 189–196.
153. Frank, J. D. (1979). The present status of outcome studies. *Journal of Consulting and Clinical Psychology* 47: 310–316.
154. Parloff, M. B. (1984). Psychotherapy research and its incredible credibility crisis. *Clinical Psychology Review* 4: 95–109.
155. Kopta, S. M., Lueger, R. J., Saunders, S. M. & 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.
156. Demeza, K. F. (1998). Getting personal: the business consulting approach to solving personal problems. *Marketeer Birmingham Business Today* November: 7.
157. Szasz, T. S. (1961). *The Myth of Mental Illness*. New York: Hoeber-Harper.
158. Foucault, M. (1972). *Histoire de la Folie à l'Âge Classique*. Paris: Gallimard.
159. Pennebaker, J. W. & Graybeal, A. (2001). Patterns of natural language use: disclosure, personality, and social integration. *Current Directions in Psychological Science* 10: 90–93.
160. Nikitopoulou, G. & Crammer, J. L. (1976). Change in diurnal temperature rhythm in manic-depressive illness. *British Medical Journal* 272: 1311–1314.
161. Pflug, B., Johnsson, A. & Ekse, T. (1981). Manic-depressive states and daily temperature: some circadian studies. *Acta Psychiatrica Scandinavica* 63: 277–289.
162. Wehr, T. A. & Wirz-Justice, A. (1982). Circadian rhythm mechanism in affective illness and in antidepressant drug action. *Pharmacopsychiatry* 15: 31–39.
163. Souetre, E., Salvati, E., Wehr, T. A., Sack, D. A., Krebs, B. & 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.
164. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K. & Takahashi, S. (1990). Circadian rhythms in depression. Part II. Circadian rhythms in inpatients with various mental disorders. *Journal of Affective Disorders* 18: 199–210.
165. Nagayama, H., Hasama, N., Tsuchiyama, K., Yamada, K., Ihara, J. & Yanagisawa, T. (1992). Circadian temperature and sleep-wake rhythms in depression. *Japanese Journal of Psychiatry and Neurology* 46: 244–245.
166. Yehuda, R., Teicher, M. H., Trestman, R. L., Levengood, R. A. & Siever, L. J. (1996). Cortisol regulation in posttraumatic stress disorder and major depression: a chronobiological analysis. *Biological Psychiatry* 40: 79–88.
167. 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.
168. Crasson, M., Kjiri, S., Colin, A., Kjiri, K., L'Hermite-Baleriaux, M., Ansseau, M. & Legros, J. J. (2004). Serum melatonin and urinary 6-sulfatoxymelatonin in major depression. *Psychoneuroendocrinology* 29: 1–12.
169. Armitage, R., Hoffman, R., Emslie, G., Rintelman, J., Moore, J. & Lewis, K. (2004). Rest-activity cycles in childhood and adolescent depression. *Journal of the Academy of Child and Adolescent Psychiatry* 43: 761–769.
170. Koenigsberg, H. W., Teicher, M. H., Mitropoulou, V., Navalta, C., New, A. S., Trestman, R. & 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.
171. Kripke, D. F., Mullaney, D. J., Atkinson, M. & Wolf, S. (1978). Circadian rhythm disorders in manic-depressives. *Biological Psychiatry* 13: 335–351.
172. Goodwin, F. K., Wirz-Justice, A. & 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. & Racagni, G. (Eds.). *Typical and Atypical Antidepressants: Clinical Practice*. New York: Raven, pp. 1–11.
173. Ehlers, C. L., Frank, E. & Kupfer, D. J. (1988). Social zeitgebers and biological rhythms. *Archives of General Psychiatry* 45: 948–952.

174. Szuba, M. P., Yager, A., Guze, B. H., Allen, E. M. & Baxter, L. R. (1992). Disruption of social circadian rhythms in major depression: a preliminary report. *Psychiatry Research* 42: 221–230.
175. Healy, D. & Waterhouse, J. M. (1995). Ther circadian system and the therapeutics of the affective disorders. *Pharmacology and Therapeutics* 65: 241–263.
176. Willner, P. (1984). The validity of animal models of depression. *Psychopharmacology* 83: 1–16.
177. Baltzer, V. & Weiskrantz, L. (1975). Antidepressant agents and reversal of diurnal activity cycles in the rat. *Biological Psychiatry* 10: 199–209.
178. Aschoff, J. (1989). Circadian activity rhythms in hamsters and rats treated with imipramine in the drinking water. *Chronobiologia* 16: 9–20.
179. Greco, A. M., Gambardella, P., Sticchi, R., D'Aponte, D. & de Franciscis, P. (1990). Chronic administration of imipramine antagonizes deranged circadian rhythm phases in individually housed rats. *Physiology and Behavior* 48: 67–72.
180. Refinetti, R. & Menaker, M. (1993). Effects of imipramine on circadian rhythms in the golden hamster. *Pharmacology, Biochemistry and Behavior* 45: 27–33.
181. Kripke, D. F. & Wyborney, V. G. (1980). Lithium slows rat circadian activity rhythms. *Life Sciences* 26: 1319–1321.
182. McEachron, D. L., Kripke, D. F., Sharp, F. R., Lewy, A. J. & McClellan, D. E. (1985). Lithium effects on selected circadian rhythms in rats. *Brain Research Bulletin* 15: 347–350.
183. Hafen, T. & 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.
184. 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.
185. Possidente, B., Lumia, A. R., McEldowney, S. & Rapp, M. (1992). Fluoxetine shortens circadian period for wheel running activity in mice. *Brain Research Bulletin* 28: 629–631.
186. Duncan, W. C., Tamarkin, L., Sokolove, P. G. & 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.
187. Duncan, W. C. & 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.
188. Hallonquist, J., Lindegger, M. & Mrosovsky, N. (1994). Rubidium chloride fuses split circadian activity rhythms in hamsters housed in bright constant light. *Chronobiology International* 11: 65–71.
189. Jacobsen, F. M., Wehr, T. A., Sack, D. A., James, S. P. & 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.
190. Chung, Y. S. & Daghestani, A. N. (1989). Seasonal affective disorder: shedding light on a dark subject. *Postgraduate Medicine* 86: 309–314.
191. Harmatz, M. G., Well, A. D., Overtree, C. E., Kawamura, K. Y., Rosal, M. & Ockene, I. S. (2000). Seasonal variation of depression and other moods: a longitudinal approach. *Journal of Biological Rhythms* 15: 344–350.
192. 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.
193. Albert, P. S., Rosen, L. N., Alexander, J. R. & Rosenthal, N. E. (1991). Effect of daily variation in weather and sleep on seasonal affective disorder. *Psychiatry Research* 36: 51–63.
194. Rosenthal, N. E., Levendosky, A. A., Skwerer, R. G., Joseph-Vanderpool, J. R., Kelly, K. A., Hardin, T., Kasper, S., DellaBella, P. & Wehr, T. A. (1990). Effects of light treatment on core body temperature in seasonal affective disorder. *Biological Psychiatry* 27: 39–50.
195. Magnusson, A. & 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.
196. Eastman, C. I., Gallo, L. C., Lahmeyer, H. W. & Fogg, L. F. (1993). The circadian rhythm of temperature during light treatment for winter depression. *Biological Psychiatry* 34: 210–220.
197. Stewart, J. W., Quitkin, F. M., Terman, M. & Terman, J. S. (1990). Is seasonal affective disorder a variant of atypical depression? Differential response to light therapy. *Psychiatry Research* 33: 121–128.
198. Wehr, T. A., Jacobsen, F. M., Sack, D. A., Arendt, J., Tamarkin, L. & Rosenthal, N. E. (1986). Phototherapy of seasonal affective disorder. *Archives of General Psychiatry* 43: 870–875.
199. Jacobsen, F. M., Wehr, T. A., Skwerer, R. A., Sack, D. A. & Rosenthal, N. E. (1987). Morning versus midday phototherapy of seasonal affective disorder. *American Journal of Psychiatry* 144: 1301–1305.
200. Lewy, A. J., Sack, R. L., Miller, K. S. & Hoban, T. M. (1987). Antidepressant and circadian phase-shifting effects of light. *Science* 235: 352–354.
201. Avery, D. H., Khan, A., Dager, S. R., Cox, G. B. & Dunner, D. L. (1990). Bright light treatment of winter depression: morning versus evening light. *Acta Psychiatrica Scandinavica* 82: 335–338.
202. Ruhrmann, S., Kasper, S., Hawellek, B., Martinez, B., Höflich, G., Nickelsen, T. & Möller, H. J. (1998). Effects of fluoxetine *versus* bright light in the treatment of seasonal affective disorder. *Psychological Medicine* 28: 923–933.
203. Terman, M., Terman, J. S. & 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.

204. Lewy, A. J., Bauer, V. K., Cutler, N. L., Sack, R. L., Ahmed, S., Thomas, K. H., Blood, M. L. & Jackson, J. M. L. (1998). Morning vs. evening light treatment of patients with winter depression. *Archives of General Psychiatry* 55: 890–896.
205. Terman, J. S., Terman, M., Lo, E. S. & Cooper, T. B. (2001). Circadian time of morning light administration and therapeutic response in winter depression. *Archives of General Psychiatry* 58: 69–75.
206. Avery, D. H., Kizer, D., Bolte, M. A. & Hellekson, C. (2001). Bright light therapy of subsyndromal seasonal affective disorder in the workplace: morning vs. afternoon exposure. *Acta Psychiatrica Scandinavica* 103: 1–8.
207. Burgess, H. J., Fogg, L. F., Young, M. A. & Eastman, C. I. (2004). Bright light therapy for winter depression: is phase advancing beneficial? *Chronobiology International* 21: 759–775.
208. Levendosky, A. A., Joseph-Vanderpool, J. R., Hardin, T., Sorek, E. & 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.
209. Koorengevel, K. M., Beersma, D. G. M., den Boer, J. A. & 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.
210. Koorengevel, K. M., Beersma, D. G. M., den Boer, J. A. & 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.
211. Terman, M. (1993). Overview: light treatment and future directions of research. In: Wetterberg, L. (Ed.). *Light and Biological Rhythms in Man*. Cambridge, UK: Pergamon, pp. 421–436.
212. Schwartz, P. J., Brown, C., Wehr, T. A. & 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.
213. Eastman, C. I., Young, M. A., Fogg, L. F., Liu, L. & Meaden, P. M. (1998). Bright light treatment of winter depression. *Archives of General Psychiatry* 55: 883–889.
214. Avery, D. H., Eder, D. N., Bolte, M. A., Hellekson, C. J., Dunner, D. L., Vitiello, M. V. & Prinz, P. N. (2001). Dawn simulation and bright light in the treatment of SAD: a controlled study. *Biological Psychiatry* 50: 205–216.
215. Martin, S. K. & 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.
216. Laberge, L., Carrier, J., Lespérance, P., Lambert, C., Vitaro, F., Tremblay, R. E. & Montplaisir, J. (2000). Sleep and circadian phase characteristics of adolescent and young adult males in a naturalistic summertime condition. *Chronobiology International* 17: 489–501.
217. Shinkoda, H., Matsumoto, K., Park, Y. M. & Nagashima, H. (2000). Sleep-wake habits of schoolchildren according to grade. *Psychiatry and Clinical Neurosciences* 54: 287–289.
218. Ghata, J., Reinberg, A., Lagoguey, M. & 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.
219. 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.
220. Horne, J. A. & Östberg, O. (1977). Individual differences in human circadian rhythms. *Biological Psychology* 5: 179–190.
221. Binkley, S., Tome, M. B., Crawford, D. & Mosher, K. (1990). Human daily rhythms measured for one year. *Physiology and Behavior* 48: 293–298.
222. Park, Y. M., Matsumoto, K., Seo, Y. J. & 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.
223. Medeiros, A. L. D., Mendes, D. B. F., Lima, P. F. & Araujo, J. F. (2001). The relationships between sleep-wake cycle and academic performance in medical students. *Biological Rhythm Research* 32: 263–270.
224. Roenneberg, T., Wirz-Justice, A. & Merrow, M. (2003). Life between clocks: daily temporal patterns of human chronotypes. *Journal of Biological Rhythms* 18: 80–90.
225. Taillard, J., Philip, P., Chastang, J. F. & 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.
226. Giannotti, F., Cortesi, F., Sebastiani, T. & Ottaviano, S. (2002). Circadian preference, sleep and daytime behaviour in adolescence. *Journal of Sleep Research* 11: 191–199.
227. Bellamy, N., Sothern, R. B. & Campbell, J. (2004). Aspects of diurnal rhythmicity in pain, stiffness, and fatigue in patients with fibromyalgia. *Journal of Rheumatology* 31: 379–389.
228. Robilliard, D. L., Archer, S. N., Arendt, J., Lockley, S. W., Hack, L. M., English, J., Leger, D., Smits, M. G., Williams, A., Skene, D. J. & von Schantz, M. (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.

17 Pet Selection and Veterinary Medicine

CHAPTER OUTLINE

17.1 Pet Selection
17.2 Veterinary Medicine

17.1 PET SELECTION

The three preceding chapters demonstrated 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. This chapter shows that circadian physiology also can assist 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 (70 million cats) is larger than the dog population (60 million dogs). Ownership of multiple pets is even higher for fish, so that the fish population is larger than the dog population even though far fewer households have fish as pets. 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). In this area, circadian physiology can help people select a pet.

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 indoors or outdoors,

FIGURE 17.1 Furry friends. Dogs (*Canis familiaris*) and cats (*Felis catus*) are the favorite pets in most households. (Source: Photographs by R. Refinetti.)

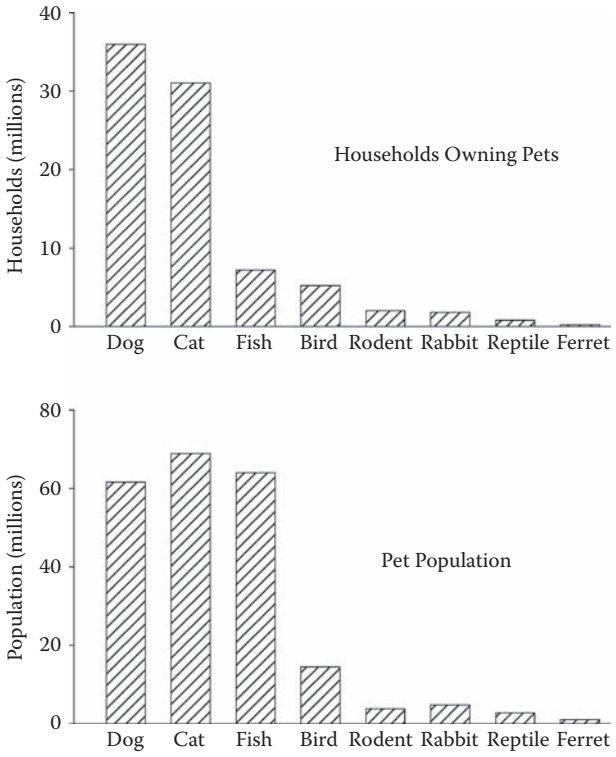


FIGURE 17.2 The most popular pets. The graphs show the number of U.S. households that own various species of pets (top panel) and the total populations of these pets (bottom panel). Although dogs are owned by the largest number of households, the populations of cats and fish are slightly larger because cat and fish owners tend to own multiple pets. (Source: *U.S. Pet Ownership and Demographics Sourcebook*. (2002). Schaumburg, IL: American Veterinary Medical Association.)

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. The figure describes fours groups of diurnal (day-active) pets and four groups of nocturnal (night-active) pets. For each group, information is provided about body size in kilograms (1 kg ≈ 2 lb), usual housing, longevity, and temperament.

and are affectionate and resourceful ("man's best friend"). Dogs can be adopted at a nominal cost from animal shelters or purchased from dog breeders at prices ranging from a few hundred dollars to several thousand dollars (in the case of purebred dogs). The long process of domestication of dogs over the centuries resulted in the selection of human-oriented canine characteristics. For example, dogs — but not wolves or chimpanzees — can understand human communication signals, such as finger pointing to indicate location.[2] It also has 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 Retriever, Rottweiler, German Shepherd, and Golden Retriever) are relatively large in size. Many breeds also kept as pets 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

TABLE 17.1
The 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, NY, and American Veterinary Medical Association, Schaumburg, IL.)

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 may be asleep when the pet owner is awake. Cats sleep about 16 hours a 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 at a nominal cost from 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 birds are diurnal, their indifferent temperament

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. (Source: Garfield © 1988 Paws, Inc. Reprinted with permission of Universal Press Syndicate. All rights reserved.)

TABLE 17.2
Pet Population: Dogs and Cats

	Dogs (million)	Cats (million)
Europe	41	47
United States	61	76

(Sources: Pet Food Institute, Washington, DC, and European Pet Food Industry Federation, Brussels, Belgium. Values are for 2002.)

FIGURE 17.5 Cottontail and sparrow. Rabbits and birds are not uncommon pets. (Source: National Image Library, U.S. Fish and Wildlife Service.)

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 animals.

Reptiles (lizards, snakes, turtles, and tortoises) and fish are the other two common diurnal pets. Among the nocturnal pets, ferrets (*Mustela putorius*) are very affectionate, while rodents (hamsters, gerbils, rats, mice, and guinea pigs) are more indifferent. Because rodents are truly nocturnal, many rodent pet owners complain that their pets are always asleep during the day. In addition, because rodents are often provided with running wheels for entertainment, they run on the wheel all night long, 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) (*Arvicanthis niloticus*), a small (120 g) diurnal mammal indigenous to north Africa.[5] Nile grass rats are *not* available at pet stores, however.

One pet that you are encouraged *not* to choose is a tiger (Figure 17.7). Tigers (*Panthera tigris*) are large, wild animals that, unlike dogs and cats, have *not* been domesticated and selectively bred for thousands of years.

FIGURE 17.6 A Nile grass rat. This small diurnal rodent (*Arvicanthis niloticus*) may be a good pet for some people. (Source: Photograph by R. Refinetti.)

FIGURE 17.7 Wild beast. Tigers (*Panthera tigris*) cannot be fully domesticated and are not safe pets. (Source: National Image Library, U.S. Fish and Wildlife Service.)

Approximately 10,000 tigers are kept in the United States, many of them as pets.[6]

17.2 VETERINARY MEDICINE

Veterinary medicine is concerned with the prevention and treatment of disease in nonhuman animals. Veterinary professionals in the United States oversee more than 200 million head of livestock that have an economic value of over $50 billion, care for over 60 million dogs at a cost of $8 billion per year, and perform numerous other tasks including food inspection and control of environmental hazards.[7] 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 17.8).

As was the case for human medicine (Chapter 16), veterinary medicine can benefit from advances in circadian physiology. Knowledge of the daily rhythmicity of body temperature can provide greater accuracy in the diagnosis of febrile states. As shown in Figure 17.9, the body temperature rhythm of the horse has a mean level of

FIGURE 17.8 Horse race. Race horses constitute one of many groups of animals that require regular veterinary care. (Source: Photograph by Bill Tarpenning, U.S. Department of Agriculture's Photography Center.)

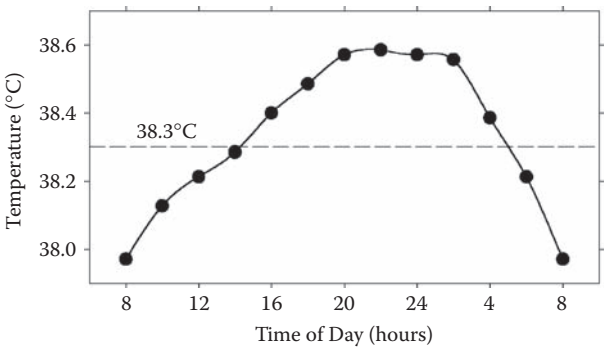


FIGURE 17.9 What is a fever? The graph shows the daily rhythm of rectal temperature of a normal healthy English Thoroughbred horse. Because the temperature oscillates from 37.9 to 38.6°C daily, it would be inaccurate to use the mean value of 38.3°C (100.9°F) as a criterion for ascertaining the presence of a fever. (Source: Piccione, G., Caola, G. & Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.)

38.3°C and a range of oscillation of 0.6°C. If the daily oscillation were ignored, a horse might 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. This variability in the characterization of a febrile state also applies to all other domestic animals. Figure 17.10 shows the normal body temperature ranges for the ten animals most commonly encountered in veterinary practice. Birds (chickens and turkeys) are consistently warmer than mammals, but considerable overlap occurs in the normal ranges for the mammalian species. Generally, core temperatures below 37.6°C characterize a hypothermic state, while temperatures above 39.7°C characterize a febrile state in any of the eight mammalian species. Note that the hypothermic threshold for these animals (37.6°C) is higher than the mean core temperature of humans (37.0°C).

Throughout this book, I mentioned many studies of circadian rhythms conducted on farm animals such as chickens,[8–22] sheep,[23–37] goats,[38–43] pigs,[44–47] horses,[48–56] and cattle.[57–62] However, the number of studies is very small when compared with the number of studies conducted on laboratory animals. Furthermore, research on circadian rhythms in farm animals too often has lacked the rigor of scientific investigation found in laboratory research.[63,64] As a consequence, the exact parameters of circadian rhythms in farm animals have not been adequately determined. 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 17.11). One of many phenomena that we have investigated together is the disruption of the body temperature rhythm caused by parturition (giving birth). Figure 17.12 shows the mean rectal temperatures of groups of mares (female horses) and ewes (female sheep), measured at dusk and dawn for

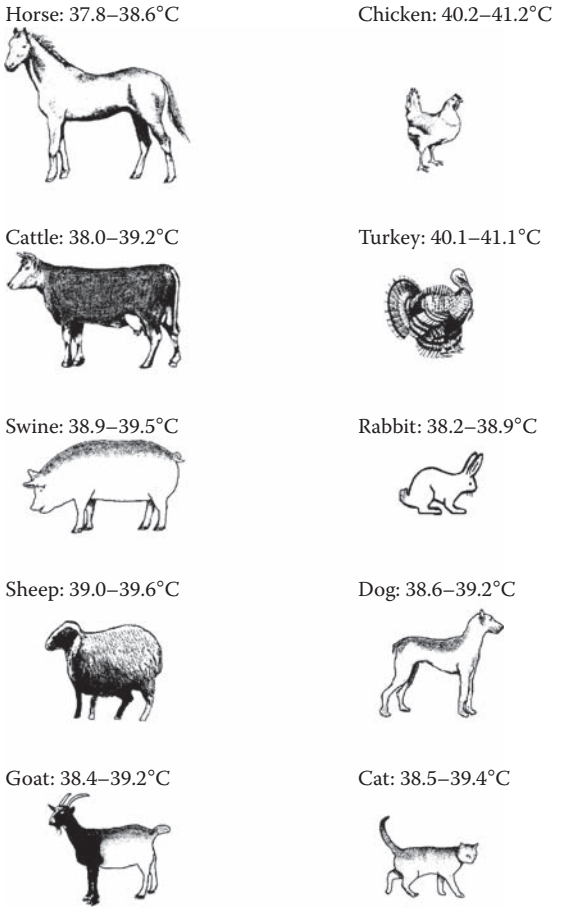


FIGURE 17.10 Normal range of body temperature. This figure shows the normal ranges of oscillation of the body temperature rhythms of the ten animals most commonly encountered in veterinary practice.

FIGURE 17.11 Giuseppe Piccione. This veterinary researcher from the University of Messina (Italy) is one of the few veterinarians in the world who specialize in the study of circadian rhythms. (Source: Photograph courtesy of Giuseppe Piccione.)

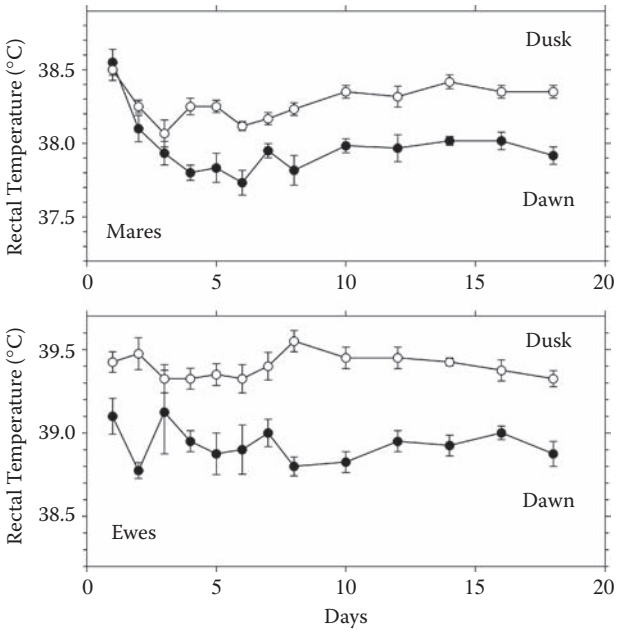


FIGURE 17.12 Maternity and circadian rhythms. The graphs show the mean rectal temperatures at dusk and dawn of mares and ewes for 18 days after parturition. The data points correspond to the means (± SE) of six mares and four ewes. Animals gave birth on Day 0. Note that — except for a transient hyperthermia, 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. (Source: Piccione, G., Caola, G. & Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.)

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 affected less by parturition.[36]

Figure 17.13 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 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[65–84] and contrasts with the sudden temperature rise upon awakening seen in rodents such as the rat.[85–103]

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.[104] The age of a horse is counted by the calendar year of birth, regardless of the exact birth date. Thus, horse breeders want their mares to give birth early in the year. As early as 1947, it was shown that the breeding season can be advanced by manipulation of the photoperiod to which the mares are exposed.[105] 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).[106] 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 also can be used to synchronize the daily rhythm of oviposition (egg-laying) of domestic fowl (chickens 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.[13,107,108] To save energy costs on a chicken farm, light–dark cycles with as little as 2 hours of light per day can be instituted.[13] 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 light–dark cycle, the farmer could obtain an egg from each hen every 21 hours instead of every 24 hours. Researchers have found that the oviposition rhythm can be entrained by a 21-hour light–dark cycle, but egg production per cycle is reduced, thus resulting in no net gain.[13]

Livestock production is a major industry. Figure 14.14 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

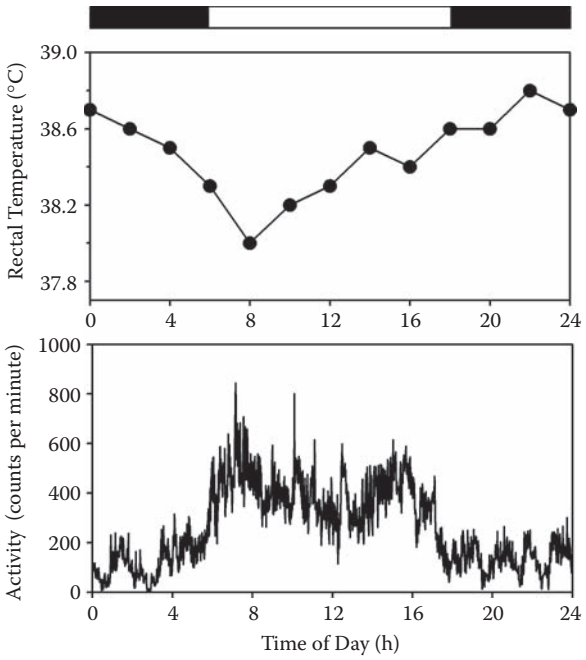


FIGURE 17.13 Rhythms of activity and body temperature of the horse. The graphs show 1-day segments of the records of body temperature (measured every 2 hours with a rectal probe) and locomotor activity (measured at 1-minute intervals with a data logger) of a representative female horse housed in a stall 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. (Source: Data courtesy of Giuseppe Piccione, University of Messina, Italy.)

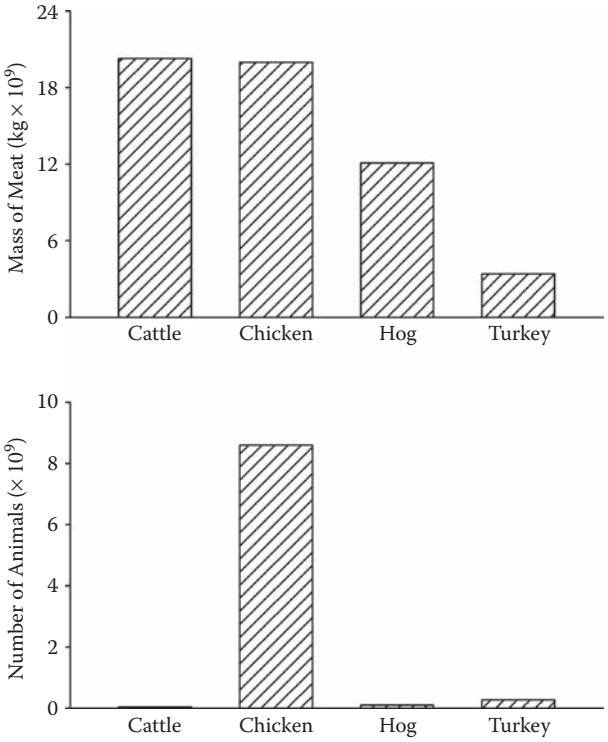


FIGURE 17.14 Livestock production. The graphs show the annual production of the four major sources of meat in the United States in terms of mass of meat (top panel) and of number of animals (bottom panel). The production of chicken meat (20 billion kilograms) is comparable with that of cattle meat; however, 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 the number of cattle (35.7 million) slaughtered. (Sources: *Livestock Slaughter — 2002 Summary* (2003). Washington, DC: National Agricultural Statistics Service; *Poultry Production and Value — 2002 Summary* (2003). Washington, DC: National Agricultural Statistics Service.)

chicken meat are produced each year (20 billion kilograms, or 44 billion pounds), and swine meat (pork) and turkey meat are the third and fourth most produced meats. However, because chickens weigh much less than the other meat-producing animals, many more chickens must be slaughtered to equal the same amount of meat (bottom panel). Each year, almost 9 billion chickens are slaughtered in the United States alone. Cattle (Figure 17.15) provide meat and also milk (dairy products). Chickens (Figure 17.16) contribute both meat and eggs to the human food supply. Pigs (Figure 17.17) and turkeys (Figure 17.18) provide meat almost exclusively, while sheep (Figure 17.19) contribute meat and fleece (wool). 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 consider 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.[7]

Some veterinarians work in zoological parks (Figure 17.20), although few of them are employed exclusively by the zoos. Most zoo veterinarians are private practitioners who have a contract with the zoo to provide care to the captive animals.[7] In zoos, knowledge of circadian physiology is helpful not only in the regular treatment of disease but also in the implementation of exhibits for nocturnal animals. Many 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,[109] the Berlin Zoological Park (in Germany) established its Nocturnal House exhibit in 1975,[110] and the Bristol Zoological Park (in England) opened its Twilight World exhibit in 1996.[111] Proper organization of these exhibits requires that the curators be familiar with the nocturnal habits of different species as well as with the physical characteristics of the photic environment (photoperiod length, illuminance levels, spectral composition, and so on).

Over 45,000 veterinarians in the United States are involved in private clinical practice, and 60 to 70% of

FIGURE 17.15 Grazing cattle. Cattle are the number one source of edible meat in the United States. (Source: Photograph by Scott Bauer, Agricultural Research Service, U.S. Department of Agriculture.)

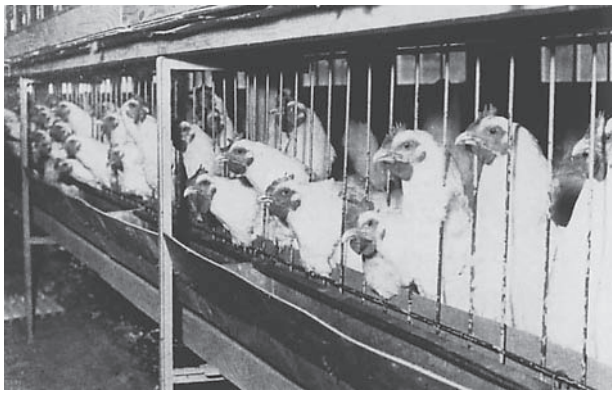


FIGURE 17.16 Chicken farm. Although production of chicken meat is slightly lower than production of beef (cattle meat), more chickens are slaughtered each year than any other farm animal in the United States. (Source: Image courtesy of Giuseppe Piccione.)

them specialize in small animals, such as cats and dogs[7] (Figure 17.21). For these practitioners, knowledge of circadian physiology can help prevent and treat disease. For example, surgical procedures for spaying and neutering, removal of tumors, or extirpation of anal glands are commonly performed in small-animal practice. These procedures require adequate anesthesia. The combination of ketamine and diazepam (or midazolam), which is commonly used in veterinary anesthesia, produces longer sedation during the inactive phase of the circadian cycle than during the active phase.[112] Thus, the anesthetic dose must be adjusted to ensure optimal action at different times of the day (although few veterinarians currently follow this recommendation).

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 fish. If nocturnality of the pet is an undesired characteristic, pets such as cats, rabbits, ferrets, and rodents may not be good choices.
2. Veterinarians provide health care for animals in private clinical practices as well as in industrial settings. Knowledge of circadian physiology can help veterinarians to improve health care and can assist industrial veterinarians and farmers in the improvement of livestock management.

EXERCISES

Exercise 17.1 Circadian discrepancy between pets and pet owners

The program Health, which you have used in exercises in the preceding chapters, includes 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.

FIGURE 17.17 Sow with piglet. Pork (swine meat) is the third most produced meat in the United States. (Source: Photograph by Scott Bauer, Agricultural Research Service, U.S. Department of Agriculture.)

FIGURE 17.18 Turkey farm. Turkey meat is the fourth most produced meat in the United States. (Source: Photograph by Scott Bauer, Agricultural Research Service, U.S. Department of Agriculture.)

Exercise 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 first on the right in the Circadian banner), you may do so 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). 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*.

FIGURE 17.19 Sheep farm. Sheep are farmed for both meat and wool. (Source: Agricultural Research Service, U.S. Department of Agriculture.)

FIGURE 17.20 At the zoo. This figure shows the Brick Elephant House, built in 1903 at the U.S. National Zoological Park. (Source: Smithsonian Institution Archives, Washington DC (Record Unit 95, Box 47, Folder 13, Neg. # 15533).)

FIGURE 17.21 My puppy. Many veterinarians specialize in the health care of household pets such as this Toy Poodle. (Source: Photograph by R. Refinetti.)

SUGGESTIONS FOR FURTHER READING

For more detailed information about the topics covered in this chapter, refer to the source articles listed in the *Literature Cited* section. For more general reading, the following sources may be useful.

Fogle, B. (2000). *The New Encyclopedia of the Dog*. New York: D.K. Publishing. This approximately 400-page general-audience book written by a veterinarian provides enough 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.

Aiello, S. E. & Mays, A. (Eds.). (1998). *Merck Veterinary Manual* (8th Edition). West Trenton, NJ: Merck & Co. Encompassing over 2300 pages, this introductory book for veterinary students is a comprehensive treatise on veterinary medicine, covering practically everything from animal behavior to zoonoses.

McCurnin, D. M. & Bassert, J. M. (Eds.). (2001). *Clinical Textbook for Veterinary Technicians* (5th Edition). New York: W. B. Saunders. At 832 pages, this textbook is, by design, not as comprehensive as the *Merck Manual*. However, it provides the essential material needed by veterinary technicians.

WEB SITES TO EXPLORE

American Animal Hospital Association:
http://www.healthypet.com
American Kennel Club:
http://www.akc.org
American Veterinary Medical Association:
http://www.avma.org
International Livestock Research Institute:
http://www.cgiar.org/ilri/
PETsMART:
http://www.petsmart.com
Virtual Livestock Library at Oklahoma State University:
http://www.ansi.okstate.edu/library/

LITERATURE CITED

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. & Tomasello, M. (2002). The domestication of social cognition in dogs. *Science* 298: 1634–1636.
3. Roy, M. M. & 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. Lemonick, M. D. (2003). Never trust a tiger. *Time* 162(16): 63–64.
7. Swope, R. E. (2001). *Opportunities in Veterinary Medicine Careers*. Chicago: VGM Career Books.
8. Winget, C. M., Averkin, E. G. & Fryer, T. B. (1965). Quantitative measurement by telemetry of ovulation and oviposition in the fowl. *American Journal of Physiology* 209: 853–858.
9. Winget, C. M. & Card, D. H. (1967). Daily rhythm changes associated with variations in light intensity and color. *Life Sciences and Space Research* 5: 148–158.
10. Aschoff, J. & von Saint Paul, U. (1973). Brain temperature as related to gross motor activity in the unanesthetized chicken. *Physiology and Behavior* 10: 529–533.
11. Aschoff, J. & Von Saint Paul, U. (1976). Brain temperature in the unanesthetized chicken: its circadian rhythm of responsiveness to light. *Brain Research* 101: 1–9.
12. Bobr, L. W. & 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.
13. Bhatti, B. M. & Morris, T. R. (1978). Entrainment of oviposition in the fowl using light–dark cycles. *British Poultry Science* 19: 333–340.
14. Kadono, H. & 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: Springer, pp. 91–99.
15. Kadono, H., Besch, E. L. & Usami, E. (1981). Body temperature, oviposition, and food intake in the hen during continuous light. *Journal of Applied Physiology* 51: 1145–1149.
16. Michels, H., Herremans, M. & 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.
17. Schenda, J. & 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.
18. Kasahara, T., Okano, T., Haga, T. & Fukada, Y. (2002). Opsin-G_{11}-mediated signaling pathway for photic entrainment of the chicken pineal circadian clock. *Journal of Neuroscience* 22: 7321–7325.
19. Willbold, E., Huhn, J., Korf, H. W., Voisin, P. & 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.
20. Chong, N. W., Chaurasia, S. S., Haque, R., Klein, D. C. & 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.
21. Bailey, M. J., Beremand, P. D., Hammer, R., Bell-Pedersen, D., Thomas, T. L. & Cassone, V. M. (2003). Transcriptional profiling of the chick pineal gland, a photoreceptive circadian oscillator and pacemaker. *Molecular Endocrinology* 17: 2084–2095.
22. Zawilska, J. B., Berezinska, M., Lorenc, A., Skene, D. J. & 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.
23. Bligh, J., Ingram, D. L., Keynes, R. D. & 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.
24. Recabarren, S. E., Vergara, M., Llanos, A. J. & Serón-Ferré, M. (1987). Circadian variation of rectal temperature in newborn sheep. *Journal of Developmental Physiology* 9: 399–408.
25. Mohr, E. & Krzywanek, H. (1990). Variations of core-temperature rhythms in unrestrained sheep. *Physiology and Behavior* 48: 467–473.
26. Mohr, E. G. & Krzywanek, H. (1995). Endogenous oscillator and regulatory mechanisms of body temperature in sheep. *Physiology and Behavior* 57: 339–347.
27. Houghton, D. C., Young, I. R. & 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.
28. Parraguez, V. H., Valenzuela, G. J., Vergara, M., Ducsay, C. A., Yellon, S. M. & Serón-Ferré, M. (1996). Effect of constant light on fetal and maternal prolactin rhythms in sheep. *Endocrinology* 137: 2355–2361.

29. Guerin, M. V. & 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.
30. Parraguez, V. H., Sales, F., Valenzuela, G. J., Vergara, M., Catalán, L. & Serón-Ferré, M. (1998). Diurnal changes in light intensity inside the pregnant uterus in sheep. *Animal Reproduction Science* 52: 123–130.
31. Lincoln, G. A., Rhind, S. M., Pompolo, S. & 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.
32. Lowe, T. E., Cook, C. J., Ingram. J. R. & Harris, P. J. (2001). Impact of climate on thermal rhythm in pastoral sheep. *Physiology and Behavior* 74: 659–664.
33. Souza, M. I. L., Bicudo, S. D., Uribe-Velásquez, L. F. & Ramos, A. A. (2002). Circadian and circannual rhythms of T3 and T4 secretions in Polwarth-Ideal rams. *Small Ruminant Research* 46: 1–5.
34. Lincoln, G., Messager, S., Andersson, H. & 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 U.S.A.* 99: 13890–13895.
35. Piccione, G., Caola, G. & Refinetti, R. (2002). Circadian modulation of starvation-induced hypothermia in sheep and goats. *Chronobiology International* 19: 531–541.
36. Piccione, G., Caola, G. & Refinetti, R. (2002). Maturation of the daily body temperature rhythm in sheep and horse. *Journal of Thermal Biology* 27: 333–336.
37. Lincoln, G. A., Andersson, H. & 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.
38. Jessen, C. & Kuhnen, G. (1996). Seasonal variations of body temperature in goats living in an outdoor environment. *Journal of Thermal Biology* 21: 197–204.
39. Jessen, C., Dmi'el, R., Choshniak, I., Ezra, D. & Kuhnen, G. (1998). Effects of dehydration and rehydration on body temperatures in the black Bedouin goat. *Pflügers Archiv* 436: 659–666.
40. Ayo, J. O., Oladele, S. B., Ngam, S., Fayomi, A. & 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.
41. Alila-Johansson, A., Eriksson, L., Soveri, T. & 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.
42. Alila-Johansson, A., Eriksson, L., Soveri, T. & Laakso, M. L. (2003). Serum cortisol levels in goats exhibit seasonal but not daily rhythmicity. *Chronobiology International* 20: 65–79.
43. Piccione, G., Caola, G. & 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.
44. Ingram, D. L. & 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.
45. Verstegen, M. W. A., van der Hel, W., Duijghuisen, R., & Geers, R. (1986). Diurnal variation in thermal demand of growing pigs. *Journal of Thermal Biology* 11: 131–135.
46. Kemp, B., de Greef-Lammers, F. J., Verstegen, M. W. A. & van der Hel, W. (1990). Some aspects of daily pattern in thermal demand of breeding boars. *Journal of Thermal Biology* 15: 103–108.
47. Henken, A. M., Brandsma, H. A., van der Hel, W. & 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.
48. Gill, J. (1991). A new method for continuous recording of motor activity in horses. *Comparative Biochemistry and Physiology A* 99: 333–341.
49. Hamra, J. G., Kamerling, S. G., Wolfsheimer, K. J. & Bagwell, C. A. (1993). Diurnal variation in plasma ir-β-endorphin levels and experimental pain thresholds in the horse. *Life Sciences* 53: 121–129.
50. Irvine, C. H. G. & Alexander, S. L. (1994). Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domestic Animal Endocrinology* 11: 227–238.
51. Guerin, M. V., Deed, J. R., Kennaway, D. J. & 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.
52. Piccione, G., Caola, G. & Refinetti, R. (2002). The circadian rhythm of body temperature of the horse. *Biological Rhythm Research* 33: 113–119.
53. Piccione, G., Caola, G. & Refinetti, R. (2004). Feeble weekly rhythmicity in hematological, cardiovascular, and thermal parameters in the horse. *Chronobiology International* 21: 571–589.
54. Piccione, G., Assenza, A., Grasso, F. & 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.
55. Piccione, G., Bertolucci, C., Foà, A. & Caola, G. (2004). Influence of fasting and exercise on the daily rhythm of serum leptin in the horse. *Chronobiology International* 21: 405–417.
56. Refinetti, R. & 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.
57. Bligh, J. & 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.
58. Araki, C. T., Nakamura, R. M., & Kam, L. W. G. (1987). Diurnal temperature sensitivity of dairy cattle in a naturally cycling environment. *Journal of Thermal Biology* 12: 23–26.

59. Hahn, G. L., Eigenberg, R. A., Nienaber, J. A. & 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.
60. Macaulay, A. S., Hahn, G. L., Clark, D. H. & Sisson, D. V. (1995). Comparison of calf housing types and tympanic temperature rhythms in Holstein calves. *Journal of Dairy Science* 78: 856–862.
61. Lefcourt, A. M., Huntington, J. B., Akers, R. M., Wood, D. L. & 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.
62. Piccione, G., Caola, G. & Refinetti, R. (2003). Daily and estrous rhythmicity of body temperature in domestic cattle. *BMC Physiology* 3: art. 7.
63. Hahn, G. L. (1989). Body temperature rhythms in farm animals: a review and reassessment relative to environmental influences. In: Driscoll, D. & Box, E. O. (Eds.). *Proceedings of the 11th ISB Congress*. The Hague: SPB Academic Publishing, pp. 325–337.
64. Piccione, G. & Refinetti, R. (2003). Thermal chronobiology of domestic animals. *Frontiers in Bioscience* 8: 258–264.
65. Kleitman, N. & Ramsaroop, A. (1948). Periodicity in body temperature and heart rate. *Endocrinology* 43: 1–20.
66. Mellette, H. C., Hutt, B. K., Askovitz, S. I. & Horvath, S. M. (1951). Diurnal variations in body temperature. *Journal of Applied Physiology* 3: 665–675.
67. Lobban, M. C. (1960). The entrainment of circadian rhythms in man. *Cold Spring Harbor Symposia on Quantitative Biology* 25: 325–332.
68. Sharp, G. W. G. (1961). Reversal of diurnal temperature rhythms in man. *Nature* 190: 146–148.
69. Aschoff, J., Gerecke, U. & Wever, R. (1967). Phasenbeziehungen zwischen den circadianen Perioden der Aktivität und der Kerntemperatur beim Menschen. *Pflügers Archiv* 295: 173–183.
70. Aschoff, J., Gerecke, U. & Wever, R. (1967). Desynchronization of human circadian rhythms. *Japanese Journal of Physiology* 17: 450–457.
71. Marotte, H. & Timbal, J. (1981). Circadian rhythm of temperature in man: comparative study with two experiment protocols. *Chronobiologia* 8: 87–100.
72. Weitzman, E. D., Moline, M. L., Czeisler, C. A. & Zimmerman, J. C. (1982). Chronobiology of aging: temperature, sleep-wake rhythms and entrainment. *Neurobiology of Aging* 3: 299–309.
73. Gander, P. H., Connell, L. J. & 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.
74. Lee, K. A. (1988). Circadian temperature rhythms in relation to menstrual cycle phase. *Journal of Biological Rhythms* 3: 255–263.
75. Scales, W. E., Vander, A. J., Brown, M. B. & Kluger, M. J. (1988). Human circadian rhythms in temperature, trace metals, and blood variables. *Journal of Applied Physiology* 65: 1840–1846.
76. Tsujimoto, T., Yamada, N., Shimoda, K., Hanada, K. & Takahashi, S. (1990). Circadian rhythms in depression. Part I: Monitoring of the circadian body temperature rhythm. *Journal of Affective Disorders* 18: 193–197.
77. Cisse, F., Martineaud, R. & 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.
78. Nakazawa, Y., Nonaka, K., Nishida, N., Hayashida, N., Miyahara, Y., Kotorii, T. & Matsuoka, K. (1991). Comparison of body temperature rhythms between healthy elderly and healthy young adults. *Japanese Journal of Psychiatry and Neurology* 45: 37–43.
79. Lee, Y. H. & 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.
80. Pollak, C. P. & Wagner, D. R. (1994). Core body temperature in narcoleptic and normal subjects living in temporal isolation. *Pharmacology, Biochemistry and Behavior* 47: 65–71.
81. Kräuchi, K. & 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.
82. Van Dongen, H. P. A., Kerkhof, G. A. & Souverijn, J. H. M. (1998). Absence of seasonal variation in the phase of the endogenous circadian rhythm in humans. *Chronobiology International* 15: 623–632.
83. Callard, D., Davenne, D., Lagarde, D., Meney, I., Gentil, C. & 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.
84. Gradisar, M. & 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.
85. Roussel, B., Chouvet, G. & Debilly, G. (1976). Rythmes circadiens des températures internes et ambiance thermique chez le rat. *Pflügers Archiv* 365: 183–189.
86. Thornhill, J. A., Hirst, M. & 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.
87. Honma, K. & Hiroshige, T. (1978). Simultaneous determination of circadian rhythms of locomotor activity and body temperature in the rat. *Japanese Journal of Physiology* 28: 159–169.
88. Meinrath, M. & D'Amato, M. R. (1979). Interrelationships among heart rate, activity, and body temperature in the rat. *Physiology and Behavior* 22: 491–498.
89. Kittrell, E. M. W. & Satinoff, E. (1988). Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiology and Behavior* 42: 477–484.

90. Berkey, D. L., Meeuwsen, K. W. & Barney, C. C. (1990). Measurements of core temperature in spontaneously hypertensive rats by radiotelemetry. *American Journal of Physiology* 258: R743–R749.
91. Morley, R. M., Conn, C. A., Kluger, M. J. & Vander, A. J. (1990). Temperature regulation in biotelemetered spontaneously hypertensive rats. *American Journal of Physiology* 258: R1064–R1069.
92. Refinetti, R., Ma, H. & Satinoff, E. (1990). Body temperature rhythms, cold tolerance, and fever in young and old rats of both genders. *Experimental Gerontology* 25: 533–543.
93. Shido, O., Sakurada, S. & Nagasaka, T. (1991). Effect of heat acclimation on diurnal changes in body temperature and locomotor activity in rats. *Journal of Physiology* 433: 59–71.
94. Shido, O., Sakurada, S., Kohda, W. & Nagasaka, T. (1994). Day-night changes of body temperature and feeding in heat-acclimated rats. *Physiology and Behavior* 55: 935–939.
95. 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.
96. Li, H. & Satinoff, E. (1995). Changes in circadian rhythms of body temperature and sleep in old rats. *American Journal of Physiology* 269: R208–R214.
97. Refinetti, R. (1996). Comparison of the body temperature rhythms of diurnal and nocturnal rodents. *Journal of Experimental Zoology* 275: 67–70.
98. Yang, Y. & 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.
99. Mistlberger, R. E., Lukman, H. & Nadeau, B. G. (1998). Circadian rhythms in the Zucker obese rat: assessment and intervention. *Appetite* 30: 255–267.
100. Ikeda, M. & 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.
101. Gordon, C. J. & Rezvani, A. H. (2001). Genetic selection of rats with high and low body temperatures. *Journal of Thermal Biology* 26: 223–229.
102. Ray, B., Mallick, H. N. & 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.
103. Leon, L. R., Walker, L. D., DuBose, D. A. & Stephenson, L. A. (2004). Biotelemetry transmitter implantation in rodents: impact on growth and circadian rhythms. *American Journal of Physiology* 286: R967–R974.
104. Nagy, P., Guillaume, D. & Daels, P. (2000). Seasonality in mares. *Animal Reproduction Science* 60: 245–262.
105. Burkhardt, J. (1947). Transition from anoestrus in the mare and the effects of artificial lighting. *Journal of Agricultural Science* 37: 64–68.
106. Palmer, E., Driancourt, M. A. & Ortavant, R. (1982). Photoperiodic stimulation of the mare during winter anoestrus. *Journal of Reproduction and Fertility* 32(S): 275–282.
107. Konishi, T. (1980). Circadian rhythm of ovipositional time in Japanese quail. In: Tanabe, Y. (Ed.). *Biological Rhythms in Birds: Neural and Endocrine Aspects*. Berlin: Springer, pp. 79–90.
108. Zivkovic, B. D., Underwood, H. & Siopes, T. (2000). Circadian ovulatory rhythms in Japanese quail: role of ocular and extraocular pacemakers. *Journal of Biological Rhythms* 15: 172–183.
109. House, H. B. & Doherty, J. G. (1975). The World of Darkness at the New York Zoological Park. *International Zoo Yearbook* 15: 31–34.
110. Klös, U. (1977). The new Nocturnal House at the West Berlin Zoo. *International Zoo Yearbook* 17: 229–231.
111. Partridge, J. (1997). The Twilight World of Bristol Zoo. *International Zoo News* 44(2): 80–84.
112. Rebuelto, M., Ambros, L., Waxman, S. & Montoya, L. (2004). Chronobiological study of the pharmacological response of rats to combination ketamine-midazolam. *Chronobiology International* 21: 591–600.

Dictionary of Circadian Physiology

OUTLINE

Introduction
English Dictionary
Language Equivalency
Units of Measurement

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

- Aschoff, J. (Ed.). *Biological Rhythms*. New York: Plenum, 1981.
- Binkley, S. *Biological Clocks: Your Owner's Manual*. Amsterdam: Harwood Academic, 1997.
- Dunlap, J. C., Loros, J. J. & DeCoursey, P. J. (Eds.). *Chronobiology: Biological Timekeeping*. Sunderland, MA: Sinauer, 2004.
- Halberg, F. & Watanabe, H. (Eds.). *Chronobiology and Chronomedicine*. Tokyo: Medical Review, 1992.
- 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 Alphabetical Order table.

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*, *analyse* for *analyze*, *defence* for *defense*). Orthography, phonology, and etymology were derived from:

- *The American Heritage Dictionary of the English Language*, 4th Edition. (2000). Boston: Houghton Mifflin.
- *New Webster's Dictionary of the English Language*. (1986). New York: Delair.
- *Academic Press Dictionary of Science and Technology.* San Diego: Academic Press, 1992.
- *PDR Medical Dictionary.* (1995) Montavale, NJ: Medical Economics.

The language equivalency table that follows the dictionary was compiled with the assistance of Frank Scheer (Dutch), Patrick Vuillez (French), Franz Halberg and the late Jürgen Aschoff (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 Edition). (1985). Boca Raton, FL: CRC Press.
- *Publication Manual of the American Psychological Association* (5th Edition). (2001). Washington, DC: American Psychological Association.

Phonetic symbols used in the dictionary are listed in the Phonetic Symbols table. Abbreviations used in the dictionary are as follows.

ABBREVIATIONS

adj.	adjective
adv.	adverb
Cf.	confer (see also)
n.	noun
pl.	plural
v.	verb

ALPHABETICAL ORDER

	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	o	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	Y	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

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, **b**i**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**dge**
k	**k**eep, **c**at, fli**ck**
l	**l**ove, a**l**ong
m	**m**other, da**mn**
n	**n**ice, sudd**en**
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**ea**r**
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, f**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
/	*Indicates primary stress*
’	*Indicates secondary stress*
.	*Delimits syllables*

ENGLISH DICTIONARY

α (al/·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·mâ/·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·zâ/·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 (a/·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·spæk/·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 (ak/·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·tâ/·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 non-climatic changes in the environment. // *v.* = **adapt** // *adj.* = **adaptive**, **adapted**, **adaptable**.

Ad libitum (ad·lï'b/·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·rï**ð**/·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·rï**ð**/·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·bĭ/·lĭ·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.

Bal1 see **Cycle** [2]

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).

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 multi-frequency 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 to 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 to 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* **Clock** [2]

Clock [1] (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.

Clock [2] *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 non-shiver-

ing 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 vec**tor**] *n*. A procedure for the analysis of biological rhythms based on the fitting of a cosine wave to the raw data. *Note:* The *single-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* **Cycle [2]**

Cycle [1] (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.

Cycle [2] *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* and *Mop3* 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, Siderial 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 early April to late October in the Northern hemisphere). *Note:* In the United States, three-letter abbreviations are used to indicate daylight-saving time according to time zones; e.g., 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·fi/) [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.

Electromagnetic spectrum (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 non-primates. *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**.

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 Illuminace.

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 non-technical 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. // *Cf*. Siderial day and Solar day.

Masking (mas/·kĭñ) [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/·ĭk·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/·tĭk) [Greek *monos*, single + *khroma*, color] *adj*. Pertaining to light of a single or narrow-band wavelength.

Mop3 *see* **Cycle [2]**

Morning-evening typology (mòr'·nĭñ·ev'·nĭñ·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/·dĭr') [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/·tĭk) [Latin *non*, negation prefix + Greek *phos*, light] *adj*. Of or relating to stimuli other than light. // *Cf*. Photic.

Nycthemeral (nĭk·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/·rĭk) [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] *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)

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. // *n*. = **periodicity**. // *Cf*. Frequency.

Period [2] *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, 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 1906 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.

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*. A light-dark cycle. *Usage*: Photoperiod is frequently used in connection with light-dark cycles that vary in the relative duration of the light and dark segments. // *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.

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'·în·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·ör'·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.

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 non-traditional working hours, usually during the evening and night.

Siderial 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*. As 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 Siderial 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 (spriñ/·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 synonymous with *entrainment*. // *v.* = **synchronize**. // *adj.* = **synchronized**. // *n.* = **synchronizer**. // Cf. Entrainment.

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 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.

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.

Waveform (wâv/·fòrm') [Old English *wafian*, wave + Latin *forma*, form] *n*. The shape of a wave (such as square, sinusoid, etc.). // *Cf*. Wave.

Wavelength (wâv/·læñkþ') [Old English *wafian*, wave + *lengthu*, length] *n*. The distance between two successive peaks (or troughs, etc.) of a regular wave. *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**

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

LANGUAGE EQUIVALENCY

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
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

LANGUAGE EQUIVALENCY (CONTINUED)

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

UNITS OF MEASUREMENT

Base units

The International System of Units recognizes seven base units associated with the seven fundamental physical quantities. The seven quantities, and the names and symbols of the units used to measure them, are

Quantity	Name	Symbol
Amount of substance (î·mânt'·îv·sûb/·stîns)	mole (mœl)	mol
Electric current (ï·læk'·trïk·kûr/·înt)	ampere (am/·pïr)	A
Length (læñkþ)	meter (me/·tîr)	m
Luminous intensity (lú'·mî·nîs·în·tæn/·sï·te)	candela (kan·dæl/·î)	cd
Mass (mas)	kilogram (kï/·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).

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 mole may be expressed either as 7 nmol or as 7×10^{-9} mol.

Supplementary and Derived Units

Supplementary and 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 table below:

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 (dĭ·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 liter = 1 dm^3)	

Conversions

Conversion factors for the most common units that are not recognized by the International System of Units are given in the table below. 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 kiloseconds.

Quantity	Unit	Conversion
Amount of substance	gram of salt	1 g NaCl = 17.112 mmol
	liter of oxygen	1 liter 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 liter O_2 (STPD) $\approx$ 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 (U.S.)	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
	quilocalories 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 (U.S.)	1 fl oz = 29.57353 ml
	pint	1 pt = 0.473177 liter
	gallon	1 gal = 3.785412 liter
Temperature	degree Fahrenheit	°F = 1.8 × °C + 32
Time	minute	1 min = 60 s
	hour	1 h = 3.6 ks
	year	1 yr $\approx$ 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.

Organisms Used

Organisms cited by name and discussed in 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 by Latin name, followed by common English name and major phylogenetic group. Except for the orders Monotremata and Marsupialia (within the class Mammalia), phylogenetic groups are indicated at the class level or above, in English.

Latin Name	English Name	Group
Abutilon theophrasti	Velvetleaf	Plant
Acanthis flammea[a]	Common redpoll	Bird
Acanthis hornemanni	Hoary redpoll	Bird
Acheta domestica	House cricket	Insect
Acomys cahirinus	Common spiny mouse	Mammal
Acomys russatus	Golden spiny mouse	Mammal
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
Amieurus 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
Anolis carolinensis	Green anole	Reptile
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 lemurinus	Owl monkey	Mammal
Aotus trivirgatus	Night monkey	Mammal
Apis cerana	Wax bee	Insect
Apis mellifera	Honey bee	Insect
Apis mellifica	Black bee	Insect
Aplodontia rufa	Mountain beaver	Mammal
Aplysia californica	Marine snail	Mollusk
Apodemus flavicollis	Yellow-necked field mouse	Mammal

(continued)

Latin Name	English Name	Group
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
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 nasutum	Pill bug	Crustacean
Armadillidium vulgare	Pill bug	Crustacean
Arvicanthis ansorgei	Kusu rat	Mammal
Arvicanthis niloticus	Nile grass rat	Mammal
Astacus astacus	Crayfish	Crustacean
Asterias forbesi	Common sea star	Echinoderm
Asterocampa leilia	Desert hackberry butterfly	Insect
Atta sexdens	Sauba ant	Insect
Auriparus flaviceps	Verdin	Bird
Autographa gamma[f]	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
Blarina brevicauda	Short-tailed shrew	Mammal
Bombyx mori	Domestic silkworm	Insect
Boreogadus saida	Polar cod	Fish
Bos taurus	Domestic cattle	Mammal
Brassica kaber	Wild mustard	Plant
Bufo cognatus	Great Plains 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
Calcinus laevimanus	Hermit crab	Crustacean
Calidris canutus	Red knot	Bird
Calidris mauri	Western 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
Cambarus bartoni	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
Campylorhyncus brunneicampillus	Cactus wren	Bird
Canis familiaris	Domestic dog	Mammal
Canis lupus	Wolf	Mammal
Canis mesomelas	Blackbacked jackal	Mammal
Capra hircus	Domestic goat	Mammal

Latin Name	English Name	Group
Carassius auratus	Goldfish	Fish
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
Cervus elaphus	Red deer	Mammal
Cetraria islandica	Iceland moss	Plant
Charadrius dubius	Little ringed plover	Bird
Chelonia mydas	Green sea turtle	Reptile
Chelydra serpentina	Snapping turtle	Reptile
Chenopodium album	Common lambsquarter	Plant
Chlorocebus aethiops	Vervet monkey	Mammal
Christinus marmoratus	Marbled gecko	Reptile
Chrysemys scripta	Turtle	Reptile
Chrysophrys auratus	Australian snapper	Fish
Cicindela hybrida	Tiger beetle	Insect
Citheronia sepulcralis	Pine devil moth	Insect
Cladonia alpestris	Reindeer lichen	Plant
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 bowringi	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
Cricetulus migratorius	Ratlike hamster	Mammal
Cricetus cricetus	European hamster	Mammal
Crotalus cerastes	Colorado desert sidewinder	Reptile
Crotalus mitchelli	Southwestern speckled rattlesnake	Reptile
Crotaphytus collaris	Collard lizard	Reptile
Cryptomys damarensis	Damara molerat	Mammal
Cryptomys hottentotus	Highveld molerat	Mammal
Cuculus canorus	Common cuckoo	Bird
Cydia pomonella	Codling moth	Insect
Cynomys leucurus	White-tailed prairie dog	Mammal
Cynomys ludovicianus	Black-tailed prairie dog	Mammal
Cynops pyrrhogaster	Japanese newt	Amphibian

Latin Name	English Name	Group
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
Dentex dentex	Common dentex	Fish
Dermochelys coriacea	Leatherback turtle	Reptile
Desmodillus auricularis	Short-tailed gerbil	Mammal
Diceros bicornis	Black rhinoceros	Mammal
Dicrostonyx groelandicus	Collared lemming	Mammal
Didelphis marsupialis	American opossum	Marsupial
Didelphis virginiana	Virginia opossum	Marsupial
Dimorphostylis asiatica	Zooplankton	Crustacean
Dipodomys deserti	Desert kangaroo rat	Mammal
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
Echinochloa crusgalli	Barnyardgrass	Plant
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 przewalski	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
Euphausia pacifica	Zooplankton	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

Latin Name	English Name	Group
Fringilla coelebs	Chaffinch	Bird
Fringilla montifringilla	Brambling	Bird
Funambulus palmarum	Striped palm squirrel	Mammal
Funambulus pennanti	Striped palm squirrel	Mammal
Fundulus majalis	Striped killifish	Fish
Galaxias maculates	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	Spider	Arachnid
Georychus capensis	Cape molerat	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 palludum	Waterstrider	Insect
Gillichthys mirabilis	Mud sucker	Fish
Giraffa camelopardalis	Giraffe	Mammal
Glaucomys volans	Flying squirrel	Mammal
Glis glis	Edible dormouse	Mammal
Glyphiulus cavernicolus	Glyphiulus	Millipede
Glyptonotus antarcticus	Giant Antarctic isopod	Crustacean
Gnathopogon caerulescens	Honmoroko	Fish
Gonyaulax polyedra[b]	Plankton	Protist
Gopherus agassizii	Desert tortoise	Reptile
Gorilla gorilla	Gorilla	Mammal
Grus canadensis	Sandhill crane	Bird
Gryllus bimaculatus	Cricket	Insect
Hantzschia virgata	Alga	Protist
Helianthus annuus	Sunflower	Plant
Heloderma suspectum	Gila monster	Reptile
Hesperiphona vespertina	Evening grosbeak	Bird
Heterocephalus glaber	Naked molerat	Mammal
Heteropneutes fossilis	Catfish	Fish
Hipposideros speoris	Schneider's roundleaf bat	Mammal
Hirundo rustica	Barn swallow	Bird
Homo sapiens	Human	Mammal
Hyalophora cecropia	Cecropia moth	Insect
Hydropsyche contubernalis	Caddisfly	Insect
Hylocichla ustulata	Swainson's thrush	Bird
Ictalurus natalis	Yellow bullhead	Fish
Ictalurus nebulosus	Brown bullhead	Fish
Iguana iguana	Green iguana	Reptile
Imbrasia belina	Mopane worm	Insect
Ischnura elegans	Damselfly	Insect
Isoodon auratus	Golden bandicoot	Marsupial
Isoodon macrouros	Short-nosed bandicoot	Marsupial
Ixodes scapularis	Deer tick	Arachnid
Lacerta viridis	European green lizard	Reptile
Lacerta vivipara	Viviparous lizard	Reptile

Latin Name	English Name	Group
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
Leipoa ocellata	Mallee fowl	Bird
Lemmus lemmus	Norwegian lemming	Mammal
Lemna perpusilla	Duckweed	Plant
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	Cockroach	Insect
Leuresthes tenuis	California grunion	Fish
Lexodonta africana	African elephant	Mammal
Lingulodinium polyedrum[b]	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
Lycopersicum esculentum	Common tomato plant	Plant
Lycopodium annotinum	Stiff clubmoss	Plant
Lycosa tarentula	Wolf spider	Arachnid
Lymnaea stagnalis	Pond snail	Mollusk
Lynx canadensis	Lynx	Mammal
Macaca arctoides[c]	Stumptail 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[c]	Stumptail monkey	Mammal
Macrobrachium acanthurus	Freshwater prawn	Crustacean
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
Marmota flaviventris	Yellow-bellied marmot	Mammal
Marmota marmota	Alpine marmot	Mammal
Marmota monax	Woodchuck	Mammal
Megascolex mauritii	Earthworm	Annelid
Meleagris gallopavo	Turkey	Bird
Melopsittacus undulatus	Parakeet (lovebird)	Bird

Latin Name	English Name	Group
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 arvalis	Common 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
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
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
Nannospalax ehrenbergi[d]	Mediterranean blind molerat	Mammal
Nassa obsoleta	Mud snail	Mollusk
Nasua nasua	Coati	Mammal
Navodon scaber[e]	Leatherjacket	Fish
Nectarinia famosa	Malachite sunbird	Bird
Nelumbo nucifera	Sacred lotus	Plant
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
Nyctereutes procyonoides	Raccoon dog	Mammal
Nyctophilus geoffroyi	Lesser long-eared bat	Mammal
Octodon degus	Degu	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 orca	Killer whale	Mammal
Orconectes immunis	Crayfish	Crustacean
Oreochromis niloticus	Nile tilapia	Fish
Ornithorhynchus anatinus	Platypus	Monotreme

Latin Name	English Name	Group
Oryctolagus cuniculus	Domestic rabbit	Mammal
Oryx beisa	Oryx	Mammal
Oryzomys palustris	Marsh rice rat	Mammal
Ovibos moschatus	Muskox	Mammal
Ovis aries	Domestic sheep	Mammal
Ovis musimon	Mouflon	Mammal
Oxyria digyna	Mountain sorrel	Plant
Pachnoda marignata	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 papio	Western baboon	Mammal
Papio ursinus	Chacma baboon	Mammal
Paramecium aurelia	Paramecium	Protist
Paramecium caudatum	Paramecium	Protist
Paraserianthes lophantha	Crested wattle	Plant
Parika scaber[e]	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
Pelecanoides urinatrix	Common diving petrel	Bird
Peltigera canina	Dog lichen	Plant
Periplaneta americana	Cockroach	Insect
Perisoreus canadensis	Gray jay	Bird
Perla burmeisteriana	Stonefly	Insect
Perognathus longimembris	Pocket 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
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
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

Latin Name	English Name	Group
Plecoglossus altivelis	Ayu	Fish
Plethodon cinereus	Red-backed salamander	Amphibian
Pleuronectes platessa	Plaice	Fish
Plodia interpunctella	Indian meal moth	Insect
Plusia gamma[f]	Gamma moth	Insect
Pluvialis apricaria	Eurasian golden plover	Bird
Podarcis muralis	Common wall lizard	Reptile
Podarcis sicula	Ruin lizard	Reptile
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
Procyon lotor	Raccoon	Mammal
Protophormia terraenovae	Blow fly	Insect
Pseudantechinus macdonnellensis	Fat-tailed antechinus	Marsupial
Pseudechis porphyriacus	Australian blacksnake	Reptile
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
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
Riparia riparia	Bank swallow (Sand martin)	Bird
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 fontinalis	Speckled trout	Fish
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
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

Latin Name	English Name	Group
Scistocerca 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
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
Sigmodon hispidus	Cotton rat	Mammal
Siphlonurus armatus	Mayfly	Insect
Sminthopsis crassicaudata	Fat-tailed dunnart	Marsupial
Sminthopsis macroura	Stripe-faced dunnart	Marsupial
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[d]	Mediterranean blind molerat	Mammal
Sparrmannia flava	Beetle	Insect
Spermophilus beecheyi[h]	California ground squirrel	Mammal
Spermophilus citellus	European ground squirrel	Mammal
Spermophilus lateralis	Golden-mantled ground squirrel	Mammal
Spermophilus parryii	Arctic ground squirrel	Mammal
Spermophilus richardsonii	Richardson's ground squirrel	Mammal
Spermophilus saturatus	Golden-mantled ground squirrel	Mammal
Spermophilus tereticaudus	Round-tailed squirrel	Mammal
Spermophilus tridecemlineatus	Thirteen-lined ground squirrel	Mammal
Spermophilus undulatus	Arctic 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
Taeniopygia guttata	Zebra finch	Bird
Tamarindus indica	Tamarind tree	Plant
Tamias striatus	Eastern American chipmunk	Mammal
Tamiasciurus hudsonicus	Red squirrel	Mammal
Tarentola boettgeri	Canary Island gecko	Reptile

Latin Name	English Name	Group
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[g]	Polar bear	Mammal
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 obesus	Bigeye tuna	Fish
Thunnus thynnus	Atlantic bluefin tuna	Fish
Thymelicus lineola	European skipper	Insect
Tiliqua scincoides	Blue-tongued lizard	Reptile
Tinca tinca	Tench	Fish
Toxostoma dorsale	Crissal thrasher	Bird
Trematomus bernacchii	Emerald rock cod	Fish
Trematomus borchgrevinki	Antarctic cod	Fish
Triatoma infestans	Kissing bug	Insect
Trichosurus vulpecula	Brush-tailed possum	Marsupial
Trigonoscelis gigas	Black-bodied beetle	Insect
Tupaia belangeri	Tree shrew	Mammal
Tupaia glis	Tree shrew	Mammal
Tupinambis teguixin	Tupinambi	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
Urocolius macrourus	Blue-naped mousebird	Bird
Urodacus armatus	Scorpion	Arachnid
Ursus americanus	Black bear	Mammal
Ursus arctos	Brown bear	Mammal
Ursus maritimus[g]	Polar bear	Mammal
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

Latin Name	English Name	Group
Wyeomyia smithii	Pitcher-plant mosquito	Insect
Xantusia riversiana	Island night lizard	Reptile
Xenopus laevis	African clawed frog	Amphibian
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] *Gonyaulax polyedra* has been renamed as *Lingulodinium polyedrum*.
[c] *Macaca speciosa* has been renamed as *Macaca arctoides*.
[d] *Nannospalax ehrenbergi* is also known as *Spalax ehrenbergi*.
[e] *Navodon scaber* is also known as *Parika scaber*.
[f] *Plusia gamma* is also known as *Autographa gamma*.
[g] *Thalarctos maritimus* is better known as *Ursus maritimus*.
[h] The genus *Spermophilus* was called *Citellus* before the 1980s.

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('i' indicates an illustration; 't' indicates a table)

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