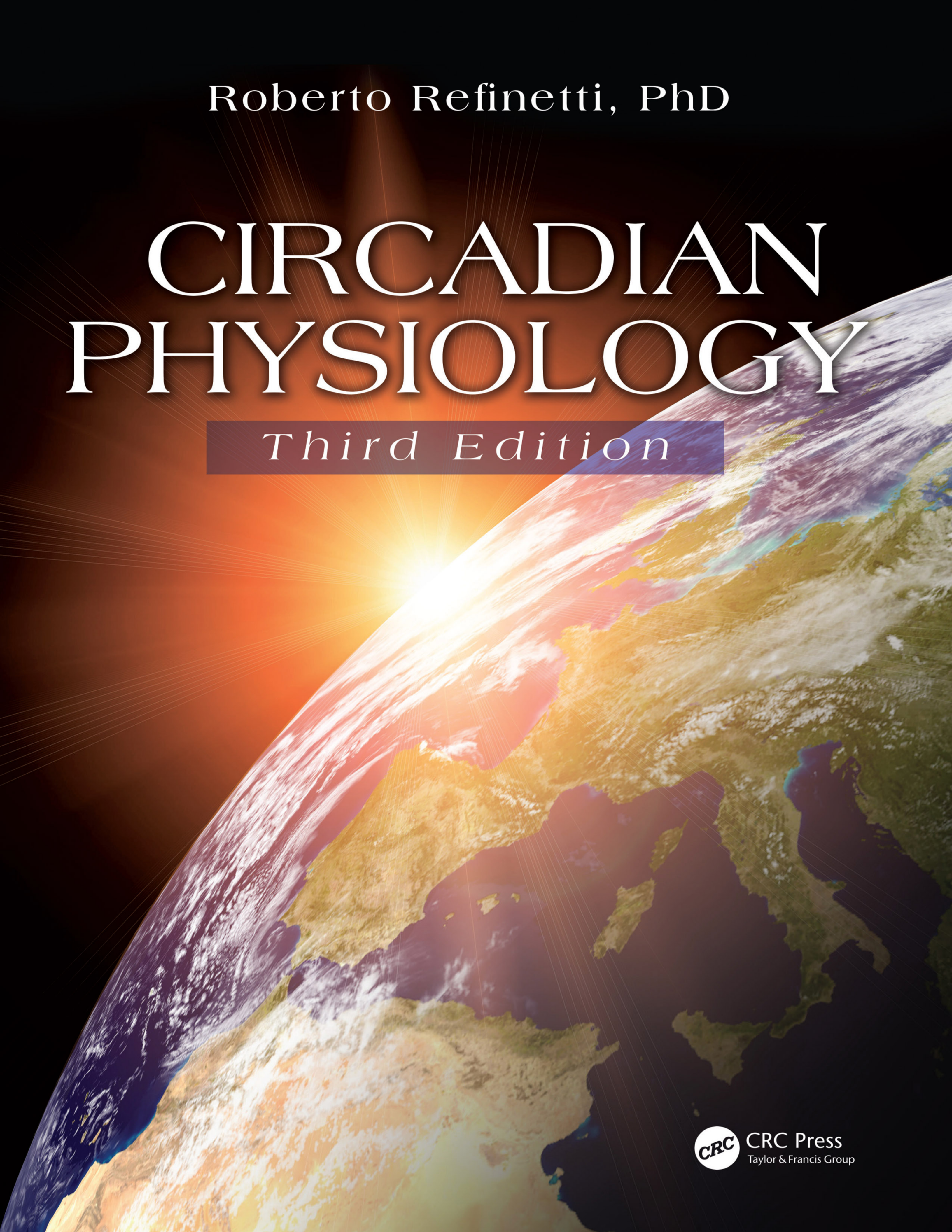
Roberto Refinetti, PhD

CIRCADIAN PHYSIOLOGY

Third Edition



CRC Press
Taylor & Francis Group

CIRCADIAN PHYSIOLOGY

Third Edition

This page intentionally left blank

CIRCADIAN PHYSIOLOGY

Third Edition

Roberto Refinetti, PhD
Boise State University
Idaho, USA

CRC Press
Taylor & Francis Group
Boca Raton London New York

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

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

Version Date: 20160202

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

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

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

Contents

SECTION II Phenomenology

SECTION III Mechanisms

SECTION IV Physical Substrates

SECTION V Applications

Supplementary Resources Disclaimer

Additional resources were previously made available for this title on CD. However, as CD has become a less accessible format, all resources have been moved to a more convenient online download option.

You can find these resources available here: www.routledge.com/9781466514973

Please note: Where this title mentions the associated disc, please use the downloadable resources instead.

Preface

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

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

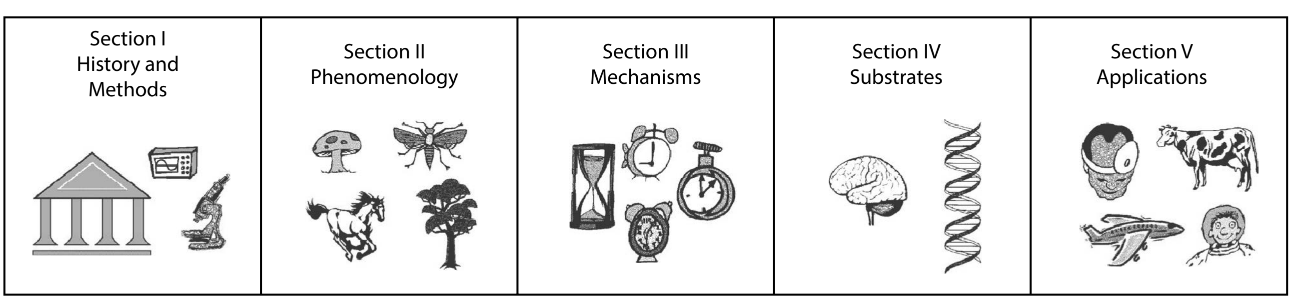
The organization of this edition is similar to that of the first and second editions because it remains the most logical and didactical. The book is divided into five sections, each with several chapters (see figure). Section I covers historical and methodological topics in the study of circadian rhythms.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

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

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

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

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



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

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

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

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

Acknowledgments

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

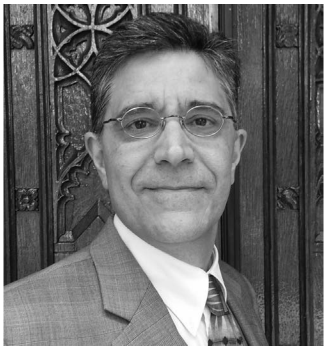
Exchanges of letters with Professor Jürgen Aschoff and Professor Franz Halberg, both pioneers in the field of circadian rhythms who are unfortunately no longer with us, helped me gain a broader historical perspective of the field.

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

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

This page intentionally left blank

Author



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

This page intentionally left blank

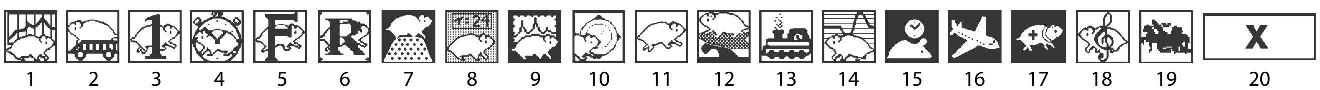
Software Installation

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

HOW TO INSTALL THE SOFTWARE

Requirements

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



Procedure

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

HOW TO USE THE SOFTWARE

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

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

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

(*Continued*)

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

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

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

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

TROUBLESHOOTING

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

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

This page intentionally left blank

Section I

History and Methods

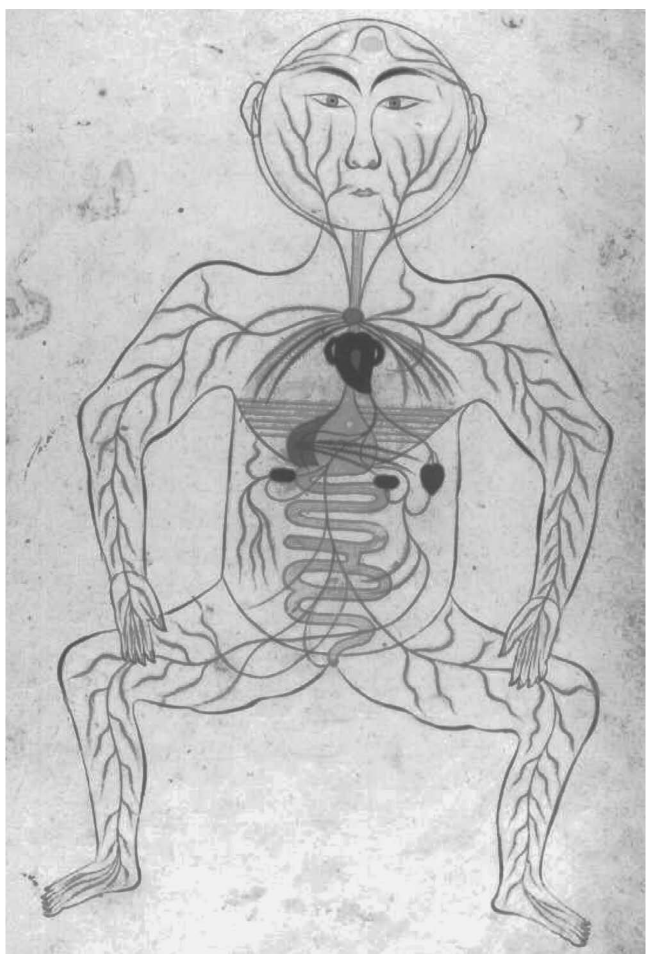


Illustration of human anatomy drawn by Persian physician Mansur ibn Mohammed in 1396. (Image courtesy of the Clendening Library at the University of Kansas Medical Center, Kansas City, MO.)

This page intentionally left blank

1 Early Research on Circadian Rhythms

1.1 REMOTE PAST

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

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

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

1.1.1 Biological Rhythms

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

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

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

It is only three centuries later, in the fourth century BC, that we find an unambiguous written record of observations in circadian physiology. This was when *Androsthenes of Thasus*, a ship captain under the command of Alexander, the Great, (Figure 1.4), recorded his observations of daily movements in plants.[9] Androsthenes traveled through North Africa and India and had the chance to observe the daily movement of the leaves of the tamarind tree (*Tamarindus indica*).

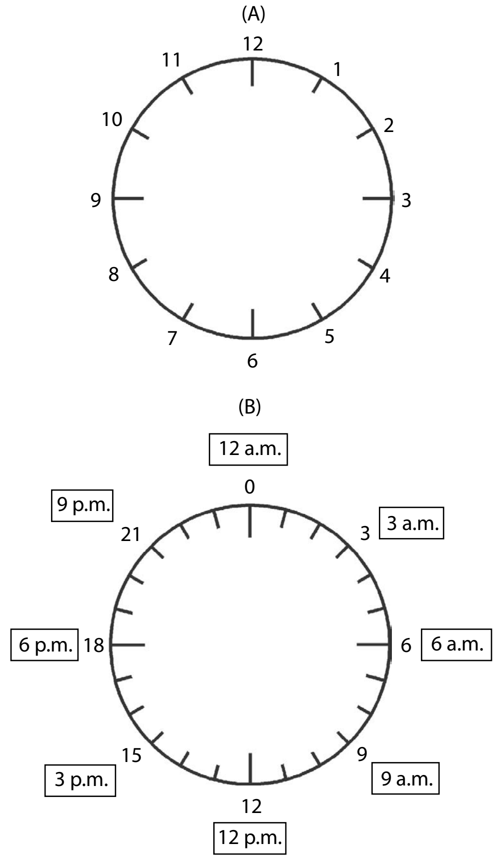


FIGURE 1.2 Diagrams of (A) a 12-hour clock and (B) a 24-hour clock. Although analog clocks almost always have 12-hour dials, a full day has twice as many hours. In most of the world, time of day is expressed according to a 24-hour clock. In the United Sates, a day is divided into 12 hours before noon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

Although most of us think of plants as dull, motionless organisms, Androsthenes noticed that the tamarind leaves exhibit an impressive daily cycle of movement, wherein the leaves move up during the day and down at night. Even if not as impressive, a similar daily movement of leaves can also be seen in the common bean plant (Figure 1.5). For those interested, Exercise 1.2 (at the end of this chapter) provides instructions on how to monitor the leaf movement of the bean plant.

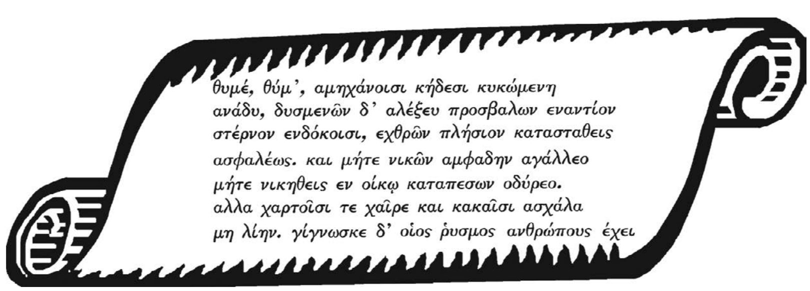


FIGURE 1.3 Is it "Greek" to you? It is Greek. This is a fragment of a poem written around 650 BC by the Greek poet Archilochus. It talks about the rhythms of life.

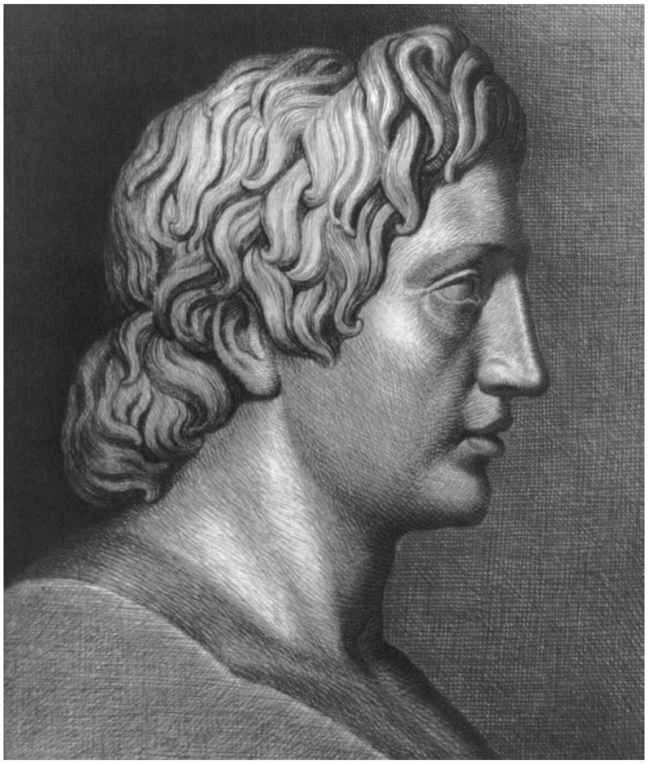


FIGURE 1.4 Alexander the Great (356–323 BC). The great Greek (Macedonian) general conquered most of the civilized world in the fourth century BC. One of the many ship captains in his fleet was Androsthenes of Thasus, who wrote the first description of daily movements in plants. (Courtesy of Library of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

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

1.1.2 Endogenous Rhythmicity

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

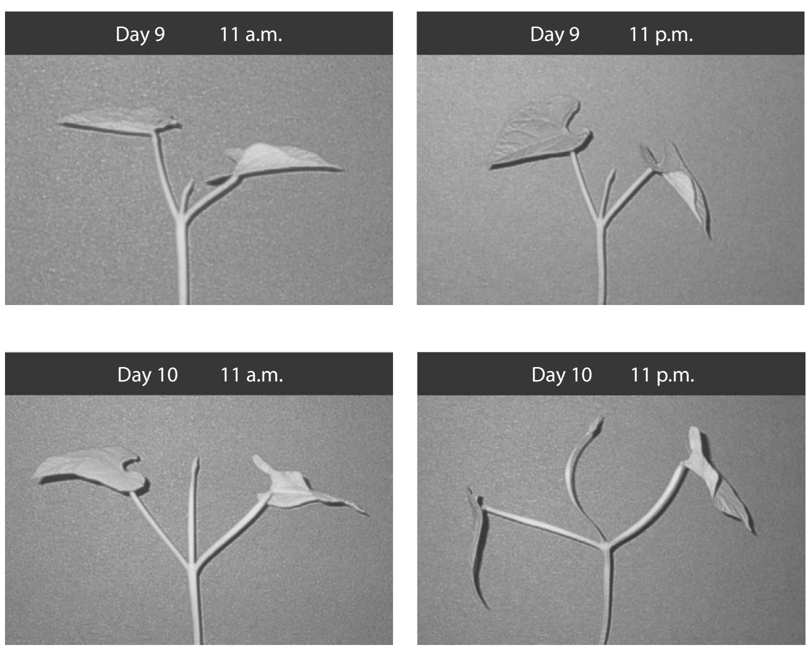


FIGURE 1.5 The "sleep" cycle of the bean plant. The leaves of the common bean plant (*Phaseolus vulgaris*) rise during the day and drop at night. (Photo and montage by R. Refinetti.)

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

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

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

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

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

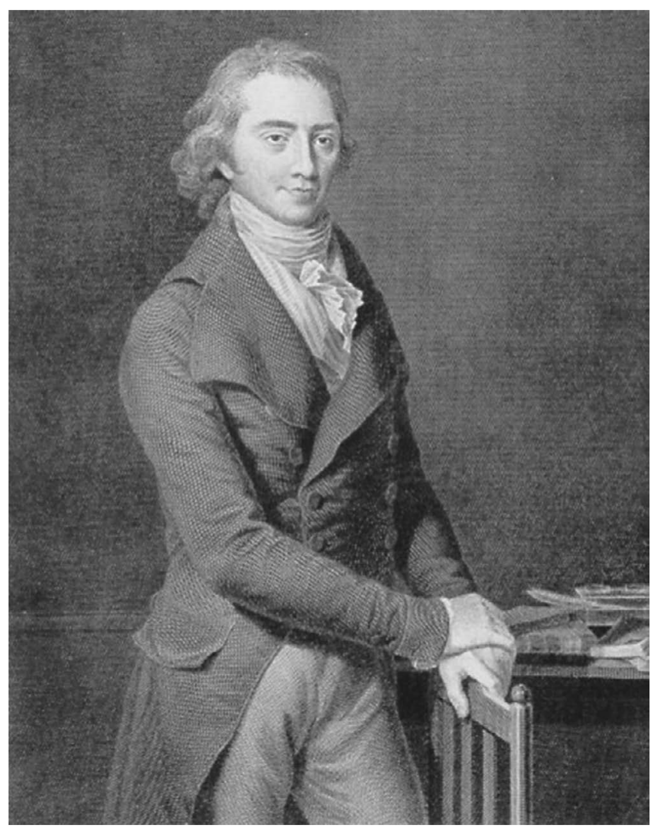


FIGURE 1.8 Christoph Wilhelm Hufeland (1762–1836). This German physician wrote the first systematic account of daily rhythmicity in human physiology as part of a larger book. (Courtesy of Clendening Library, University of Kansas Medical Center, Kansas City, MO.)

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

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

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

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

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

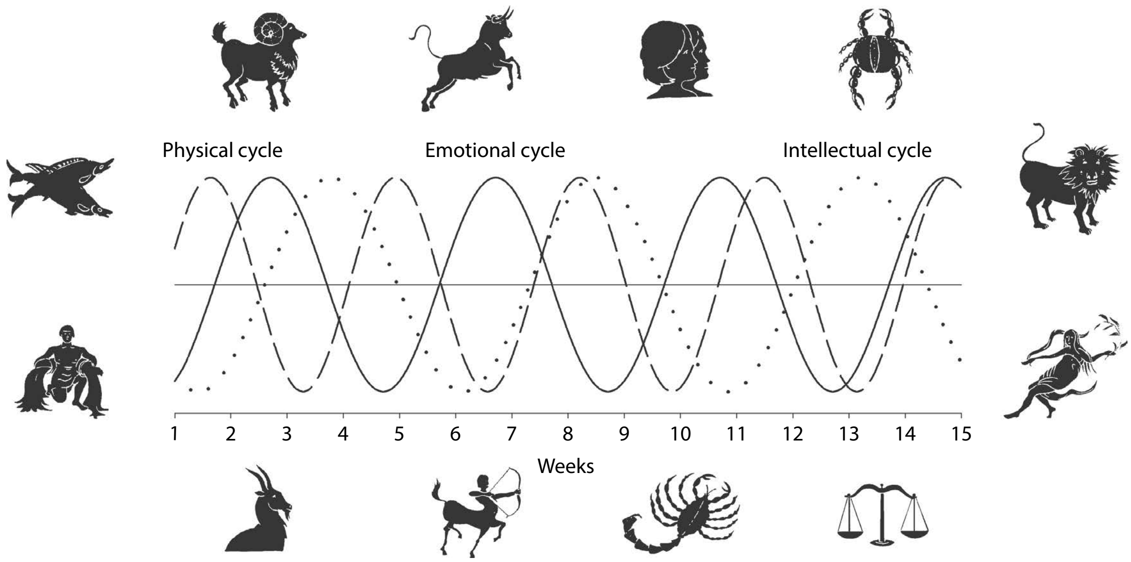


FIGURE 1.11 Biorhythms and horoscope? The concept of biorhythms was developed by W. Fliess and H. Swoboda in the late 1800s. Although they claimed no connection with the signs of the zodiac, their notion of biorhythms was just as unscientific as horoscopes are. (Data from Crawley, J., *The Biorhythm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

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

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

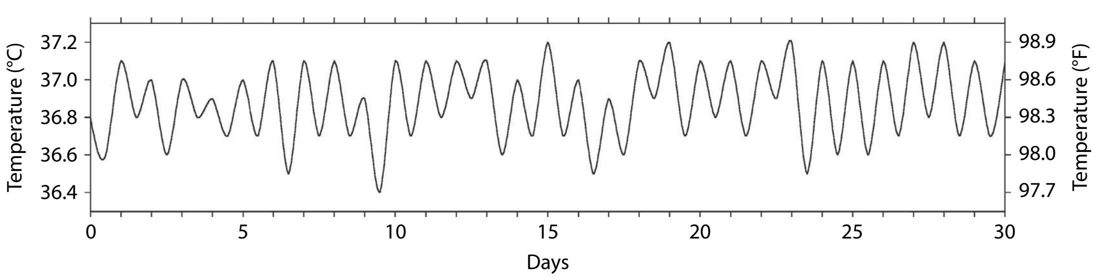


FIGURE 1.12 A real biological rhythm. In 1844, British physician John Davy made accurate measurements of the day-to-day variation of his own body temperature. (Data from Davy, J., *Philos. Trans. R. Soc. Lond.*, 135, 319, 1845.)

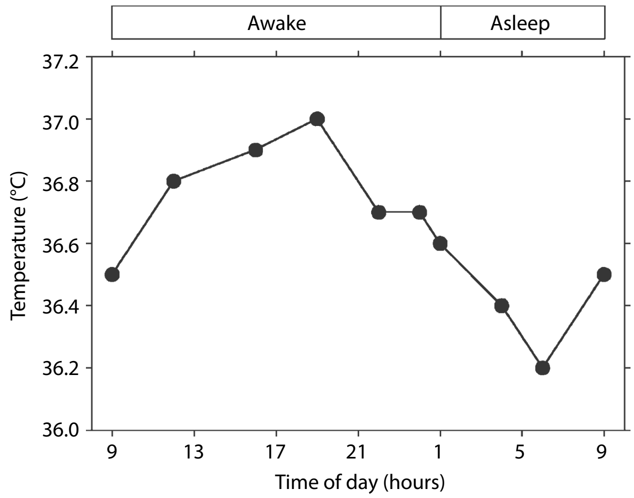


FIGURE 1.13 An early record of the daily rhythm of body temperature. In 1865, physician William Ogle conducted measurements of his own oral temperature with temporal resolution high enough to allow the characterization of a daily rhythm. (Data from Ogle, W., *St. George's Hosp. Rep.*, 1, 221, 1866.)

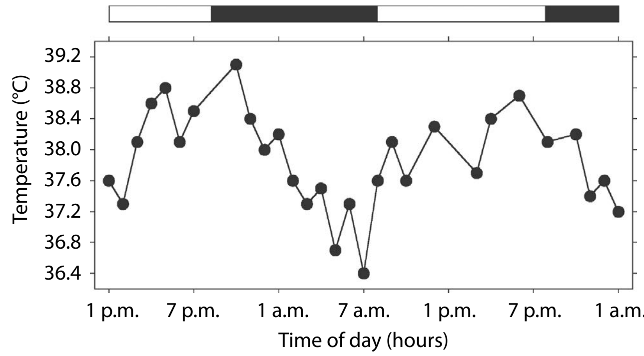


FIGURE 1.14 Old records of body temperature of a monkey. From 1902 to 1905, Simpson and Galbraith conducted numerous measurements of the body temperature of rhesus monkeys, as exemplified in these records from Monkey #31. The light and dark bars at the top of the figure indicate the approximate duration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

1.2 TWENTIETH CENTURY

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

1.2.1 1901–1950

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

The first demonstration of the endogenous nature of circadian rhythms in an animal species was provided by Maynard Johnson, in Illinois, in 1926.[42] Johnson studied the rhythm of locomotor activity (i.e., the rhythm of moving around) of deer mice (*Peromyscus leucopus*). He kept the mice in constant darkness in an environment without temporal cues and examined the time at which the animals became active each day (the "onset time"). As shown in Figure 1.15, the activity onsets drifted 4 hours (from 4 p.m. to 8 p.m.) in about a month—again, with some day-to-day variability. Thus, the activity onsets were delayed by about 6 minutes each day. This means that the mice were running on a 24.1-hour clock rather than on a 24.0-hour clock. Because all potential geophysical time cues would be expected to run on a 24.0-hour clock (the period of Earth's rotation), Johnson justifiably concluded that the clock responsible for the activity rhythm of the mice was

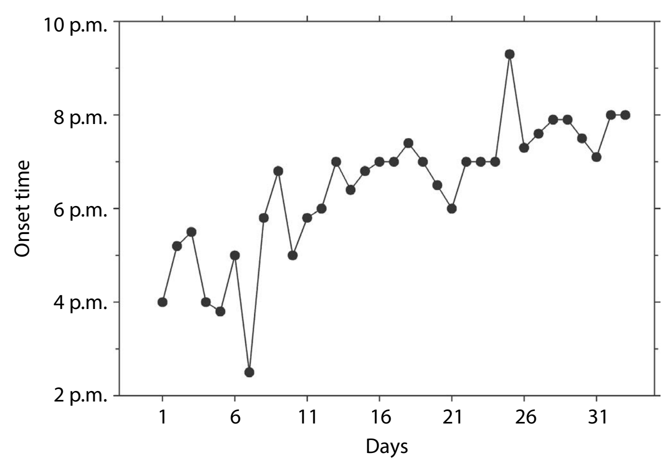


FIGURE 1.15 "Free-running" mouse. In 1925, Maynard Johnson documented a circadian rhythm of locomotor activity in deer mice (*Peromyscus leucopus*) maintained in constant darkness. The rhythm exhibited a period longer than 24 hours and, therefore, could not be attributed to geophysical factors. "Onset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

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

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

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

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

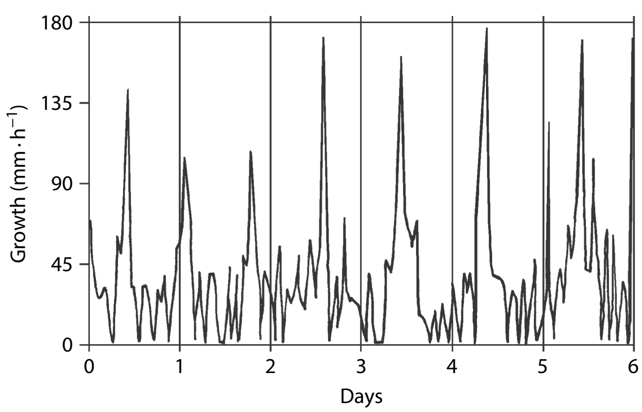


FIGURE 1.16 Daily rhythmicity in prokaryotes. In 1929, Rogers and Greenbank demonstrated the existence of daily rhythmicity in the growth of bacteria, which are prokaryotes (i.e., organisms whose cells do not have a separate nucleus). Except in the second of the 6 days shown, clear daily peaks of growth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

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

1.2.2 1951–2000

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

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

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

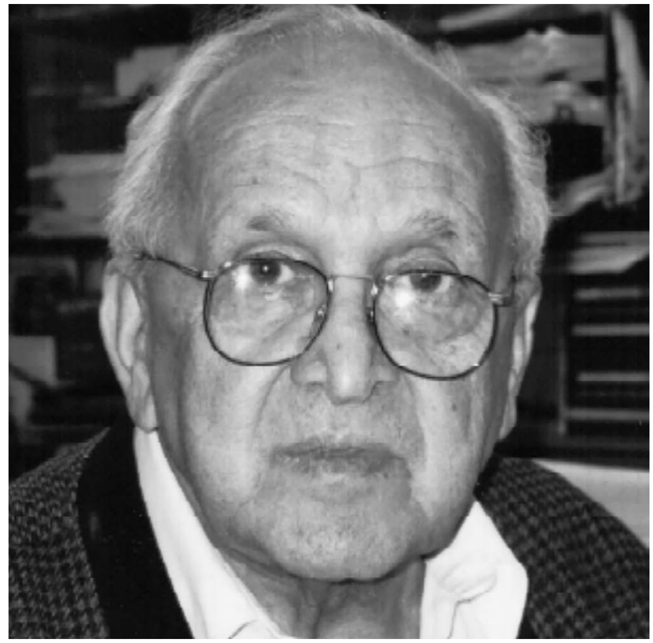


FIGURE 1.19 Franz Halberg (1919–2013). This American physician (originally from Romania) created the terms *circadian* and *chronobiology* and was a life-long advocate of the establishment of chronobiology as a separate discipline. (Photo courtesy of Franz Halberg.)

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

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

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

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

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

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

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

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

I should hasten to point out that antagonisms are not peculiar to circadian physiology—or to science more generally. In the musical arts, for instance, the renowned classical composer and conductor Rimsky-Korsakov had this to say about the no-less renowned Ludwig van Beethoven: "His music abounds in countless leonine leaps of orchestral imagination, but his technique, viewed in detail, remains much inferior to his titanic conception."[81] In plain words: "Beethoven is a bad composer!" Needless to say, I and millions of others beg to differ. In any event, small but sincere attempts at reconciliation of the two factions were made over the years. A conference held at the Cold Spring Harbor Laboratory (in Long Island, New York) in 1960 had already brought together Bünning, Aschoff, Halberg, Pittendrigh, and others under one roof,[82] although the division of the factions had not been yet strongly established at that time. Thirty-five years later, in 1995, a conference was organized at Dartmouth Medical School by members of the clocks faction (Figure 1.21). Although most participants were members of the clocks faction, members of the chronome faction were welcomed as well. A few years later, in 1999, an eclectic group of circadian physiologists organized a congress sponsored by nine different professional organizations dedicated to the study of biological rhythms. Participants in this congress—held in Washington, DC (Figure 1.22)—included basic researchers as well as medical practitioners and provided the opportunity for the exchange of ideas between individuals with quite different professional interests. After the turn of the century, in 2001, a World Federation of Societies for Chronobiology was established, bringing together 13 professional associations with diverse interests related to biological rhythms. The federation held its first congress in 2003.[83]

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

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

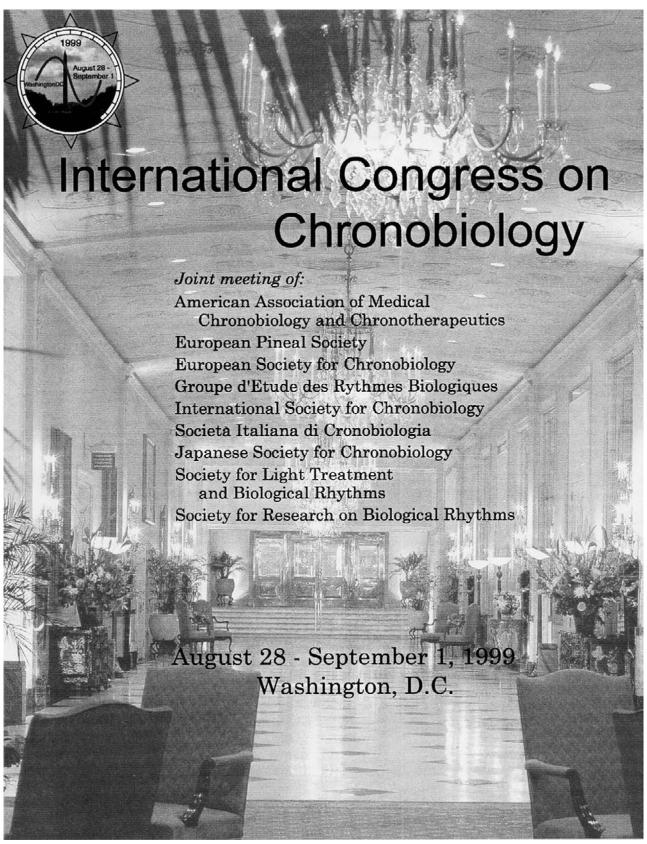


FIGURE 1.22 **First major attempt to unify the field.** An International Congress on Chronobiology, held in Washington, DC, in 1999, was the first major attempt to unify the field of studies of biological rhythms. (Image: Cover of the Congress' Program.)

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

1.3 CURRENT TRENDS

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

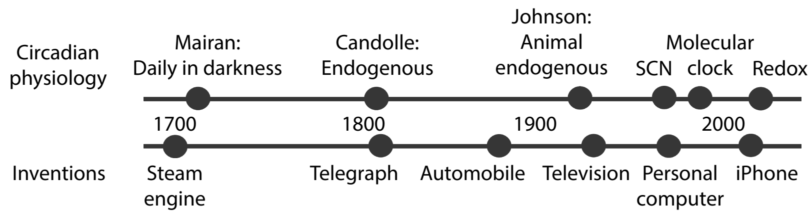


FIGURE 1.23 Circadian timeline. This diagram provides a timeline of major events in circadian physiology and major inventions in the past 300 years. Information about the suprachiasmatic nucleus (SCN), molecular clock, and redox will be provided in Chapter 12.

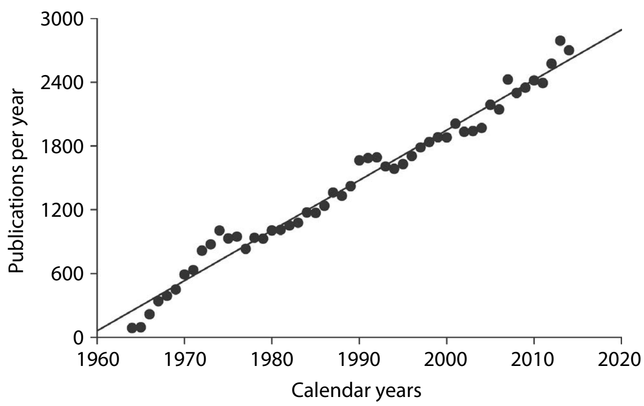


FIGURE 1.24 Growth in the number of journal articles on circadian rhythms published during the past 50 years. The number of articles on circadian rhythms catalogued by PubMed (U.S. National Library of Medicine) grew from fewer than 100 articles per year in 1964 to almost 2700 articles per year in 2014.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

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

1.3.1 Circadian Physiology and the World

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

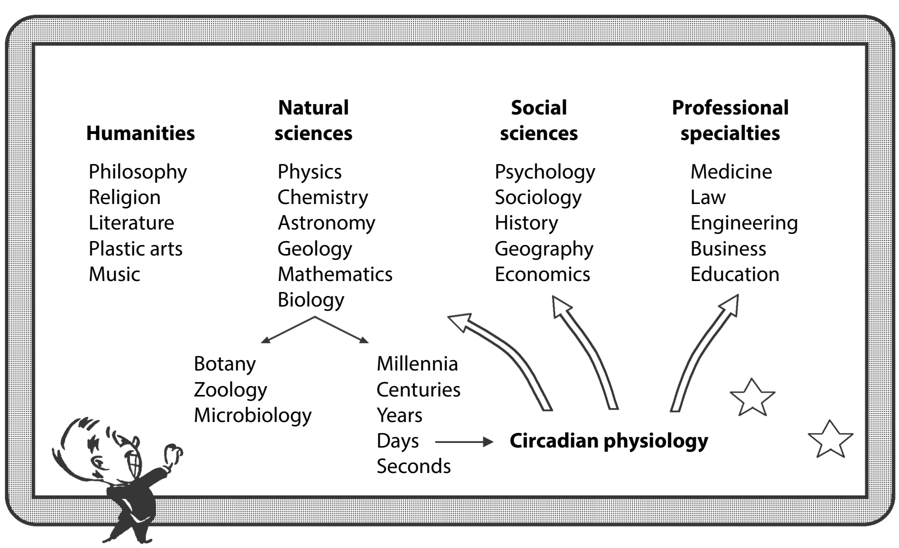


FIGURE 1.25 The "big picture." Circadian physiology has a definite place in the universe of human knowledge, and its implications are widespread.

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

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

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

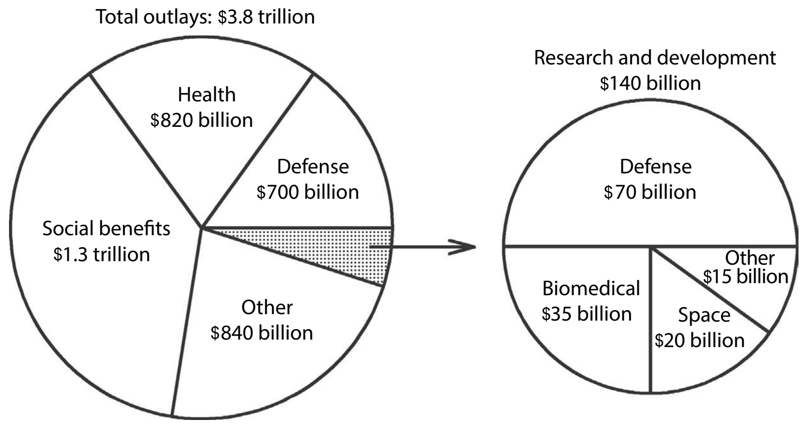


FIGURE 1.27 The money chest. Shown is the breakdown of outlays in the United States federal budget (year 2014). (From Janssen, S., ed. *The World Almanac and Book of Facts*, Simon & Schuster, New York, 2014; Mervis, J. and Malakoff, D., *Science*, 346, 1437, 2014.)

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

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

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

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

1.3.2 Current Researchers

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

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

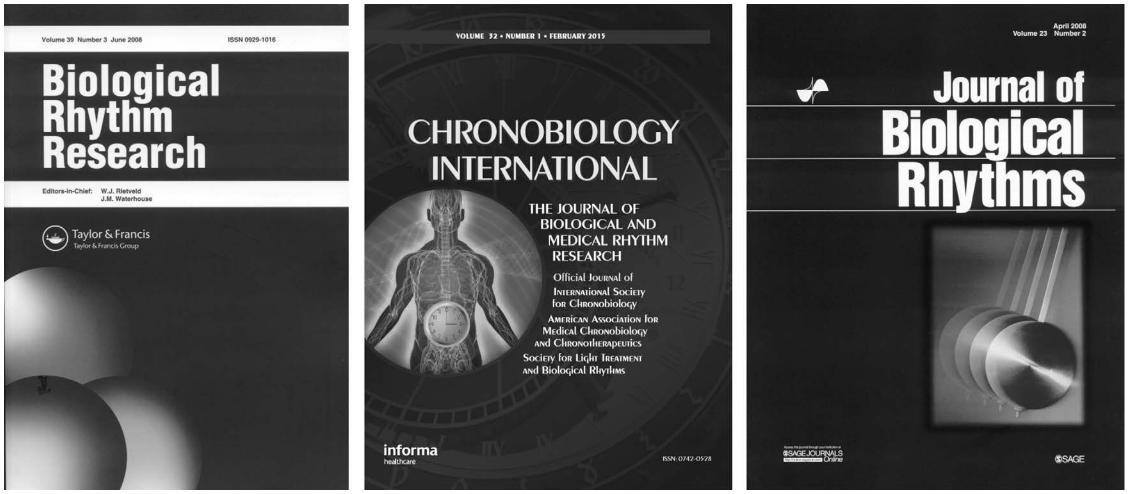


FIGURE 1.28 The three print journals specialized in biological rhythms. Currently, there are three print journals specialized in biological rhythms: *Biological Rhythm Research* (published by Taylor & Francis), *Chronobiology International* (published by Taylor & Francis), and the *Journal of Biological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

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

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

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

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

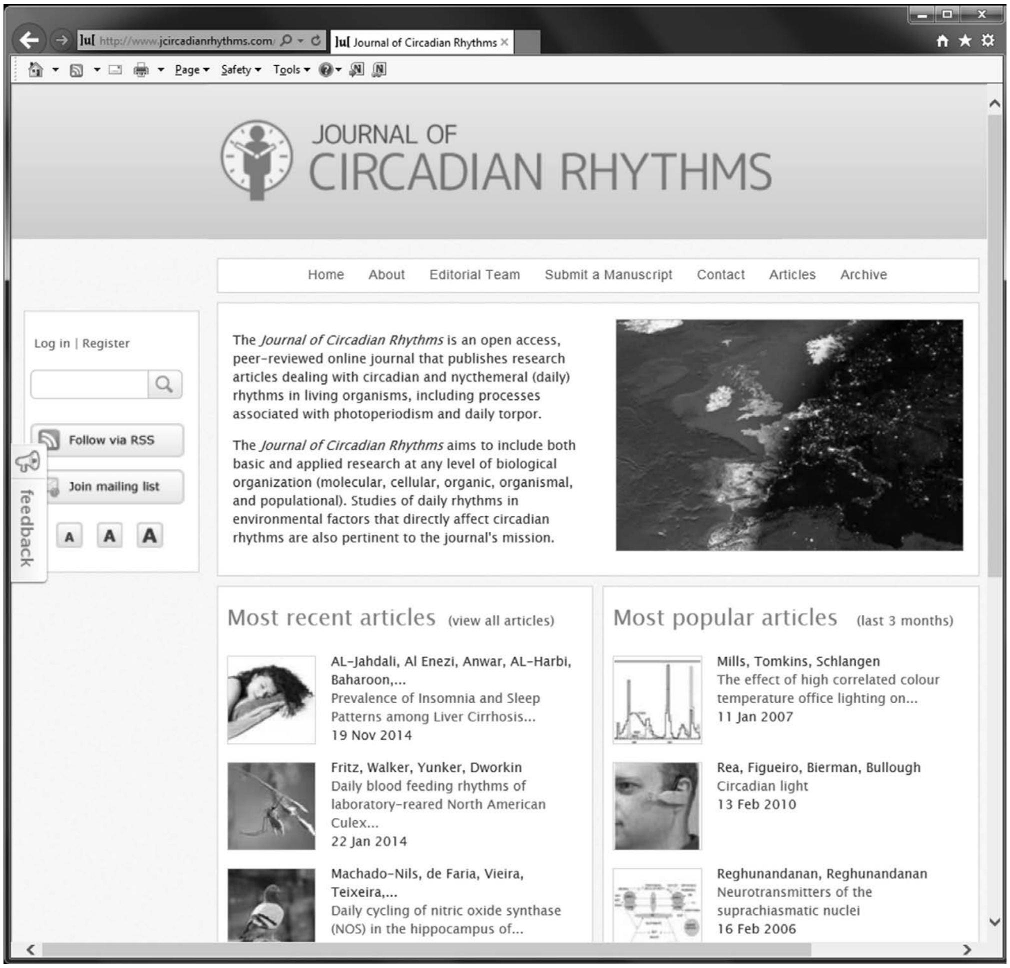


FIGURE 1.29 **The *Journal of Circadian Rhythms*.** The first journal specialized in circadian rhythms was launched in 2003. The *Journal of Circadian Rhythms*, published by Ubiquity Press, is an open-access electronic journal (with no print version) in which articles are freely available to readers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

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

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

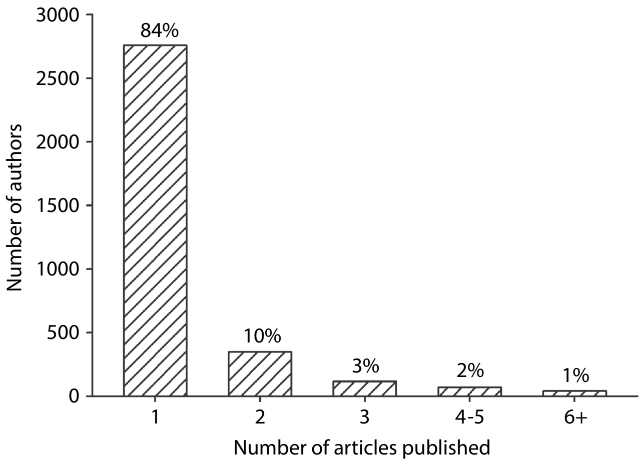


FIGURE 1.30 How many people publish research articles on circadian rhythms? In a 12-month interval not long ago, 3330 authors published journal articles containing the word *circadian* in the title or as an indexed key word. The figure shows how many of these authors published how many articles. (Data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

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

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

TABLE 1.3
The Most Prolific Circadian Physiologists Today[a]

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

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

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

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

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

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

TABLE 1.4
Contemporary Circadian Physiologists[a]

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

(*Continued*)

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

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

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

1.4 ETHICS OF ANIMAL RESEARCH

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

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

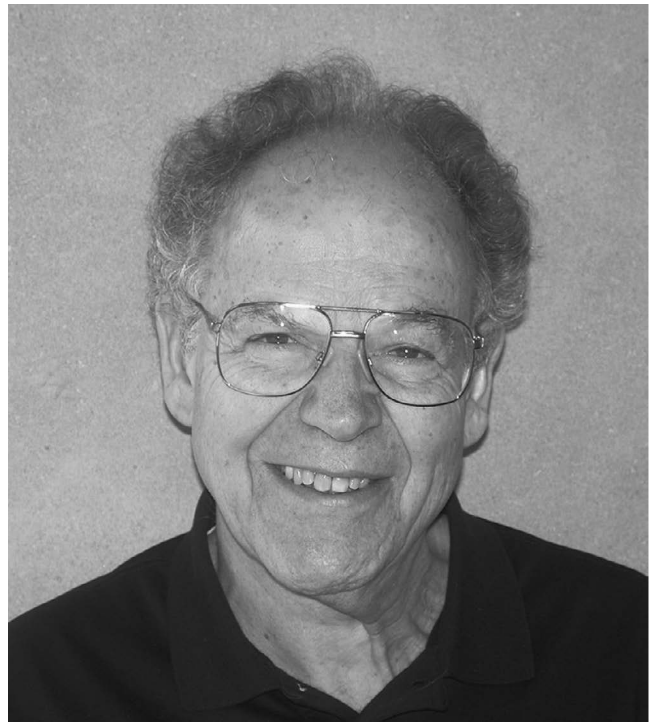


FIGURE 1.35 Michael Menaker (1934–). This American biologist specializes in the neurobiology of circadian rhythms in vertebrates. (Photo courtesy of Rebecca Arrington.)

1.4.1 Use of Animals

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

TABLE 1.5
Courses on Biological Rhythms Offered at North American Universities

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

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

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

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

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

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

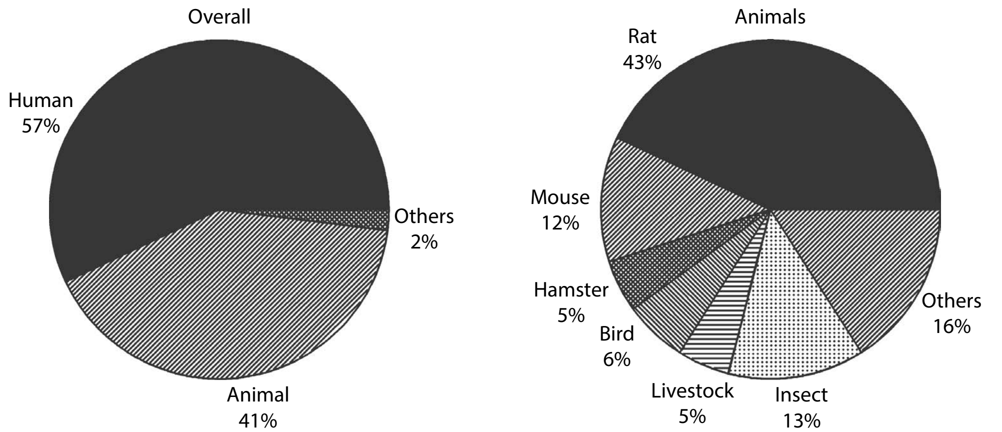


FIGURE 1.37 Proportions of experimental subjects in studies of circadian rhythms. More than half of all studies dealing with circadian rhythms are conducted on human subjects. More than half of the studies conducted in animals involve rats and mice. (PubMed database searched by R. Refinetti in October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

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

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

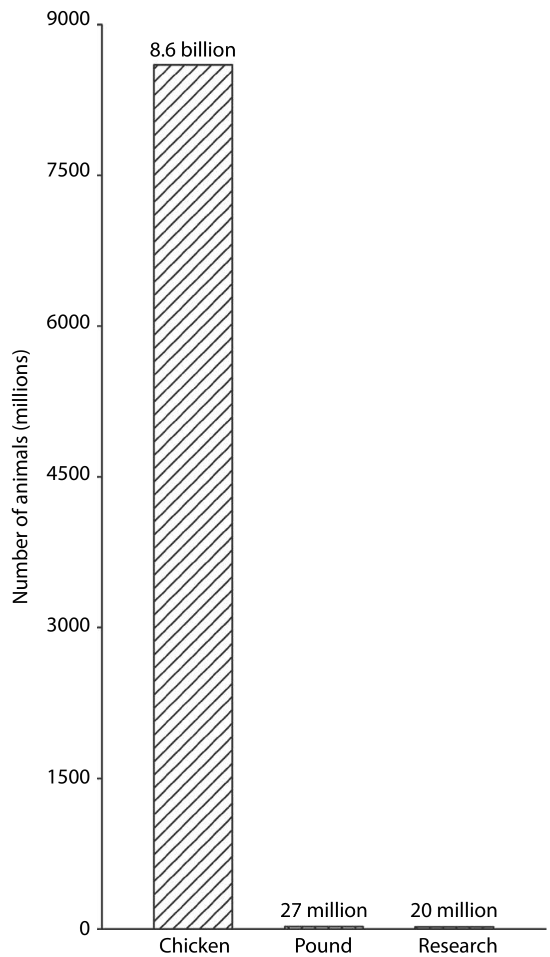


FIGURE 1.38 Comparative figures of animal use by humans. The figure shows the approximate number of animals killed each year in the United States in three sectors: chicken (used as food), pound (cats and dogs euthanized in animal shelters because of shortage of adoptions), and research (mostly rats and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

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

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

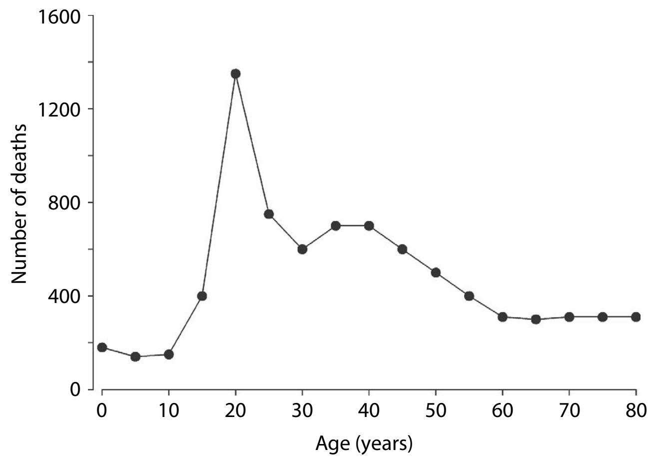


FIGURE 1.39 Deaths due to motor vehicle accidents in the United States. The figure shows the actual number of human deaths resulting from motor vehicle accidents in the year 1999 for various ages (ages between multiples of 5 are not shown). (Data from U.S. National Safety Council, *Injury Facts 2002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

1.4.2 Ethical Issue

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

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

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

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

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

SUMMARY

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

EXERCISES

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

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

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

Across

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

Down

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

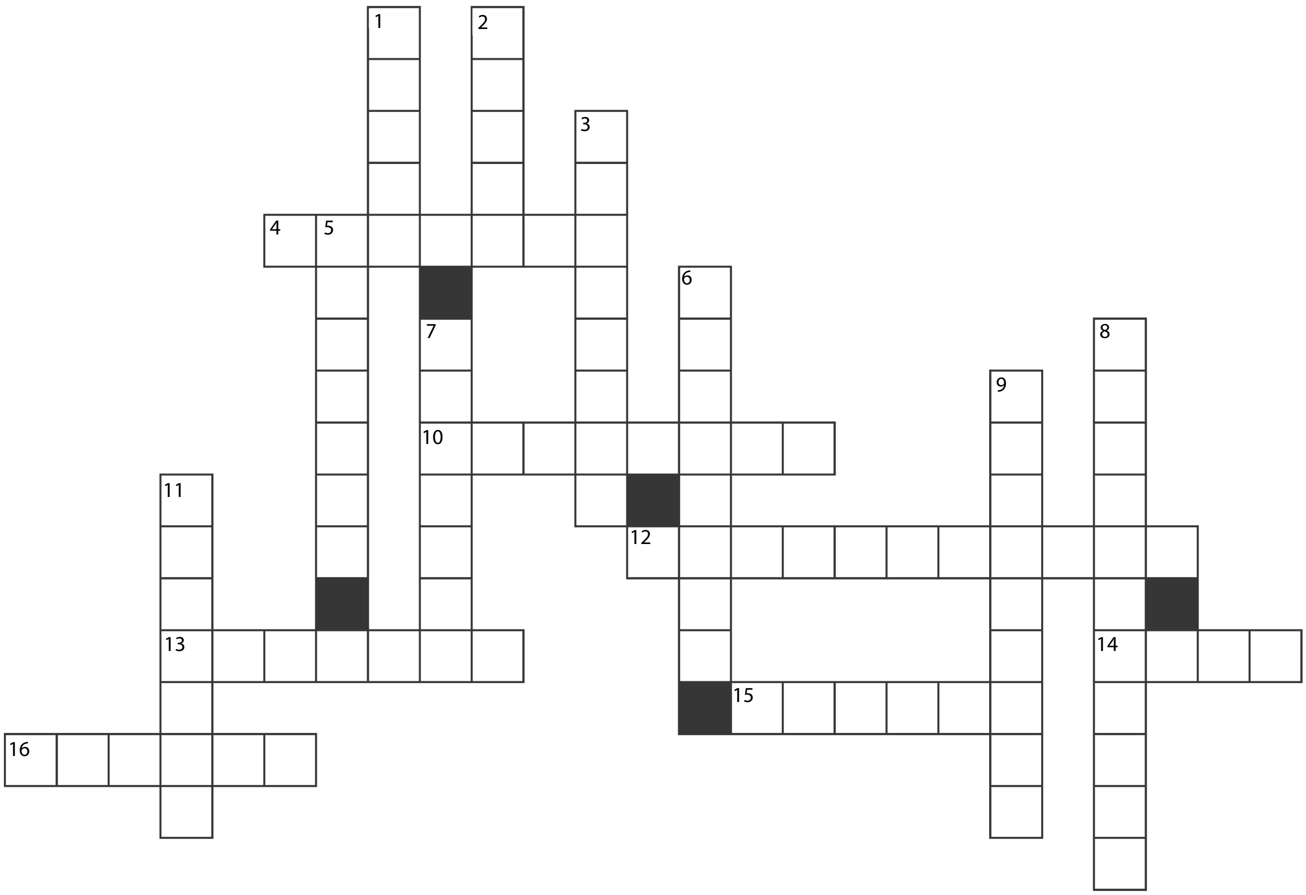


FIGURE 1.40 Crosswords. See Exercise 1.3.

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

SUGGESTIONS FOR FURTHER READING

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

WEBSITES TO EXPLORE

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

KEY TO CROSSWORDS (FIGURE 1.40)

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

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

REFERENCES

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

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

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

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

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

2 Research Methods in Circadian Physiology

2.1 SCIENTIFIC METHOD

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

2.1.1 Philosophy and Science

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

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

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

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

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

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

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

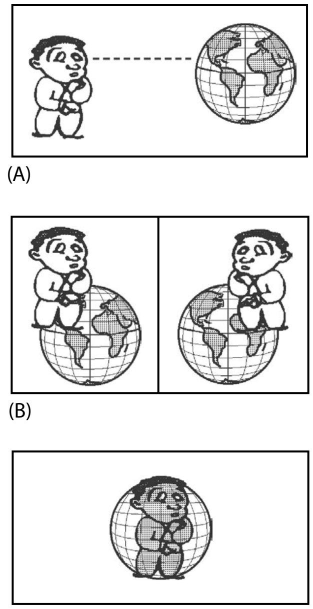


FIGURE 2.3 Three different worldviews. The drawings symbolize the three main epistemological perspectives: realism (A), relativism (B), and dialectics (C).

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

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

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

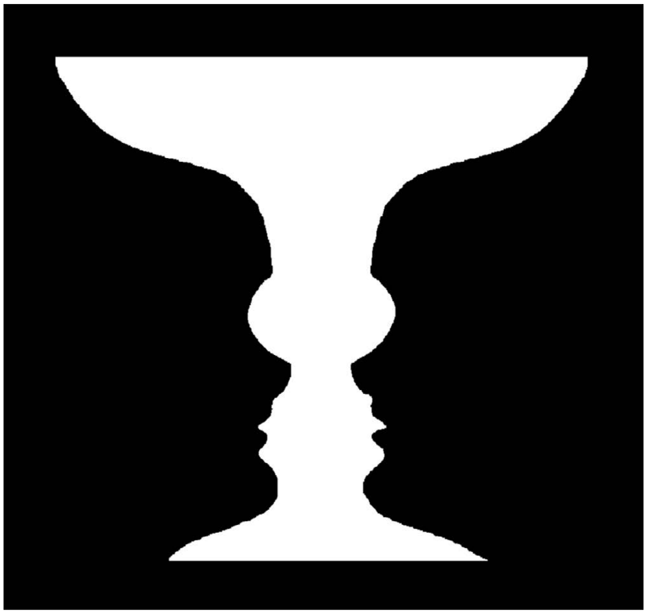


FIGURE 2.4 A goblet or two faces? This figure shows that the perception of an image may depend on how one looks at it. (Adapted from Levine, M.W. and Shefner, J.M., *Fundamentals of Sensation and Perception*, Addison-Wesley, Reading, MA, 1981.)

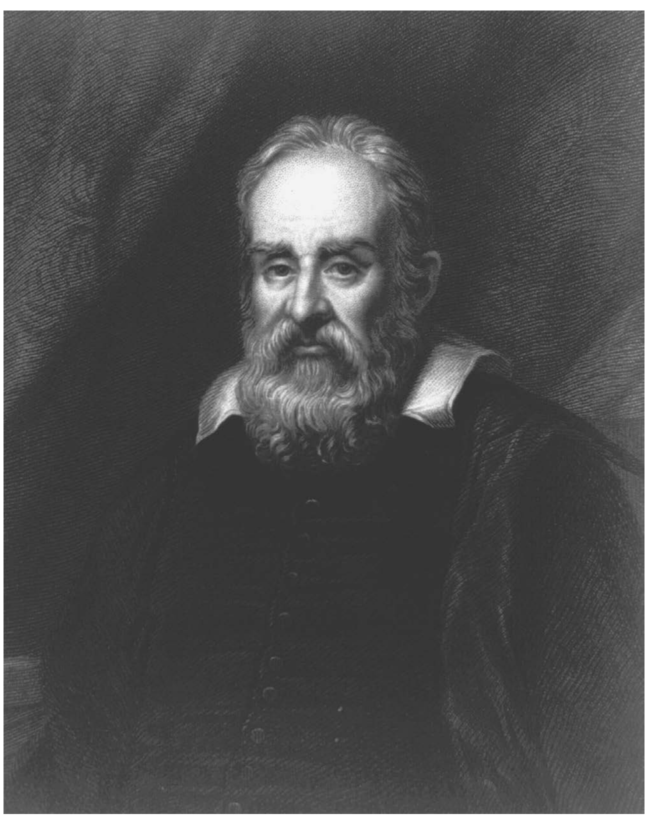


FIGURE 2.5 Galileo Galilei (1564–1642). This famous Italian astronomer and physicist endorsed epistemological realism. (Courtesy of Library of Congress, Washington, DC.)

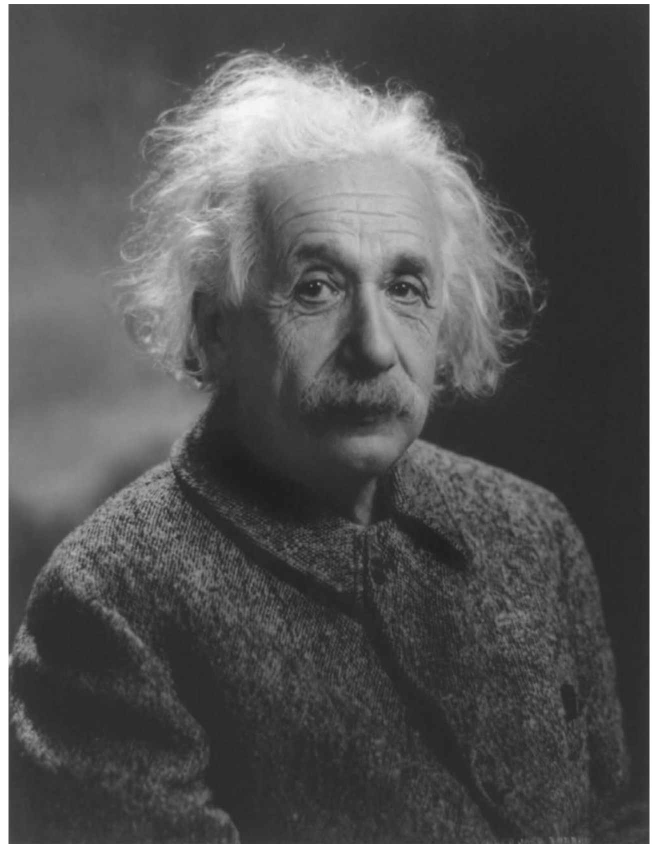


FIGURE 2.6 Albert Einstein (1879–1955). This German theoretical physicist, the most famous scientist of the twentieth century, is widely known for his theory of relativity. Nonetheless, he was an epistemological realist. (Courtesy of Library of Congress, Washington, DC.)

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

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

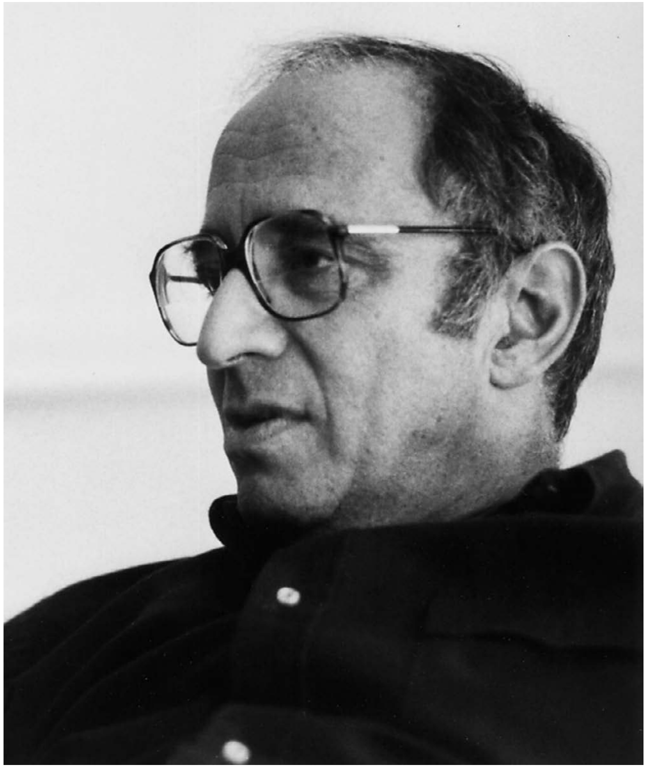


FIGURE 2.7 Thomas Kuhn (1922–1996). This American philosopher of science was greatly influential in the resurgence of epistemological relativism in the late twentieth century. (Courtesy of MIT Museum, Massachusetts Institute of Technology, Cambridge, MA.)

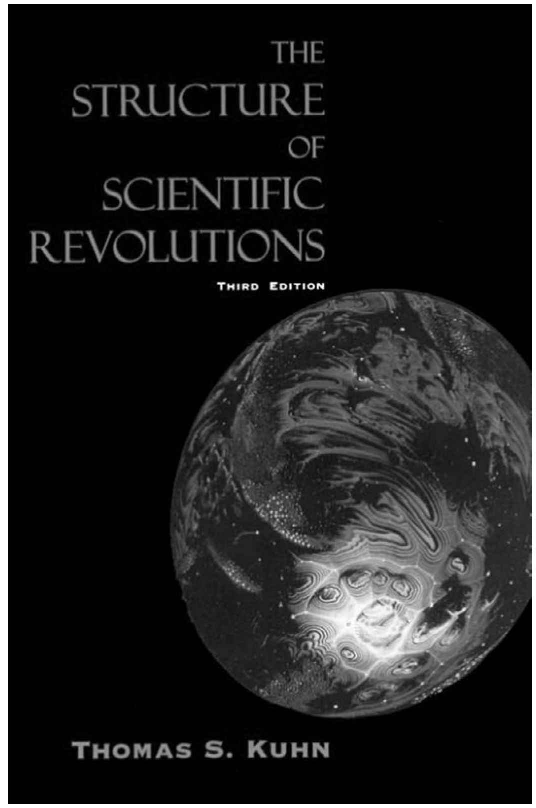


FIGURE 2.8 Cover of the 3rd edition of Kuhn's *The Structure of Scientific Revolutions*. This book by Thomas Kuhn, first published in 1962, is probably the best known philosophy of science book ever published. The third edition was published by the University of Chicago Press in 1996.

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

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

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

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

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

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

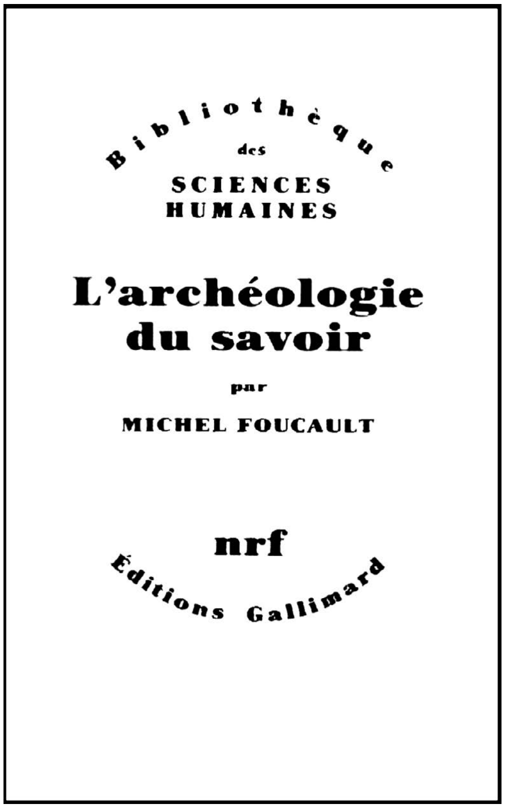


FIGURE 2.9 Cover of the original edition of Michel Foucault's *The Archeology of Knowledge*. This book by French philosopher Michel Foucault, published by Éditions Gallimard in 1969, delineated the method that leads to relativism through structuralism.

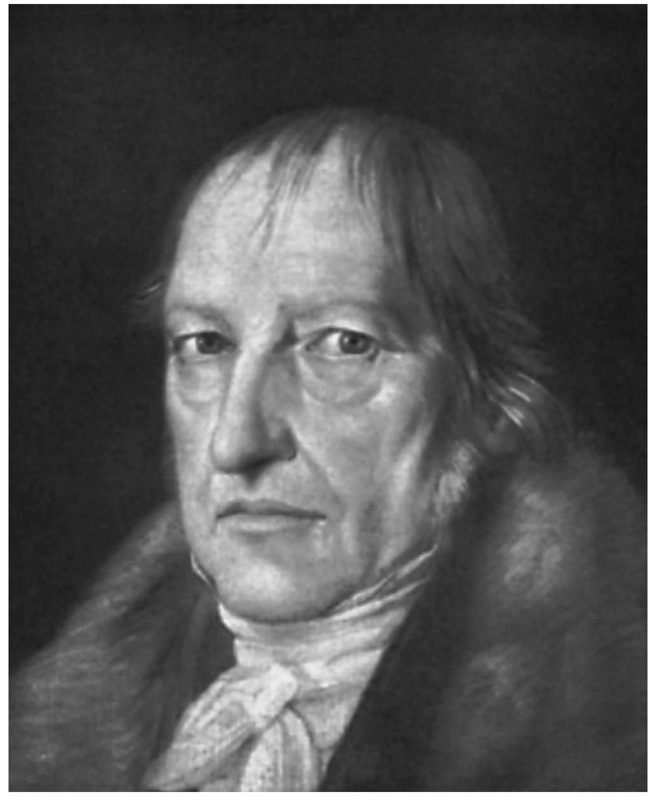


FIGURE 2.10 Georg Wilhelm Friedrich Hegel (1770–1831). This German philosopher is considered by many as the greatest philosopher of modern times. He was the father of modern dialectics. (Courtesy of The North American Fichte Society, University of Pennsylvania, Philadelphia, PA.)

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

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

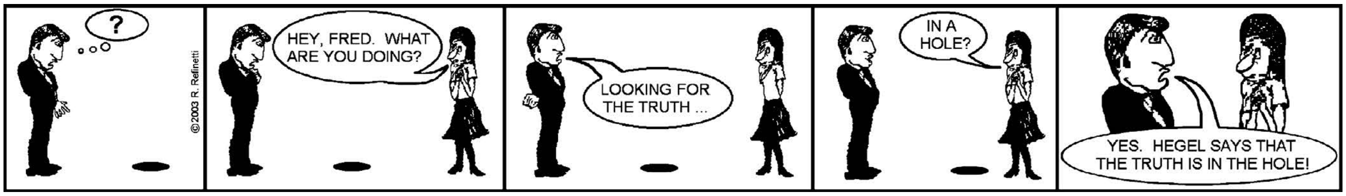


FIGURE 2.11 Looking for the truth. This comic strip is a pun upon Hegel's dialectics.

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

2.1.2 Rules of the Method

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

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

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

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

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

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

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

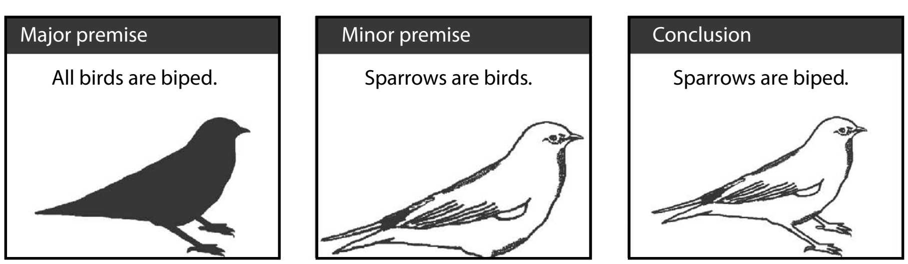


FIGURE 2.12 Syllogism. A basic syllogism consists of deriving a conclusion from a major premise and a minor premise.

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

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

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

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

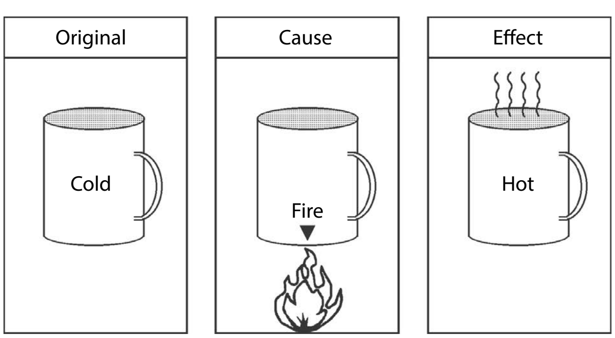


FIGURE 2.13 The principle of determinism. The principle of determinism asserts that every change in nature requires a cause. To warm up some coffee, heat is needed. No heat, no hot coffee.

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

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

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

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

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

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

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

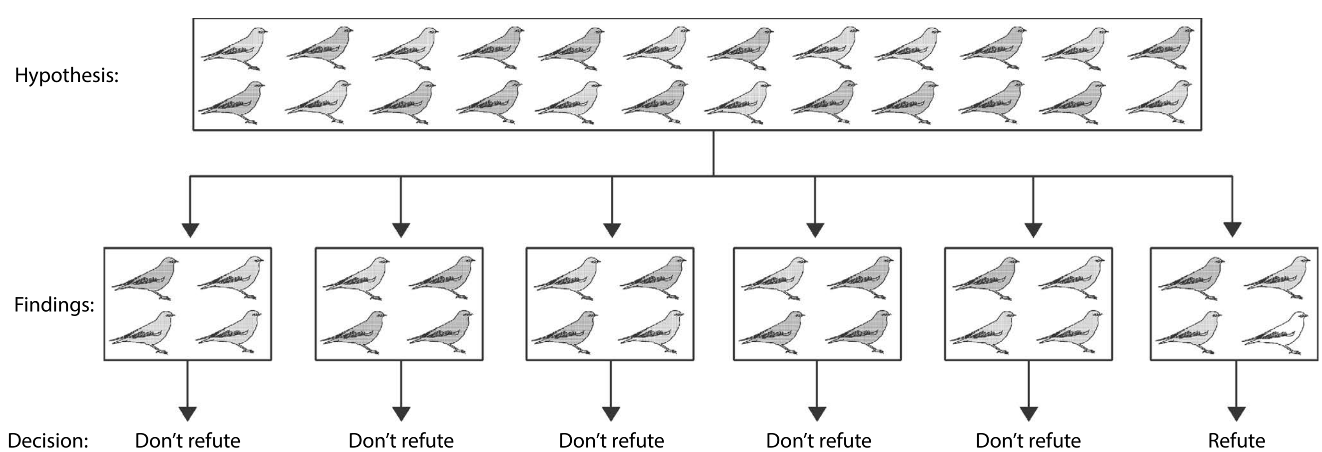


FIGURE 2.14 The refutability of hypotheses. If one has the hypothesis that all sparrows are gray, one can hold on to the hypothesis so long as one sees only gray sparrows. However, the sight of a single white sparrow is enough to refute the hypothesis.

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

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

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

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

2.1.3 Science and Religion

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

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

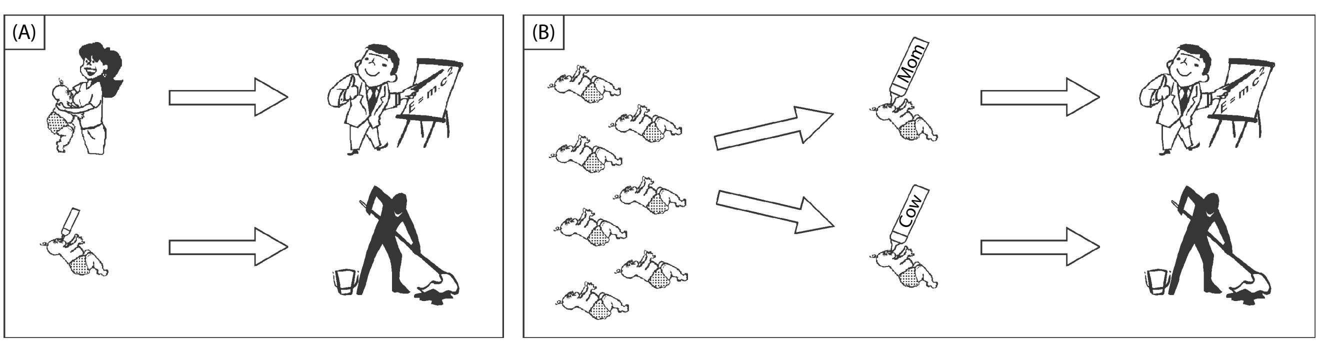


FIGURE 2.15 Correlation and causation. Although a correlation is often suggestive of causation (A), only controlled experiments allow reliable inferences about causality (B). See text for details.

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

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

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

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

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

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

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

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

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

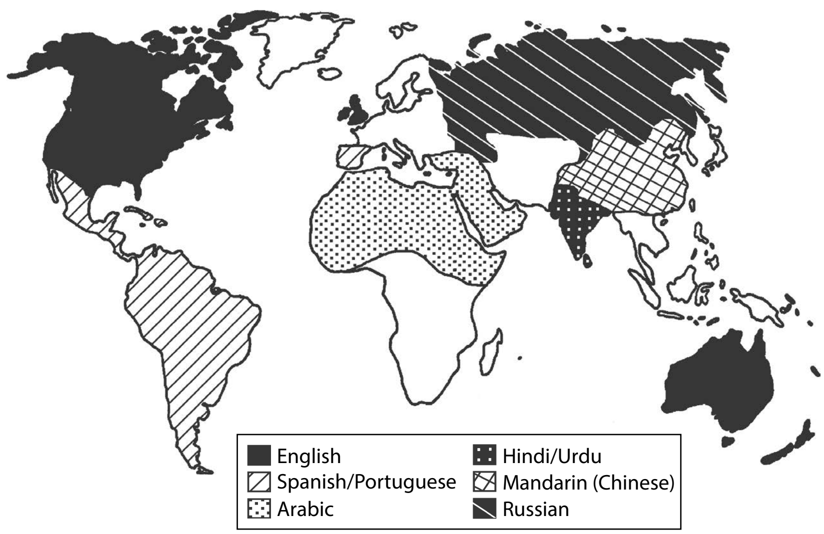


FIGURE 2.17 Languages of the world. The world distribution of languages is very similar to the world distribution of religions. The predominant language and predominant religion of different parts of the world are paired thus: English with Christian Protestantism, Spanish/Portuguese with Christian Catholicism,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

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

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

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

And yet, despite the ultimate incompatibility of science and religion, it often happens that a religious scientist can appeal to faith in religious matters but resort to scientific procedures in empirical matters. In such cases, religion and science can coexist peacefully in the same individual—or at least so claimed a few well-known scientists and philosophers who had religious faith.[91,92] In other instances—such as that of members of some Protestant denominations who perceive a conflict between *creationism* and *evolution*—religious faith requires abandonment of the scientific approach. In these cases, religious and scientific interests have irreconcilable differences.[93–95]

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

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

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

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

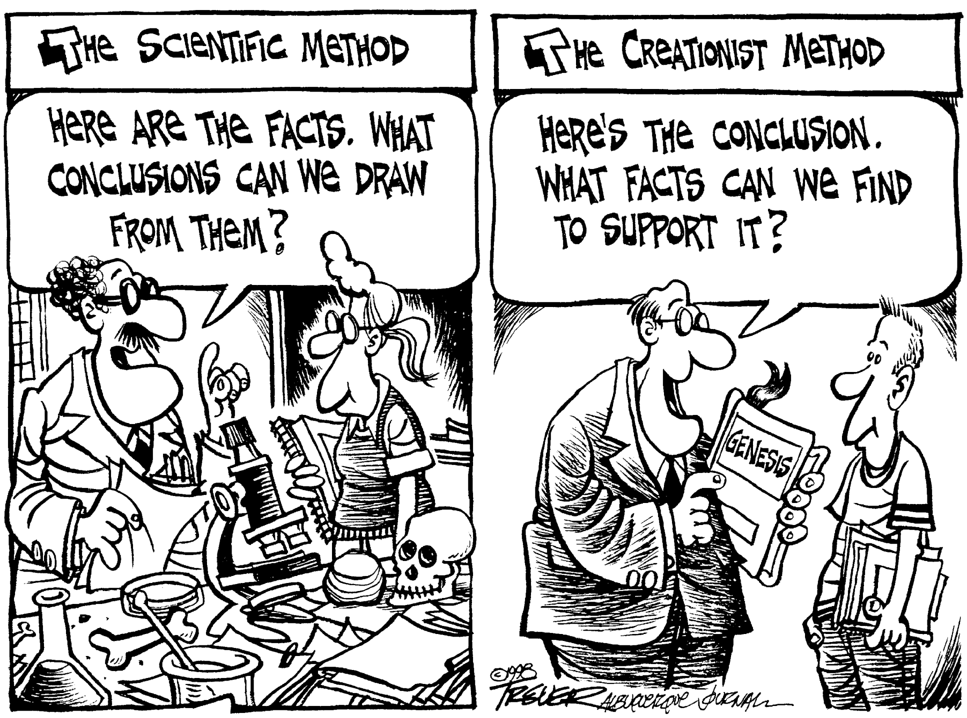


FIGURE 2.18 Different approaches to learning. This cartoon by John Trever characterizes well the difference in the approaches that scientists and creationists use to learn about the world. (Copyright 1998 by John Trever. Reproduced by permission of PoliticalCartoons.com.)

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

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

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

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

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

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

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

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

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

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

2.2 RESEARCH ON POPULATIONS AND ORGANISMS

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

Circadian physiologists seldom study actual populations, such as a group of horses in the wild (Figure 2.21). When they do study populations, they may make use of *satellite telemetry* (Figure 2.22). In this case, a signal emitter is attached to one or more of the subjects, and the location of the emitter is tracked by an orbiting satellite.[109] More commonly, individual subjects are studied via land-based *radiotelemetry*[110] (Figure 2.23) or by the satellite-assisted Global Positioning System (GPS).[111,112] Manufacturers of equipment for satellite telemetry or radiotelemetry for use in the wild include Lotek

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

Wireless Co. (Newmarket, Canada), North Star Science and Technology (Baltimore, Maryland), Sirtrack Ltd. (Havelock North, New Zealand), Telenax Ltda. (Playa del Carmen, Mexico), Telemetry Solutions Inc. (Concord, California), and Telonics Inc. (Mesa, Arizona).

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

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

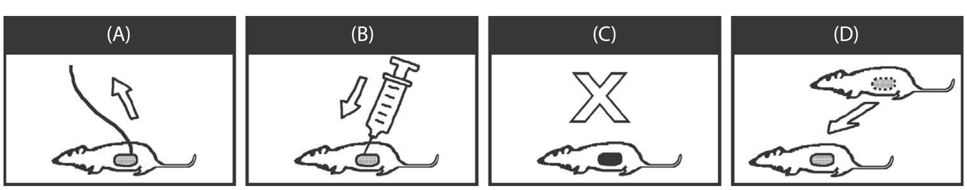


FIGURE 2.20 Research methods in physiology. Research on the vital processes of living organisms always involves one or more of four basic methods: (A) recording, (B) stimulation, (C) lesioning, and (D) transplantation.

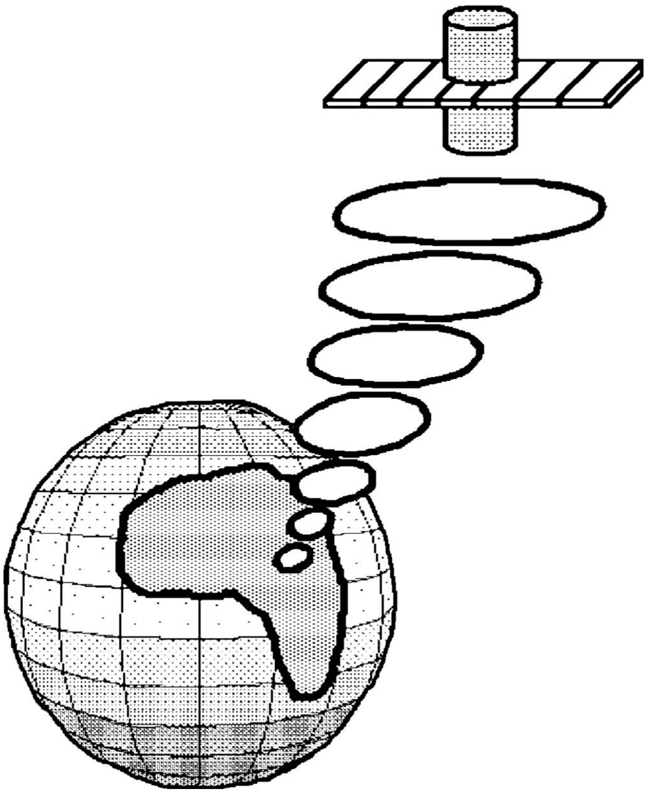


FIGURE 2.22 Satellite telemetry. Signals traced by satellite can be used for the study of populational rhythms in natural settings.

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

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

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

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

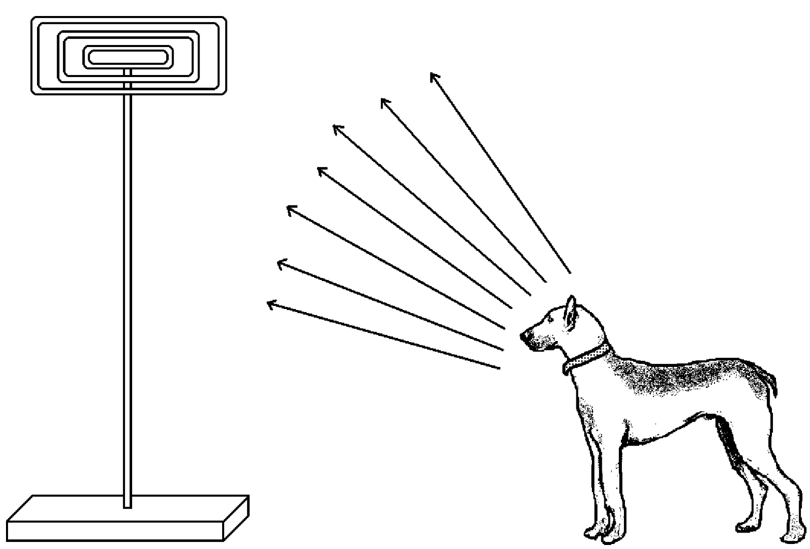


FIGURE 2.23 Radiotelemetry. Radio signals emitted by a transmitter attached to an organism (such as a collar worn by a dog) allow for the monitoring of rhythms over relatively wide areas.

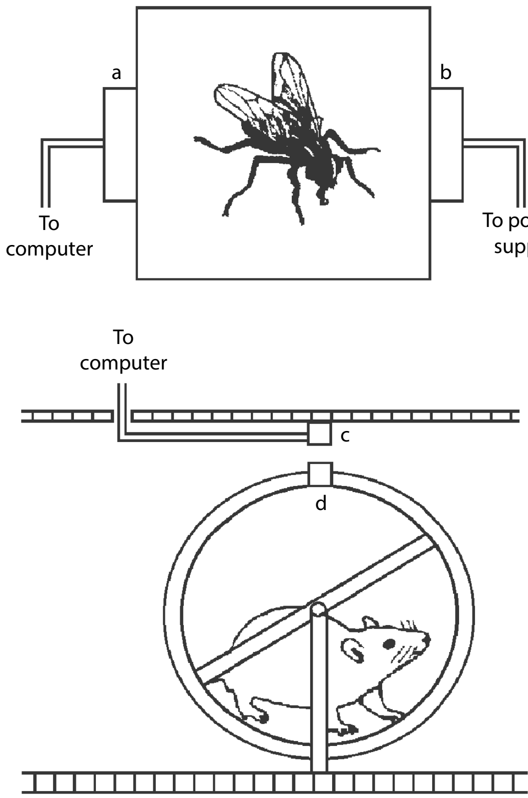


FIGURE 2.24 Monitoring activity rhythms in the laboratory. In laboratory settings, locomotor activity of individual organisms is often monitored by infrared movement detectors (a, sensor; b, emitter) or by mechanic or magnetic switches attached to running wheels (c, magnetic switch; d, magnet).

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

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

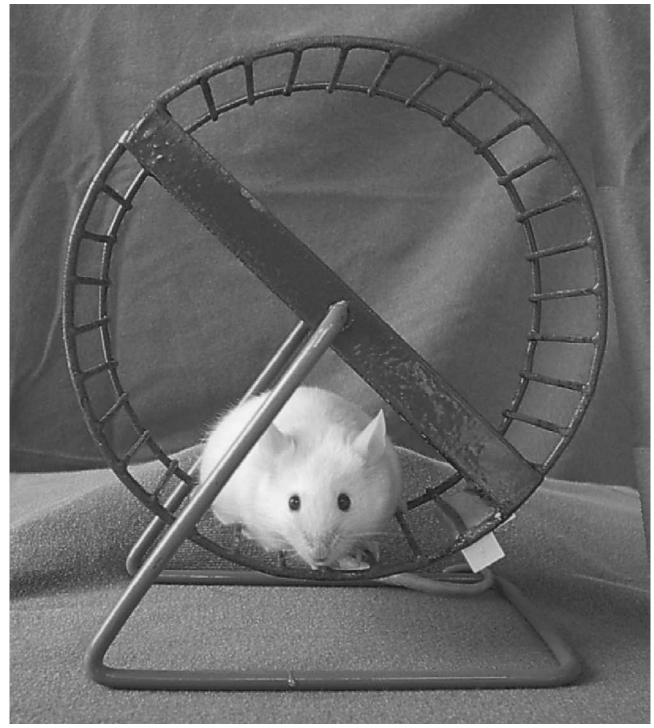


FIGURE 2.25 Mouse on wheels. An albino mouse stares at you from his running wheel.

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

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

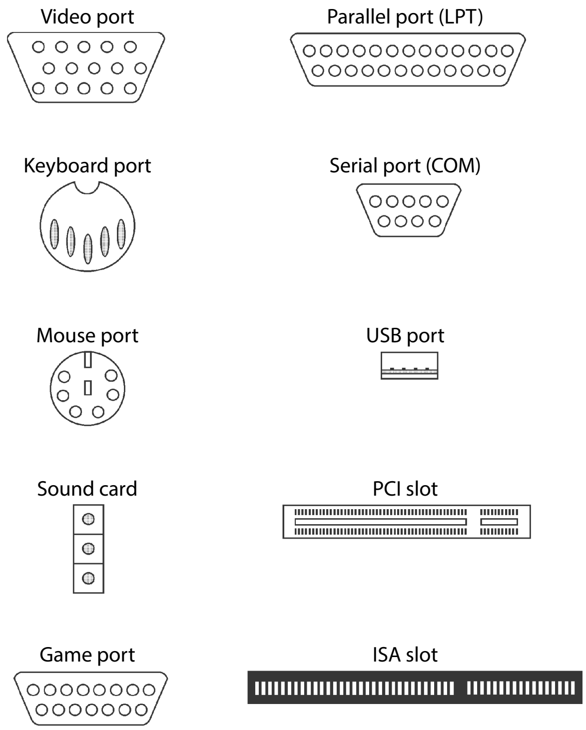


FIGURE 2.26 Standard computer interfaces. These diagrams identify the 10 interface connectors available in most personal computers. Access to PCI slots and ISA slots usually requires opening of the computer cover. In recent years, there has been a trend to replace the directly accessible interfaces with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

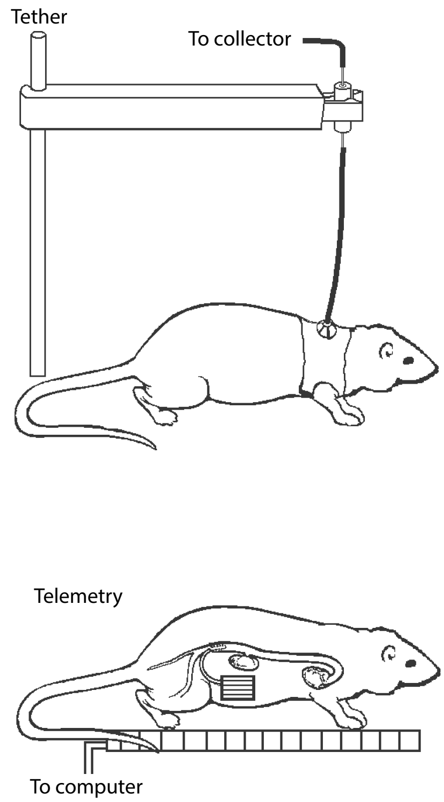


FIGURE 2.27 Monitoring physiological variables: tether and telemetry. Monitoring of physiological variables such as body temperature, heart rate, and concentration of hormones in the blood requires tether or short-range telemetry devices.

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

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

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

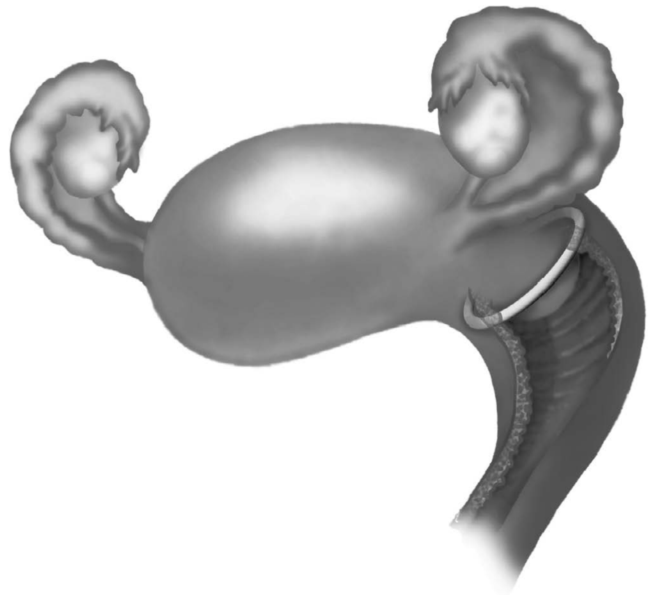


FIGURE 2.28 OvuRing. This wireless temperature sensor was developed to help women monitor body temperature changes along the menstrual cycle. (Image courtesy of Prima-Temp Inc., Boulder, CO.)

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

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

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

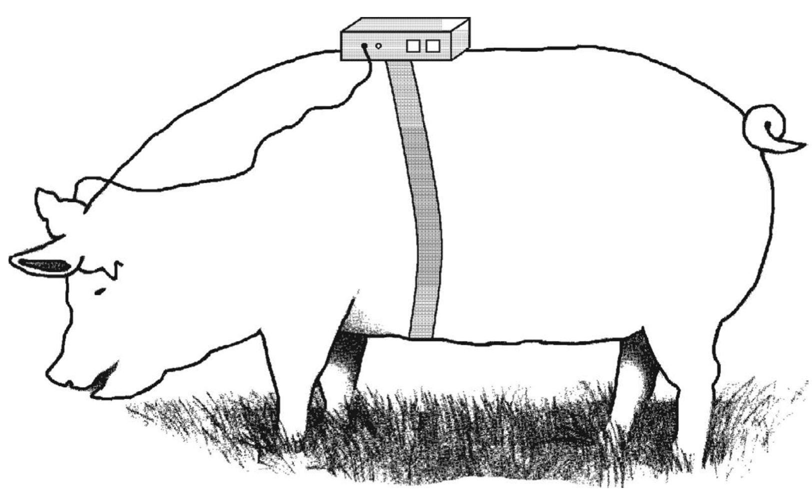


FIGURE 2.29 Monitoring physiological variables: data loggers. Data loggers are an alternative for the use of tether and telemetry devices in the monitoring of physiological variables.

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

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

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

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

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

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

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