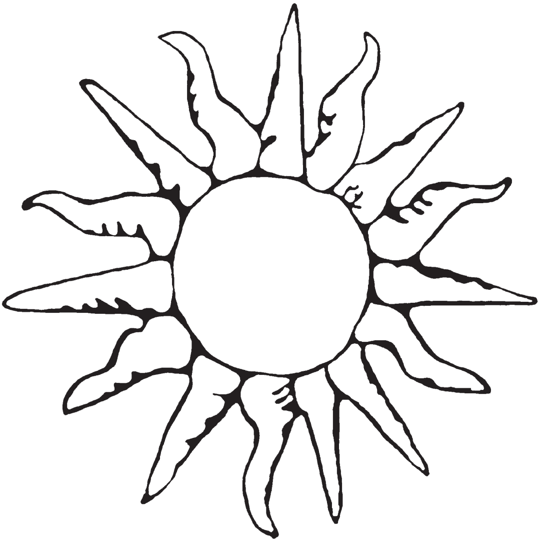

# Life Under the Sun

### Peter A. Ensminger

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# *Contents*

# *Preface*

I wrote *Life Under the Sun* to share with people who have an enthusiasm for science in general and biology in particular my fascination with animals, plants, fungi, and microbes that respond to light. I hope that this book will appeal to professional biologists as well, for biology is such a diverse field that few professionals know all about the light-induced responses in the various organisms discussed in this book. A specialist is often just a layperson when outside his or her own discipline.

Peter Medawar has argued that the scientific paper is an exercise in deception because it misleads readers about the true thought processes that underlie scientific discovery. According to Medawar, scientists lead us to believe that their research is conducted in the same logical and sequential manner in which it is presented in their papers. True, nonscientists might be deceived. But no professional scientist is fooled, because we all practice this so-called deception regularly in our own papers.

In any case, this utterly undeceptive book is a collection not of scientific papers but of informal essays. In *Essais* (1580), the collection generally credited with introducing the form, Michel de Montaigne wrote, "I am myself the matter of my book." Although there is more of me in the essays of *Life Under the Sun* than would be appropriate in formal scientific papers, the organisms themselves are the matter of my book.

The first three essays delve into vision in humans and other animals. The next three consider the effects of light on human health. The topics then diverge to describe light effects in plants, fungi, and other organisms. There is no need for readers to progress sequentially through the essays. In fact, I anticipate that after taking in the Introduction, readers will migrate to the essays they find most personally interesting. One person may turn to

### *Some Basic Photochemistry*

Any molecule that absorbs light or radiation is a potential chromophore — light-absorbing component — for photochemical reactions. Photochemistry begins when the absorption of radiation excites a molecule to a higher energy level. Sometimes this excitation energy is dissipated as heat or as light of a longer wavelength (fluorescence or phosphorescence); in this case, the energy has been "wasted," because it did not cause any permanent chemical changes. Under certain conditions, the excitation energy can cause chemical transformations of the molecule; in this case, the energy has performed "photochemical work." Some examples of photochemical work discussed in this book are decomposition (photolysis; see Chapters 4 and 8) and structural rearrangement (photoisomerization; see Chapters 1, 2, 7, and 14).

Chapter 5, "A SAD Tale," about the effects of light on Seasonal Affective Disorder; another may begin with Chapter 12, "Turning on a Butterfly," about special photoreceptors that are located on the genitalia of butterflies; and another may jump to Chapter 15, "Too Much of a Good Thing," about methods that plants use to cope with excessive sunlight.

Some of these essays have appeared elsewhere. Chapter 8, "Light and Beer," an account of the deleterious effect of light on beer, is adapted from an article published in *Zymurgy,* a magazine for home brewers (of which I am one). Chapter 7, "A Novel Method of Weed Control," which describes the use of nighttime plowing to control weeds, and Chapter 4, "A Burning Issue," about the harmful effects of ultraviolet radiation, were adapted from articles that originally appeared in *Biology Digest,* a monthly periodical for biology students. I am grateful to the publishers of these essays for permission to reprint the revised versions here.

Why essays? In *Night Life,* a collection of essays about wildlife at nighttime, Diana Kappel-Smith has compared writers to carpenters, some of whom are expert at making furniture, others at making buildings. Like her, I am more a maker of furniture than of buildings, and consequently my book, like hers, is a collection of essays. This probably has something to do with my beginning as a biologist at the laboratory bench, composing brief, narrowly focused articles. Although I have written in many other forms since my conversion to a biologist at the computer keyboard, I still feel most comfortable composing small pieces about specific topics. I hope that readers will appreciate this collection as a whole, much as we can appreciate the arts-and-crafts ambience in a room full of Stickley furniture.

Anyone whose appetite has been whetted by an essay can consider it a stepping stone to pursue the topic further by looking at the Suggested Reading list at the close of each essay. In addition, I have included a Glossary for readers who may be stymied by a technical term, and I have compiled extensive endnotes for readers who want to pursue the scientific literature. I have also prepared a Web site with supplementary information and lists of related sites. My site is accessible from the Yale University Press site for *Life Under the Sun* (www.yale.edu/yup/books/088043.htm) and will be regularly updated. I encourage you all to contact me through this site.

# *Acknowledgments*

I must thank many individuals for their help in preparing this book. In addition to my wife, Lisa, who provided encouragement throughout, and our dog Sandy (now deceased), who lay at my side as I wrote, I feel lucky to have had constructive feedback from the foremost experts in the various fields covered. In particular, I thank Jeff Palmer (Indiana University) for discussing the phylogeny depicted in figure 1; Robert B. Barlow (State University of New York Upstate Medical Center, Syracuse) for discussing his research on horseshoe crabs and *Rimicaris* shrimp and for his comments on Chapter 1; Steve Chamberlain (Syracuse University) for providing videotapes of black smokers and *Rimicaris* shrimp, discussing *Rimicaris* shrimp with me, and commenting on Chapter 2; Thomas W. Cronin (University of Maryland, Baltimore County) for discussing his research on mantis shrimps and commenting on Chapter 3; Jack Werner (University of Colorado, Boulder) for discussing his work on impressionism and the vision of Claude Monet; Richard Setlow (Brookhaven National Laboratory) for his comments on Chapter 4; Betsy Sutherland (Brookhaven National Laboratory) for discussing photoreactivation in humans; Norman E. Rosenthal (National Institute of Mental Health, NIH, Bethesda) for commenting on Chapter 5; Martin J. Warren (University College, London) for commenting on Chapter 6; Karl M. Hartmann (Erlangen University, Germany) for help in preparing figure 2 and table 1 and for discussing his research on seed germination and commenting on Chapter 7; Denis De Keukeleire (University of Ghent, Belgium) for discussing his research on hop bitter acids and commenting on Chapter 8; Morten Meilgaard (Stroh Brewery), John Paul Maye (Pfizer), and David Hysert (John I. Haas) for discussing photostable hop compounds; Rudy Held (Kalsec) for sending lit-

erature on photostable hop compounds; David Dennison (Dartmouth College) for discussing his experience as a student in Max Delbrück's lab and for commenting on Chapter 9; Paul R. Fisher (LaTrobe University, Melbourne, Australia) and Donat P. Häder (Erlangen University, Germany) for discussing their research on *Dictyostelium;* Paul R. Fisher for commenting on Chapter 10; Pill Soon Song (University of Nebraska, Lincoln) for commenting on Chapter 11; Kentaro Arikawa (Yokohama City University, Japan) for discussing his research on butterfly genitalia and for commenting on Chapter 12; Woody Hastings (Harvard University) for commenting on Chapter 13; Jeff Stuart (Syracuse University) for discussing his research on *Halobacterium* and commenting on Chapter 14; and William W. Adams III (University of Colorado, Boulder) for commenting on Chapter 15. Lastly, I thank my editors at Yale University Press: Dan Heaton, for his meticulous attention to detail, and Jean Thomson Black, for gently ushering me into the world of book publishing.

# Introduction

*Of Physiology from top to toe I sing*
*— Walt Whitman*

The earth, which is 93 million miles from the sun, receives a small fraction of the light that the sun radiates into space, but that small fraction serves as the energy source that supports life on our planet. People have long appreciated the great significance of the sun. Many ancient cultures in Africa, Asia, and the Americas put it at the center of their religions. Many other cultures, both ancient and modern, have recognized that the sun provided them with the light and warmth that sustained them and their crops, and have used the sun as a cultural or religious motif.[1]

Modern science has provided us with a more complete understanding of why the sun is so important (see sidebar, "Some Basic Photochemistry," p. viii). Plants use chlorophyll and other pigments to absorb the energy in sunlight, and the biochemical reactions of photosynthesis transform solar energy into biochemical energy. Plants then use this biochemical energy for growth, while giving off oxygen as a waste product. Animals eat plants, or they eat other animals that eat plants, and they breathe in the oxygen expelled by the plants.

But sunlight provides more than the *energy* for photosynthesis, and thereby the food we need to eat and the oxygen we need to breathe. Sunlight also provides *information* to plants, animals, fungi, and microbes about their environments. Thus life on earth — which has evolved under the influence of sunlight for billions of years — has become specialized to sense the quantity, direction, polarization, wavelength, and periodicity of light. Sunlight allows animals to see, and it controls movement and morphogenesis in plants, fungi, and microbes. It tells organisms the time of day and the time of year by resetting their circadian and annual rhythms. But sunlight can also be injurious: the ultraviolet radiation in sunlight harms many animals by suppressing their immune systems and it can damage the DNA of nearly all organisms.

These essays do not catalogue all the different biological responses to light; nor do they provide a comprehensive coverage of photobiology, the branch of biology that deals with the interactions of light and living organisms. Many of the books listed under Suggested Reading provide a more complete coverage of photobiology and more complete descriptions of the myriad biological effects of light.

Instead, this book presents intriguing pieces of the overall picture by describing specific responses to light in diverse organisms, providing a cross-section of photobiology. These responses include *vision,* the perception of the overall appearance of objects that is mediated by the eyes and brains of animals; *photosynthesis,* the conversion of light energy into biochemical energy that is used for food and occurs in plants and some lower life forms; *phototaxis,* the movement of an organism according to the direction of light that occurs in many single-celled organisms; *phototropism,* the orientation of a sessile organism or one of its parts according to the direction of light; and *photosensitization,* a reaction to light that can lead to red and swollen tissues in humans and other animals. This collection of essays describes these and other responses to light in a diversity of organisms whose evolutionary relationships are depicted in figure 1. By sampling these very different responses in these very different organisms, the reader gets a glimpse of the larger picture, for nature often reveals itself in its individual creations.

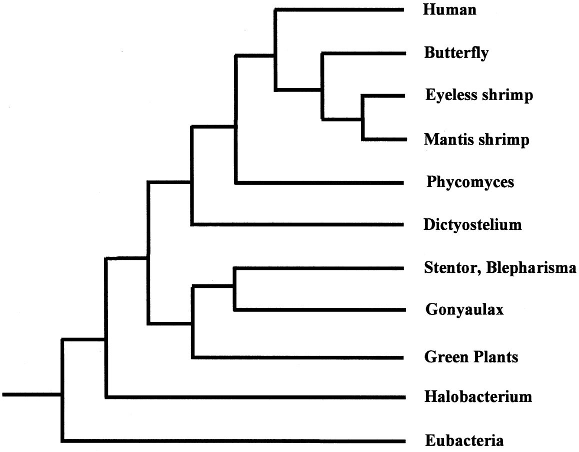

Figure 1. Evolutionary tree.
The evolutionary branching pattern of organisms featured in *Life Under the Sun,* based on data presented by Joel Dacks and Andrew J. Roger (J. Dacks and A. J. Roger, 1999, The first sexual lineage and the relevance of facultative sex, *Journal of Molecular Evolution* 48, 779–83). This tree groups together related organisms and shows, for example, that the mantis shrimp and eyeless shrimp are more closely related to each other than either is to the yellow swallowtail butterfly. Branch lengths do not reflect evolutionary distance.

## *Levels of Light on Earth*

Light can be measured in different ways and with different types of instruments. Photobiologists use many technical terms to characterize environmental light levels because there are so many different types of biological responses to light.[2] Thus researchers studying vision, photosynthesis, phototaxis, phototropism, and photosensitization must use different types of light measurements. Unfortunately, some of the terminology used to describe environmental light levels has changed over time, so different researchers may use the same term for slightly different types of measurements.[3] Adding to the confusion, some photobiology research papers do not specify the aspect of a light field that has been measured, nor the methods used for measurement.

The indivisible unit of light or electromagnetic radiation is the photon. One simple way to express environmental light levels is *photon flux,* which is defined as the number of photons that strike a flat surface of a specified area in a given unit of time. A similar measurement is *spherical photon flux,* which is defined as the number of photons that strike a sphere of a specified cross-section per unit of time. Spherical photon flux, which is often used by plant biologists, takes into account the three-dimensional structure of organisms that can sense light from all directions. Under the special circumstance in which all light comes from a single direction—which is often the case in laboratory experiments—the photon flux is equal to the spherical photon flux. Under natural conditions, however, the spherical photon flux is higher than the photon flux, and the relation between the two values varies according to the time of day.[4] In a somewhat Procrustean manner, table 1 classifies many of the diverse responses described in the essays of this book according to spherical photon flux.

Throughout the course of a day, the range of the spherical photon flux typically encountered on earth spans seven or eight orders of magnitude.[5] At the high end of this scale—noon on a clear day—most of the light on earth obviously comes from the sun. But what about the small amount of ambient light present at nighttime, long after sunset? Aside from artificial sources, this residual light comes from the moon, the stars, and air glow, a natural phenomenon caused by chemical reactions driven by electron currents in the upper atmosphere.[6]

The enormous range of light levels that are encountered during the day is similar in magnitude to the difference between the height of a small child (about 1 meter) and the diameter of the earth (about 12,800 kilometers). Because we are — in the words of vision scientist and Nobel laureate Haldan K. Hartline — "children of the sun," it should not be surprising that our own eyes can function over this entire range.[7] In fact, our eyes function over an even broader range, and so do the light-sensing systems of many other organisms (table 1).

## *Wavelengths of Light on Earth*

We are children of the sun in more ways than one. Not only do our eyes and the photosensory systems of many other organisms function over the range of light levels present on earth, but we and other organisms are also most sensitive to the range of wavelengths that are brightest on earth, between 400 and 700 nm (see figure 2).[8] As Newton showed with a simple prism, the white light from the sun is composed of many colors and extends from violet to blue, green, yellow, orange, and then red. Very little ultraviolet radiation below 300 nm reaches the earth because the sun's emission falls off very sharply below 300 nm and because stratospheric ozone absorbs much of the radiation in this region of the spectrum. Very little infrared radiation above 1000 nm reaches the earth because the emission from the sun declines at long wavelengths and because water in the earth's atmosphere absorbs strongly above 1000 nm.[9]

Although most photobiological responses are elicited by light between 400 and 700 nm, there are significant differences in the pigments used to absorb light and in the particular wavelengths that these pigments most effectively absorb (see figure 2). Thus certain butterflies have photoreceptors on their genitalia that absorb maximally at about 380 nm (ultraviolet radiation); during daytime, human vision is most sensitive to yellow-green light of about 555 nm; and photosynthesis in higher plants is most effectively stimulated by red light of about 675 nm. Three different photoreceptive molecules are responsible for these different

Table 1
Levels of light on earth and biological responses

Column 1 lists different environmental light levels;[1] column 2, spherical photon flux (number of visible photons [400 to 700 nm] intercepted by a sphere of one meter cross-section per second) associated with these light levels;[1] column 3, biological responses associated with these light levels; column 4, photoreceptive pigments for the different biological responses; column 5, chapters that discuss the biological responses; and column 6, notes and references for the biological responses.

| Environmental light level | Spherical photon flux (photons $m^{-2}s^{-1}$) | Biological response | Photoreceptive pigment | Chapter | Notes and references |
|---|---|---|---|---|---|
| Clear midday | $\sim 10^{21}$ | Photosynthesis saturation, sun-loving plants (continuous exposure to sunlight) | Chlorophyll | 15 | Light level is species dependent[2] |
| Cloudy midday | $\sim 10^{20}$ | Photosynthesis saturation, shade-loving plants (continuous exposure to sunlight) | Chlorophyll | 15 | Light level is species-dependent[2] |
| Clear sunset | $\sim 10^{19}$ | Lengthening of *Gonyaulax* circadian period (single four-hour red-light pulse) | Chlorophyll (?) | 13 | Threshold for bioluminescence rhythm[3] |
| Early twilight, clear | $\sim 10^{18}$ | Seed germination (LFR) (one-second red-light pulse) | Phytochrome | 7 | Threshold for low-fluence response[4] |
| Late twilight, clear | $\sim 10^{17}$ | Negative phototaxis in *Dictyostelium* amoebas (continuous white-light exposure) | Porphyrin (?) | 10 | Higher light levels: negative phototaxis (AX-2 strain)[5]<br>Lower light levels: positive phototaxis (AX-2 strain)[5] |

| | | | | | |
|---|---|---|---|---|---|
| Midnight, clear, full moon | $\sim 10^{16}$ | Photophobic response in *Stentor coeruleus* (continuous red-light exposure) | Stentorin | 11 | Threshold level for light avoidance in "stella" strain[6] |
| Midnight, clear, new moon | $\sim 10^{15}$ | Human color vision | Iodopsin | 1, 2 | Threshold corresponding to $\sim 10^{-2}$ milli-Lamberts[7] |
| Midnight, cloudy, new moon | $\sim 10^{14}$ | Seed germination (VLFR) (one-second red-light pulse) | Phytochrome | 7 | Threshold for very low–fluence response[4] |
| | $\sim 10^{12}$ | Positive phototaxis in *Dictyostelium* slugs (continuous white-light exposure) | Porphyrin (?) | 10 | Threshold level (NC4 strain)[8]<br>Threshold for AX2 strain is ~100-fold lower[9] |
| | $\sim 10^{10}$ | Human vision (one-millisecond green-light pulse) | Rhodopsin | 1 | Threshold corresponding to $\sim 10^{-6.5}$ milli-Lamberts[7,10] |
| | $\sim 10^{9}$ | Phototropism in *Phycomyces* (continuous blue-light exposure) | Flavin | 9 | Threshold level[11] |
| | $\sim 10^{7}$ | Adaptation acceleration in *Phycomyces* (35-minute red-light pulse) | Flavin (?) | 9 | Threshold level[12] |

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1. Based on measurements in Erlangen, Germany given in K. M. Hartmann et al., 1998, Photocontrol of germination by

(*continued*)

Table 1 Continued

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moon and starlight, *Zeitschrift Pflanzen Krankheit Pflanzen Schutz,* Sonderheft 16, 119–27; K. M. Hartmann, 1995, Harrowing at night is half weeded, *International Symposium on Weed and Crop Resistance to Herbicides.* Córdoba, Spain. Simultaneous measurements of spherical illuminance, a measure of the light level sensed by the human eye, range from about $10^5$ lux during a clear midday to about $10^{-3}$ lux during a cloudy midnight at new moon.

2. F. B. Salisbury and C. W. Ross, 1985, *Plant Physiology,* Belmont, Calif., Wadsworth.
3. T. Roenneberg and J. W. Hastings, 1991, Are the effects of light on phase and period of the Gonyaulax clock mediated by different pathways? *Photochemistry and Photobiology* 53, 525–33.
4. K. M. Hartmann and W. Nezadal, 1990, Photocontrol of weeds without herbicides, *Naturwissenschaften 77*, 158–63.
5. D.-P. Häder and B. Vollersten, 1991, Phototactic orientation in *Dictyostelium discoideum* amoebae, *Acta Protozoologica* 30, 19–24.
6. C. B. Hong et al., Light-adaptation in the photophobic response by *Stentor coeruleus, Archives of Microbiology* 147, 117–20.
7. P. Buser and M. Imbert, 1995, *Vision,* Cambridge, MIT Press.
8. K. L. Poff and D.-P. Häder, 1984, An action spectrum for phototaxis by pseudoplasmodia of *Dictyostelium discoideum, Photochemistry and Photobiology* 39, 433–36.
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10. S. Hecht et al., 1942, Energy, quanta, and vision, *Journal of General Physiology* 25, 819–40.
11. K. Bergman et al., 1969, *Phycomyces, Bacteriological Reviews* 33, 99–150; P. A. Ensminger et al., 1990, Action spectra for photogravitropism in *Phycomyces* wild type and three behavioral mutants (L150, L152, and L154), *Photochemistry and Photobiology* 51, 681–87.
12. P. Galland et al., 1989a, Subliminal light control of dark adaptation kinetics in *Phycomyces* phototropism, *Photochemistry and Photobiology* 49, 485–91; X.-Y. Chen et al., 1993, Action spectrum for subliminal light control of adaptation in *Phycomyces* phototropism, *Photochemistry and Photobiology* 58, 425–31.

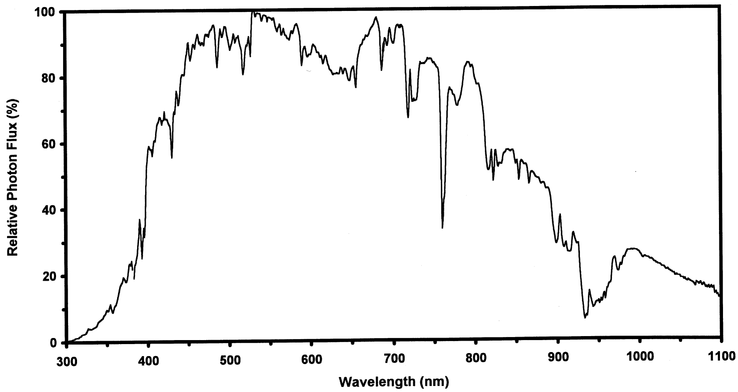

Figure 2. Daylight spectrum.

The spectrum of sunlight on earth, from 300 to 1100 nm, scanned in 1 nm steps with 2 nm bandwidth and recorded on a clear summer day (August 7, 1991) by Karl Hartmann and Wolfgang Kaufmann at 12:00 Central European Time on the flat roof of the Biologikum at Erlangen University, Germany (49°35′N, 11°02′E; 300 m above sea level). The wavelength maxima of biological responses discussed in *Life Under the Sun* are:

~260 nm — DNA damage, *in vitro* (Chapter 4)
~280 nm — Photo-degradation of isoalpha acids in beer (Chapter 8)
~310 nm — Sunburn (Chapter 4)
~380 nm — Genitalic photoreception in butterflies (Chapter 12)
~410 nm — Phototaxis in *Dictyostelium* slugs and amoebas (Chapter 10)
~450 nm — Phototropism in plants and *Phycomyces* (Chapter 9)
~475 nm — *Gonyaulax* bioluminescence (Chapter 13)
~505 nm — Human vision at low light levels (Chapters 1, 2, 3)
~555 nm — Human vision at high light levels (Chapters 1, 2, 3)
~568 nm — Photosynthesis in *Halobacterium* (Chapter 14)
~610 nm — Photophobic response & negative phototaxis in *Stentor* (Chapter 11)
~668 nm — Formation of the Pfr form of phytochrome (Chapter 7)
~675 nm — Photosynthesis in land plants (Chapter 15)
~730 nm — Formation of the Pr form of phytochrome (Chapter 7)

responses. The chemical structures of all the photoreceptive molecules discussed in this book appear in the appendix. Even readers with little training in chemistry can look at these figures and appreciate the basic similarity of these molecules, for they all have one or more ringlike structures and alternating single and double bonds between carbon atoms, which chemists call "conjugated double bonds."

Our own eyes and the eyes of other animals do not simply measure light's wavelength; they judge colors according to wavelength combinations. Plants can also sense wavelength combinations with phytochrome, a photosensory pigment that behaves like photochromic eyeglasses, which change color under different types of light. Certain fungi and microbes may also be able to sense wavelength combinations. Plants, fungi, and microbes lack nervous systems, so none could be considered to have color vision. But the diverse pigments and the exquisite specializations that have evolved for absorbing different wavelengths of light evince the importance of light's wavelength.

Light is so important to us that Diane Ackerman has called our eyes "the great monopolists of our senses."[10] The same might be said of the photosensory and energy-transducing systems of the many organisms discussed in this book, for they truly seem to dominate the lives of these organisms.

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Björn, L. O. (1976) *Light and Life.* New York, Crane, Russak.

Buser, P., and M. Imbert (1992) *Vision.* Cambridge, MIT Press.

Fein, A., and E. Z. Szuts (1982) *Photoreceptors: Their Role in Vision.* New York, Cambridge University Press.

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Smith, K. C., ed. (1989) *The Science of Photobiology.* New York, Plenum.

Wolken, J. J. (1995) *Light Detectors, Photoreceptors, and Imaging Systems in Nature.* New York, Oxford University Press.

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# 1 Vision at the Threshold

> *Obviously the amount of energy required to stimulate any eye must be large enough to supply at least one quantum to the photosensitive material. No eye need be so sensitive as this. But it is a tribute to the excellence of natural selection that our own eye comes so remarkably close to the lowest limit.*
>
> *—Selig Hecht, Simon Shlaer,*
> *and Maurice Pirenne*

Vision is surely the most important of our senses. A single glance instantly gives us information about our surroundings that is much more sophisticated than that from our other senses. Our eyes perform many amazing feats. They focus on objects at different distances, adapt to ambient light levels that vary more than a billionfold in brightness, and discriminate among many different colors, from the violet to the red.[1] One of the most remarkable features of our eyes is that they can function at extraordinarily low light levels.

How much light do we need to see? Experiments performed under certain controlled conditions have shown that we can perceive a one-millisecond flash of blue-green light that contains about 100 photons.[2] Other experiments have shown that we can perceive a steady point source of blue-green light that has a flux of about 800 photons per second.[3] Because the photosensitive cells of the retina absorb only about 10 percent of the blue-green light that actually enters the eye, it follows that the threshold for vision occurs when the retina absorbs about 10 photons from a one-millisecond flash or about 80 photons per second from a continuous point source of light.[4]

The retinas of certain animals that are renowned for their night vision absorb a higher percentage of the light that enters their eyes because they have larger pupils, which allow more light to reach the retina, and reflective tapetums at the back of their eyes, which reflect light that was not absorbed during its first pass through the photoreceptor cells.[5] Thus cats and owls have a threshold that is about five times lower than our own, even though their retinas have about the same photosensitivity.

What limits our sense of vision at low light levels? This simple question is not so simply answered. Indeed, vision scientists have seriously investigated this question since the late nineteenth century.[6]

We know that at low light levels, such as on a starry night (see table 1, p. 6), vision is due mostly to light absorption by the visual pigment rhodopsin in the rod cells of the retina. Each rhodopsin molecule is composed of a retinal molecule (a vitamin A analog) that is attached to a protein called opsin (see figure A1, Appendix). Each of our rod cells contains about 100 million molecules of rhodopsin, and the retina of each eye has about 100 million rod cells. Thus each of our eyes has about $10^{16}$ (ten thousand trillion) molecules of rhodopsin. Having so much rhodopsin is a definite advantage for vision at low light levels, for it increases the probability that a photon that enters the eye will be captured. We also know that the key event in vision is a rapid light-induced activation (photoisomerization) of the rhodopsin molecule.[7] The efficiency of this process is also a definite advantage for vision at low light levels.

To really understand what limits our sense of vision at low light levels, we need to look at a few key experiments that have been performed in the past fifty years.

## *Detection of a Single Photon*

One of the best known experiments in vision science was performed at Columbia University in the 1940s by Selig Hecht, Simon Shlaer, and Maurice Pirenne.[8] These researchers gave one-millisecond flashes of light of variable fluence rate ("intensity") to one another and a few volunteers under highly controlled conditions. The light flashes were administered following thirty minutes of dark adaptation and were so narrowly focused that they fell upon only about 500 of the more than 100 million rod cells in each observer's retina. Hecht, Shlaer, and Pirenne then recorded how frequently the different observers perceived the flashes of light.

They used a relatively simple mathematical method, known as Poisson statistics, to analyze the data. Their results showed that a person can perceive a light flash if 500 nearby rod cells of the retina absorb about six photons within one millisecond. If six photons are absorbed by 500 rod cells, then the probability that any single rod cell has absorbed two photons is very small ($P = 6/500 \times 6/500$, or about one in seven thousand). Thus Hecht, Shlaer, and Pirenne concluded that activation of about six nearby rod cells within one millisecond is sufficient for the perception of a light flash.

These experiments are renowned because they demonstrated very simply and elegantly—yet indirectly—that a single photon that is absorbed by a single molecule of rhodopsin can chemically excite a rod cell. Unfortunately, some students and biologists who are not well-versed in vision science occasionally misunderstand these results to mean that we can actually perceive a single photon. This is not at all correct. In fact, rhodopsin molecules in several nearby rod cells must absorb photons for the perception of a light flash. Moreover, the photons must be absorbed within a very small area of the retina and must be administered within a critical period of time. If a larger area of retina is stimulated, or the timing of the light flash is significantly greater than one millisecond, then more photons are necessary to trigger light perception.

A limitation of these experiments is that they are indirect. How can it possibly be proven that a single photon can excite a single rod cell?

The answer to this question came in the early 1960s from electrical recordings of the retina of the horseshoe crab *(Limulus polyphemus)*.[9] In particular, experiments performed on horseshoe crabs maintained in complete darkness showed that their retinas exhibit random electrical fluctuations, similar to the static on an untuned radio. Illumination increases the number of these fluctuations, a bit like turning up the volume on the same untuned radio. Analysis of the effect of the fluence rate of light on the electrical noise of the horseshoe crab retina showed that absorption of a single photon causes a single "bump" of electrical noise. This was an important confirmation of the conclusion of Hecht, Shlaer, and Pirenne.

Unfortunately, such experiments could not be performed with human retinas, because the rod cells of vertebrates are electrically coupled ("linked"). Denis Baylor and colleagues at Stanford University overcame this difficulty in 1979 by developing the "suction-electrode method," in which the outer segment of a single rod cell is sucked into the tip of a very small pipette. This elegant method allows measurement of the electrical current from isolated rod cells. The original experiments were performed with rod cells from the toad *Bufo marinus,* but subsequent experiments were performed with rod cells from primates.[10] The results showed that absorption of a single photon by a single rhodopsin molecule electrically activates a rod cell by blocking the entry of about one million positively charged ions into the cell. This further confirmed the conclusion of Hecht, Shlaer, and Pirenne that human rod cells respond to the absorption of a single photon.

## *A New Question Arises*

Just as these important electrophysiological experiments answered one important question, they gave rise to another. What causes the electrical noise that occurs in rod cells held in complete darkness?

Early vision scientists realized that whatever the source of this noise, it was significant to the visual process.[11] Rod cells seem to have a basal level of electrical fluctuations, also known as "dark light," and illumination merely increases the number of these fluctuations.[12] The disadvantage of this electrical noise is that it places a lower limit on the amount of light that can be perceived. But there is also an advantage of this electrical noise. If excitation of a single rod cell, rather than several rod cells, led to perception of light, then we would constantly be confusing the electrical noise inherent to the rod cells with actual light signals.

Examination of the eyes of different animals provides a hint about the source of this electrical noise. Among different animal species, there is a strong correlation between the electrical noise level and the rhodopsin content of the photosensitive rod cells.[13] This suggests that rhodopsin itself is the source of the noise. Moreover, different species appear to have evolved different mechanisms for reducing the level of electrical noise in their photoreceptor cells. Humans and other primates have relatively small rod cells; small cells have less rhodopsin and are therefore less noisy.[14] Many cold-blooded animals, such as amphibians, have very large photoreceptor cells; they achieve low noise levels by living at low temperatures, where the noise level is greatly reduced.[15] This gives cold-blooded animals better night vision than humans and most other warm-blooded animals.

An early hypothesis was that the electrical noise in rod cells is caused by "thermal activation" of rhodopsin, the visual pigment.[16] An explanation of this early hypothesis went something like this: Rhodopsin and all other molecules have a certain amount of thermal energy that causes them to bend and twist about at random. Every now and then, the thermal energy of a rhodopsin molecule is large enough to cause an activation of the same sort that occurs following absorption of a photon. The probability of thermal activation in any single rhodopsin molecule is extremely small. Each rod cell has 100 million rhodopsin molecules, however, so the probability of thermal activation occurring anywhere in a single rod cell is much greater.

There is a problem with this thermal activation hypothesis. Thermodynamic measurements showed that light activation of rhodopsin required crossing a large energy barrier (activation energy, 45 kcal per mole), whereas thermal activation of rhodopsin required crossing a small energy barrier (activation energy, 25 kcal per mole).[17] Thus the mechanism that causes electrical fluctuations of rod cells kept in darkness is different from that which causes electrical fluctuations following illumination.

Robert Barlow and colleagues from Syracuse University and Rockefeller University have proposed a novel solution to this problem.[18] Light-activation of a rod cell has long been known to involve activation of rhodopsin, a molecule that consists of retinal bound to an opsin protein by a "protonated Schiff-base linkage" (see figure A2, Appendix). Based on theoretical calculations and experimental results, Barlow and colleagues suggest that dark activation of rhodopsin occurs as a two-step process:

1. The positive charge between the retinal and opsin is removed, creating a bond that lacks a positive

charge, known technically as a "deprotonated Schiff-base linkage."

2. This rare, deprotonated form of rhodopsin is thermally activated, thus causing electrical noise in darkness.

This controversial hypothesis predicts that increasing the proton concentration (acidity) inside photoreceptor cells should increase the amount of positively charged (protonated) rhodopsin, decrease the amount of uncharged (deprotonated) rhodopsin, and therefore reduce the electrical noise level of the cell. Thus the researchers tested their hypothesis by performing experiments on the horseshoe crab, the same organism that was previously used to demonstrate that absorption of a single photon causes a single "bump" of electrical noise.

The brain of the horseshoe crab normally generates a circadian rhythm that includes transmission of nerve signals to the photoreceptor cells, reducing their noise level at nighttime. Thus in their first test, Barlow and colleagues mimicked this brain signal by delivering electrical current shocks to the photoreceptor cells and noted a rapid increase in the proton concentration of the photoreceptor cells and a subsequent reduction in photoreceptor noise level. In their second test, they injected the eye of the horseshoe crab with a mildly acidic solution and noted a rapid and dramatic reduction in the electrical noise level. Both of these results are consistent with the researchers' novel hypothesis for the generation of photoreceptor noise.[19]

A more recent study has concluded that the same novel mechanism is the most likely source of noise in the photoreceptor cells in the eyes of all invertebrates and vertebrates, including those of humans.[20] Indeed, the rod cells of some vertebrates become more acidic with roughly the same time course as the decrease in overall electrical noise.[21] Thus stabilization of the positively charged form of rhodopsin during prolonged exposure to darkness appears to contribute to the high photosensitivity that humans and other vertebrates attain during dark adaptation.

A rod cell in the retina of our eyes can sense the absorption of a single photon, and absorption of photons by several rod cells allows us to perceive a flash of light. Because the photon is the indivisible unit of light energy, our eyes have achieved a nearly optimal solution for detection of low levels of light. In a review of the different sensory systems of many different organisms, William Bialek showed that many of these sensory systems also approach the limits imposed upon them by the laws of physics for detection of low stimulus levels.[22]

This degree of biological perfection may seem surprising, because some evolutionary biologists have maintained that natural selection does not allow organisms to achieve perfect solutions but allows only solutions that are compromises, solutions that result from many trials and errors over the course of many generations.[23]

As indicated by the classic paper of Hecht, Shlaer, and Pirenne, examination of the physical limits to the perception of light has not only taught us a great deal about the biochemistry and physiology of vision, it has also taught us something important about evolution and natural selection. Perhaps we can learn more about biology by examining how the laws of physics constrain the function of organisms as well as individual biological molecules.

## Suggested Reading

Barlow, R. B. et al. (1993) On the molecular origin of photoreceptor noise. *Nature* 366, 64–66.

Bialek, W. (1987) Physical limits to sensation and perception. *Annual Review of Biophysics and Biophysical Chemistry* 16, 455–78.

Birge, R. R., and R. B. Barlow (1995) On the molecular origins of thermal noise in vertebrate and invertebrate photoreceptors. *Biophysical Chemistry* 55, 115–26.

Hecht, S., et al. (1942) Energy, quanta, and vision. *Journal of General Physiology* 25, 819–40.