How Photoreceptor Cells Respond to Light

New information about how light energy is changed into neural signals shows how an individual photoreceptor cell of the eye registers the absorption of a single photon, or quantum of light

by Julie L. Schnapf and Denis A. Baylor

Vision begins with the conversion of packets of electromagnetic energy called photons, or quanta, into neural signals the brain can analyze. The translation is accomplished by the photoreceptor cells of the eye. They lie in a mosaic at the back surface of the retina, the plate of neurons lining the inside of the eyeball. The cornea and lens of the eye form an image of the outside world on the layer of photoreceptors. Each cell absorbs the light at one point of the image and generates an electrical signal that encodes how much light has been absorbed. The signals are transmitted through an elaborate array of synapses, or neural junctions, in the retina and brain. At these junctions signals from the population of photoreceptors are pooled and compared. The process enables the visual system to obtain information about form, movement and color in the outside world.

Given the key role of the photoreceptors in vision, it is surprising that for a long time not much was known about how they operate. The situation has changed dramatically over the past quarter century or so. Improved methods for making electrical recordings from individual photoreceptors have provided detailed information about the mechanism by which light energy is transduced into neural signals. The new techniques have made it possible to observe directly the signal triggered by the absorption of a single photon. Such measurements have also led to simple explanations for several features of overall visual performance, such as why we perceive dim stimuli more slowly than bright ones, why we sometimes see light in complete darkness and why certain mixtures of different wavelengths evoke the same color sensation as light of a single wavelength does.

In the eyes of most vertebrates there are two types of photoreceptors: rod cells and cone cells. Rods mediate vision in dim light but are so sensitive that they become overloaded and incapable of signaling in ordinary daylight. Daylight vision is mediated by cones, which operate successfully at high light levels. Cone vision is richer in spatial and temporal detail and makes it possible to sense colors.

Rods and cones bear specialized organelles for transducing and transmitting signals. At one end of the cell (farthest from the lens) is the so-called outer segment, which absorbs light and generates electrical signals. At the other end of the cell is the synaptic ending, which relays the signals to other neurons (bipolar and horizontal cells) in the retina by secreting a chemical transmitter. Between the outer segment and the synaptic ending lies a region called the inner segment.

The outer segment of a rod is cylindrical, whereas the outer segment of a cone usually tapers—hence the names rod and cone. Both kinds of outer segment contain a large expanse of photosensitive membrane studded with light-absorbing pigment molecules. Rods contain the reddish pigment rhodopsin. In the human retina there are three kinds of cone, each of which contains a pigment that absorbs strongly in the short-, middle- or long-wavelength region of the visible spectrum. The differences in the absorption bands of the three cone pigments provide the basis for color vision. In starlight, when vision is mediated by rods, all objects appear colorless.

In the rods the photosensitive membrane consists of an orderly pile of disks inside a separate surface membrane, resembling a stack of coins inside a test tube. In the cones, on the other hand, the photosensitive membrane consists of one large, elaborately folded sheet that also serves as the surface membrane. The membrane topology of the rods indicates that a diffusible substance, an "internal transmitter," relays information from the disks, where light is absorbed, to the surface membrane, where the electrical signal is generated. Evidence from many laboratories now indicates that the transmitter is a nucleotide, cyclic guanosine monophosphate (cGMP), which also takes part in transduction in cones.

How does the absorption of light by a rod or a cone generate an electrical signal? The answer requires an understanding of how the photoreceptor behaves in darkness. One might naively think the cell would be dormant in the absence of light; in reality, however, the cell is abuzz with activity. The membrane of a photoreceptor, like the membrane of other cells, separates solutions that have different concentrations of ions (atoms with a net electric charge). The solutions both outside and inside a photoreceptor contain positively charged sodium ions and positively charged potassium ions.

ROD CELLS AND CONE CELLS in the retina of the tiger salamander are enlarged 2,000 diameters in a scanning electron micrograph made by Scott Mittman and Maria T. Maglio of the University of California at San Francisco. The cylindrical cells are the rods, the smaller conical cells the cones. The photoreceptor cells of the human retina are roughly four times as thin.

Outside the cell the concentration of sodium ions is high and of potassium ions low; inside the cell the concentration of potassium ions is high and of sodium ions low. The concentration differences are maintained by the action of a "pump" that uses metabolic energy to extrude sodium and draw in potassium.

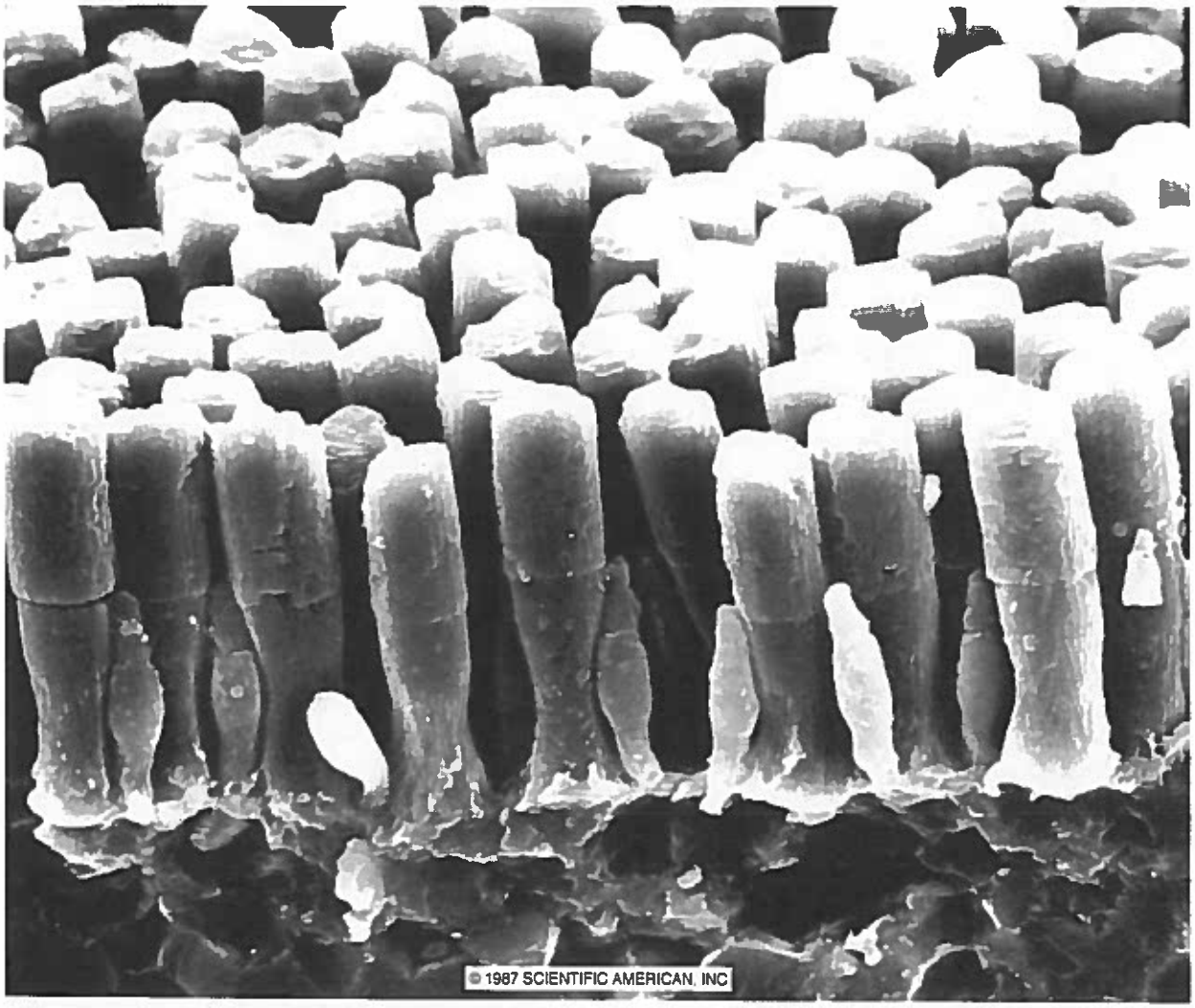
In the resting state the membranes of most neurons allow potassium ions to cross them more freely than other ions. Because potassium ions are more concentrated inside the cell, they tend to diffuse across the membrane to the outside. As the diffusion proceeds, charge is moved from the inner to the outer surface of the membrane. The transfer of charge causes the internal potential to become negative with respect to the outside, typically by as much as .1 volt. In a photoreceptor the permeability to the potassium ions is highest at the inner segment and synaptic ending.

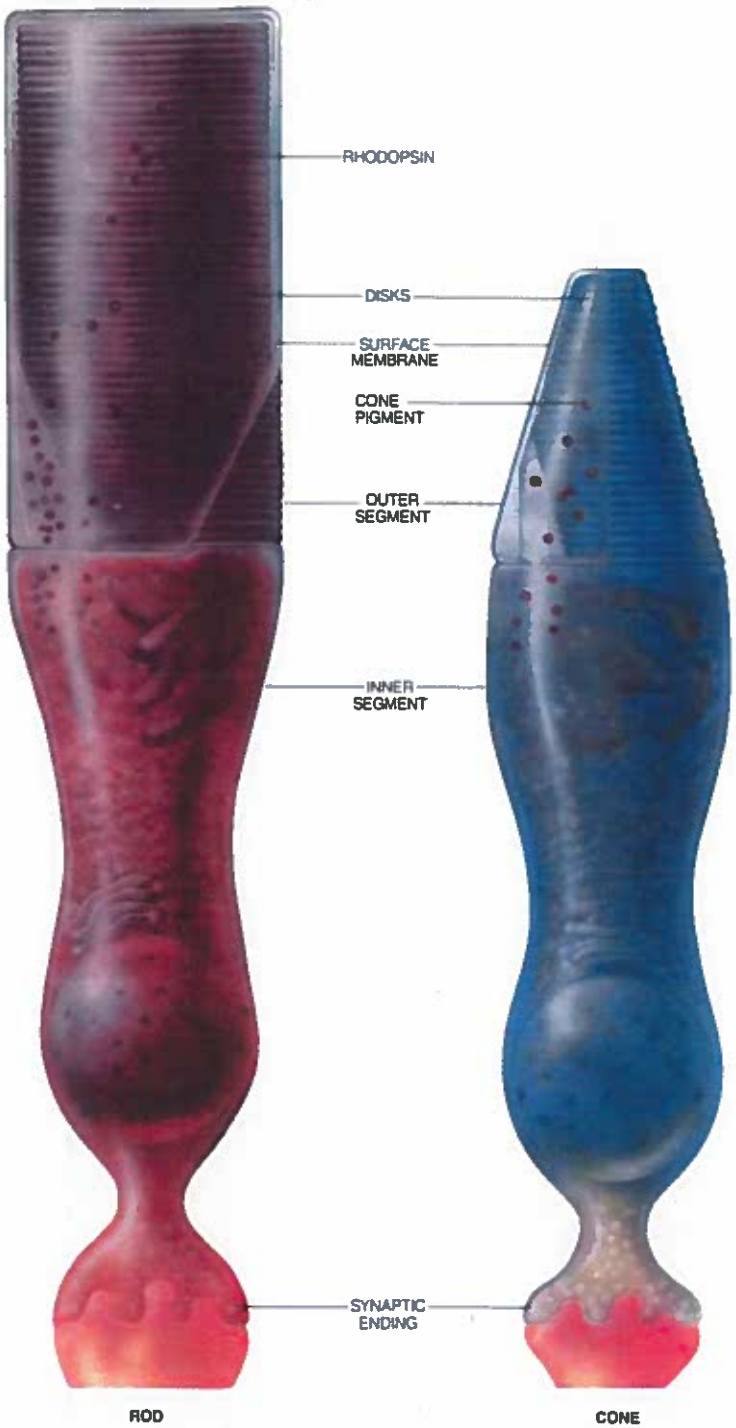
In darkness a photoreceptor also has an appreciable permeability to sodium ions. The sodium ions flow from the more concentrated external solution into the outer segment, carrying an inwardly directed electric current. The inward current is balanced by the outward current of potassium ions from the rest of the cell. The loop of current is called the dark current.

When a rod or a cone absorbs light, the influx of sodium is blocked. This reduces the dark current and allows the negative polarization of the cell interior to increase. The negative swing of the transmembrane voltage is called a hyperpolarization and the reduction in dark current is known as a photocurrent. The dark current and photocurrent were first described in about 1970 by William A. Hagins, Richard D. Penn and Shuko Yoshikami, then at the National Institute of Arthritis and Metabolic Diseases. They measured the currents around a large population of rods.

The light-evoked hyperpolarization is generated at the outer segment but spreads to the synaptic ending, where it is communicated to other retinal cells. The hyperpolarization can be recorded with a microelectrode placed inside one of the relatively large rods or cones found in the retina of certain fishes, amphibians and reptiles. Experiments of this type were pioneered in the mid-1960's by Tsuneo Tomita and his colleagues at Keio University in Japan. These recordings show that the transmembrane voltage is about −40 millivolts (mV) in the dark. A flash of light causes a hyperpolarization that increases with the strength of the flash. After a very bright flash the response reaches a limiting size of about 30 mV, at which the membrane voltage is −70 mV.

In 1975 Robert Fettiplace and one of us (Baylor) at the Stanford Univer-





RODS AND CONES differ in both form and function but have certain similarities. The upper part of the cells, which is called the outer segment, contains light-absorbing pigment molecules; the lower part, the inner segment, contains mitochondria and the nucleus. The synaptic ending links the photoreceptors to other retinal cells. Rods mediate vision in dim light; cones mediate vision in daylight. In the human retina there are three types of cone. Each of them incorporates a pigment that absorbs strongly in the blue, green or red region of the visible spectrum, providing the foundation for color vision.

sity School of Medicine showed that the hyperpolarization is indeed necessary and sufficient for controlling the flow of information across synapses to other visual neurons. In order to simulate and prevent the light-evoked hyperpolarization, we employed an intracellular electrode to pass an electric current into a single photoreceptor. Simultaneously we monitored the responses of another cell, called a ganglion cell, farther along in the chain of retinal neurons. We successfully reproduced the response of the ganglion cell to a small spot of light applied to the photoreceptor by artificially hyperpolarizing the photoreceptor in darkness. Moreover, the ganglion cell failed to respond when we blocked the light-evoked hyperpolarization by injecting a depolarizing current.

How does the absorption of light block the influx of sodium ions at the outer segment? In the dark both rods and cones have a high concentration of cyclic guanosine monophosphate. This substance binds to pores in the surface membrane and opens them, allowing sodium ions to enter. In the light the concentration of cGMP drops, cGMP leaves the binding sites and the pores close. The permeability of the membrane to sodium atoms is thereby decreased and the membrane hyperpolarizes.

The chain of molecular events leading to the reduction of cGMP consists of three steps. George Wald and his colleagues at Harvard University showed some years ago that the pigments in both rods and cones contain a light-absorbing component called 11-*cis* retinal, coupled to a protein that "tunes" the absorption to a particular region of the visible spectrum; the proteins of the rod pigment rhodopsin and the three cone pigments are different. When the retinal in rhodopsin absorbs a photon of light, it changes configuration, causing the protein part of the molecule to become enzymatically active. As Lubert Stryer and his fellow workers at Stanford subsequently showed, the active form of rhodopsin catalytically activates many molecules of a protein Stryer and his co-workers named transducin. The activated transducin molecules in turn activate an enzyme that cleaves cGMP. The system behaves like a chemical photomultiplier. Absorption of a single photon by rhodopsin causes the rapid breakdown of hundreds of molecules of cGMP and blocks the entry of a million sodium ions.

Recently strong evidence in support of the idea that cGMP does indeed control the transport of sodium ions through the surface membrane was

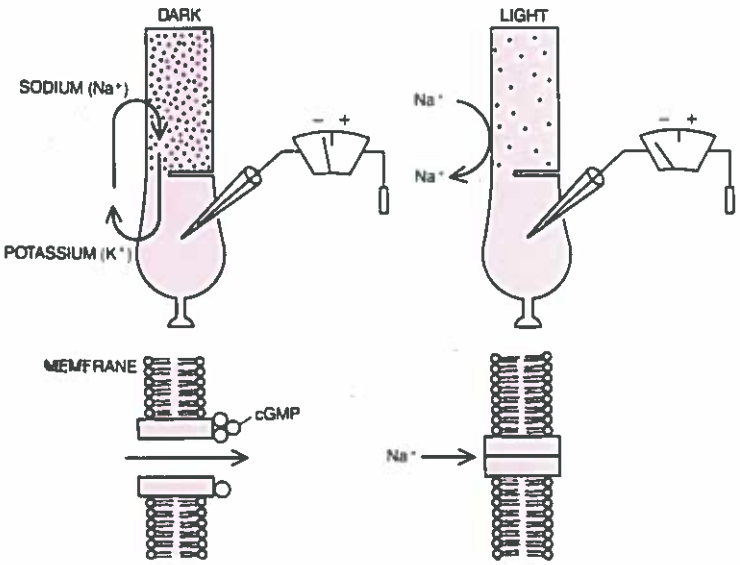
obtained by Evgeniy Fesenko and his colleagues at the Academy of Sciences of the U.S.S.R. They touched a patch pipette—a glass capillary with a tip about a micrometer (one millionth of a meter) in diameter—to the surface membrane of the outer segment of a rod from a frog retina. By applying gentle suction to the pipette and rapidly withdrawing it, they excised the patch of membrane that adhered to the tip. They found that when they exposed the patch to cGMP, it became permeable to sodium.

The molecular mechanism of the cGMP-regulated sodium movement was not clear until Anita Zimmerman in our laboratory and Lawrence Haynes and King-Wai Yau of the University of Texas Medical Branch at Galveston succeeded in showing that the current passing through a single permeation site can exceed a million sodium ions per second. Such a flux surpasses by two orders of magnitude the transport rates of membrane carrier molecules that must undergo a configuration change to translocate ions one or a few at a time. The large flux shows instead that ions cross the membrane by diffusing through water-filled pores. The opening of an individual pore appears to be triggered by the cooperative binding of three or more molecules of cGMP. In other words, each pore behaves like an efficient molecular switch designed to detect infinitesimal changes in the concentration of cGMP.

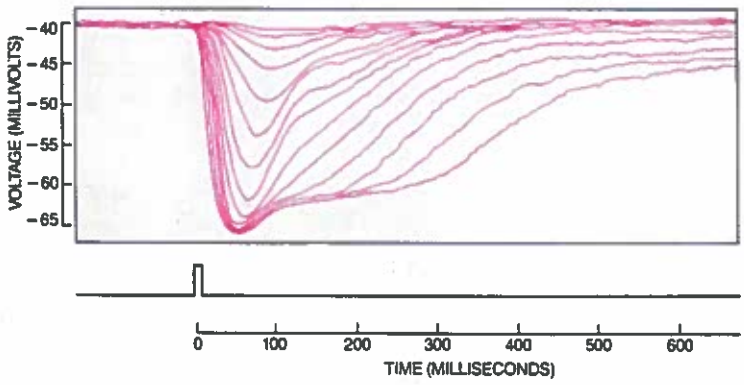
Under appropriate conditions a rod in the human retina signals the absorption of a single photon, which activates only one of the 100 million rhodopsin molecules in the rod. This remarkable performance was first demonstrated in psychophysical experiments done in the early 1940's by Selig Hecht, Simon Shlaer and Maurice H. Pirenne of Columbia University. They directed dim flashes of light into one eye of a subject who was sitting in complete darkness. By varying the strength of the flash they found that the subject usually perceived a flash when only seven photons were absorbed. Because a population of 500 rods absorbed the photons in a random spatial pattern, there was virtually no chance that any rod had absorbed more than one photon. The investigators therefore concluded that a rod must produce a detectable signal when it absorbs a single photon.

What are the amplitude and form of the electrical signal that is triggered by the absorption of a photon? How likely is it to occur after an absorption occurs? Do similar signals occur in darkness? In the early 1970's workers in several laboratories attempted to record the quantal voltage response of rods. The initial efforts failed. The reason is that rods "pool" their signals: specialized connections called gap junctions link neighboring rods in the retina and allow electric currents to flow freely among their interiors. As a consequence the hyperpolarizing response to a single photon is distributed to 10 or more rods, making it too small to detect.

In order to overcome the problem created by pooling, Yau, Trevor D. Lamb and one of us (Baylor) decided to use a different indicator of the rod



ELECTRICAL RESPONSE TO LIGHT of a rod or cone results from a reduction in the outer-segment surface membrane's permeability to sodium ions. In darkness the sodium ions, which carry a positive charge, flow into the cell and steadily reduce the negative charge density on the inside of the cell membrane (*top left*). An outward flow of potassium ions through the inner segment and the synaptic ending completes a continuous loop of "dark current." The high sodium permeability is maintained by the action of the nucleotide cyclic guanosine monophosphate (cGMP), whose concentration in darkness is high (*stippling*). In darkness several cGMP molecules bind to a pore and cause it to open (*bottom left*). In light the concentration of cGMP drops, the nucleotide leaves the binding sites and the pore closes (*bottom right*). The influx of sodium ions is thereby blocked and the internal voltage of the cell hyperpolarizes: it becomes more negative (*top right*).



HYPERPOLARIZING VOLTAGE RESPONSES from a red cone in a turtle retina were recorded with an intracellular electrode. The traces are superposed responses to brief flashes of increasing strength. The voltage difference across the membrane is plotted as a function of the elapsed time after the flash, which is shown in the lower trace. The strengths of the flashes were increased by factors of two; the weakest flash activated about 50 molecules of the light-absorbing pigment in the cone. Bright flashes caused the response amplitude to saturate, the membrane potential reaching about −65 millivolts.

response. We measured a rod's photocurrent rather than its voltage. Our choice proved to be a good one, because the photocurrent is effectively independent of membrane voltage and is therefore not influenced by coupling among rods.

To identify the response to a single photon, we observe a rod's response to very dim light. At first we worked with rods from toad retinas; more recently the two of us, in collaboration with Brian J. Nunn of Stanford, have worked with rods and cones from retinas of the macaque monkey (*Macaca fascicularis*). To make the measurements we draw an individual outer segment into a close-fitting glass capillary tube. We then record the rod's photocurrent with a sensitive amplifier connected to the capillary.

We repeatedly shine on the rod a flash so weak that it activates on the average one molecule of rhodopsin. The resulting photocurrent varies, assuming values near zero, one, two and three picoamperes (trillionths of an ampere). Such variation is expected, because the emission of photons from the source fluctuates randomly: sometimes the flash fails to activate a rhodopsin molecule and at other times it activates one, two or three molecules. Statistical analysis and calibration of the flash strength show that the one-picoampere response is triggered by the activation of a single rhodopsin molecule. The size and shape of the response are remarkably constant, suggesting that the gain of the enzyme cascade is subject to an elegant control. Measurements also show that there is a good chance—about 50 percent—that a photon will trigger a response when it is absorbed.

Photon counting by rods is impressive but not quite perfect. Even in complete darkness rods give an occasional signal identical with that triggered by the absorption of a photon. In a rod from a monkey retina, for example, signals appear randomly at a rate of one every two and a half minutes on the average. The signals seem to arise because thermal energy, or heat, can activate a rhodopsin molecule just as light can. This process sets the ultimate limit on a rod's ability to reliably encode very dim light. Fortunately, however, thermal activation proceeds quite slowly: from the frequency of the signals and the number of rhodopsin molecules in the rod we find that the half-life of the process is 420 years at body temperature. (Only because a rod is packed with rhodopsin molecules can the error signals be studied experimentally.) Nevertheless, the signals are perceived by the visual system and give rise to sensations of very dim light in complete darkness. Psychophysicists have quantified such "dark light" and find an activation rate similar to what we have measured.

The response of a cone to a single photon cannot be measured because it is too small. Background fluctuations overwhelm it. The quantal response can nonetheless be estimated from the response of a cone to flashes that activate many of its pigment molecules. We estimate that in a cone one absorbed photon produces a photocurrent approximately 10 femtoamperes (10×10^{-15} ampere) in size. This is about 100 times smaller than a rod's quantal response. The characteristic difference in the response sizes helps to explain why human cone-mediated daylight vision is less sensitive than rod-mediated night vision.

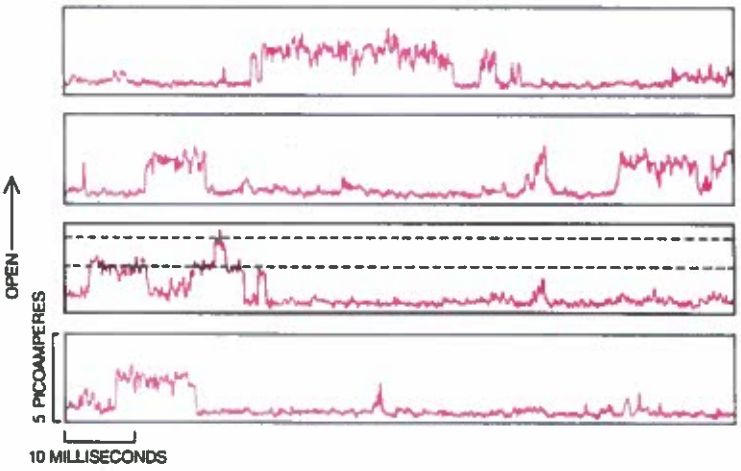
On the other hand, a cone's quantal response is roughly four times as fast as that of a rod. A primate rod, for instance, takes 300 milliseconds to finish signaling the absorption of a photon. In that time a pitched baseball travels most of the way to home plate. Because of their greater response speed, cones are better at encoding rapidly changing visual stimuli.

Visual transduction appears, then, to involve a tradeoff between sensitivity and temporal resolution. The small, fast quantal responses of cones enable the visual system to detect rapid changes in intensity or rapid movement of objects when the level of illumination is high and the rods are saturated. The slower and larger rod signals, on the other hand, are optimal for counting photons when the level of illumination is low.

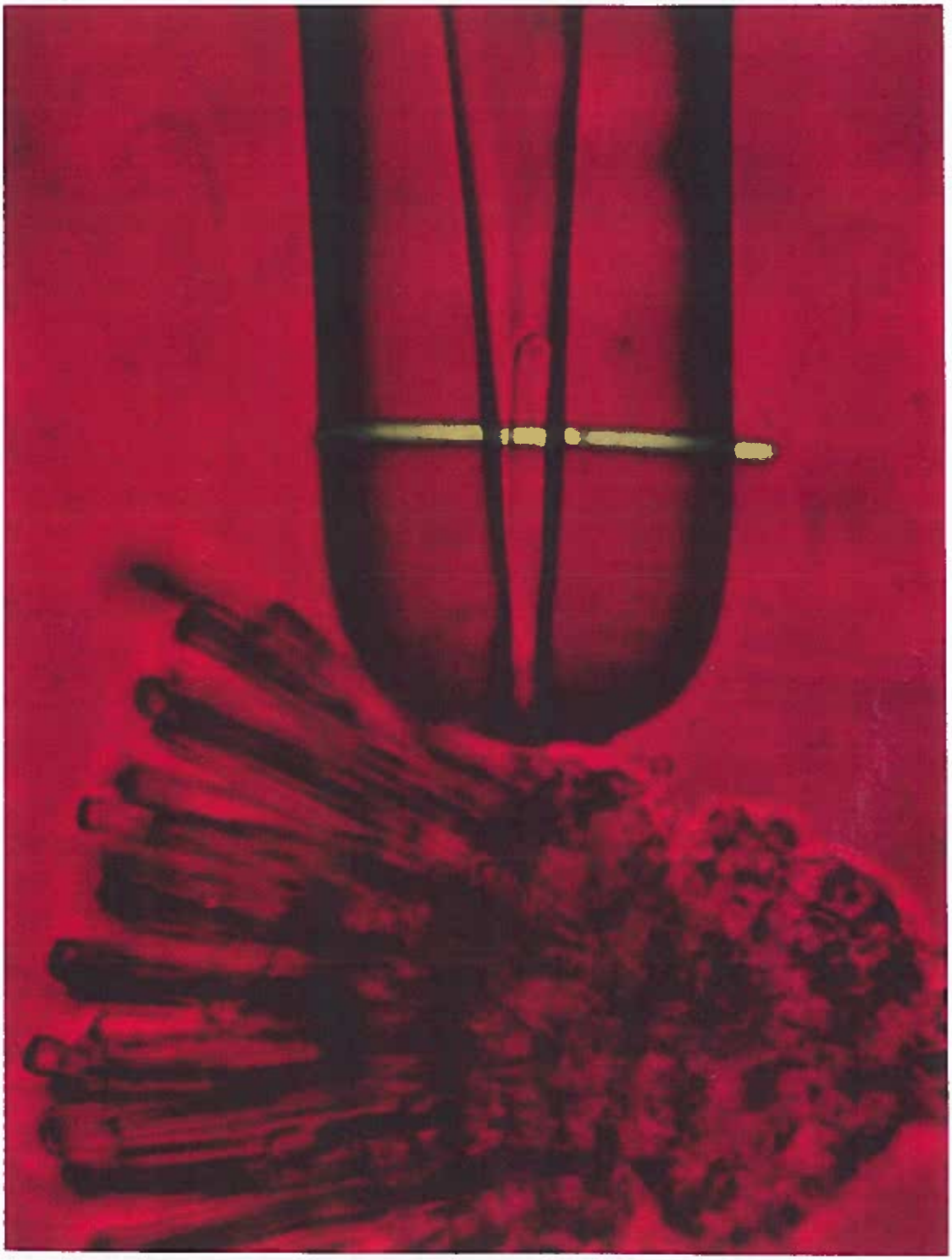
A striking increase in visual sensitivity occurs at low levels of illumination because of a switch from cone vision to rod vision. On entering a dimly lighted room, for example, we are initially blind because of the insensitivity of the cone system. Slowly the rod system becomes more sensitive, and as it assumes the primary role objects become visible. Even in pure rod vision, however, visual sensitivity rises as the level of background light falls. Does this change in sensitivity take place within the rods or in other neurons that process the rod signals?

The effect of background light on the sensitivity of a primate rod can be determined by recording responses to dim flashes from a rod that has been adapted to darkness. The peak amplitude of the response is divided by the flash strength to yield a measure called the flash sensitivity. Steady background lights are then turned on and the sensitivity is again determined. The sensitivity drops as the intensity of the background lights increases. The desensitization is accounted for by a simple saturation mechanism. As the background intensity rises, more of the sodium channels in the surface membrane close, making fewer channels available to be closed by the flash.

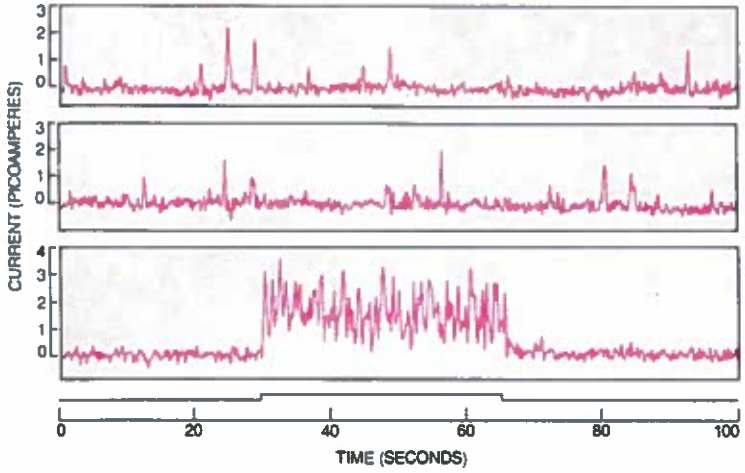
In psychophysical experiments the sensitivity of the human rod system has been measured by determining the strength of a flash that is barely de-



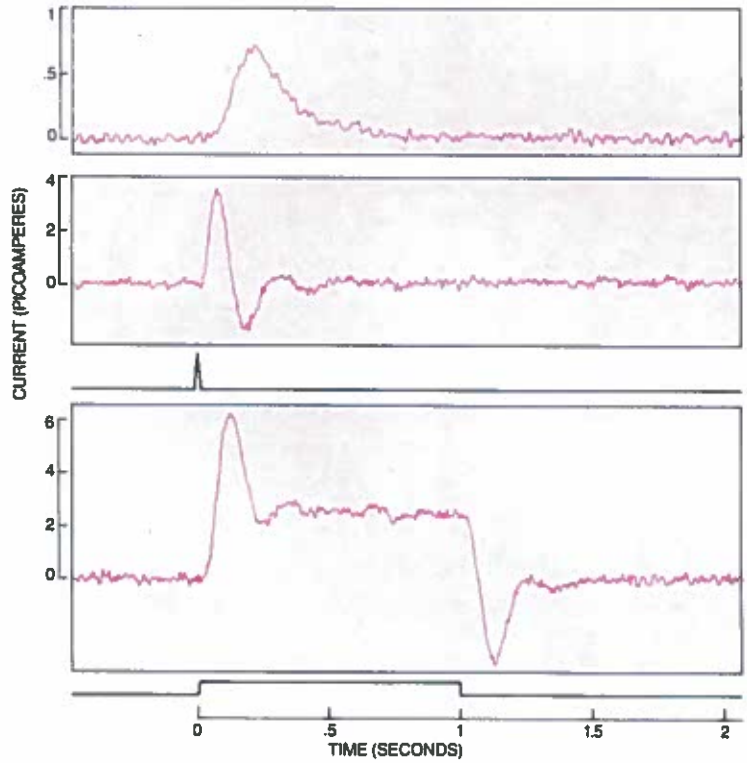
MEASURABLE ELECTRIC CURRENT results when a single pore in the outer segment of a rod opens. Here a patch of the membrane of a salamander rod was exposed to a solution containing cGMP while the membrane voltage was held at +75 millivolts. Upward deflections in the traces correspond to the opening of a single pore. In the third trace from the top two pores open simultaneously. In order to improve the resolution of the measurement the concentrations of calcium and magnesium ions were made very low.



SINGLE-ROD OUTER SEGMENT of a toad is drawn into a suction pipette for recording electric currents induced by light. The outer segment in the pipette is about 50 micrometers (millionths of a meter) long and six micrometers in diameter. The rod is stimulated by the transverse slit of light, and the electrical response is amplified and recorded. To preserve the adaptation of the cell to the dark, the work is done under infrared light and is viewed on a video monitor. The piece of retina is from the toad *Bufo marinus*.



RESPONSE OF MONKEY ROD to a single photon is monitored by drawing the rod into a suction pipette. In the upper traces dim flashes activating on the average one pigment molecule were delivered to the outer segment. The response of the rod fluctuated, its amplitude ranging from zero to one or two picoamperes (trillionths of an ampere). The activation of a single pigment molecule triggers a response of about one picoampere; the fluctuations in amplitude are caused by random variations in the emission of photons from the light source. The lower trace shows the response of the same rod to a dim, steady light, which activated on the average about 10 pigment molecules per second.



WAVEFORM OF SINGLE-PHOTON EFFECT in a rod and cone from the macaque monkey retina is revealed by averaging responses to dim flashes. The change in current through the membrane is plotted as a function of elapsed time after the flash. The response of the rod (*top*) was elicited by a flash that activated an average of one pigment molecule; the response of the cone (*middle*) was triggered by a flash that activated about 200 molecules. (The response is a scaled-up version of the response to a single photon.) The bottom trace shows the response of the cone to a pulse of light one second long.

tectable in the presence of a diffuse background light. The sensitivity to the flash decreases as the background intensity is increased. A background that strongly desensitizes rod vision, however, may have little effect on the measured sensitivity of an individual rod. For example, a steady light activating 40 molecules of rhodopsin per second per rod causes a 10,000-fold drop in the sensitivity of rod vision but reduces the sensitivity of a rod outer segment by only about 20 percent. The measurements support the conclusion of the late William A. H. Rushton of the University of Cambridge that desensitization of human rod vision by background light is due to neuronal processing beyond the rod outer segments. The mechanism of the effect remains to be determined.

When the background intensity exceeds the level that corresponds approximately to the blue sky at noon, the flash sensitivity of human rod vision falls precipitously: it saturates. In such bright light, changes in the rate of photon absorption in the rods are not detected by the visual system. Electrical measurements show that single rods become unresponsive to a flash at about the same background intensity that saturates rod vision. This limit in vision therefore expresses a property of the rod transduction.

The sensitivity of a single photoreceptor to light of different wavelengths is determined by the probability that its visual pigment will absorb photons of those wavelengths. Measurements of the spectral sensitivity of single cells from the macaque, which is thought to have photoreceptors like those of man, provide a physiological basis for the spectral characteristics of human vision. The wavelengths of visible light lie roughly between 400 and 750 nanometers (billionths of a meter). Light of longer wavelengths (near-infrared) is poorly absorbed by the visual pigments; light of shorter wavelengths (near-ultraviolet) can be absorbed by the visual pigments but fails to reach the retina because it is absorbed in the cornea and lens.

Macaque rods show a peak sensitivity in the blue-green region of the spectrum, near 490 nanometers. The measured spectral sensitivity agrees with the spectral sensitivity of human rod vision determined by psychophysical experiments.

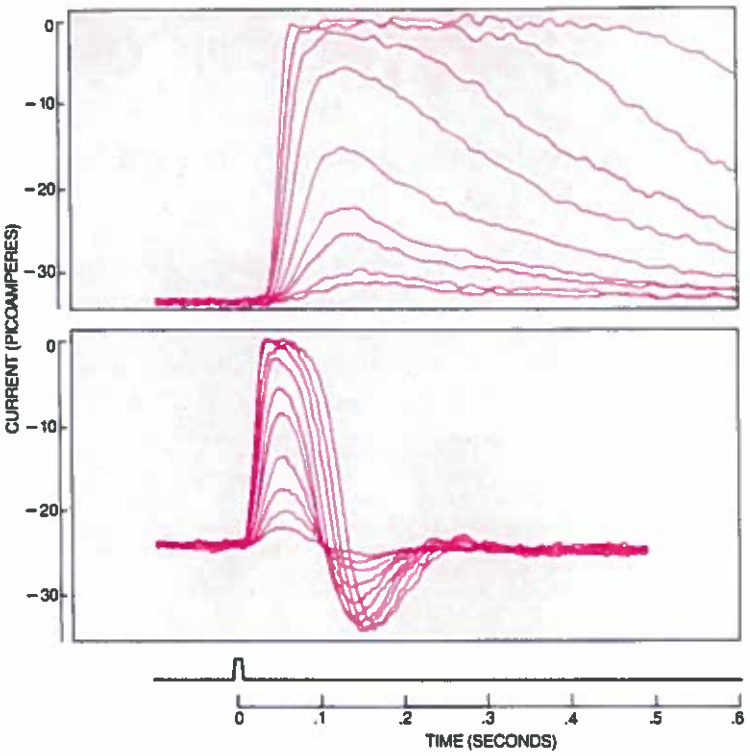
Monkey cones fall into three groups that have peak sensitivities at roughly 430, 530 and 560 nanometers. The groups, which correspond closely to those of humans, may be called blue, green and red to indicate the relative positions of the spectral maxima. Each

type of cone is sensitive to light over a broad range of wavelengths, and the sensitivities of the groups show considerable overlap. Nevertheless, the segregation of pigments into the appropriate cones appears to be quite strict. From the form of the sensitivity curves we conclude that less than one in 100,000 pigment molecules in a blue cone is of the red or green type.

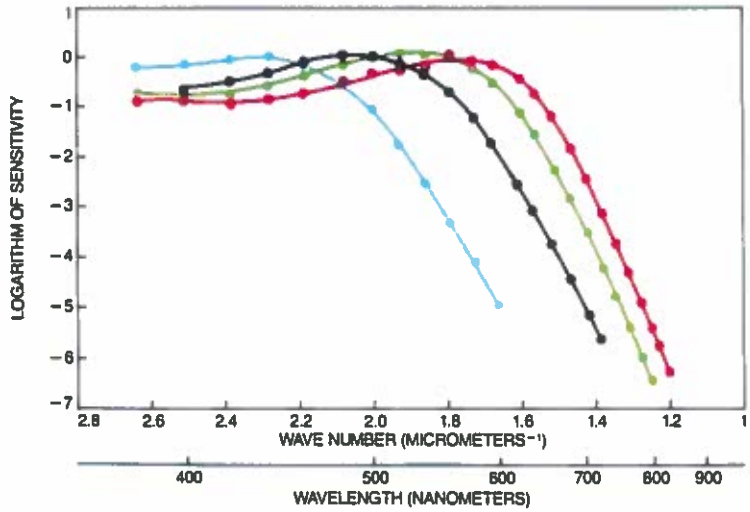
A cone's response does not depend on the wavelength of the photon that was absorbed; all stimuli that elicit identical absorptions give identical responses. By sensing the ratio of excitations in the three kinds of cone, however, the visual system is able to compute color from wavelength. It has been known for many years from psychophysical experiments that two stimuli of different wavelength composition will appear identical if both stimuli evoke, within each kind of cone, the same number of absorptions. Although the trichromacy of color vision has been well established by such observations, its exact basis has been unclear because of uncertainty about the specific cone spectral sensitivities. These sensitivities are now known for monkey cones, and it is satisfying that they predict the rules governing the light intensities a human requires to make color matches.

At long wavelengths the perception of hue is determined by the relative absorption in the red and green cones alone. As the wavelength increases beyond about 600 nanometers, the perceived hue changes from orange to a progressively deeper red. Beyond 700 nanometers a curious reversal occurs and the hue becomes more orange. This phenomenon, the "paradoxical hue shift," was discovered in 1955 by Giles S. Brindley of Cambridge. It is explained by the form of the spectral sensitivities of the red and green cones. The ratio of the red- and green-cone sensitivities has a maximum at 700 nanometers, and so this wavelength appears reddest.

The molecular mechanism of visual transduction and the central processing of photoreceptor signals are still far from being completely understood. Although the internal transmitter for visual excitation has been identified, the operation of the nucleotide cascade and the control of sodium permeability are only beginning to be characterized. Much also remains to be learned about how small signals generated by single photons are transmitted across synapses, separated from noise and processed by the visual system. The years ahead promise to be an exciting time for experiments in both areas of investigation.



MEMBRANE CURRENTS from a monkey rod (*top*) and cone (*bottom*) were recorded with a suction pipette. The outer segments were illuminated uniformly by flashes of light. The superposed recordings show the current of the outer segment as a function of time after the flash. The strength of the flashes was progressively doubled until the responses reached their maximum amplitude, and the inward current shut off completely. In the rod the response was half its maximum when 30 rhodopsin molecules were activated; in the cone the response was half its maximum when 1,200 pigment molecules were activated.



RELATIVE SENSITIVITY TO A PHOTON for rods and cones of the macaque monkey is plotted against the wavelength of the photon. The spectral sensitivities are quite similar to those of human receptors. The black curve is the spectrum of the rods, and the red, green and blue curves are respectively the spectra of the red, green and blue cones.