# Principles and Practice of Clinical Electrophysiology of Vision


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11830 Westline Industrial Drive
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1 2 3 4 5 6 7 8 9 0 CL CL MV 95 94 93 92 91

**Library of Congress Cataloging-in-Publication Data**
Principles and practice of visual electrophysiology / [edited by] John R. Heckenlively, Geoffrey B. Arden.
p. cm.
Includes bibliographical references.
Includes index.
ISBN 0-8151-4290-0
1. Electroretinography. 2. Electrooculography. 3. Visual evoked response. I. Heckenlively, John R. II. Arden, Geoffrey B. (Geoffrey Bernard)
[DNLM: 1. Electrooculography. 2. Electrophysiology. 3. Electroretinography. 4. Evoked Potentials, Visual. 5. Vision Disorders—physiopathology. WW 270 P957]
RE79.E4P75 1991 91-13378
617.7 1547—dc20 CIP
DNLM/DLC
for Library of Congress

# 7

# Retinal Physiology

Gertrude Falk

Most of our knowledge about how signals are generated in rods and cones and transmitted along the pathway to the optic nerve dates from about 1970, and the pace of advances has increased rapidly. Much of this progress is due to the use of intracellular microelectrodes to record directly from retinal cells and to inject dyes that identify them unambiguously. Although most new observations were made on the retinas of lower vertebrates where the cells are of larger size, much of what has been learned appears to apply to mammalian retinas as well. The elucidation of the underlying biochemistry of phototransduction, that is, the mechanism by which light absorption leads to an electrical signal, also owes much to new ideas and techniques.

## PHOTOTRANSDUCTION IN RODS AND CONES

### Electrical Changes Produced by Light

As with all cells, there is a potential difference across the surface membrane of photoreceptors such that the inside of the cell is more negative than the outside. The membrane potential arises as a result of the selective permeability of the membrane to ions and the difference in concentration of ions whereby the intracellular concentration of K ions is much greater than extracellular, and the reverse is the case for Na ions. These ionic gradients are maintained by a Na-K exchange pump whose energy is supplied by adenosine triphosphate (ATP). This pump is located in the inner segment where the mitochondria that generate ATP are also located. In the dark, channels in the surface membrane of the outer segments are open and allow the passage of cations, mainly Na ions, which move down their electrochemical gradient. This inward current keeps the cell somewhat depolarized. The membrane of the inner segment has channels that selectively allow the passage of K ions. Consequently, there is a circulating dark current (Fig 7–1). The absorption of light by the visual pigment causes the channels of the outer segment to close, thereby suppressing the inward dark current and causing the visual cell to hyperpolarize, i.e., the inside of the cell becomes more negative with respect to the outside than in the dark.

The voltage changes in a cone in response to brief flashes of light of increasing intensity are illustrated in Figure 7–2. The response amplitude is continuously graded with light intensity. As the light becomes brighter, the response saturates, and further increasing the brightness simply increases the duration of the response. Similar but slower responses can be recorded from rods. Figure 7–3 shows the change in the current through the outer segment membrane of a single monkey rod and a cone. The convention is that inward current has a negative sign. Increasing the intensity of a light flash suppresses increasing amounts of the inward dark current until, when the light is bright enough, all of the dark current is suppressed. The transient decrease in circulating current, as ion channels in the outer segment close, gives rise to the leading edge of the

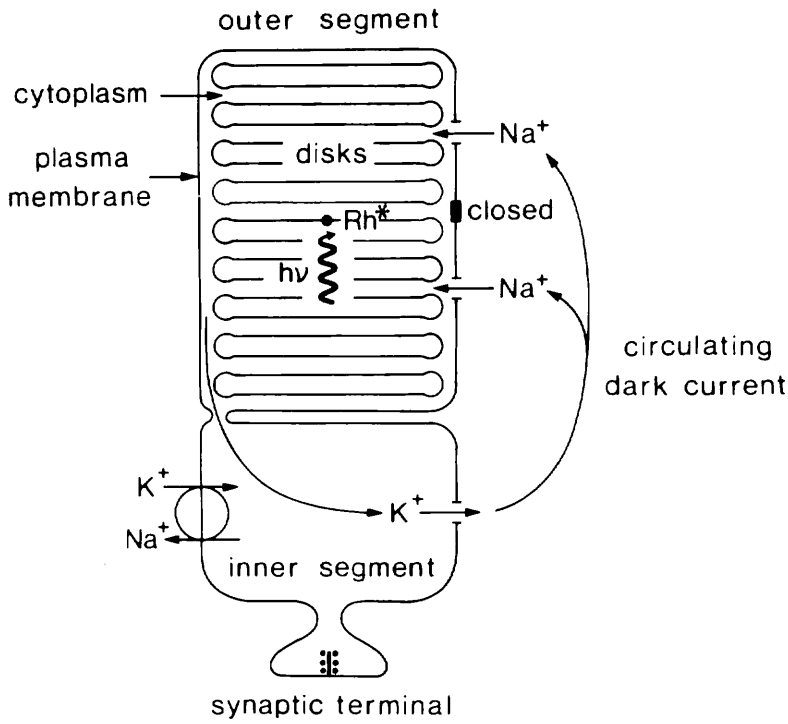



**FIG 7–1.**
Ion movements across the surface membrane of a rod. In the dark there is a circulating current as cations, mainly Na ions, cross the outer segment membrane while K ions exit from the inner segment. Rhodopsin is embedded in stacks of disc membranes that are separated from each other and from the surface membrane. Light, absorbed by rhodopsin, leads to a change in the concentration of a diffusible substance and the closure of ion channels in the outer segment surface membrane, and this reduces the circulating current. (From Lamb TD: *Trends Neurosci* 1986; 9:224–228. Used by permission.)

a-wave of the electroretinogram (ERG) and contributes to its entire time course.

Several features of the responses of rods and cones illustrated in Figure 7–3 stand out. The response of the cone has a much faster time course than the rod and is less sensitive to light. Some 40 times as many photons must be absorbed by a cone to give the same photocurrent as a rod. The action spectrum of the photocurrents recorded from individual primate cones corresponds to the absorption

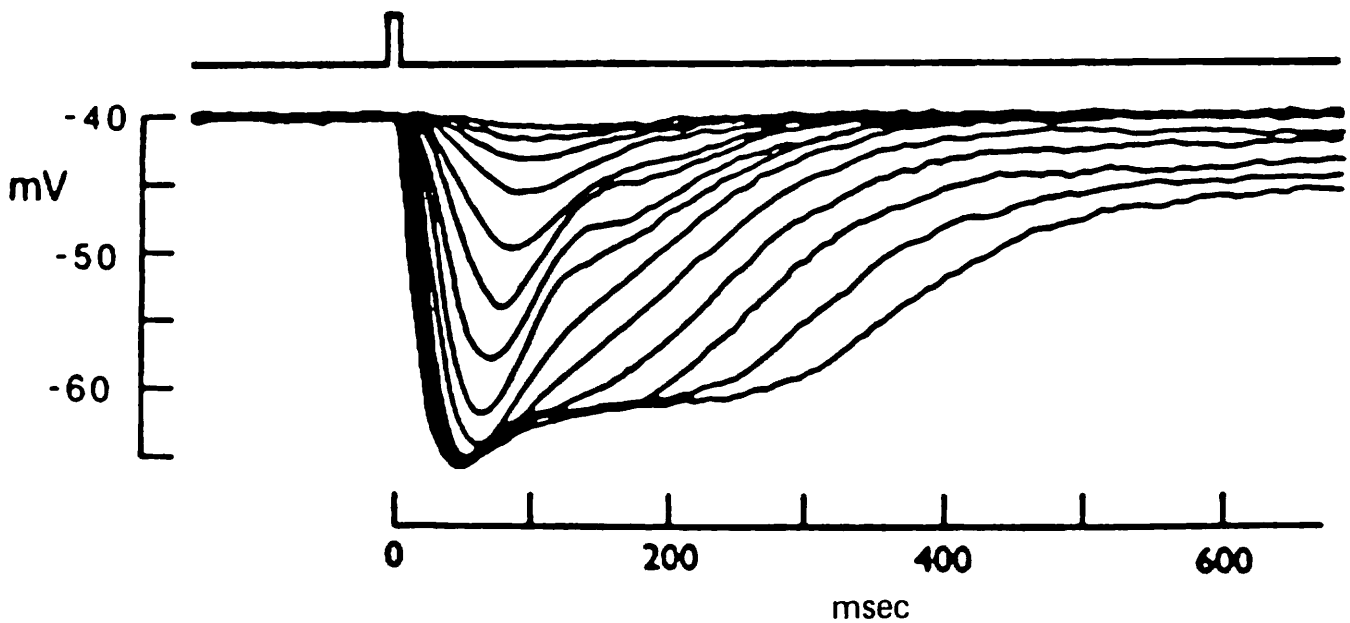



**FIG 7–2.**
Voltage changes in a turtle cone that are produced by light flashes of increasing intensity. Downward deflection indicates hyperpolarization (greater internal negativity). The timing of the flash is shown in the *top* trace. (Adapted from Baylor DA, Fuortes MGF: *J Physiol* 1970; 207:77–92.)

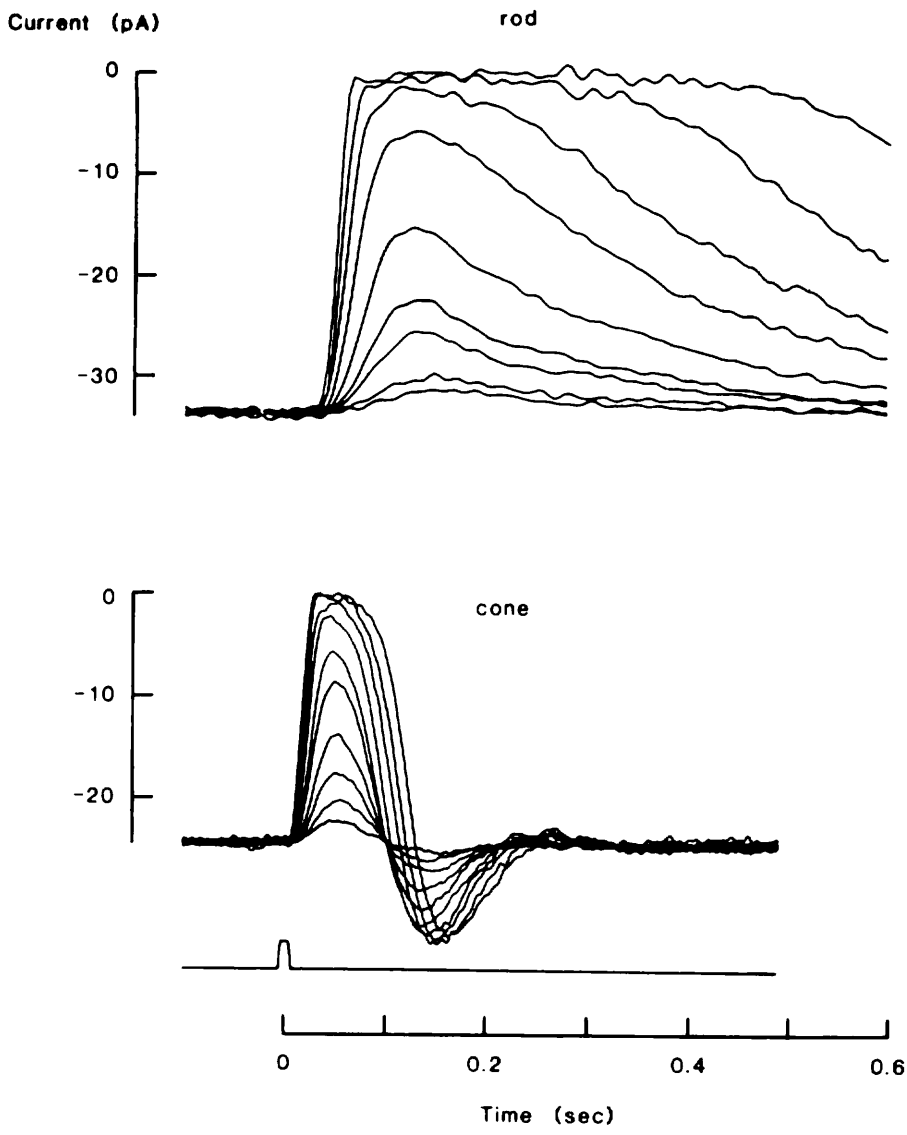



**FIG 7–3.**
Reduction in circulating current through a monkey rod and a cone that is produced by light flashes. In the dark there is an inward current through the outer segment membranes, and this current is reduced by light. The flash intensity was increased by a factor of about 2, ranging between 3 and 900 photoisomerizations in the rod and between 200 and 36,000 in the cone. Timing of the flash is shown *below.* (From Baylor DA: *Invest Ophthalmol Vis Sci* 1987; 28:34–49. Used by permission.)

spectrum of three different pigments that have maxima at 460, 530, and 568 nm (Fig 7–4). The responses of rods peak at 491 nm and closely match the human scotopic sensitivity curve.

The response of rods reaches half-saturation when each rod has absorbed about 30 photons. Each primate rod contains about $10^9$ rhodopsin molecules, so the probability of a photon that enters the rod being absorbed is high (30% to 50%). It is possible to record the rod electrical response even to single photons. Because rods are electrically coupled in the retina such that the effect of a single photon captured by a rod is attenuated and spread over many rods (see the later section on functional connections of rods and cones), it is necessary to isolate rods physically or electrically within the retina in order to demonstrate single-photon responses (Fig 7–5). Because light is quantized, the light stimulus itself and hence the response will fluctuate statistically according to a Poisson distribution. If the light flash is very

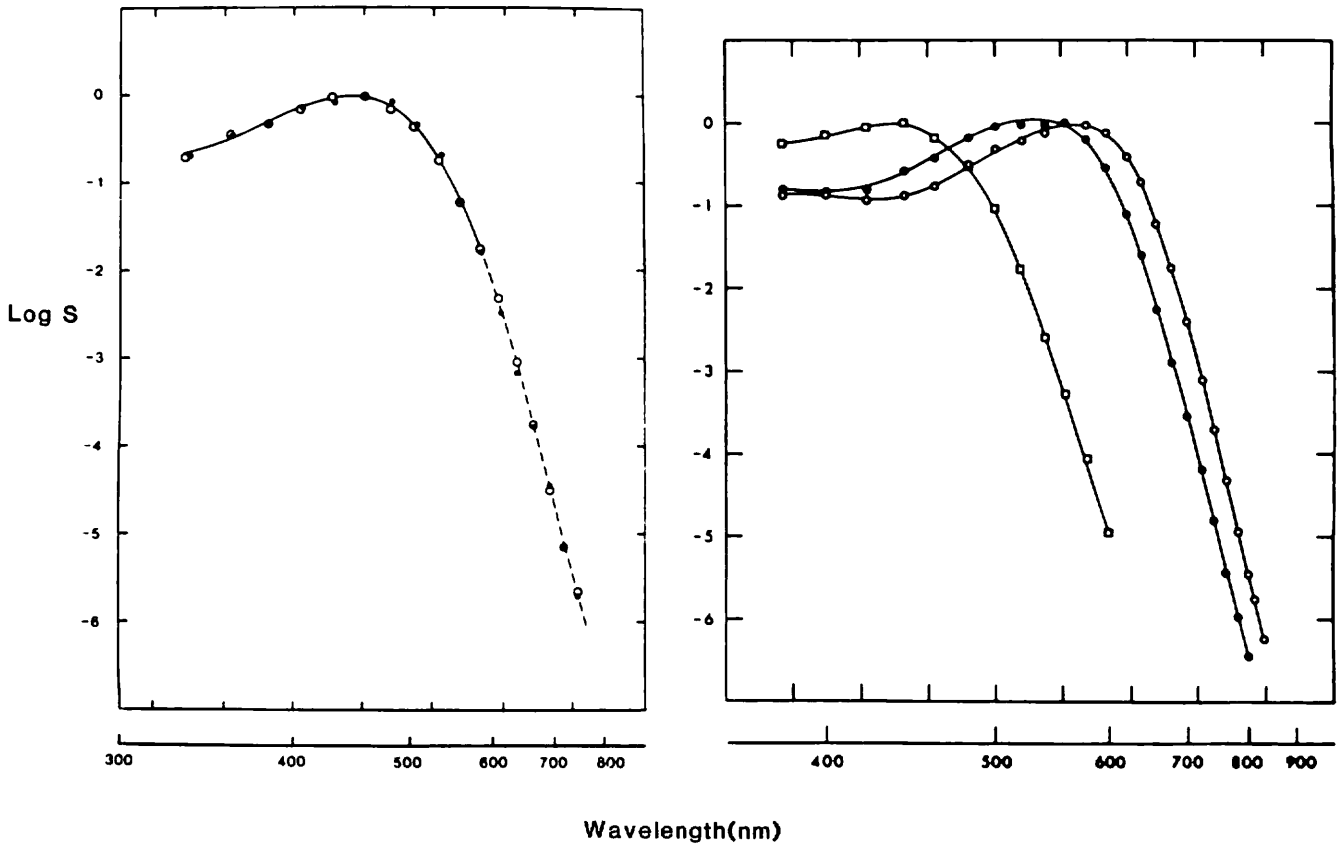



**FIG 7–4.**
Spectral sensitivity *(S)* of the photoresponse of individual cones and rods of the monkey retina. The cones (shown on the *right*) have their peak sensitivity in the red, green, or blue. *Open circles* in the graph on the *left* represent the averaged spectral sensitivity of individual rods, while the *closed circles* show the human scotopic spectral sensitivity. (Adapted from Baylor DA: *Invest Ophthalmol Vis Sci* 1987; 28:34–49.)

dim, say of such intensity that on average one photon would be absorbed, then on individual presentations of the stimulus, 37% would give no response, 37% would give the response to a single photon, 18% to two photons, and 6% to three photons. The expected variability in the response to a dim light flash is seen in Figure 7–5. On those occasions when a single photon is absorbed, the photocurrent amounts to about 1 pA at the peak, and this corresponds to the closure of about 4% of the membrane channels that had been open in the dark, i.e., many channels close as a result of a single photoisomerization of rhodopsin. Thus, there is a large amplification in phototransduction that transforms the energy of a photon into electrical energy with a gain of about $10^6$. Since rhodopsin is embedded in the stacks of disc membranes physically isolated from the surface membrane, there must be an internal transmitter involved in mediating the effect of light.

### The Cyclic Nucleotide Cascade

The biochemical changes underlying phototransduction are now fairly well understood. The internal transmitter is a cyclic nucleotide, cyclic guanosine monophosphate (cGMP), that is present in the outer segments of the photoreceptors and that in darkness opens ion channels in the surface membrane. A sequence of reactions initiated by the photoisomerization of the visual pigment leads to the hydrolysis of cGMP. As a consequence of the decreased concentration of cGMP, membrane channels of the outer segment close.

The sequence of events that intervene between the *cis-trans* photoisomerization of retinal, the chromophore of rhodopsin, and the amplified hydrolysis of cGMP is shown in Figure 7–6. Photolyzed rhodopsin (R*), probably in the form of metarhodopsin II, diffuses laterally within the internal

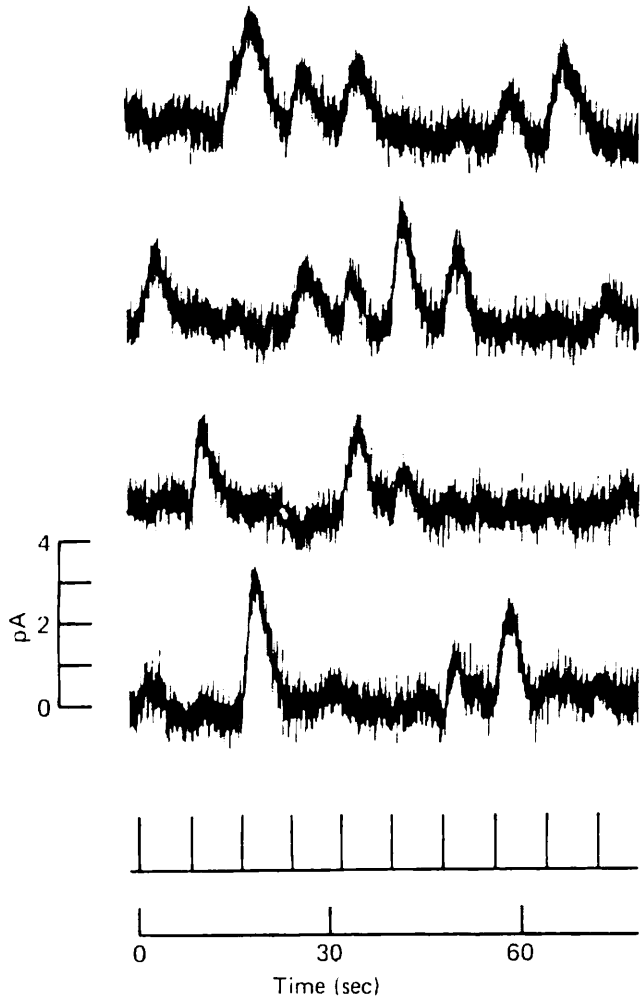



**FIG 7–5.**
Fluctuations of the photocurrent of a single rod in response to dim light flashes. These records show that rods respond to single photons. Each row is part of the same continuous record. The timing of the flashes is shown at the *bottom* trace. (From Baylor DA, Lamb TD, Yau K-W: *J Physiol* 1979; 288:613–634. Used by permission.)

disc membrane where it binds to a G protein called transducin (T). G proteins bind guanyl nucleotides, and in the dark transducin is bound to guanosine diphosphate (GDP). R* catalyzes the rapid exchange of guanosine triphosphate (GTP) for GDP. Then R* dissociates from the complex R*-T GTP to interact with another molecule of T-GDP while transducin bound to GTP (T-GTP) activates an enzyme, phosphodiesterase, that rapidly catalyzes the hydrolysis of cGMP. During the lifetime of a single R*, 500 molecules of transducin undergo GTP-GDP exchange; a similar number of phosphodiesterase molecules are activated with the consequent hydrolysis of about $10^5$ molecules of cGMP. Hence the term *cGMP cascade* is used to describe biochemical phototransduction.

Several factors are involved in restoring the system to the dark state. R* is phosphorylated by a kinase that may be activated by light. Phosphorylated R* binds to another protein, arrestin,† and when bound has a low affinity for T-GDP. Transducin itself is a guanosine triphosphatase (GTPase) that catalyzes the splitting of a phosphate group from GTP to yield GDP, i.e., it degrades itself into an inactive form. The conversion occurs on a time scale of a few seconds.

The return to the dark state involves restoration of the cGMP concentration by synthesis requiring another enzyme, guanylate cyclase (see Fig 7–6). On a very much slower time scale are the thermal decomposition of rhodopsin to opsin and all-*trans* retinal and subsequent reisomerization of the chromophore of rhodopsin.

The cGMP cascade also operates in cones but is quantitatively different from rods. It would appear that the stages of the cascade are kinetically more rapid and operate at lower gain than in rods. One possibility is that the intermediates in the cascade have shorter lifetimes, and this might account for the lower sensitivity of cones.

### Light Adaptation

The term *light adaptation* means a time-dependent process in which light leads to desensitization but the system recovers responsiveness, although requiring light of higher intensity. There appears to be a gain control mechanism by which the gain varies with the intensity of the ambient light. Light adaptation occurs even at intensities at which fewer than $10^{-7}$ of the visual pigments are bleached per second, so it is clear that it cannot be attributed to any significant pigment loss reducing the quantum catch.

Light adaptation occurs in the rods and cones via a negative-feedback mechanism controlling the concentration of cGMP in the outer segments. The ion channels that are opened by cGMP in the dark admit not only Na and K but also Ca ions. The free calcium concentration inside the cell is low, less than $10^{-6}$M, as compared with an extracellular concentration of 2

*†Arrestin is also known as the S antigen and the 48-kilodalton (kD) protein (although its true molecular weight is about 45 kD). S antigen is probably involved in autoimmune uveitis.*

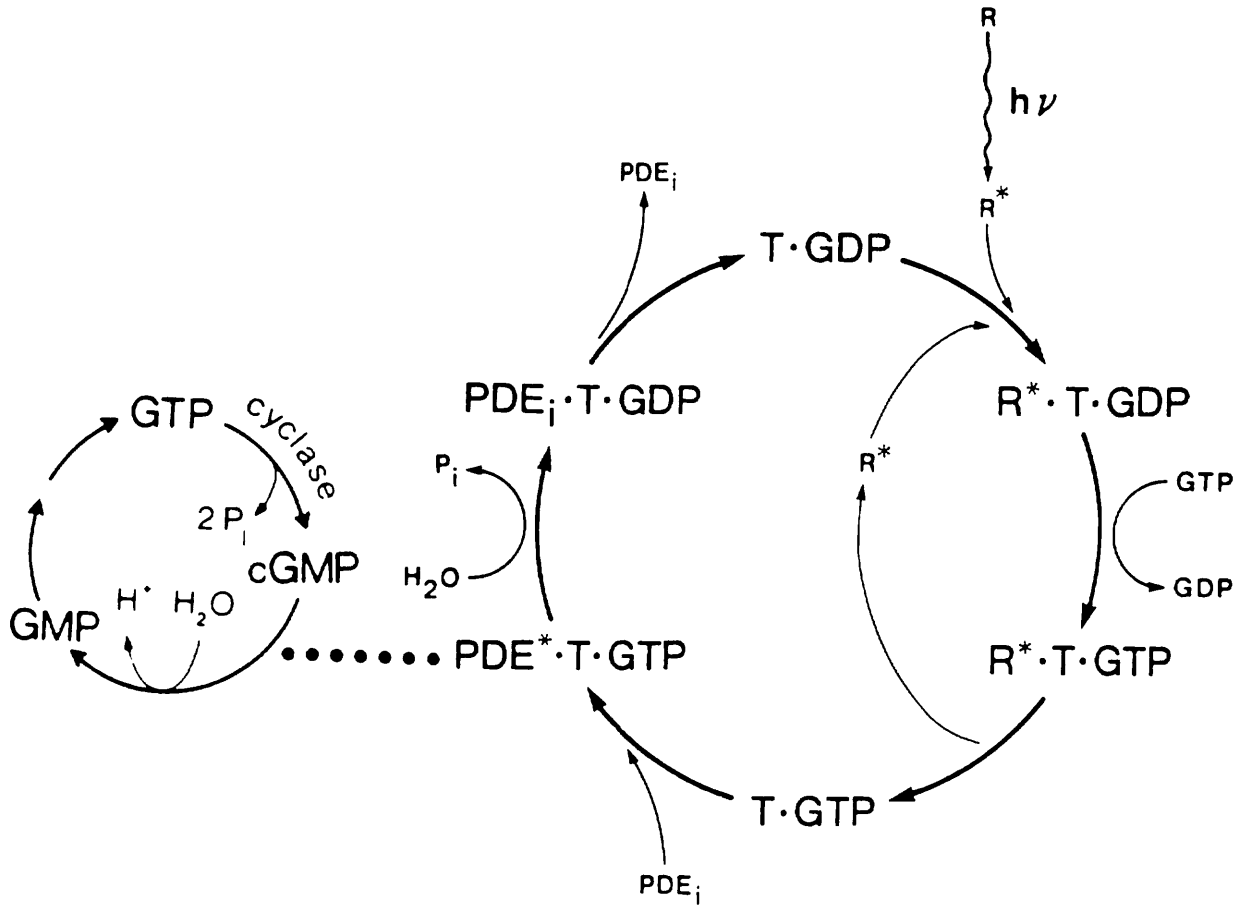



**FIG 7–6.**
The cGMP cascade of phototransduction. The cycle on the *right* shows the steps by which the absorption of light by rhodopsin leads to the activation of a phosphodiesterase *(PDE)* hydrolyzing cGMP. *T* represents the GTP-binding protein transducin.

to $4 \times 10^{-3}$M, and thus Ca ions tend to move into the outer segment in the dark. The level of free internal Ca in the visual cell is kept low by binding, by being internalized in the mitochondria located in the inner segment, and by a Ca-Na exchange mechanism located in the surface membrane of the outer segment. One Ca ion and one K ion move out of the cell in exchange for four Na ions. The internal free calcium level controls the activity of the synthesizing enzyme, the cyclase, such that in the dark the enzyme is not fully active. There is a balance between synthesis and hydrolysis of cGMP to maintain its concentration constant. This is necessary because otherwise fluctuations in concentration would give rise to a large dark noise impairing visual detection.

In the light the activity of the phosphodiesterase increases markedly so that cGMP concentration falls, and this leads to the closure of ion channels, thereby impeding the entry of Ca. The Ca-Na exchange continues, however, so the internal concentration of Ca falls. Since Ca is an inhibitory regulator of the cyclase, the fall in Ca concentration results in increased synthesis of cGMP that offsets the fall in cGMP concentration resulting from light-induced hydrolysis by phosphodiesterase. Hence, fewer channels will be closed by a given light intensity in the light-adapted state than would otherwise be the case. Thus more light will be needed than when dark-adapted to produce a response of a criterion magnitude.

However, for reasons not as yet understood, primate photoreceptors do not appear to light adapt so as to have an extended range of operation. Instead, the response saturates. This saturation probably arises from the finite number of light-controlled channels in the surface membrane. At low light levels the number of channels closed is proportional to the light intensity. At higher light levels there are fewer open channels to be closed, so a given increment in light intensity has a lower probability of

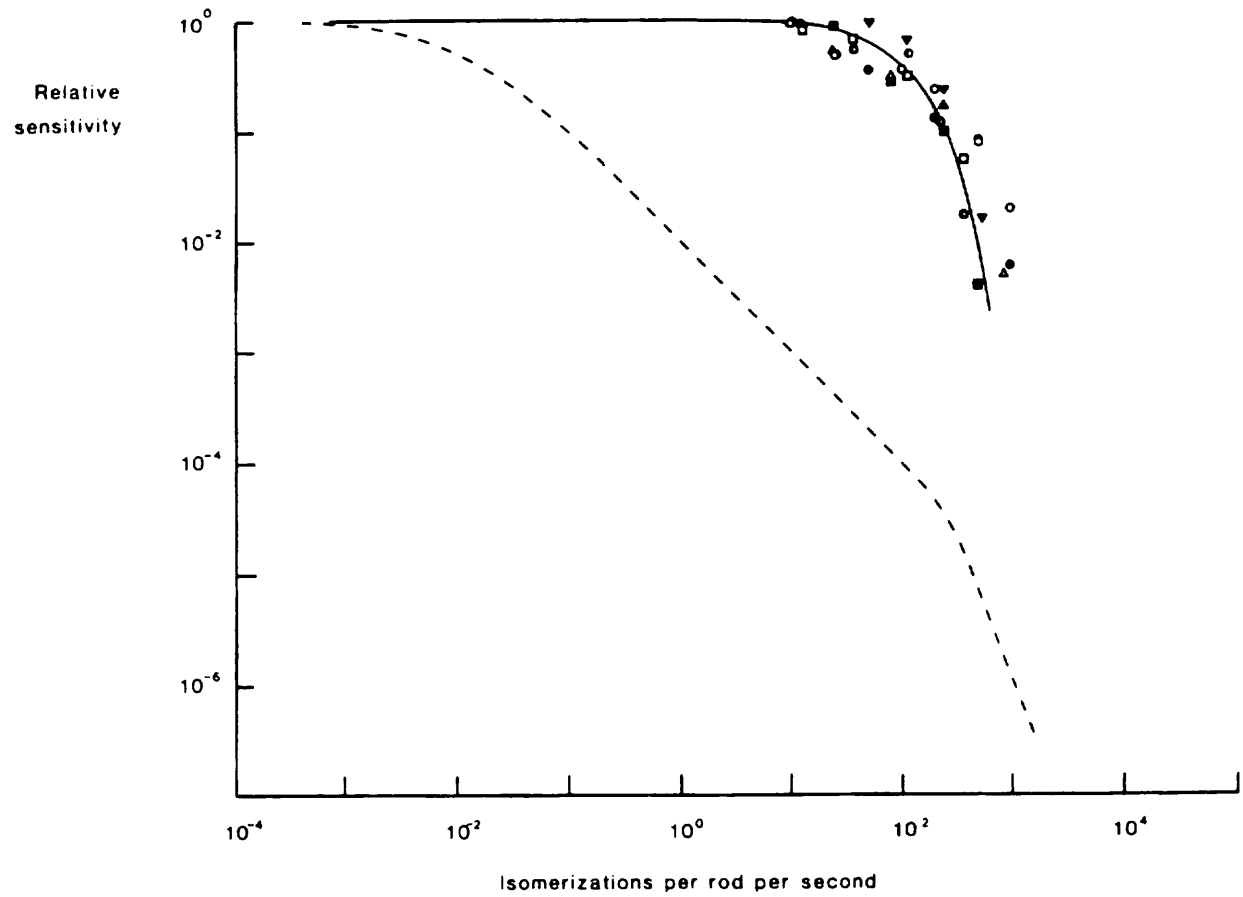



**FIG 7–7.**
The desensitization of monkey rod outer segments *(points, solid line)* and human rod vision *(dashed line)* by background light. The sensitivity, relative to its value in darkness, is plotted against the background light intensity (both on logarithmic scales). (From Baylor DA: *Invest Ophthalmol Vis Sci* 1987; 28:34–49. Used by permission.)

closing channels. When all channels are closed, the system is fully saturated. Yet there is much evidence that the retina has an extended range of operation, i.e., it light-adapts. By implication this adaptation must occur more proximally, possibly at the bipolar cell level. Saturation, unlike light adaptation, is not time dependent. The difference between light adaptation and saturation is strikingly illustrated in Figure 7–7, which describes the change in sensitivity with background light. The curve on the right shows the desensitization of single monkey rods by background light. The sensitivity falls to half when the background bleaches about 50 molecules of rhodopsin per second. At higher background levels the sensitivity falls precipitously as saturation occurs so that at backgrounds bleaching 1,000 molecules of rhodopsin per second the relative sensitivity has declined to about $10^{-3}$. On the other hand, human rod vision measured psychophysically (the dotted curve in Fig 7–7) obeys the Weber-Fechner relation over a wide range of light intensities such that sensitivity varies inversely with background over nearly 4 log units. It is interesting to note that the deviation from this relation when scotopic sensitivity falls abruptly at backgrounds greater than $10^3$ isomerizations per rod per second had been termed *rod saturation.* The question of light adaptation in primate rods is currently being reinvestigated in the light of conflicting reports.

## FUNCTIONAL INTERCONNECTIONS OF RODS AND CONES

### Rod-rod Coupling

Soon after the voltage changes of photoreceptors in response to light were first recorded directly, it was realized that rods do not operate independently

of their neighbors. The signal induced in a rod that has absorbed light spreads to other rods at some distance from the site of light absorption. The receptive field of a rod is therefore much greater than the dimension of a single rod. By injecting current into a single rod artificially, it is possible to study the way in which the signal spreads to other rods. The conclusion from such experiments and from experiments mapping the responses to spots or bars of light is that rods are electrically coupled by low-resistance contacts. These contacts, known as gap junctions, can be observed in the electron microscope.

There are some interesting consequences of coupling. Coupling results in spatial averaging, thereby reducing fluctuations in membrane potential such as might arise from the random absorption of light quanta. Because current can spread to other rods, the response to a single photon in a rod in a coupled network is much smaller than it would be in an isolated rod. However, many rods converge at the synapses with bipolar and horizontal cells, which then act as the summing point for the distributed rod signals, so the transmitted signal is at least as large as in the absence of coupling. In fact, as will be discussed in the section on synaptic transmission, there may even be an advantage conferred by rod coupling in ensuring that the absorption of light is efficiently signaled to postsynaptic cells.

There is a further feature of signal spread through the coupled rod network. As the signal spreads, the waveform of the voltage response becomes briefer in duration. This striking behavior results from the presence of voltage-gated ionic channels in the inner segment membrane. An important consequence of this nonlinear behavior of the inner segment membrane is that the rod voltage response is more transient than the photocurrent. Although phototransduction is an inherently slow process, the faster voltage change in the rods results in an improvement of temporal resolution.

### Cone-cone Coupling

Cones are also electrically coupled, although the extent of coupling varies among species. It has been found that only single cones bearing the same visual pigment are coupled together. Gap junctions exist between cones in the primate retina, and this implies electrical coupling, although it is likely that at the fovea cones are independent. Interactions between cones of different classes occur postsynaptically.

### Rod-cone Coupling

The conclusion that rods and cones are electrically coupled is based on observation of the mixing of rod and cone photoresponses by recording the spread in the photoreceptor layer of current injected into one photoreceptor and by electron microscopy showing the presence of gap junctions between rods and cones (in the primate retina as well). These observations are in agreement in showing that rod-cone coupling is much weaker than rod-rod coupling.

## SIGNAL SHAPING AT THE FIRST SYNAPSE

### Control of Transmitter Release by Membrane Potential

The graded hyperpolarization of rod and cone outer segments by light spreads to the synaptic terminals largely without significant decrement because the distances are short. The release of a transmitter stored in vesicles at the synaptic terminal is controlled by the membrane potential. The transmitter, L-glutamate, acts on the postsynaptic cells, the bipolar and the horizontal cells, and this causes their membrane potential to change.

At all synapses in the nervous system the rate of release of transmitter varies with membrane potential and increases steeply as the presynaptic membrane is depolarized. Transmitter release is strongly dependent on the free intracellular concentration of Ca ions, and there is an influx of Ca into the cell when depolarized. The dependence of intracellular Ca concentration on the rod membrane potential and the dependence of glutamate release on rod membrane potential are illustrated in Figure 7–8. Recent estimates indicate that there is a 10-fold change in transmitter release for a 6-mV change in rod membrane potential. Since transmitter release changes exponentially with membrane potential, this means that a 12-mV change will alter transmitter release by 100-fold.

Rods and cones are somewhat depolarized in the dark (membrane potential of −40 mV) and hyperpolarize in light (see Fig 7–2). Hence they release transmitter in the dark, and light causes a fall in transmitter release. Photoreceptors hyperpolarize in bright light by about 25 mV, which is sufficient to suppress transmitter release completely. From an inspection of Figure 7–8 it would be also likely that, for the detection of nonuniform illumination, it would be an advantage if the rods were electrically coupled so that the membrane potential changes were spread over many rods. If rods were not cou-

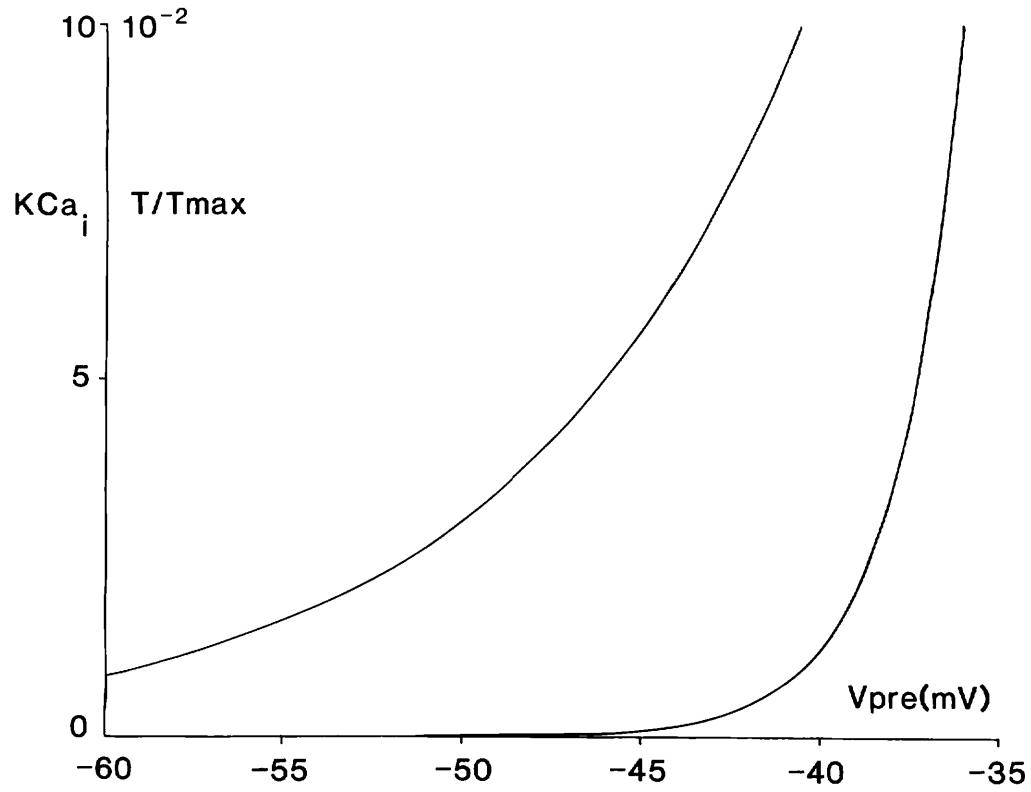



**FIG 7–8.**
Relations between the free calcium concentration $Ca_i$ at the synaptic terminal of a rod and the rod membrane potential $V_{pre}$ and between the rate of transmitter release $T$ and rod membrane potential. $K$ and $T_{max}$ are constants. The curves are based on the measured voltage dependence of the calcium current of the rod inner segment and the fourth-power relation between internal calcium concentration and transmitter release, which is found at other synapses. (From Falk G: *Prog Ret Res* 1988; 8:255–279. Used by permission.)

pled, then even if a single photon absorbed by a rod were sufficient to suppress transmitter release completely by that rod, all the other rods converging onto a postsynaptic cell would continue to release transmitter at the dark rate. However, because of the steep nonlinear relation between membrane potential and transmitter release, the sharing of the signal with rods that have not absorbed quanta will lead to a much greater total change in transmitter release by the rods in the region and thus a larger postsynaptic response. It can also be shown that by biasing the membrane potential of rods and cones in the depolarizing direction in the dark so that they operate on the steeper part of the relation for transmitter release, the system is optimized for transmission of dim light signals.

### Postsynaptic Responses

The responses to light of varying intensity of bipolar and horizontal cells in the virtually all-rod retina of the dogfish are illustrated in Figure 7–9. It will be seen that there are two kinds of responses that arise in different bipolar cells. One type depolarizes when the center of its receptive field is illuminated. It is called an on-bipolar cell because it drives ganglion cells that fire at the onset of light. The other type of bipolar cell hyperpolarizes when the center of its receptive field is illuminated. Because it drives ganglion cells that are inhibited during light and that fire at light offset, it is called an off-bipolar cell. The retina is thus organized in two parallel paths, the on-pathway, which responds to a local increase in brightness, and an off-pathway, which responds to a decrease in brightness. It is known that this parallel processing enhances the contrast sensitivity of the eye. The on- and the off-bipolar cells synapse with amacrine and ganglion cells in different parts of the inner plexiform layer. An on-bipolar cell injected with a fluorescent dye after its light responses had been recorded is shown in Figure 7–10, Plate 1. The axon terminals of on-bipolar cells extend

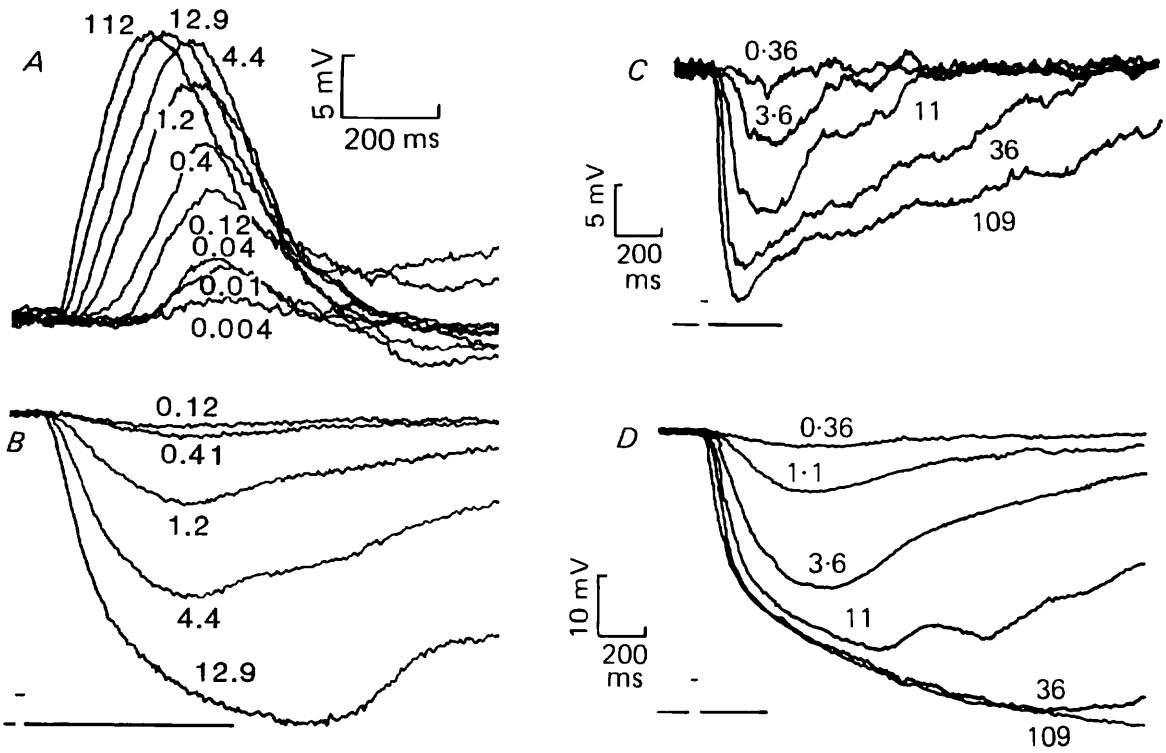



**FIG 7–9.**
Responses of rod bipolar and horizontal cells of the dogfish retina to brief flashes. **A,** an on-bipolar cell; **B,** an adjacent horizontal cell; **C,** an off-bipolar cell; and **D,** an adjacent horizontal cell. Timing of the flash is shown under each set of records. Numbers by the records show the mean number of rhodopsin molecules bleached in each rod by the flash. (From Falk G: *Prog Ret Res* 1988; 8:255–279. Used by permission.)

to the proximal part of the inner plexiform layer, while the off-bipolar cells extend only to the distal layers of the inner plexiform layer.

Except in some lower vertebrates where the responses of some horizontal cells may be color coded, the light response of horizontal cells is a graded hyperpolarization dependent on light intensity. The maximum response of horizontal cells can be very large, about 60 mV, or about twice as large as the maximum for bipolar cells. Horizontal cells have a very large receptive field because they are extensively coupled electrically. For this reason large responses can only be obtained with wide-field illumination. Focal illumination gives only very small responses because of electrical loading by the rest of the coupled horizontal cell network.

In the mammalian retina, there are two kinds of horizontal cells. Type A has a large soma with relatively thick dendrites radiating to form a more or less circular field. Type B has a smaller soma and densely branching, fine dendrites. Emerging from the type B soma is a fine long axon ending in an elaborate arborization terminating in synaptic contact with rod spherules. The dendrites of both A and B horizontal cells make synapses with cones. Rod and cone signals can be recorded from both types of horizontal cells, although the A horizontal cell makes no synapses with rods. It is likely that this mixing of rod and cone signals arises from low-resistance paths (gap junctions) between rod spherules and cone pedicles.

Because of their large-field characteristics, horizontal cells are well suited to provide the antagonistic surround that opposes the center responses of bipolar cells. Horizontal cells make synaptic contact with bipolar cells and also feed back synaptically onto cones in such a way as to oppose the light-induced center response of the bipolar cells. The antagonistic center-surround organization of the bipolar cell receptive field is illustrated in Figure 7–11. In the salamander retina illustrated and in many other species the bipolar cells have input from both rods and cones. The responses to small centered spots of light are shown for an on- and an off-bipolar cell. The response to light selectively absorbed by the cones is much faster than the rod-elicited response

**FIG 7–10.**
A radial section through the retina of the dogfish as viewed in a fluorescence microscope. In the center of the field is a rod on bipolar cell that had been injected with a nontoxic fluorescent dye while in situ in the living retina and that responded to light as in Figure 7–9,A. The rod layer is in the *upper part* of the figure, with the outer segments being strongly autofluorescent. A fine axon of the bipolar cell can be traced deep into the inner plexiform layer and terminates as a bulbous process (calibration bar, 25 μm). (From Ashmore JF, Falk G: *J Physiol* 1980; 300:115–150. Used by permission.) (See also Plate 1.)

largely because of more rapid cone phototransduction. The cone-driven response is also much less sensitive (per absorbed photon).

The response to an annulus of light, concentric with the center of the bipolar cell's receptive field, is of opposite polarity to the center response. The cell may not respond at all to large-field illumination because the center response is offset by the surround. The antagonistic surround is abolished by procedures such as applying dopamine to horizontal cells, which removes the electrical coupling of horizontal cells. Center-surround antagonism is important for edge detection and in enhancing contrast sensitivity. It is much reduced in the dark-adapted retina, possibly because transmission of the center bipolar response occurs at a much higher gain than does the surround response mediated by the horizontal cells (see the later section on synaptic gain).

### Conductance Changes Mediated by L-glutamate

The following question naturally arises: how does lowering the concentration of the transmitter L-glutamate in the synaptic region of bipolar and horizontal cells as a result of the hyperpolarization of photoreceptors lead to characteristic postsynaptic responses that may even be of opposite polarity? Glutamate, like other neurotransmitters, alters the conductance of ion channels, and these conductance changes lead to changes in the membrane potential of the postsynaptic cell. To understand how a conductance change leads to a change in voltage across the cell membrane, it is necessary to consider the electric circuit in Figure 7–12. The properties of the postsynaptic cell can be represented by two batteries in parallel, each of which has an internal conductance. The battery $E_0$ is determined in most cells by the ratio of the K ion concentration between the inside of the cell and the outside, with a value that ranges between −70 and −100 mV. The K conductance $G_0$ is constant in the sense that it does not depend directly on the concentration of the neurotransmitter. $E_r$ and the variable conductance $g_r$ represent the properties of channels that are controlled by neurotransmitters. The value of $E_r$ depends on the relative permeability of these channels to ions and the relative internal and external concentration of the ions. Glutamate-controlled channels in the retina allow the passage of cations rather nonselectively, and because of the opposite transcellular distribution of K and Na, $E_r$ has a value near 0 mV. The potential across the cell membrane in the model of Figure 7–11 depends on $E_r$, $E_0$, and the relative conductances $g_r$ and $G_0$, and this can be analyzed in an exact way. Qualitatively it can be seen that if $g_r$ becomes very large as compared with $G_0$, then the membrane potential will approach $E_r$, and if $G_0 >> g_r$, the membrane potential approaches $E_0$.

Glutamate opens ion channels of horizontal and off-bipolar cells and depolarizes them (Fig 7–13). In the dark when rods and cones release glutamate, some of the synaptic channels are opened so that the membrane potential lies about midway between $E_r$ and $E_0$. Hence these cells hyperpolarize in the

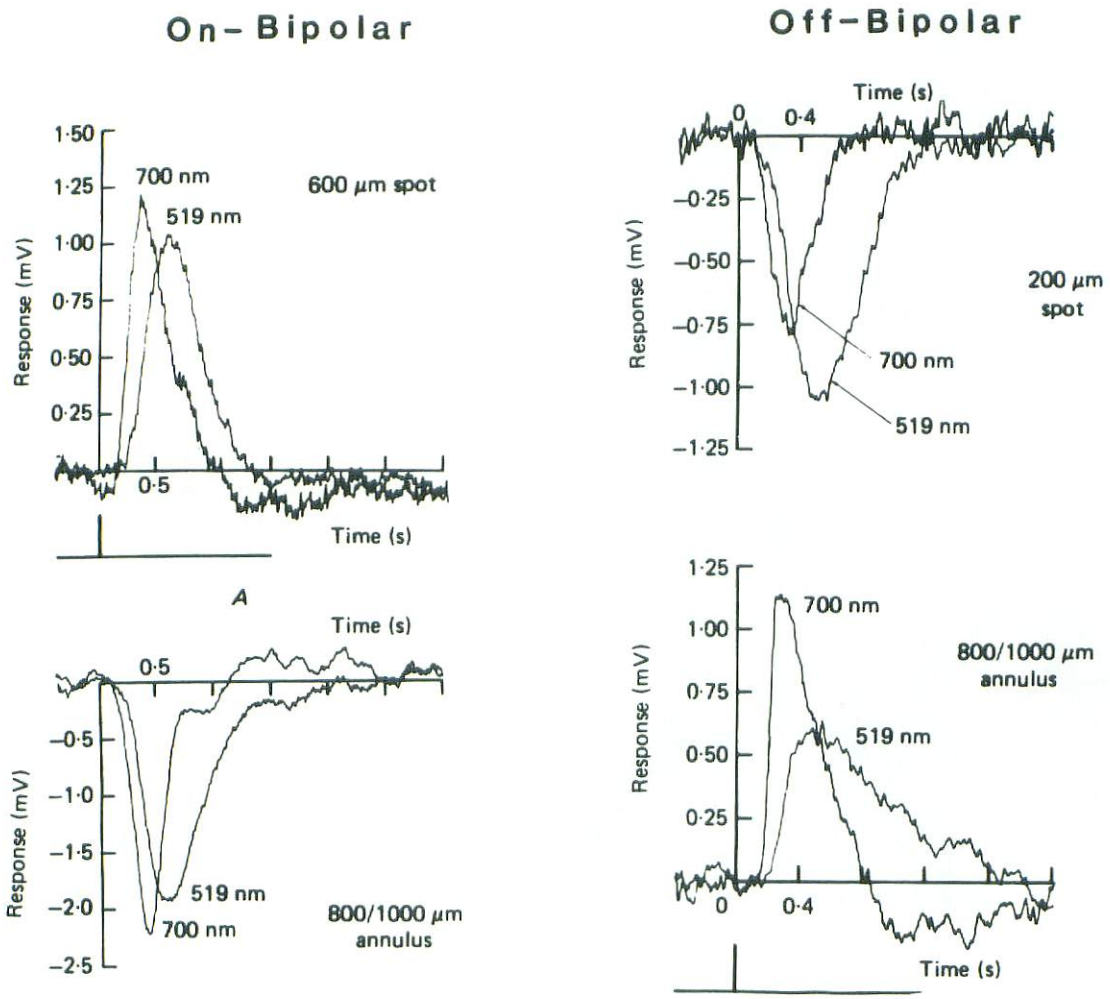



**FIG 7–11.**
Center-surround organization of the receptive field of bipolar cells. The *left-hand panel* shows the responses of an on-bipolar cell in the salamander retina to centered spots of light *(upper set)* selectively stimulating rods or cones. The *lower set* shows the responses of opposite polarity when the surround is stimulated by an annulus of light. The *right-hand panel* shows the responses of an off-bipolar cell in the same retina. (Adapted from Capovilla M, Hare WA, Owen WG: *J Physiol* 1987; 391:125–140.)

light so that the membrane potential approaches $E_0$ as a result of the light-induced decrease in glutamate release by the photoreceptors. In contrast, glutamate closes ionic channels of on-bipolar cells and drives the membrane potential of these cells closer to $E_0$ in the dark. In the light when glutamate release is reduced, these channels open and drive the membrane potential closer to $E_r$.

### Synaptic Gain and the Light Sensitivity of Postsynaptic Cells

Not only is there gain in phototransduction, but photoreceptor signals are also amplified by synaptic events. For transmission to horizontal cells and off-bipolar cells the gain is about 10, but for on-bipolar cells it is several hundred. This difference is reflected in the much greater sensitivity of the on-bipolar cells to light such that an appreciable signal is generated when a photon is absorbed by only 1 out of 100 rods making synaptic contact with the on-bipolar cell (see Fig 7–9). It is most likely that this large gain in synaptic transmission to on-bipolar cells accounts for the very large difference in the light required to elicit an a-wave as compared with a b-wave. When on-bipolar cells depolarize in response to light, K ions leave the cell driven by the electrochemical gradient. Müller cells, sensing the

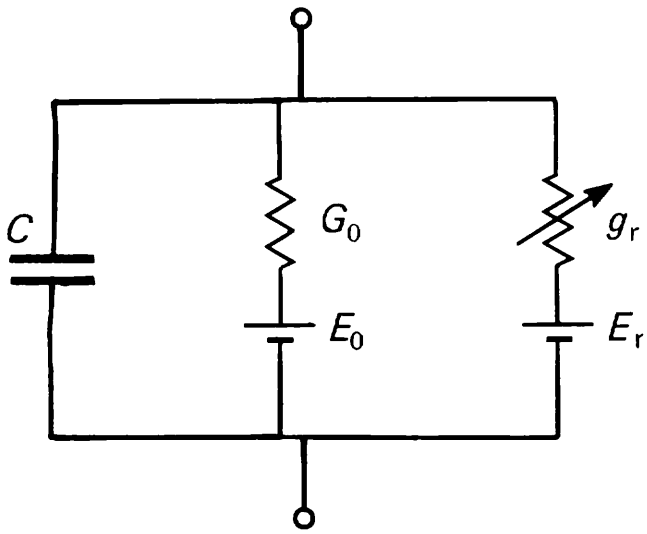



**FIG 7–12.**
Equivalent circuit of a postsynaptic cell. The *right-hand* branch shows the transmitter-controlled pathway. The *middle* pathway shows the properties of a nonsynaptic membrane. See the text. (From Falk G: *Prog Ret Res* 1988; 8:255–279. Used by permission.)

raised K concentration in the extracellular space, generate extracellular current flow, which is recorded as the b-wave. It is possible to selectively block the response of on-bipolar cells, and this results in loss of the b-wave.

Synaptic gain depends on how much of a signal is required to alter presynaptic transmitter release and on the nature of the conductance change produced by the transmitter (glutamate). Since rods and cones are somewhat depolarized in the dark, thus operating on the steep part of the relation between membrane potential and transmitter release (see Fig 7–8), a small change in potential, produced by dim light, will result in a large change in the amount of transmitter released. The higher gain at the synapse with on-bipolar cells may result from the fact that in the dark most of the synaptic channels controlled by the transmitter are closed. The consequence is that the voltage change produced in the bipolar cell by a given change in membrane conductance will be larger than would be the case if there were many channels open in the dark. The presence of open channels would otherwise shunt out the voltage produced by a given change in transmitter concentration.

In addition to amplifying the photoreceptor signals, the synapse with rod bipolar cells also behaves as a high-pass filter that improves temporal resolution in the rod pathway.

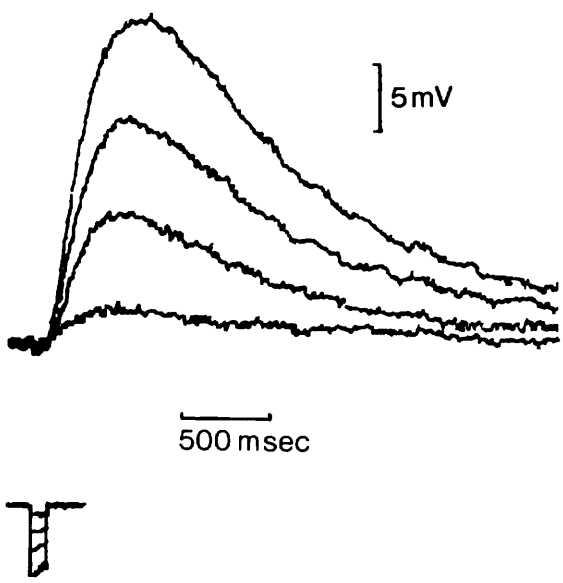



**FIG 7–13.**
Depolarization of a rod horizontal cell by pulses of glutamate.

## SIGNALS IN THE INNER RETINA

It is only in the inner retina that action potentials are generated. Figure 7–14 shows the spike trains from a single on-center ganglion cell recorded from the cat retina. As is typical for mammalian ganglion cells, there is a maintained discharge in the dark, probably resulting from the spontaneous isomerization of a few of the very many rhodopsin molecules in the rods within the receptive field of the ganglion cell (although rhodopsin is very stable). As shown in Figure 7–14,A, the ganglion cell firing rate promptly increases when a small spot of light is centered within its receptive field. An annulus of light (B) suppresses the spontaneous discharge, and when the annulus is removed, there is a transient increase in the firing rate. Uniform illumination (C) has a lesser effect on the firing rate. When the observations are repeated with off-ganglion cells, the firing pattern is reversed. A small central spot of light inhibits firing, but at light offset, there is a brisk increase in firing. A bright annulus increases firing, which is then inhibited at the offset of the light. Uniform illumination again has much less effect on firing. Within the categories of on- and off-ganglion cells, there is a further subdivision of types, those that respond with a sustained change of firing with light and those that respond only transiently. Figure

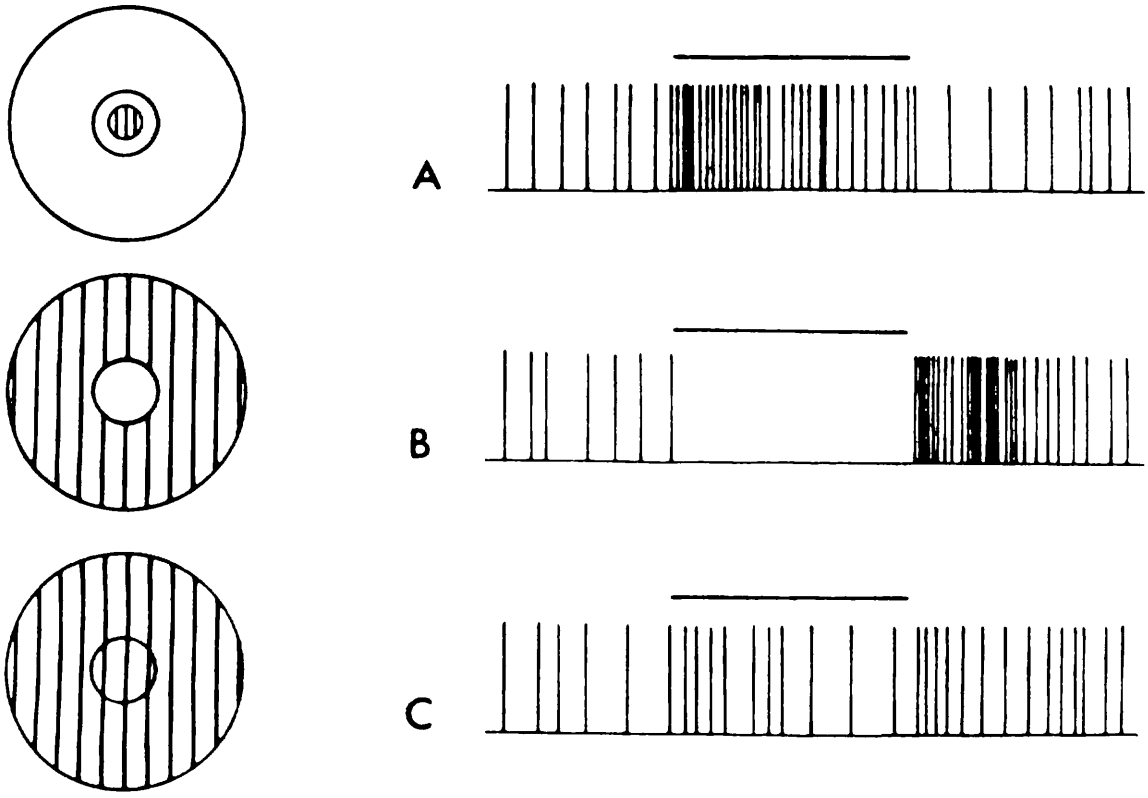



**FIG 7–14.**
Firing of an on-center ganglion cell in the retina of an anesthetized cat. The *upper* record shows the response to a centered spot of light, the *middle* record shows the response to an annulus, and the *lowest* record shows the response to large-field stimulation. (From Kuffler SW: *Invest Ophthalmol* 1973; 12:794–813. Used by permission.)

7–15 summarizes the properties of the on- and off-ganglion cells in the cat retina. There is another class of ganglion cells, the directionally sensitive cells, that respond only to light moving across their receptive field in a preferred direction and that do not respond to stationary light. Other cells act as simple motion detectors. Many ganglion cells are color coded, some having color opponent properties. Although the basic organization of the retina into two parallel pathways, the on- and the off-pathway, each characterized by a center-surround antagonism, is set at the outer plexiform layer, there is a large measure of further processing at the level of the ganglion and amacrine cells.

In general, the synaptic contacts between on-ganglion cells and on-bipolar and amacrine cells are made in the proximal part of the inner plexiform layer (in lamina b), while the synaptic interactions for the off-pathway occur in the distal part of the inner plexiform layer (lamina a). Most amacrine cells fire action potentials at either the onset or offset of light. There are also interplexiform cells that have their cell bodies among the amacrine cells and that send processes both to the inner plexiform layer and to the outer plexiform layer where they make synaptic contact with bipolar or horizontal cells. Thus a considerable complexity is introduced at the level of the inner plexiform layer, especially when it is considered that amacrine cells may make reciprocal synapses (feedback synapses) with bipolar cells and with each other.

For the cone system the simplest pathway is the direct pathway, cone → on-bipolar → on-ganglion cell or cone → off-bipolar → off-ganglion cell. For the rod pathway in mammals, the situation is more complicated. There appears to be only one kind of rod bipolar cell, yet both on- and off-ganglion cells are known to have rod input. Moreover, rod bipolar cells do not make direct synaptic contact with ganglion cells but synapse with a particular type of amacrine cell, the AII amacrine in lamina b. The AII amacrine cell is a narrow-field, bistratified cell with processes both in laminae a and b. Low-resistance electrical junctions exist between the AII amacrine and the on-bipolar cell of the cone pathway so that the signal conveyed to the AII amacrine cell from the

*Properties of Sustained and Transient 'On' and 'Off' Ganglion Cells*

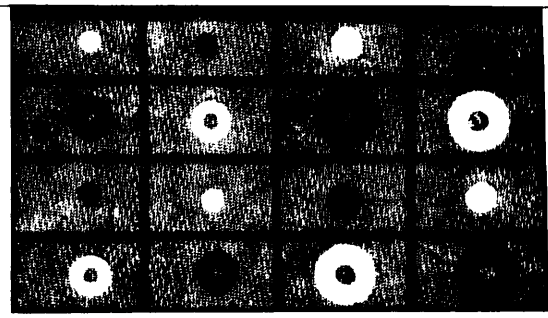
| | | Sustained (Tonic, X) | | Transient (Phasic, Y) | |
|---|---|---|---|---|---|
| | | On | Off | On | Off |
| Excitatory Visual Trigger | Centre |  | | | |
| | Surround | | | | |
| Inhibitory Visual Trigger | Centre | | | | |
| | Surround | | | | |
| Synapse Position in IPL | | subl. b | subl. a | subl. b | subl. a |
| Optimal Spot | | Small | | Large | |
| Firing to Stationary Optimal Spot | | Sustained | | Transient | |
| Receptive Field Size | | Small | | Large | |
| Spatial Summation | | Linear | | Non-Linear | |
| Peripheral Activation | | Ineffective | | Effective | |
| Relative Common Location | | Central retina | | Peripheral retina | |
| Morphology | | Small soma, dendrite, & axon ($\beta$ type) | | Large soma, dendrite & axon ($\alpha$ type) | |
| Axonal Conduction | | Slow | | Fast | |
| Destination | | LGN | | LGN<br>Superior colliculus | |
| Possible Functional Role | | Analysis of spatial detail | | Detection of novel or of movement in visual field | |

**FIG 7–15.**
Summary of the properties of ganglion cells of the cat's retina. (From Ikeda H: *Prog Ret Res* 1985; 4:1–32. Used by permission.)

rod bipolar cell is in turn fed directly to the cone on-bipolar cell and then to the on-ganglion cell. The AII amacrine cell also synapses with the cone off-bipolar cell, which is inhibited during light (scotopic) onset and which fires at light offset when the inhibition is removed. The AII amacrine cell accumulates glycine, an inhibitory transmitter. (See Chapter 5.)

## RETINAL NEUROTRANSMITTERS

As discussed at length in the section "Conductance Changes Mediated by L-Glutamate," glutamate is the transmitter released by rods and cones. Glutamate or a closely related substance is also released by some bipolar cells and probably some amacrine cells. Glutamate acts on ganglion cells to increase the visually evoked response of both on- and off-sustained ganglion cells but not of transient cells. On the other hand, acetylcholine enhances the firing of on- and off-transient ganglion cells. Acetylcholine is released by on- and off-amacrine cells of characteristic morphology, the starburst amacrine cells.

The inhibitory transmitters are $\gamma$-aminobutyric acid (GABA) and glycine. They open Cl channels and thus reduce the effect of excitatory transmitters that act on cationic channels. GABA is accumulated by horizontal cells in some species and mediates surround inhibition of bipolar cells. On-center ganglion cells receive inhibitory input from GABAergic amacrine cells, while off-center ganglion cells receive inhibitory input from glycinergic amacrine cells.

Dopamine is accumulated by some amacrine and interplexiform cells. Dopaminergic amacrine cells

make extensive connections with AII amacrine cells. Dopamine reduces the light responses of rod bipolar cells and the b-wave. Dopamine increases the resistance of gap junctions between horizontal cells so that the effective receptive field of horizontal cells is reduced and, consequently, the extent of surround antagonism of bipolar cell responses. Many of the effects of dopamine are exerted at sites that are distant from the initial site of release. The dispersion of pigment granules in the light is mediated by dopamine released more proximally in the retina.

Many other neurotransmitters found in the central nervous system, serotonin, adrenaline, substance P, and other peptides, are also found in the retina, but their role in visual processing within the retina is currently under investigation.

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