Retina I: Photoreceptors and Functional Organization

The Retina's Role in Vision

The retina detects light and tells the brain about aspects of light that are related to objects in the world

By virtue of its photoreceptors, the retina detects light in the retinal image and transforms the light energy into a communicable form of chemical energy; this process is **phototransduction**, and all retinas do it—even the simplest. The photoreceptors are laid out in a large array (about 100 million of them in the human eye), much like the molecules of silver halides or dyes in photographic films, and as a group they transform the pattern of light and shade in the retinal image into a corresponding pattern of gradations in neural activity. Also, from the different photopigments they contain, the photoreceptors begin the process of giving color to a world that has none (the sensation of color, like vision itself, is a product of the brain).

The signals from 100 million photoreceptors constitute a massive amount of information to be processed if the retinal image is to be reconstructed to form a mental image of the world; numbers in the gigabyte range are not unreasonable. Processing this information by taking into account each photoreceptor's signal would be a slow process—very much slower than the important, dynamic changes in the retinal image as we look around or as objects move. What the retina is doing, therefore, is editing the information, selecting salient features of the retinal image and packaging them in a form that can be quickly and economically conveyed to the brain by the million or so retinal ganglion cell axons in the optic nerve.

But what is a "salient feature" and how are salient features extracted from the rest? Salience, as far as the retina is concerned, appears to be related to changes

It is light above all, according to the direction from which it comes and along which our eyes follow it, it is light that shifts and fixes the undulations of the sea.

Marcel Proust, Within a Budding Grove

The eyeball ... consists primarily and essentially of a sheet of nervous matter visually endowed—that is, capable of being so affected by light, that, when duly connected with the sensorium, what we call sight or perception of light, is the result.

■ William Bowman, Lectures on the Eye

in light intensity—in space, time, or both. An abrupt spatial change in intensity, for example, is an edge or border, a temporal change is a sudden brightening or dimming in one part of the image, and a spatiotemporal change is movement of part of the image. To put it another way, the most informative aspects of the retinal image are the variations in light intensity. By signaling variations and ignoring those parts of the retinal image where light intensity is constant over space and time, the retina provides the brain with important information about the retinal image and yet does not overload it with repetitive, redundant facts.

The question of how salient features are recognized, extracted, and signaled is a question about how the retina works. In other words, how are photoreceptor signals about the *amount* of light striking them transformed to signals about *changes* in light over space and time? The general answer is that the retina doesn't make decisions about which aspects of the retinal image are important and which are not; recognition is not part of its job. Instead, its circuitry automatically gives preference to spatiotemporal changes in the retinal image by comparing signals from different photoreceptors (the spatial dimension), and signals occurring within certain periods of time (the temporal dimension). The flow of signals through the circuitry depends on these comparisons revealing differences—differences between the amounts of light falling on different photoreceptors or differences between the timing of signals arriving at a particular point in the circuit.

Catching Photons: Photoreceptors and Their Environment

Each photoreceptor contains one of four photopigments, each of which differs in its spectral absorption

The human retina has four photopigments, one rod photopigment and three cone photopigments, all of similar construction. Each photopigment has two elements: a large protein portion, which is called an **opsin**, and a small attached molecule called *retinal*, which is an aldehyde of vitamin A.* The *retinal* is the portion of the photopigment first affected by light absorption and it is considered to be the active part, or **chromophore**, of the opsin–*retinal* complex. In the human photopigments, only opsin differs from one pigment to another; the same *retinal* is used for all.

The differences among photopigment opsins have an important consequence—namely, differences among the photopigments in the wavelengths of light that they preferentially absorb. Thus a photopigment's **absorbance spectrum**, which is the amount of light absorbed as a function of wavelength, is its signature, distinguishing it from other photopigments.

In principle, an absorbance spectrum is easy to determine (Figure 13.10). It is a matter of having a solution of photopigment, passing light of a given wavelength through the solution, and measuring the amount of light incident on the solution (I_{inc}) and the light exiting the solution (the light transmitted, I_{trans}); the difference, $I_{inc} - I_{trans}$, is the amount of light absorbed (I_{abs}). The **optical density** (D) and the **absorbance** (A) are both described by the logarithm of the ratio of incident to transmitted light, that is, log (I_{inc}/I_{trans}).

But as indicated in Figure 13.10, the amount of light absorbed depends not only on the pigment's intrinsic spectral absorption properties, but also on the concentration of the pigment in solution and the length of the path that the light beam must traverse through the pigment solution. These features are expressed formally in terms of absorbance as

$$A = e_{\lambda} \cdot c \cdot d$$

where *c* is concentration, *l* is path length, and e_{λ} is the spectral **extinction coefficient**. The quantity of interest is e_{λ} because it is a property of the photopigment molecule, but it may be difficult to isolate if concentration or path length is not known. The strategy is to use the ratio $A_{\lambda}/A_{\lambda_{max}}$. In this ratio, which is the *relative* absorbance, concentration and path length cancel out. The relative absorbance is unity at the peak absorption wavelength and less than 1 at other wavelengths. Thus a plot of relative absorbance as a function of wavelength provides a unique signature for the photopigment molecule, showing how well the molecule absorbs light at different wavelengths.

Absorbance spectra for the human photopigments are shown in Figure 13.11. Each pigment absorbs maximally at a different wavelength (its λ_{max}) that can be used to characterize the pigment. The rod photopigment, rhodopsin, has its peak absorption near a wavelength of 496 nm, which would be seen as blue-green light. The three cone pigments have absorption maxima in the blue part of the spectrum (419 nm), green (531 nm), and what is usually called red when referring to the photopigment but is really yellow (558 nm).

These absorbance spectra were obtained from individual photoreceptors, not pigments in solution.[†] The method used, called microspectrophotometry, is dif-

†William A. H. Rushton (1901–1980) used his method of "retinal densitometry" to provide believable spectra for the human cone pigments in vivo, and he took the opportunity to name

^{*}The old gambit that mothers used to get children to eat carrots—"carrots are good for your eyes"—contains an element of truth. Carrots are rich in vitamin A, and vitamin A deficiencies do affect vision, since the *retinal* in the photopigments is derived from vitamin A stored in the liver. Normal diets have plenty of vitamin A from sources other than carrots.



Relative absorbance
$$\left(\frac{A_{\lambda}}{A_{\lambda_{\max}}}\right) = e_{\lambda}/e_{\lambda_{\max}}$$

Measuring Absorbance

The amount of light absorbed by a pigment in solution depends not only on the characteristics of the pigment, but also on its concentration and the distance that light travels through the solution. When neither concentration nor path length is known, the relative absorbance (or relative density) is used to factor out both concentration and path length. See text for additional explanation.

ficult because the amount of photopigment in a photoreceptor is limited. The light passing through the photoreceptor bleaches some of the pigment, which means that the concentration of the pigment changes during the course of measurements at different wavelengths. Moreover, bleached photopigment accumulates that may have absorbance characteristics different from those of the parent photopigment. Thus the raw data must be corrected to produce the curves in Figure 13.11. But once this is done, absorbance measurements from different photoreceptors always coincide with one of the four curves, which is evidence that each photoreceptor contains one, and only one, photopigment. Any mixture of photopigments would necessarily produce different absorbance spectra.

them: erythrolabe, chlorolabe, and cyanolabe (red-, green-, and blue-seizing). It is unfortunate that his names have not stuck, because the common alternatives (L, M, S; or long-, medium-, and short-wavelength-sensitive) are mundane and no more precise. Rushton's magnificent lecture *cum* performance entitled "Three Cambridge Students of Light and Colour" (Isaac Newton, Thomas Young, and James Clerk Maxwell) implied that a fourth name—his—should be added to this grand tradition.

I once questioned some of Rushton's early data on the blue-cone pigment, which prompted—in his inimitable style—a vigorous and thorough exposure of my ignorance about color vision. I was right to be skeptical, as it turned out, but I have avoided the subject ever since.

Figure 13.11 Absorbance Spectra for Human Visual Pigments

These curves are based on measurements from individual photoreceptors. Since each photoreceptor contains only one photopigment, these absorbance spectra are signatures for the photoreceptors. There is one kind of rod photoreceptor and three kinds of cone photoreceptor (red, green, and blue). (Replotted from Dartnall, Bowmaker, and Mollon 1983.)



A photoreceptor is characterized by the single photopigment it contains. All rods contain rhodopsin, so there is just one type of rod photoreceptor. Cones may have any one of the three other pigments, so there are three types of cone photoreceptors. The cones are familiarly referred to as red, green, or blue cones, on the basis of the part of the spectrum that their photopigment absorbs most strongly. (A noncommittal, less colorful designation is long-, medium-, and short-wavelength-sensitive cones, or L, M, and S cones.) Since normal trichromatic color vision utilizes all three cone photopigments, it has long been suspected that individuals with profound color deficiencies (dichromats) lack one of the photopigments. This is true, and since each cone contains only one pigment, the basis of the defect is the lack of the gene that normally allows a subset of cones to synthesize one of the pigments. (The total number of cones is not affected. What should have been red cones, for example, end up being green cones instead.)

Although the terminology "red cone" or "green cone" makes them sound very different, their absorbance spectra are not far apart, and the spectral regions in which they absorb overlap considerably. But the small difference is significant. Red cones absorb long-wavelength light to which the green cones are almost totally insensitive, and green cones absorb at shorter wavelengths that are not absorbed by the red cones. With the exception of the wavelength at which the two absorbance spectra intersect, no wavelength will have equal absorption by both photopigments. In other words, a single wavelength will have different effects, and this difference makes wavelength discrimination possible.

Color vision requires more than one photopigment

Even if we know that a cone contains a particular photopigment, the photoreceptor's signal says nothing about the wavelength of absorbed photons. Photoreceptors signal the amount of light they absorb—how many photons they catch—and as long as the wavelength is within their absorbance spectrum, only the number of photons caught is relevant. To put it another way, wavelength discrimination would not be possible with just one pigment.

The problem is illustrated in Figure 13.12. With a single photopigment, there are many possible pairs of wavelengths that produce the same absorption and the same signal from the photoreceptor. In fact, any two wavelengths can be confused because it is impossible to know if the given magnitude of a photoreceptor signal resulted from absorption of a large fraction of photons from a weak light source to which the pigment was quite sensitive or absorption of a small fraction



Figure 13.12 Wavelength Discrimination Requires More Than One Photopigment

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Photoreceptor signals depend on the number of photons absorbed, not on their wavelength. Thus signals based on a single photopigment are ambiguous; lights of different wavelength can produce the same signal strength. When two photopigments are present, it is impossible to find two wavelengths that produce identical signals from both photoreceptors.

from a strong light source to which the pigment was relatively insensitive. Thus, with just one pigment, it is impossible to say if a change in photoreceptor signal strength is the result of a change in the wavelength of light, a change in the intensity of light with no change in wavelength, or a change in both.

With more than one pigment, however, wavelength discrimination is possible. The two wavelengths that are absorbed equally by a single photopigment are absorbed differently by a second one. If the signals from the photoreceptors containing these two pigments can be compared, there is a basis for saying that the two lights have different wavelengths. Also, the trade-off between wavelength and intensity that can fool a single photopigment does not work when two pigments are present.

In general, wavelength discrimination is possible with two different photopigments and is even better with more than two. Most mammals get by with blue and green cone systems, most primates also have the third (red) system, and some birds and insects have a fourth cone system, in the ultraviolet region of the spectrum. Our system of three cone pigments allows us to discriminate wavelengths of light near the middle of the spectrum that differ by only 2 or 3 nm.

At light levels where the cones are operating, the rods are very insensitive, and the behavioral spectral sensitivity curve for the eye is the curve designated "cones" in Figure 13.13. This is the photopic (light-adapted) spectral sensitivity function for the human visual system, produced by the combination of information from all three cone systems. The curve peaks near 555 nm, meaning that the visual system is most sensitive to yellow-green light under light-adapted, conedominated conditions.

When the eye is adapted to dark, however, the spectral sensitivity function shifts along the wavelength axis so that its peak is now in the blue-green area near 500 nm. This scotopic (dark-adapted) spectral sensitivity function is almost identical to the absorbance spectrum for rhodopsin (see Figure 13.11), which is one of the pieces of evidence that the curve is determined solely by the rods. And since the rods are detecting dim lights under dark-adapted conditions, there can be no wavelength discrimination, because only one photopigment is in use. Rods and their rhodopsin have nothing to do with our color vision.

The change from maximum sensitivity in the yellow-green under lightadapted conditions to maximum sensitivity in the blue-green under darkadapted conditions is the **Purkinje shift**, named for Jan Purkinje (1787–1869), who also described the entoptic phenomenon called the Purkinje tree (see

Figure 13.13 Luminous Efficiency Functions for the Standard Human Observer

Relative luminous efficiency is closely related to relative sensitivity. The standard observer is most sensitive to light of 507 nm wavelength under scotopic (dark-adapted) conditions and to light with 555 nm wavelength under photopic (light-adapted) conditons. (Plotted from data in Wyszecki and Stiles 1967.)



Chapter 16). The Purkinje shift and subsequent observations by such anatomists as Max Schultze and Heinrich Müller (see Vignette 14.1) on the dominance of rods in nocturnal species and cones in diurnal species led to what was called the duplicity theory of vision and the description of mixed rod-andcone retinas like our own as duplex retinas.

The photopigments are stacked in layers within the outer segments of the photoreceptor

The outer segments of photoreceptors have a distinctive structure (Figure 13.14). They contain a series of **lamellae**, or **discs**, each of which is a flattened membranous sac, rather like a tiny deflated red blood cell. Discs in the outer segments of rods are not continuous with the outer segment membrane (except at the base of the outer segment, where they form; see Figure 13.33*a*); they are held in place by cytoskeletal attachment proteins (peripherins) around the rims of the discs. The outer segment membrane, and the cone discs are actually deep folds in the membrane.

Although the rod and cone outer segments illustrated here differ in shape, with the cone outer segment tapering from base to tip, this is not always the case. The outer segments of foveal cones are as cylindrical as rods. The difference in the disc structure between rods and cones is always present, however.

The photopigment molecules are embedded in the disc membranes, as shown for rhodopsin in Figure 13.15; the situation is similar for the cone pigments. Each of the two paired disc membranes is a bilayer of phospholipids into which the photopigment molecules are inserted; seven helical portions of the opsin traverse the bilipid membrane, enclosing the *retinal* chromophore within a kind of cage. Both sides of the discs in the outer segments of rods are studded with photopigment molecules. Since there are up to 1000 discs in rod outer segments and each is covered with photopigment molecules, the total number of molecules in a rod will be very large; the number is finite, however, and very bright light can bleach a substantial fraction of the resident photopigment molecules.*

*How large *is* the number? Each side of an outer segment disc on a rod has an area of about $3 \mu m^2$, giving a total disc surface area of around $6 \mu m^2$. Estimates of the density of rhodopsin molecules are on the order of $25,000/\mu m^2$, which means that each disc would have around 150,000 rhodopsin molecules. If there are 1000 discs in the outer segment of the rod, the total complement of rhodopsin molecules per rod will be around 150 million. By comparison, bright ambient sunlight produces a retinal illumination of almost a million photons per rod per second. If rhodopsin did not regenerate, it would be bleached away in about 2.5 minutes.



Figure 13.14 Structure of the Outer Segments of Rods and Cones

The outer segments of both rods and cones are filled with bilayered discs in which the photopigment molecules are embedded. Cone discs are deep folds of the membrane that remain continuous with the outer segment membrane. Rod discs are not continuous with the outer segment membrane, except at the base of the outer segment, where the discs form. Neither the membranes nor the discs are drawn to scale; they are thinner in real life than they appear here.

Light absorption produces a structural change in the photopigments

In its dark state, the *retinal* in a photopigment has a particular configuration, one of several **stereoisomeric** forms it can assume, in which it clings to a particular site on the opsin. As Figure 13.15 shows, the long opsin molecule loops in and out of the disc membrane, and the *retinal* is covalently bound to a lysine residue on transmembrane domain 7 within the disc membrane; one can think of the opsin as forming a snug, form-fitting pocket in which the *retinal* resides. Absorption of light, which is a transfer of energy from photons to the photopigment, raises the energy level of the *retinal* sufficiently that one of its double bonds breaks and the *retinal* changes its shape to another stable stereoisomer (Figure 13.16). Technically, the change is from the 11-cis isomer to the all-trans isomer of *retinal*. This change, which is the only direct effect of light on the eye and is the initiating step for vision, exposes a charged site on the opsin, which prompts a restructuring of the protein itself into its activated form. The overall change of a rhodopsin molecule from its inactivated form before absorbing a photon to its activated form is designated by the transaction $R \rightarrow R^*$.

The activated rhodopsin (R*) persists for a brief period of time, during which it can interact with other molecules on the disc membrane. Additional changes in configuration end with the separation of the all-trans *retinal* from the opsin and its conversion to all-trans retin*ol*. The photopigment is then said to be **bleached**.

Rhodopsin in the Disc Membrane

An enlarged view of a small piece of disc membrane shows the photopigment opsin with seven helices running through the layers of phospholipids that constitute the membrane. The helices form a kind of cage for the photosensitive *retinal*. The C terminus of the opsin and the other loops of amino acids on the outer surface of the disc membrane are the sites at which opsin interacts with other molecules in the phototransduction cycle. (After Stryer 1986.)



Bleached photopigment cannot be affected by light; thus the more photopigment that is bleached, the less sensitive the eye will become. Restoration of sensitivity requires that the 11-cis *retinal* be reconstructed and reattached to the opsin; this is an enzyme-driven process called **regeneration**, which we will consider in more detail shortly. One of the differences among photopigments is their regeneration rate: Cone pigments regenerate faster than rhodopsin. Under conditions of steady illumination, an equilibrium is established such that the rates of bleaching and regeneration are equal and the amount of bleached photopigment will remain constant. In reaching this equilibrium, however, the rods, with their slower regeneration rate, will have had more pigment bleached than the cones before the equilibrium is established; therefore, for any given level of steady illumination, the rod pigment will always be more bleached than the cone pigments.

Structural change in the photopigment activates an intracellular second-messenger system using cGMP as the messenger

Phototransduction begins with the transfer of energy from photons to photopigments and ends with a change in glutamate release by the photoreceptor terminal to signal the photon absorption. The initial part of the process is a cascade of intracellular events resulting from activation of a photopigment molecule, as summarized in Figure 13.17.

Photoreceptor disc membranes contain a **G protein**, a multimeric (multipart) assembly of subunits that bind opsin on one side and the nucleotides **GDP** (guanosine diphosphate) or **GTP** (guanosine triphosphate) on another. The G protein in rods is called transducin and designated G_T ; in its normal inactivated state, G_T binds a molecule of GDP to one of its subunits. When activated rhodopsin (R*) encounters a molecule of G_T , GDP is exchanged for GTP, and the GTP-carrying α subunit of G_T separates from the rest of the molecule; it is now activated and designated G^* , which is the subunit with GTP. Thus, $R^* + G_T \rightarrow G^*$.

Like activated rhodopsin, the activated G protein has a brief lifetime in which it can interact with other molecules. The molecule it chooses is the enzyme



Figure 13.16 Photon Absorption Changes the Form of Retinal

Retinal is an aldehyde of vitamin A that has several stable configurations. Before photon absorption, it is bound to opsin in its 11-cis configuration. Photon absorption breaks the double bond between carbons 11 and 12, which permits rotation to the all-trans configuration. This is the initiating step in vision. (After Stryer 1987.)

cGMP phosphodiesterase (**PDE**). PDE is a multimer with two sites at which it can interact with G*. The interaction activates PDE: $G^* + PDE \rightarrow PDE^*$. In this case, activation exposes a site on the PDE molecule that allows it to exert its catalytic effect on its target molecule, which is **cGMP** (3',5'-cyclic guanosine monophosphate).

To this point, all events have taken place on the disc membrane. Rhodopsin, G_T , and PDE all reside there, and their activated forms act there. But the ultimate effect of this chain of events is to alter the status of ion channels in the outer segment membrane, which is some distance away. What is required is a molecule residing in the cytoplasm that can act as a go-between from the discs to the outer segment membrane channels. The messenger is cGMP.

When a molecule of cGMP diffuses near PDE* on a disc, the exposed catalytic site on PDE* will effect a change from cGMP to 5'-GMP (5'-guanosine monophosphate). The important event here is not the production of 5'-GMP; this molecule has no direct role in phototransduction. What matters is eliminating a molecule of cGMP from the intracellular pool of cGMP. The status of the ion channels in the outer segment membrane depends on the concentration of cGMP within the cell; eliminating cGMP molecules from the pool reduces the intracellular cGMP concentration. Lowering the cGMP concentration closes membrane channels, thereby altering the flow of current into the photoreceptor.

A G protein–mediated second-messenger system is not unique to photoreceptors. Many G proteins can interact with various sorts of receptors (the photopigments are the receptors in the rods and cones) and then activate other intracellular messengers, such as cAMP, cGMP, or Ca²⁺. G protein–mediated secretory systems were considered, in less detail, for the lacrimal gland (see Chapter 7) and for aqueous production by ciliary epithelial cells (see Chapter 11). The neurotransmitters that normally interact directly with ion channels in cell

The Phototransduction Cascade

Events in the photoreceptor disc membrane initiated by photon absorption are shown here as interactions between molecules, which are represented by icons. There are four main steps in the cascade: (1) Rhodopsin is activated by photon absorption, (2) activated rhodopsin activates a G protein (G \rightarrow G*), (3) activated G protein activates cGMP phosphodiesterase (PDE), and (4) activated cGMP phosphodiesterase converts cGMP to GMP. The reduction in cGMP concentration will cause outer segment membrane channels to close. (After Rodieck 1998.)



membranes can also interact with other receptors that trigger second messengers via G proteins. Acetylcholine, for example, acts directly at the nicotinic synapses es and indirectly, via a G protein–linked receptor, at muscarinic synapses (see Chapter 5); a G protein–linked glutamate receptor at transmission between cones and bipolar cells will be considered later in the chapter. Also, most blood-circulated hormones and the neuromodulators work through G protein linkages, as do transduction mechanisms in the olfactory and gustatory systems.

A decrease in cGMP concentration closes cation channels, decreases the photocurrent, and hyperpolarizes the photoreceptor

Most of the ionic current flowing into the outer segment consists of Na⁺ ions, as well as some Ca²⁺ ions, that pass through channels in the membrane. These channels, one of which is illustrated schematically in Figure 13.18, are not voltage sensitive. Instead, they are ligand-gated channels whose status—open or closed—is controlled by the binding of the ligand, which in this case is cGMP. The channels are open when they bind cGMP molecules and closed when they do not. The availability of cGMP for binding depends on the concentration; as the concentration increases, the number of molecules available for binding increases. Thus the



Figure 13.18 cGMP-Gated Cation Channels in the outer segment Membrane

When the cGMP concentration is high, which is the dark condition for the photoreceptor, cGMP is available to bind to membrane channels. Channels spend more time in the open state when cGMP is bound, thereby permitting Na^+ and Ca^{2+} to flow into the cell. The phototransduction cascade initiated by photon absorption reduces the cGMP concentration, which closes the cGMP-gated channels.

number of open channels will increase as the concentration of cGMP rises, and channels will close as the cGMP concentration falls.

Since the absorption of light *reduces* the amount of cGMP, channels will close and the number of Na⁺ ions flowing into the cell will decrease. But to see the effect of this decrease in Na⁺ flow on the cell's membrane potential, we need to consider other ion channels and pumps in the photoreceptor membrane (Figure 13.19).

When the photoreceptor is in the dark, both Na⁺ and K⁺ are flowing in and out of the photoreceptor. Na⁺ enters the outer segment membrane through open cGMP-gated channels and through a cation exchanger (Na⁺ is swapped for K⁺ and Ca²⁺). It is pumped back out of the cell by metabolically driven Na⁺–K⁺ pumps in the inner segment membrane. K⁺ enters the inner segment through the Na⁺–K⁺ pump and flows out through selective K⁺ channels in the inner segment membrane and the cation exchanger in the outer segment membrane. The result of these ionic currents is a cell membrane potential of about –40 mV (inside negative); this potential is a compromise between the tendency of the Na⁺ inflow to make the inside more positive (depolarized) and the tendency of the K⁺ outflow to make it more negative (hyperpolarized).

Photon absorption upsets this equilibrium. As Na^+ channels in the outer segment membrane close following light absorption, the inward Na^+ current decreases and the K⁺ current becomes more dominant; light absorption has no effect on the cation exchanger or on the Na^+ –K⁺ pump. Positive charge is now

Figure 13.19 The Photocurrent Is Reduced by Light Absorption

Most of the cuurent flowing into the outer segment in the dark is a Na⁺ current through the cGMP-gated channels. Na⁺ outflow across the inner segment membrane is linked to K⁺ inflow by membrane pumps. Channel closure following light absorption reduces the inward Na⁺ current with little effect on other sources of inflow and outflow. This reduction in positive charge within the outer segment makes the transmembrane potential more negative; that is, the receptor hyperpolarizes.



moving out of the cell faster than it is flowing in; the interior of the cell thus becomes more negative relative to the exterior. This negative swing of the potential across the cell membrane is hyperpolarization.

Although only the current flowing into the outer segment through the cGMPgated channels is altered by photon absorption, its effects are felt thoughout the cell. The decreased Na⁺ current into the outer segment reduces the amount of resident positive charge, and the outer segment hyperpolarizes. But for the photoreceptor to alter its rate of glutamate release from its terminal, which is rather distant, the change in the outer segment membrane potential must also produce a change in the membrane potential at the terminal. This change occurs because ions can move with considerable freedom through the interior of the cell, and they will do so whenever there is an imbalance of electric charge. In this case, the increased negativity in the outer segment tends to draw positively charged ions from other parts of the cell until the electric potential is the same everywhere. Some of these positive ions will be drawn from the terminal of the photoreceptor, and their departure makes the terminal less positive, hence more negative. This hyperpolarization of the terminal reduces the rate of glutamate release.

Absorption of one photon can produce a detectable rod signal

A superb psychophysical study done more than 50 years ago by Hecht, Shlaer, and Pirenne (1942) concluded that the absorption of one photon is sufficient to excite a rod, but it took another 40 years to see the rod signal directly and to confirm that absorption of a single photon produces a detectable signal. Some responses by rods to single photons are shown in Figure 13.20. The small upward deflections are changes in current flowing across the outer segment membrane of a monkey rod; the smallest of them are produced by the absorption of single photons. (By convention, the upward deflections in the current traces represent *decreased* current flow into the cell. The method for recording photoreceptor current is illustrated in Box 14.1.)



Rod Responses to Single Photon Absorptions

Very dim light flashes produce small *decreases* in rod photocurrent (shown as upward deflections of the current traces), the smallest of which are about the same magnitude as and correspond to the absorption of a single photon (identified by dots). Larger responses indicate absorption of more than one photon; absence of response indicates no absorption. (After Baylor, Nunn, and Schnapf 1984.)

The rod's ability to signal the absorption of a single photon raises a question: How can the breaking of a single bond in one *retinal* molecule produce a detectable change in a rod's signal? Neither the amount of energy transferred nor the change in one molecule out of 150 million is significant by itself. These small changes must be amplified; Figure 13.21 outlines how.

Absorption of one photon activates only one rhodopsin molecule. But photopigment molecules are not locked in position in the disc membranes; they can move rapidly in the plane of the membrane, like molecular sailboats buffeted by shifting breezes, exposing their interaction sites to multiple G proteins in a very short time. Having activated one G protein molecule, the activated rhodopsin sails on to other G proteins; during its activated lifetime (about 50 ms), rhodopsin will activate about 800 G protein molecules; that is, $1 \text{ R}^* \rightarrow 800 \text{ G}^*$.

The step in which G^{*} activates PDE does not involve amplification. The relationship is one-to-one: 1 G^{*} \rightarrow 1 PDE^{*}, and thus 1 R^{*} \rightarrow 800 PDE^{*}. One activated PDE molecule, however, can catalyze the breakdown of more than one molecule of cGMP during its activated lifetime. Roughly 6 cGMP molecules are converted to GMP by each activated PDE. Thus, 1 PDE^{*} \rightarrow 6 cGMP \rightarrow GMP, and 1R^{*} \rightarrow 4800 cGMP \rightarrow GMP.

In terms of numbers of molecules, the original event has been amplified 4800fold. Activation of one rhodopsin molecule has taken 4800 cGMP molecules out of circulation so that they are no longer available to keep the cGMP-gated Na⁺ channels open. This may not sound like much, but in terms of the photoreceptor's pool of unbound cGMP, 4800 molecules constitute nearly 1% of the free cGMP molecules in the cell. Of more importance, this fall in the cGMP concentration causes a significant fraction of the open Na⁺ channels to close; 175 Na⁺ channels represent about 2% of the 10,000 or so channels that are open at any given moment. Since the flow of current into the outer segment is proportional to the number of open channels, closing 2% of the channels reduces the inward



Amplification in the Phototransduction Cascade

Absorption of a single photon, an event affecting only one molecule, is amplified to produce a conversion of 4800 cGMP molecules to GMP, which reduces the cGMP concentration by about 2%. Enough cGMP-gated channels close to cause a change in photocurrent of about 1 pA (2%). The main amplification stage is the activation of 800 G protein molecules by one activated rhodopsin molecule. (Data from Rodieck 1998.) current by 2%, which is on the order of 1 pA (see Figure 13.20). The corresponding voltage change across the cell membrane is about 1 mV hyperpolarization.

Amplification within the cone phototransduction cascade is similar to that in rods, and the evidence suggests that cones, when fully adapted to dark, can also signal the absorption of single photons. The change in photocurrent to single absorptions is smaller in cones than in rods, however. Although we can perceive the near simultaneous absorption of single photons by five to ten rods, cones need to be absorbing multiple photons before their signals reach threshold. Part of the difference is inherent to the photoreceptors and part to the neural wiring; the rod system is highly convergent, which allows rod signals to be pooled and summed, but the cone pathways have relatively little convergence.

Photocurrent in the outer segment decreases in proportion to the number of absorbed photons

The changes in current flow into the outer segments of a rod and a red cone from a monkey retina are shown in Figure 13.22. (The different timescale makes the smallest responses look very different from those in Figure 13.20.) In the dark, the rod has a steady inward current flow of –34 pA, which decreases transiently (becomes less negative) in response to a brief flash of light. In the series of responses shown here, the smallest response was to the absorption of just 3 photons, and the inward current decreased by about 2 pA. Increasing the number of photons per light flash increases the size of the decrease in current; for the brightest flash (860 photons) the decrease in current was a full 34 pA, effectively taking the flow of current to zero. Note that the effect of the bright stimuli lasts well beyond the duration of the light flash; if a second light flash were presented to the rod, it would have very little additional response, no matter how bright the second flash. This prolonged response represents adaptation, and for some time after a bright light flash, the rod will be less sensitive than it was before the stimulus.

If the rod always counted photon absorptions as individual events—that is, if the response were directly proportional to the number of photons absorbed—the maximum response in this series would be almost 300 times larger than the smallest response, which is clearly not the case. The actual relationship is shown when the response magnitude is plotted against the logarithm of the number of photons absorbed; over most of the range, the response magnitude (decrease in inward photocurrent) is proportional to the log number of photons absorbed. When the photon numbers are very small, the response does not increase rapidly, and in this region, the rod is counting photons. At the other extreme, where the curve flattens out for large numbers of photons, increases in flash intensity produce no increase in response. Here, the photocurrent has dropped to zero because all the membrane channels are closed and the rod is said to be *saturated*.

Recordings of photocurrents from cones show a similar dependence of response magnitude on flash intensity, with two significant differences. First, the numbers of photons per light flash are significantly larger than those of the rods, ranging from about 190 photons for the smallest cone response to 36,000 photons for the largest response. Cones signal flash intensities that are well out of the rod range. Second, the cone photocurrents lack the sustained character of the rod responses. Even when the cone photocurrent is driven to zero, it quickly returns to its original value and then swings the other way, indicating an *outward* current flow. This overshoot quickly returns to the original steady photocurrent level that existed before the light flash.

The plot of response versus flash intensity in the cone is similar in shape to that of the rod, except it is shifted to the right, reflecting the higher flash intensities needed to elicit the responses. Since the rod and the cone were fully adapted to the dark, the relative shift of the cone curve shows that rods are most sensi-



tive at low flash intensities, whereas cones operate at high intensities for which rods are saturated and insensitive. The initial rise of the cone response occurs as the rod response reaches its maximum; thus, the task of detecting light flashes is handed over from rods to cones with no break in flash detection.

The relatively transient nature of the cone response keeps the cone from saturating at high flash intensities. Although the cone response may be at its maximum (zero current) for a short time, it is restored so quickly that the cone will be sensitive to a second light flash in a way that the rod cannot be. The mechanism underlying this difference between rods and cones is not fully understood, but part of the explanation involves differences in the kinetics of the phototransduction cascade in rods and cones; events proceed more quickly in the cones.

Responses from red, green, and blue cones are similar in shape and timing, although there appear to be differences in response magnitude. The variation of their responses to light flashes that have equal numbers of photons but different wavelengths—their spectral sensitivity curves—fit very nicely with the absorbance spectra of the cone photopigments shown in Figure 13.11. And to reinforce a point made earlier, stimuli of two different wavelengths produce identical responses from a cone when equal numbers of photons are absorbed. Thus the signal from a cone, like that from a rod, relates to the number of photons captured and says nothing about their wavelength.

Photopigments activated by photon absorption are inactivated, broken down, and then regenerated

The photoreceptor responses in Figure 13.22 persist beyond the duration of the stimulus, largely because most events in the phototransduction cascade have lifetimes of 50 ms or more. These lifetimes are the durations of the activated forms of rhodopsin, G_T , and PDE. None of these activated states can be allowed

Figure 13.22

Responses of Rods and Cones to Light Flashes of Different Intensity

The magnitude of change in the photocurrent in both rods and cones increases with increasing intensity of brief light flashes (left). Responses by rods are considerably more prolonged than those of cones. The increase in response is directly proportional to the logarithm of the number of photons absorbed over a range of I log unit (right). Rods respond to much lower light levels than cones; the rod response is at its maximum before the cone response begins to increase appreciably. (After Baylor 1987.)



Figure 13.23 Rhodopsin, Transducin, and Phosphodiesterase Cycles

All the major players in phototransduction go from inactivated to activated forms and back again. The rhodopsin cycle is the longest, and part of the process of regenerating inactivated rhodopsin occurs in the pigment epithelium (shaded region). See text for discussion. G, G protein; PDE, cGMP phosphodiesterase; R, rhodopsin. to persist indefinitely; the activation must be terminated and the different molecules must be restored to their unactivated conditions so that they can take part in the cascade triggered by new photon absorptions. Figure 13.23 illustrates some of the steps in inactivation and restoration of the native states of rhodopsin, transducin, and PDE.

Activated rhodopsin (R*) is phosphorylated by an enzyme, rhodopsin kinase. The addition of phosphates to the C terminus of the opsin (see Figure 13.15) produces enough structural change in the opsin that another protein, **arrestin**, can bind to the opsin at the site where the activated rhodopsin normally interacts with the G protein. Blocking the site prevents further interaction with G_T and brings the rhodopsin's ability to activate G_T to an end. Moreover, arrestin promotes the separation of all-trans *retinal* from the opsin; this is the final stage of photopigment bleaching. When all-trans *retinal* dissociates from the opsin, it diffuses into the cytosol of the photoreceptor outer segment, leaving the opsin behind in the disc membrane. Another enzyme (all-trans retinal oxidoreductase) changes the all-trans *retinal* to all-trans retinol.

Regeneration of the photopigment requires converting the all-trans *retinal* to 11-cis *retinal* that can recombine with the opsin. All but the recombination takes place in the pigment epithelium, not in the photoreceptor; the details will be considered shortly (see Figure 13.29).

Transducin (G_T) goes through a similar cycle. The activated form, G^* , is part of the original molecule (the α subunit) with GTP attached. After binding to and activating PDE, GDP is substituted for GTP, and the now inactive fraction of the G protein separates from PDE and recombines with the β and γ subunits to form the original G_T . The specific triggers for the changes in the G_T cycle are probably enzymes, but they have not been fully characterized.

The PDE cycle is very simple, insofar as it is known. PDE is activated when G^{*} attaches and exposes one of the catalytic sites for cGMP; it returns directly to the inactivated state when the G^{*} separates from it.

Both the G_T and the PDE cycles are relatively fast, in the sense that the transition from the activated form back to the original inactivated configuration is brief compared to the lifetimes of the activated molecules. This is not the case for regenerating rhodopsin, however. Regeneration takes considerably more time than anything else, and this time course dictates the fraction of a photoreceptor's pigment bleached by a steady source of illumination. Since the regeneration rate is constant, as the rate of photon absorption and bleaching exceeds the regeneration rate, the fraction of photopigment bleached at equilibrium will rise. Thus the brighter the ambient illumination, the greater the fraction of pigment bleached. But since the regeneration rate is significant, the fraction of bleached photopigment will always be less than 1 (that is, it is impossible to bleach all of it).