

The neurobiology group in the Department of Pharmacology at Harvard Medical School, 1963, the group that later formed the Department of Neurobiology. Standing (left to right): Edwin Furshpan, Stephen Kuffler, David Hubel. Seated (left to right): David Potter, Edward Kravitz, and Torsten Wiesel.

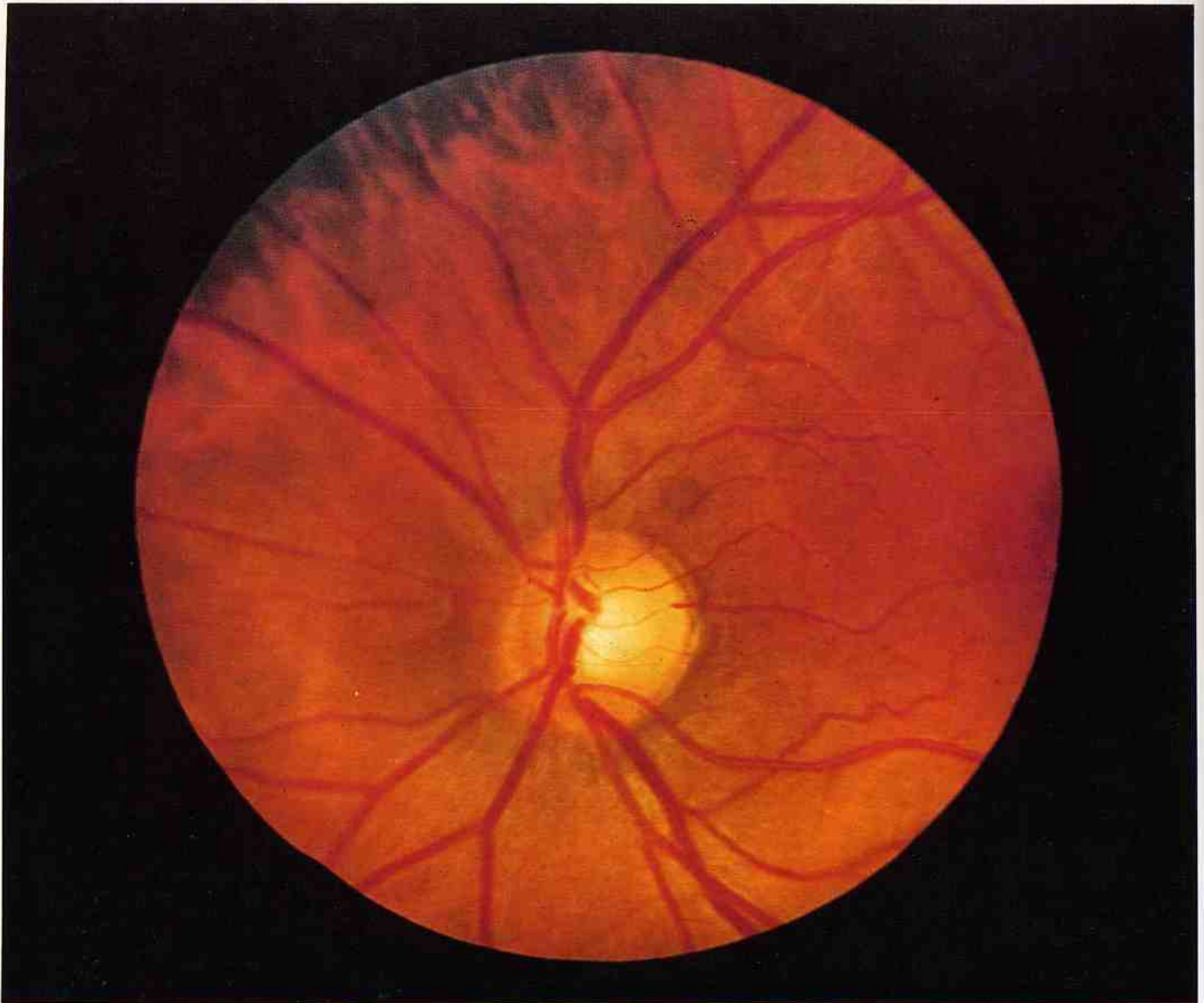


EYE, BRAIN, AND VISION

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An ophthalmologist looking into the eye would see something like this photograph of a normal retina. The large pale circle is the optic disc; here arteries enter and (darker) veins leave the retina. The darker

red pigmented area to the extreme right is the macula; in the center of this region, not shown, is the fovea. The black area at the upper left is normal melanin pigmentation.

THE EYE

The eye has often been compared to a camera. It would be more appropriate to compare it to a TV camera attached to an automatically tracking tripod—a machine that is self-focusing, adjusts automatically for light intensity, has a self-cleaning lens, and feeds into a computer with parallel-processing capabilities so advanced that engineers are only just starting to consider similar strategies for the hardware they design. The gigantic job of taking the light that falls on the two retinas and translating it into a meaningful visual scene is often curiously ignored, as though all we needed in order to see was an image of the external world perfectly focused on the retina. Although obtaining focused images is no mean task, it is modest compared with the work of the nervous system—the retina plus the brain. As we shall see in this chapter, the contribution of the retina itself is impressive. By translating light into nerve signals, it begins the job of extracting from the environment what is useful and ignoring what is redundant. No human inventions, including computer-assisted cameras, can begin to rival the eye. This chapter is mainly about the neural part of the eye—the retina—but I will begin with a short description of the eyeball, the apparatus that houses the retina and supplies it with sharp images of the outside world.

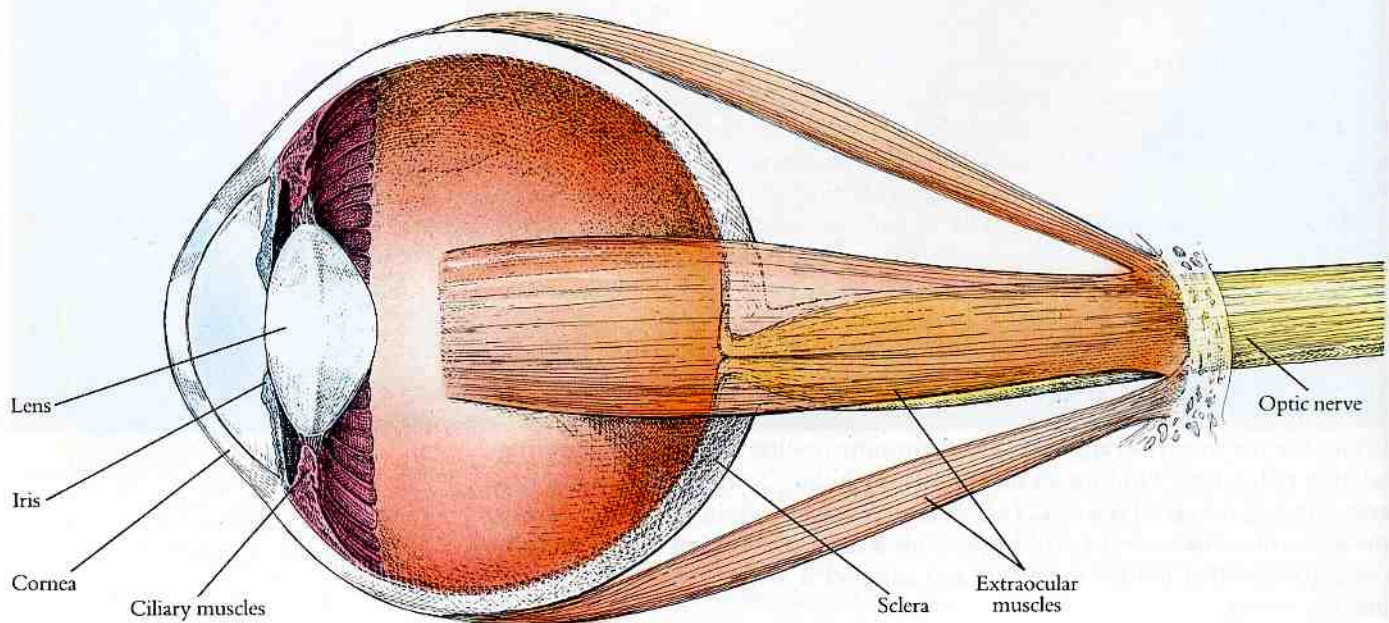


THE EYEBALL

The collective function of the nonretinal parts of the eye is to keep a focused, clear image of the outside world anchored on the two retinas. Each eye is positioned in its socket by the six small extraocular muscles mentioned in Chapter 2. That there are six for each eye is no accident; they consist of three pairs, with the muscles in each pair working in opposition, so as to take care of movements in one of three orthogonal (perpendicular) planes. For both eyes, the job of tracking an object has to be done with a precision of a few minutes of arc—or else we see double. (To see how distressing *that* can be, try looking at something and pressing on the side of one eye with your index finger.) Such precise movements require a collection of finely tuned reflexes, including those that control head position.

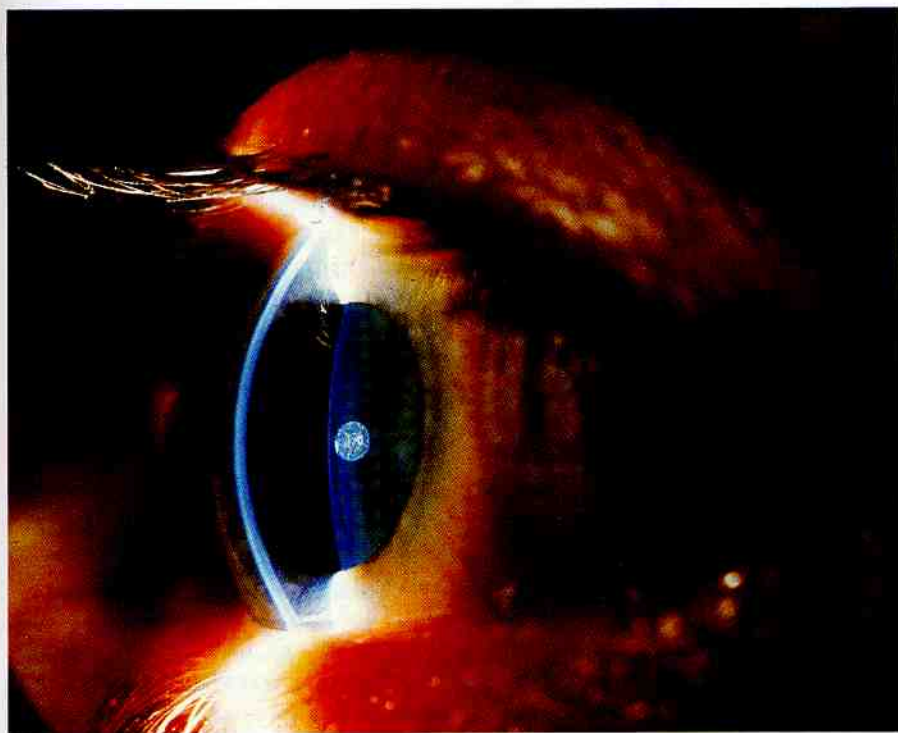
The cornea (the transparent front part of the eye) and lens together form the equivalent of the camera lens. About two-thirds of the bending of light necessary for focusing takes place at the air-cornea interface, where the light enters the eye. The lens of the eye supplies the remaining third of the focusing power, but its main job is to make the necessary adjustments to focus on objects at various distances. To focus a camera you change the distance between lens and film; we focus our eye not by changing the distance between lens and retina

The eyeball and the muscles that control its position. The cornea and the lens focus the light rays onto the back of the eye. The lens regulates the focusing for near and far objects by becoming more or less globular.



but by changing the shape of the rubbery, jellylike lens—by pulling or relaxing the tendons that hold it at its margin—so that it goes from more spherical for near objects to flatter for far ones. A set of radial muscles called *ciliary muscles* produces these changes in shape. (When we get older than about 45, the lens becomes hard and we lose our power to focus. It was to circumvent this major irritation of aging that Benjamin Franklin invented bifocal spectacles.) The reflex that contracts these ciliary muscles in order to make the lens rounder depends on visual input and is closely linked to the reflex controlling the concomitant turning in of the eyes.

Two other sets of muscles change the diameter of the pupil and thus adjust the amount of light entering the eye, just as the iris diaphragm of a camera determines the f-stop. One set, with radial fibers like the spokes of a wheel, opens the pupil; the other, arranged in circles, closes it. Finally, the self-cleaning of the front of the cornea is achieved by blinking the lids and lubricating with tear glands. The cornea is richly supplied with nerves subserving touch and pain, so that the slightest irritation by dust specks sets up a reflex that leads to blinking and secreting of more tears.



Light enters the eye through the transparent cornea, where much of the bending of light takes place. The white dot in the pupil is a reflection of light.

THE RETINA

All this intricate superstructure exists in the interests of the retina, itself an amazing structure. It translates light into nerve signals, allows us to see under conditions that range from starlight to sunlight, discriminates wavelength so that we can see colors, and provides a precision sufficient for us to detect a human hair or speck of dust a few yards away.

The retina is part of the brain, having been sequestered from it early in development but having kept its connections with the brain proper through a bundle of fibers—the optic nerve. Like many other structures in the central nervous system, the retina has the shape of a plate, in this case one about a quarter millimeter thick. It consists of three layers of nerve-cell bodies separated by two layers containing synapses made by the axons and dendrites of these cells.

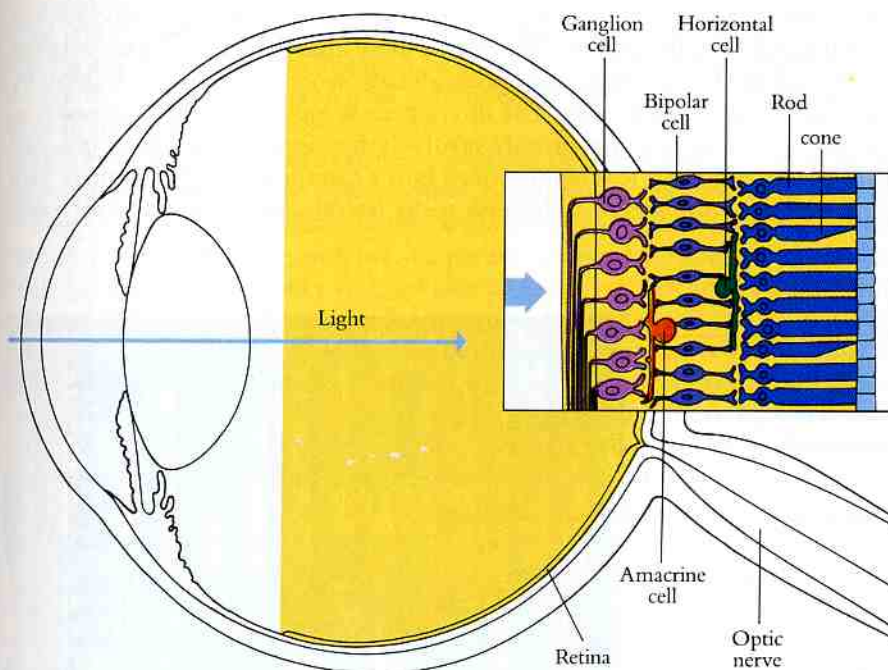
The tier of cells at the back of the retina contains the light receptors, the rods and cones. Rods, which are far more numerous than cones, are responsible for our vision in dim light and are out of commission in bright light. Cones do not respond to dim light but are responsible for our ability to see fine detail and for our color vision.

The numbers of rods and cones vary markedly over the surface of the retina. In the very center, where our fine-detail vision is best, we have only cones. This rod-free area is called the *fovea* and is about half a millimeter in diameter. Cones are present throughout the retina but are most densely packed in the fovea.

Because the rods and cones are at the back of the retina, the incoming light has to go through the other two layers in order to stimulate them. We do not fully understand why the retina develops in this curious backward fashion. One possible reason is the location behind the receptors of a row of cells containing a black pigment, melanin (also found in skin). Melanin mops up the light that has passed through the retina, keeping it from being reflected back and scattering around inside the eye; it has the same function as the black paint inside a camera. The melanin-containing cells also help chemically restore the light-sensitive visual pigment in the receptors after it has been bleached by light (see Chapter 8). For both functions, the melanin pigment must be close to the receptors. If the receptors were at the front of the retina, the pigment cells would have to be between them and the next layer of nerve cells, in a region already packed with axons, dendrites, and synapses.

As it is, the layers in front of the receptors are fairly transparent and probably do not blur the image much. In the central one millimeter, however, where our vision is most acute, the consequences of even slight blurring would be disastrous, and evolution seems to have gone to some pains to alleviate it by having the other layers displaced to the side to form a ring of thicker retina,





The enlarged retina at the right shows the relative positions of the three retinal layers. Surprisingly, the light has to pass through the ganglion-cell and bipolar-cell layers before it gets to the rods and cones.

exposing the central cones so that they lie at the very front. The resulting shallow pit constitutes the fovea.

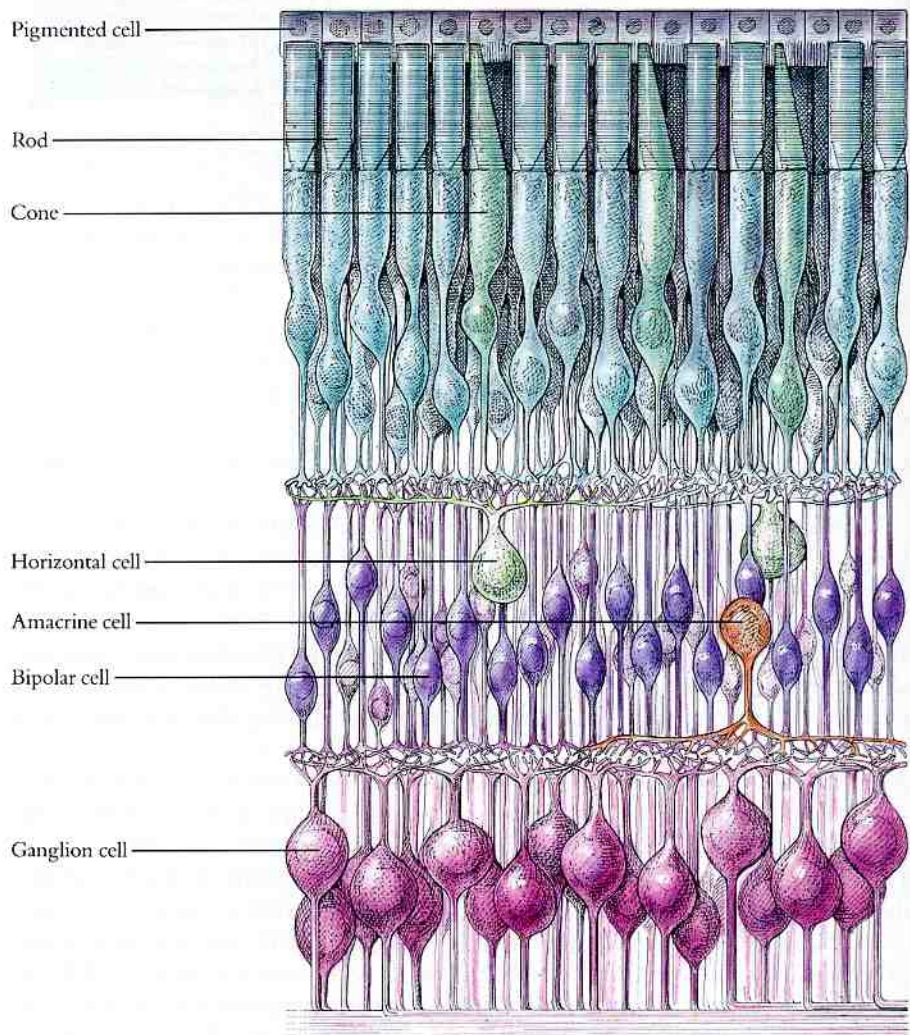
Moving from back to front, we come to the middle layer of the retina, between the rods and cones and the retinal ganglion cells. This layer contains three types of nerve cells: bipolar cells, horizontal cells, and amacrine cells. *Bipolar cells* receive input from the receptors, as the diagram on this page shows, and many of them feed directly into the retinal ganglion cells. *Horizontal cells* link receptors and bipolar cells by relatively long connections that run parallel to the retinal layers; similarly, *amacrine cells* link bipolar cells and retinal ganglion cells.

The layer of cells at the front of the retina contains the *retinal ganglion cells*, whose axons pass across the surface of the retina, collect in a bundle at the optic disc, and leave the eye to form the optic nerve. Each eye contains about 125 million rods and cones but only 1 million ganglion cells. In the face of this discrepancy, we need to ask how detailed visual information can be preserved.

Examining the connections between cells in the retina can help resolve this problem. You can think of the information flow through the retina as following two paths: a direct path, from light receptors to bipolar cells to ganglion cells, and an indirect path, in which horizontal cells may be interposed be-



tween the receptors and bipolars, and amacrine cells between bipolars and retinal ganglion cells. (See the drawing of these direct and indirect connections on this page). These connections were already worked out in much detail by Ramón y Cajal around 1900. The direct path is highly specific or *compact*, in the sense that one receptor or only relatively few feed into a bipolar cell, and only one or relatively few bipolars feed into a ganglion cell. The indirect path is more diffuse, or extended, through wider lateral connections. The total area



A cross section of the retina, about mid-way between the fovea and far periphery, where rods are more numerous than cones. From top to bottom is about one-quarter millimeter.

occupied by the receptors in the back layer that feed one ganglion cell in the front layer, directly and indirectly, is only about one millimeter. That area, as you may remember from Chapter 1, is the *receptive field* of the ganglion cell, the region of retina over which we can influence the ganglion cell's firing by light stimulation.

This general plan holds for the entire retina, but the details of connections vary markedly between the fovea, which corresponds to exactly where we are looking—our center of gaze, where our ability to make out fine detail is highest—and the far outer reaches, or periphery, where vision becomes relatively crude. Between fovea and periphery, the direct part of the path from receptor to ganglion cell changes dramatically. In and near the fovea, the rule for the direct path is that a single cone feeds a single bipolar cell, and a single bipolar in turn feeds into one ganglion cell. As we go progressively farther out, however, more receptors converge on bipolars and more bipolars converge on ganglion cells. This high degree of convergence, which we find over much of the retina, together with the very compact pathway in and near the very center, helps to explain how there can be a 125:1 ratio of receptors to optic nerve fibers without our having hopelessly crude vision.

The general scheme of the retinal path, with its direct and indirect components, was known for many years and its correlation with visual acuity long recognized before anyone understood the significance of the indirect path. An understanding suddenly became possible when the physiology of ganglion cells began to be studied.

THE RECEPTIVE FIELDS OF RETINAL GANGLION CELLS: THE OUTPUT OF THE EYE

In studying the retina we are confronted with two main problems: First, how do the rods and cones translate the light they receive into electrical, and then chemical, signals? Second, how do the subsequent cells in the next two layers—the bipolar, horizontal, amacrine, and ganglion cells—interpret this information? Before discussing the physiology of the receptors and intermediate cells, I want to jump ahead to describe the output of the retina—represented by the activity of the ganglion cells. The map of the receptive field of a cell is a powerful and convenient shorthand description of the cell's behavior, and thus of its output. Understanding it can help us to understand why the cells in the intermediate stages are wired up as they are, and will help explain the purpose of the direct and indirect paths. If we know what ganglion cells are telling the brain, we will have gone far toward understanding the entire retina.





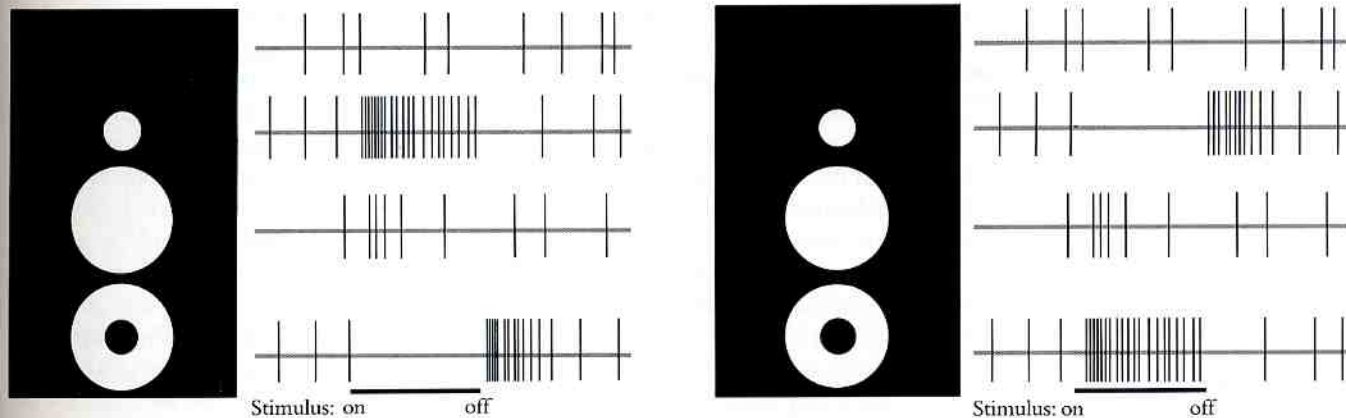
Stephen Kuffler at a laboratory picnic, taken around 1965.



Around 1950, Stephen Kuffler became the first to record the responses of retinal ganglion cells to spots of light in a mammal, the cat. He was then working at the Wilmer Institute of Ophthalmology at the Johns Hopkins Hospital. In retrospect, his choice of animals was lucky because the cat's retina seems to have neither the complexity of movement responses we find in the frog or rabbit retina nor the color complications we find in the retinas of fish, birds, or monkeys. Kuffler used an optical stimulator designed by Samuel Talbot. This optical device, a modified eye doctor's ophthalmoscope, made it possible to flood the retina with steady, weak, uniform background light and also to project small, more intense stimulus spots, while directly observing both the stimulus and the electrode tip. The background light made it possible to stimulate either rods or cones or both, because only the cones work when the prevailing illumination is very bright, and only the rods work in very dim light. Kuffler recorded extracellularly from electrodes inserted through the sclera (white of the eye) directly into the retina from the front. He had little difficulty finding retinal ganglion cells, which are just under the surface and are fairly large.

With a steady, diffuse background light, or even in utter darkness, most retinal ganglion cells kept up a steady, somewhat irregular firing of impulses, at rates of from 1 to 2 up to about 20 impulses per second. Because one might have expected the cells to be silent in complete darkness, this firing itself came as a surprise.

By searching with a small spot of light, Kuffler was able to find a region in the retina through which he could influence—increase or suppress—the retinal ganglion cell's firing. This region was the ganglion cell's receptive field. As you might expect, the receptive field was generally centered at or very near the tip of the electrode. It soon became clear that ganglion cells were of two types, and for reasons that I will soon explain, he called them *on-center cells* and *off-center cells*. An on-center cell discharged at a markedly increased rate when a small spot was turned on anywhere within a well-defined area in or near the center of the receptive field. If you listen to the discharges of such a cell over a loudspeaker, you will first hear spontaneous firing, perhaps an occasional click, and then, when the light goes on, you will hear a barrage of impulses that sounds like a machine gun firing. We call this form of response an *on response*. When Kuffler moved the spot of light a small distance away from the center of the receptive field, he discovered that the light suppressed the spontaneous firing of the cell, and that when he turned off the light the cell gave a brisk burst of impulses, lasting about 1 second. We call this entire sequence—suppression during light and discharge following light—an *off response*. Exploration of the receptive field soon showed that it was cleanly subdivided into a circular on region surrounded by a much larger ring-shaped off region.



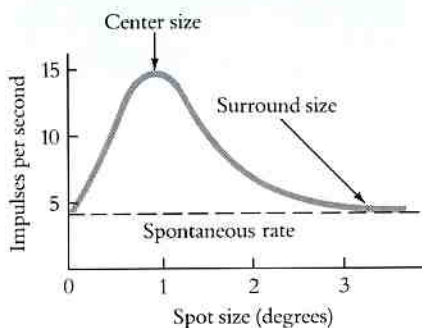
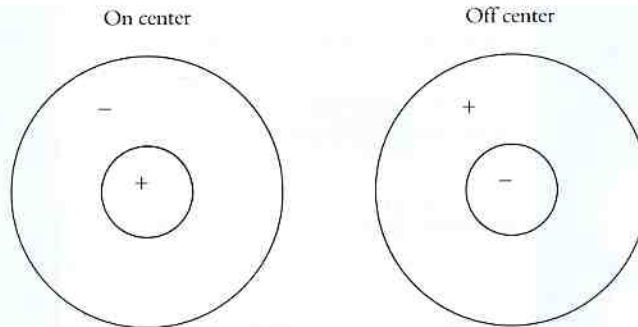
The more of a given region, on or off, the stimulus filled, the greater was the response, so that maximal on responses were obtained to just the right size circular spot, and maximal off responses to a ring of just the right dimensions (inner and outer diameters). Typical recordings of responses to such stimuli are shown on this page. The center and surround regions interacted in an antagonistic way: the effect of a spot in the center was diminished by shining a second spot in the surround—as if you were telling the cell to fire faster and slower at the same time. The most impressive demonstration of this interaction between center and surround occurred when a large spot covered the entire receptive field of the ganglion cell. This evoked a response that was much weaker than the response to a spot just filling the center; indeed, for some cells the effects of stimulating the two regions cancelled each other completely.

An *off-center* cell had just the opposite behavior. Its receptive field consisted of a small center from which off responses were obtained, and a surround that gave on responses. The two kinds of cells were intermixed and seemed to be equally common. An off-center cell discharges at its highest rate in response to a black spot on a white background, because we are now illuminating only the surround of its receptive field. In nature, dark objects are probably just as common as light ones, which may help explain why information from the retina is in the form of both on-center cells and off-center cells.

If you make a spot progressively larger, the response improves until the receptive-field center is filled, then it starts to decline as more and more of the surround is included, as you can see from the graph on the next page. With a spot covering the entire field, the center either just barely wins out over the surround, or the result is a draw. This effect explains why neurophysiologists

Left: Four recordings from a typical on-center retinal ganglion cell. Each record is a single sweep of the oscilloscope, whose duration is 2.5 seconds. For a sweep this slow, the rising and falling phases of the impulse coalesce so that each spike appears as a vertical line. To the left the stimuli are shown. In the resting state at the top, there is no stimulus: firing is slow and more or less random. The lower three records show responses to a small (optimum size) spot, a large spot covering the receptive-field center and surround, and a ring covering the surround only. *Right:* Responses of an off-center retinal ganglion cell to the same set of stimuli shown at the left.

The two main types of retinal-ganglion-cell receptive fields are on center, with inhibitory surround, and off center, with excitatory surround. “+” stands for regions giving on responses, “-” for regions giving off responses.



As we stimulate a single on-center retinal ganglion cell with ever larger spots, the response becomes more powerful, up to a spot size of about 1 degree. This is the center size. Further enlargement of the spot causes a decline, because now the spot invades the antagonistic surround. Beyond about 3 degrees there is no further decline, so that 3 degrees represents the total receptive field, center plus surround.

before Kuffler had such lack of success: they had recorded from these cells but had always used diffuse light—clearly far from the ideal stimulus.

You can imagine what a surprise it must have been to observe that shining a flashlight directly into the eye of an animal evoked such feeble responses or no response at all. Illuminating all the receptors, as a flashlight surely does, might have been expected to be the most effective stimulus, not the least. The mistake is to forget how important inhibitory synapses are in the nervous system. With nothing more than a wiring diagram such as the one on page 27, we cannot begin to predict the effects of a given stimulus on any given cell if we do not know which synapses are excitatory and which are inhibitory. In the early 1950s, when Kuffler was recording from ganglion cells, the importance of inhibition in the nervous system was just beginning to be realized.

Before I go on to describe the receptors and other retinal cells, I want to make three additional comments about receptive fields. The first is a general comment about receptive fields as a concept; the other two comments are specifically about the receptive fields of retinal ganglion cells: their overlap and their dimensions.

THE CONCEPT OF A RECEPTIVE FIELD

Narrowly defined, the term *receptive field* refers simply to the specific receptors that feed into a given cell in the nervous system, with one or more synapses intervening. In this narrower sense and for vision, it thus refers simply to a region on the retina, but since Kuffler's time, and because of his work, the term has gradually come to be used in a far broader way. Retinal ganglion cells were historically the first example of cells whose receptive fields

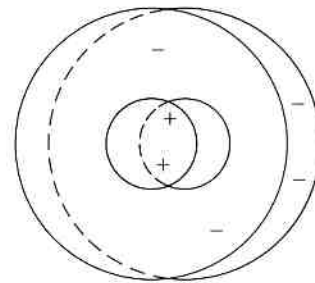
had a substructure: stimulating different parts of the receptive fields gave qualitatively different responses, and stimulating a large area resulted in cancellation of the effects of the subdivisions rather than addition. As presently used, *receptive field* tends to include a description of the substructure, or if you prefer, an account of how you have to stimulate an area to make the cell respond. When we speak of "mapping out a cell's receptive field", we often mean not simply demarcating its boundaries on the retina or the screen the animal is looking at, but also describing the substructure. As we get deeper into the central nervous system, where receptive fields tend to become more and more complex, we will find that their descriptions become increasingly elaborate.

Receptive-field maps are especially useful because they allow us to predict the behavior of a cell. In the case of retinal ganglion cells, for example, suppose we stimulate an on-center cell with a long, narrow rectangle of light, just wide enough to span the receptive-field center, and long enough to go beyond the whole field, center plus surround. We would predict from the on-center map on page 42 that such a stimulus would evoke a strong response, since it covers all the center and only a small fraction of the antagonistic surround. Furthermore, from the radial symmetry of the map we can predict that the magnitude of the cell's response will be independent of the slit's orientation. Both predictions are confirmed experimentally.

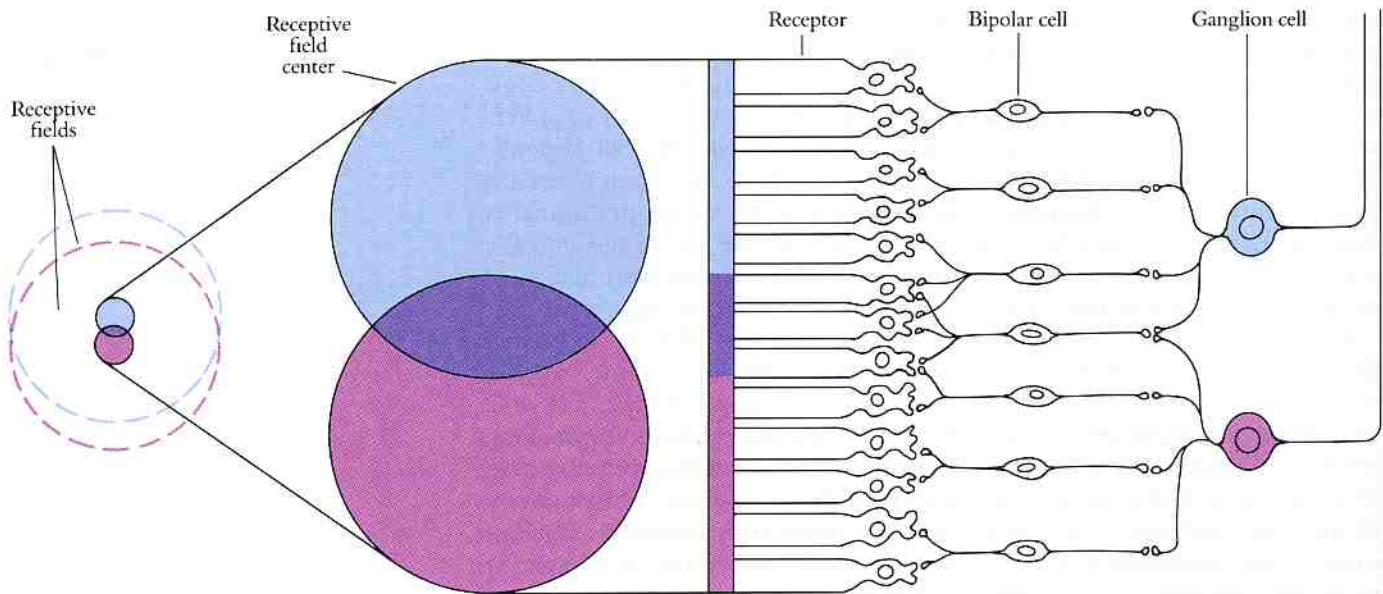
THE OVERLAP OF RECEPTIVE FIELDS

My second comment concerns the important question of what a population of cells, such as the output cells of the retina, are doing in response to light. To understand what ganglion cells, or any other cells in a sensory system are doing, we have to go at the problem in two ways. By mapping the receptive field, we ask how we need to stimulate to make one cell respond. But we also want to know how some particular retinal stimulus affects the entire population of ganglion cells. To answer the second question we need to begin by asking what two neighboring ganglion cells, sitting side by side in the retina, have in common.

The description I have given so far of ganglion-cell receptive fields could mislead you into thinking of them as forming a mosaic of nonoverlapping little circles on the retina, like the tiles on a bathroom floor. Neighboring retinal ganglion cells in fact receive their inputs from richly overlapping and usually only slightly different arrays of receptors, as shown in the diagram on this page. This is the equivalent of saying that the receptive fields almost completely overlap.



The receptive fields of two neighboring retinal ganglion cells will usually overlap. The smallest spot of light we can shine on the retina is likely to influence hundreds of ganglion cells, some off center and some on center. The spot will fall on the centers of some receptive fields and on the surrounds of others.

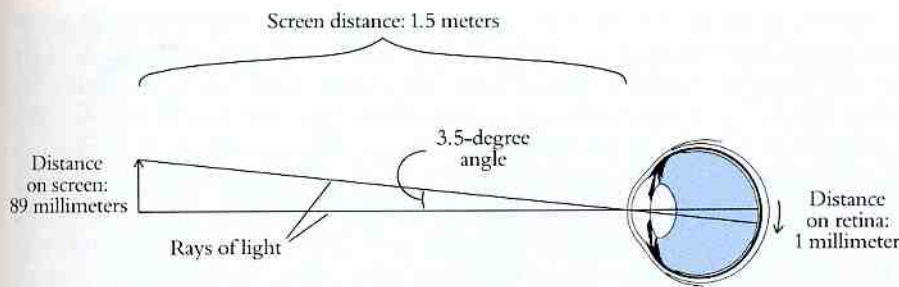


Two neighboring retinal ganglion cells receive input over the direct path from two overlapping groups of receptors. The areas of retina occupied by these receptors make up their receptive-field centers, shown face on by the large overlapping circles.

You can see why by glancing at the simplified circuit in the diagram above: the cell colored purple and the one colored blue receive inputs from the overlapping regions, shown in cross section, of the appropriate colors. Because of divergence, in which one cell makes synapses with many others at each stage, one receptor can influence hundreds or thousands of ganglion cells. It will contribute to the receptive-field centers of some cells and to the surrounds of others. It will excite some cells, through their centers if they are on-center cells and through their surrounds if they are off-center cells; and it will similarly inhibit others, through *their* centers or surrounds. Thus a small spot shining on the retina can stir up a lot of activity, in many cells.

DIMENSIONS OF RECEPTIVE FIELDS

My third comment is an attempt to relate these events in the retina to everyday vision in the outside world. Obviously our vision completely depends on information the brain receives from the eyes; all this information is conveyed to the brain by the axons of retinal ganglion cells. The finer the detail conveyed by each of these fibers, the crisper will be our image of the world.



One millimeter on the retina corresponds to 3.5 degrees of visual angle. On a screen 1.5 meters away, 1 millimeter on the retina thus corresponds to about 3.5 inches, or 89 millimeters.

This fineness of detail is best measured not by the overall size of receptive fields, but by the size of the field centers.

We can describe the size of a receptive field in two ways. The more straightforward description is simply its size on the retina. This has the disadvantage of being not very meaningful in the everyday scale of things. Alternatively, you could measure receptive-field size in the outside world, for example, by taking its diameter on a screen that an animal faces, but you would then have to specify how far the screen is from the animal's eyes. The way around this problem is to express receptive-field size as the *angle* subtended by the receptive field on the screen, at the animal's eye, as shown in the figure on this page. We calculate this angle in radians simply by dividing the field diameter by the screen distance, but I will use degrees: $(\text{radians} \times \pi)/180$. One millimeter on the human retina corresponds to an angle of about 3.5 degrees. At 54 inches screen distance, 1 inch on the screen corresponds to 1 degree. The moon and sun, seen from the earth, are about the same size, and each subtends one-half a degree.

Receptive fields differ in size from one ganglion cell to the next. In particular, the *centers* of the receptive fields vary markedly and systematically in size: they are smallest in the fovea, the central part of the retina, where our visual acuity, or ability to distinguish small objects, is greatest; they get progressively larger the farther out we go, and meanwhile our acuity falls off progressively.

In a monkey the smallest field centers yet measured subtend about 2 minutes of arc, or about 10 micrometers (0.01 millimeters) on the retina. These ganglion cells are probably outside the fovea but near it. In the fovea, cones have diameters and center-to-center spacing of about 2.5 micrometers, a figure that matches well with our visual acuity, measured in terms of our ability to separate two points as close as 0.5 minutes of arc. A circle 2.5 micrometers in diameter on the retina (subtending 0.5 minutes) corresponds to a quarter viewed from a distance of about 500 feet.

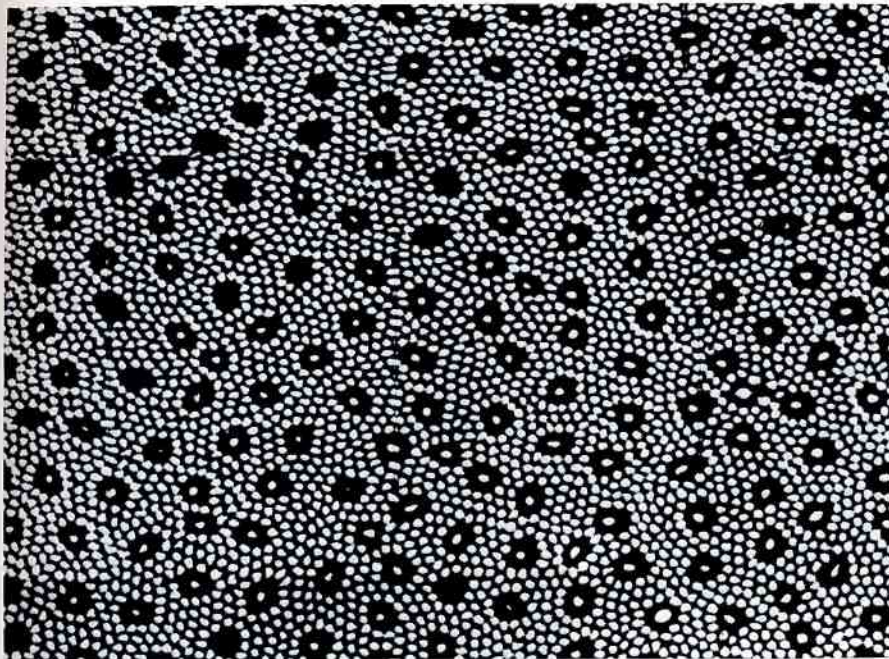
Far out in the periphery of the retina, receptive-field centers are made up of thousands of receptors and can have diameters of 1 degree or more. Thus as we go out along the retina from its center, three items correlate in an impressive way, surely not by coincidence: visual acuity falls, the size of the receptor population contributing to the direct pathway (from receptors to bipolars to ganglion cells) increases, and the sizes of receptive-field centers increase. These three trends are clues that help us understand the meaning of the direct and indirect paths from receptors to ganglion cells. The strong implication is that the center of the receptive field is determined by the direct path and the antagonistic surround by the indirect one, and that the direct path sets limits on our acuity. To obtain more evidence for this conclusion, it was necessary to record from the other cells in the retina, as I will describe in the next section.

THE PHOTORECEPTORS

It was many years before much progress was made in the physiology of the receptors, bipolars, horizontal cells, or amacrine cells. There are many reasons for this: vascular pulsations bedevil our attempts to keep microelectrodes in or close to single cells; receptors, bipolars, and horizontal cells do not fire impulses, so that recording the much smaller graded potentials requires intracellular techniques; and it is hard to be certain which of the cell types our electrode is in or near. We can circumvent some of these problems by choosing just the right animal: retinas of cold-blooded vertebrates survive when taken out of the eye and bathed in oxygenated salt water, and eliminating the blood circulation eliminates arterial pulsations; the mudpuppy (a kind of large salamander) has very large cells, easy to record from; fish, frogs, turtles, rabbits, and cats all have special advantages for one or another kind of study, so that many species have been used in the study of retinal physiology. The problem with using so many species is that the details of the organization of the retinas can differ markedly from one species to the next. Moreover, our knowledge of the primate retina, one of the most difficult to record from, has until recently had to depend largely on inferences from the results pooled from these other species. But progress in primates is accelerating as the technical difficulties are overcome.

In the past few years, our understanding of the way in which a rod or cone responds to light has dramatically increased, so much so that one has the feeling of at last beginning to understand how they work.

Rods and cones differ in a number of ways. The most important difference is in their relative sensitivity: rods are sensitive to very dim light, cones require

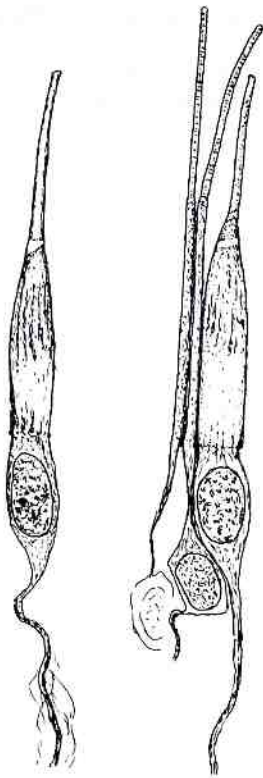


This section through the peripheral retina of a monkey passes through the layers of rods and cones. The tiny white circles are rods; the larger black regions with a white dot in the center are cones.

much brighter light. I have already described the differences in their distribution throughout the retina, the most notable being the absence of rods in the fovea. They differ in shape: rods are long and slender; cones are short and tapered. Both rods and cones contain light-sensitive pigments. All rods have the same pigment; cones are of three types, each type containing a different visual pigment. The four pigments are sensitive to different wavelengths of light, and in the case of the cones, these differences form the basis of our color vision.

The receptors respond to light through a process called *bleaching*. In this process, a molecule of visual pigment absorbs a *photon*, or single package, of visible light and is thereby chemically changed into another compound that absorbs light less well, or perhaps differs in its wavelength sensitivity. In virtually all animals, from insects to humans and even in some bacteria, this receptor pigment consists of a protein coupled to a small molecule related to vitamin A, which is the part that is chemically transformed by light. Thanks largely to the work in the 1950s of George Wald at Harvard, we now know a lot about the chemistry of bleaching and the subsequent reconstitution of visual pigments.

Most ordinary sensory receptors—chemical, thermal, or mechanical—are depolarized in response to the appropriate stimulus, just as nerves become depolarized in response to an excitatory stimulus; the depolarization leads to



A single cone (left) and two rods and a cone (right) have been teased apart and stained with osmic acid. The slender process at the top of each cell is the outer segment, which contains the visual pigment. The fibers at the bottom go to the synaptic regions, not shown.

release of transmitter at the axon terminals. (Often, as in visual receptors, no impulse occurs, probably because the axon is very short.) Light receptors in invertebrates, from barnacles to insects, behave in this way, and up to 1964 it was assumed that a similar mechanism—depolarization in response to light—would hold for vertebrate rods and cones.

In that year Japanese neurophysiologist Tsuneo Tomita, working at Keio University in Tokyo, first succeeded in getting a microelectrode inside the cones of a fish, with a result so surprising that many contemporaries at first seriously doubted it. In the dark, the potential across the cone membrane was unexpectedly low for a nerve cell: roughly 50 millivolts rather than the usual 70 millivolts. When the cone was illuminated, this potential *increased*—the membrane became *hyperpolarized*—just the reverse of what everyone had assumed would happen. In the dark, vertebrate light receptors are apparently more depolarized (and have a lower membrane potential) than ordinary resting nerve cells, and the depolarization causes a steady release of transmitter at the axon terminals, just as it would in a conventional receptor during stimulation. Light, by increasing the potential across the receptor-cell membrane (that is, by *hyperpolarizing* it), cuts down this transmitter release. Stimulation thus turns the receptors off, strange as that may seem. Tomita's discovery may help us to understand why the optic-nerve fibers of vertebrates are so active in the dark: it is the receptors that are spontaneously active; presumably, many of the bipolar and ganglion cells are simply doing what the receptors tell them to do.

In the ensuing decades, the main problems have been to learn how light leads to hyperpolarization of the receptor, especially how bleaching as little as a *single* molecule of visual pigment, by a single photon of light, can lead, in the rod, to a measureable change in membrane potential. Both processes are now reasonably well understood. Hyperpolarization by light is caused by the shutting off of a flow of ions. In darkness, part of the receptor membrane is more permeable than the rest of the membrane to sodium ions. Consequently, sodium ions continually flow into the cell there, and potassium ions flow out elsewhere. This flow of ions in the dark, or *dark current*, was discovered in 1970 by William Hagins, Richard Penn, and Shuko Yoshikami at the National Institute of Arthritis and Metabolic Diseases in Bethesda. It causes depolarization of the receptor at rest, and hence its continual activity. As a result of the bleaching of the visual pigment in response to light, the sodium pores close, the dark current decreases, and the membrane depolarization declines—the cell thus hyperpolarizes. Its rate of activity (that is, transmission release) decreases.

Today, as a result of the work of Evgeniy Fesenko and co-workers at the Academy of Sciences in Moscow, Denis Baylor at Stanford University, King-Wai Yau of the University of Texas, and others, we are much closer to understanding the linkage between the bleaching and the closing of the sodium

pores. For example, it had been very hard to imagine how the bleaching of a single molecule could lead to the closing of the millions of pores that the observed potential changes would require. It now appears that the pores of the receptor are kept open by molecules of a chemical called cyclic guanosine monophosphate, or *cGMP*. When the visual pigment molecule is bleached, a cascade of events is let loose. The protein part of the bleached pigment molecule activates a large number of molecules of an enzyme called transducin; each of these in turn inactivates hundreds of *cGMP* molecules, with consequent closing of the pores. Thus as a result of a single pigment molecule being bleached, millions of pores slam shut.

All this makes it possible to explain several previously puzzling phenomena. First, we have long known that a fully dark-adapted human can see a brief flash of light so feeble that no single receptor can have received more than 1 photon of light. Calculations show that about six closely spaced rods must be stimulated, each by a photon of light, within a short time, to produce a visible flash. It now becomes clear how a single photon can excite a rod enough to make it emit a significant signal.

Second, we can now explain the inability of rods to respond to changes in illumination if the light is already bright. It seems that rods are so sensitive that at high illumination levels—for example, in sunlight—all the sodium pores are closed, and that any further increase in light can have no further effect. We say the rods are *saturated*.

Perhaps a few years from now students of biology will regard this entire story of the receptors as one more thing to learn—I hope not. To appreciate fully its impact, it helps to have spent the years wondering how the receptors could possibly work; then suddenly, in the space of a decade or less of spectacular research, it all unfolds. The sense of excitement still has not subsided.

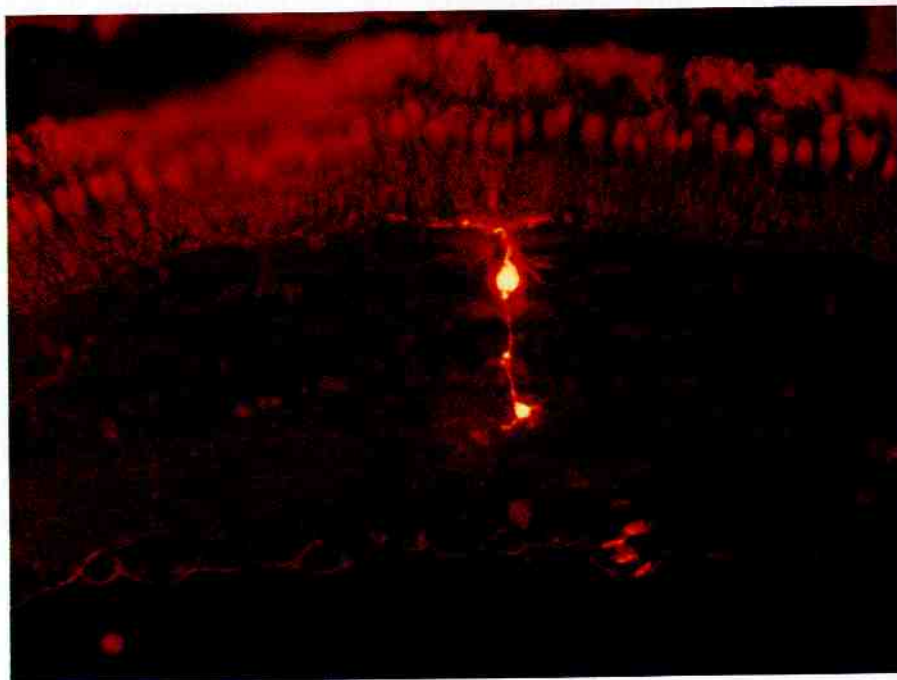
BIPOLAR CELLS AND HORIZONTAL CELLS

Horizontal cells and bipolar cells occur, along with amacrine cells, in the middle layer of the retina. The bipolar cells occupy a strategic position in the retina, since all the signals originating in the receptors and arriving at the ganglion cells must pass through them. This means that they are a part of both the direct and indirect paths. In contrast, horizontal cells are a part of the indirect path only. As you can see from the diagram on page 152, horizontal cells occur much less frequently than bipolar cells, which tend to dominate the middle layer.

Before anyone had recorded from bipolar cells, the big unknown was whether they would prove to have center-surround receptive fields, as ganglion cells do, and come in two types, on center and off center. If the answer was yes, it would almost certainly mean that the organization discovered by Kuffler for ganglion cells was a passive reflection of bipolar-cell organization. The knowledge that the receptive fields of bipolar cells were indeed center-surround and were of two types came from intracellular recordings first made by John Dowling and Frank Werblin at Harvard Biological Laboratories and by Akimichi Kaneko at Harvard Medical School. The next question is how these receptive fields are built up. To answer it we have to begin by examining the connections of receptors, bipolar cells, and horizontal cells.

The bipolar cell sends a single dendrite in the direction of the receptors. This either synapses with one receptor (always a cone) or it splits into branches that synapse with more than one receptor. When more than one receptor feeds into a single bipolar cell, they collectively occupy a relatively small area of retina. In either case, these receptors must account for the receptive-field center, because the area they occupy matches the field center in size. The next question is whether the synapses between receptors and bipolar cells are excitatory or inhibitory, or both.

To record from a cell in the nervous system is one thing; it is another to record from a cell and know exactly what kind of cell it is. This microscopic picture shows a single bipolar cell in the retina of a goldfish, recorded in 1971 by Akimichi Kaneko, then at Harvard Medical School. The fact that it is a bipolar cell and not an amacrine or horizontal cell was proven by injecting a fluorescent dye, procyon yellow, through the microelectrode. The dye spread throughout the cell, revealing its shape. In this cross section, receptors are on top.



Bipolar cells, like receptors and horizontal cells, do not fire impulses, but we still speak of an on response, meaning a depolarization to light and therefore increased transmitter release from the cell's terminals, and an off response, to imply hyperpolarization and decreased release. For the off-center bipolars these synapses must be excitatory, because the receptors themselves are turned off (hyperpolarized) by light; for the on-center bipolars the synapses must be inhibitory. To see why (if you, like me, find this confusing), you need only think about the effects of a small spot of light. Receptors are active in the dark: light hyperpolarizes them, turning them off. If the synapse is excitatory, the bipolar will have been activated in the dark, and will likewise be turned off by the stimulus. If the synapse is inhibitory, the bipolar will have been suppressed in the dark, and the light, by turning off the receptor, will relieve the suppression of the bipolar cell—that is, the bipolar cell will be activated. (No one said this would be easy.)

Whether the receptor-to-bipolar synapse is excitatory or inhibitory could depend on either the transmitter the receptor releases or the nature of the channels in the bipolar cell's postsynaptic membrane. At present no one thinks that one receptor releases two transmitters, and much evidence favors the idea that the two bipolar types have different receptor molecules.

Before we discuss where the receptive-field surrounds of the bipolar cells come from, we have to consider the horizontal cells.

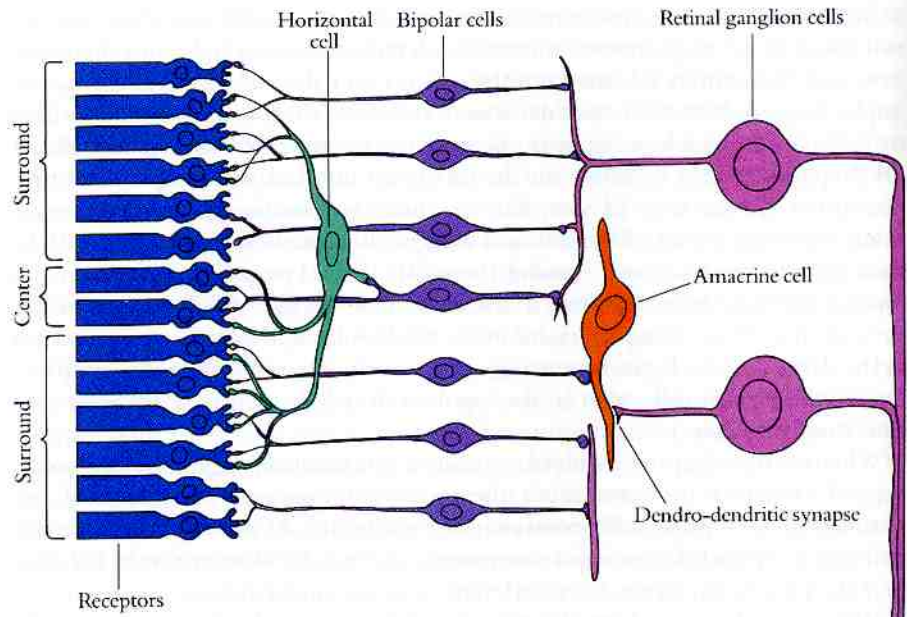
Horizontal cells are important because they are probably at least in part responsible for the receptive-field surrounds of retinal ganglion cells; they represent the part of the indirect pathway about which we know the most. They are large cells, and among the strangest in the nervous system. Their processes, or nerve fibers, make close contact with the terminals of many photoreceptors, distributed over an area that is wide compared with the area directly feeding a single bipolar cell. Every receptor contacts both types of second-order cell, bipolar and horizontal.

Horizontal cells come in several subtypes and can differ greatly from species to species; their most unusual feature, which they share with amacrine cells, is their lack of anything that looks like an ordinary axon. From the slightly simplified account of nerve cells given in the last chapter you may rightly wonder how a nerve without an axon could transmit information to other neurons. When the electron microscope began to be used in neuroanatomy, we soon realized that dendrites can, in some cases, be presynaptic, making synapses onto other neurons, usually onto their dendrites. (For that matter, axon terminals can sometimes be postsynaptic, with other axons ending on them.) The processes that come off the cell bodies of horizontal cells and amacrine cells apparently serve the functions of both axons and dendrites.

The synapses that horizontal cells make with receptors are likewise unusual, lacking the electron-microscopic features that would normally tell us which

A hypothetical circuit shows how center-surround receptive fields are thought to be built up. The center, an off center in this example, is produced by a small patch of receptors, making powerful excitatory synaptic contacts on a single bipolar cell. One or several such cells feed into a ganglion cell to form its center. The surround of the bipolar cell's receptive field is produced by a much larger number of receptors (including those in the central patch), which feed a horizontal cell with excitatory synapses. The horizontal cell may contact the bipolar cell or project back onto the receptors.

If the bipolar cell is off center, the synapses onto the bipolar cell from the central patch of receptors are presumed to be excitatory. (The receptors are turned *off* by light.) The horizontal cell is presumed to inhibit either the bipolar cell or the receptors themselves. Note two input paths to ganglion cells, one directly from bipolars and the other from bipolar to amacrine to ganglion cell.



way the information is conveyed. It is clear that receptors feed information to horizontal cells through excitatory synapses because horizontal cells, like receptors, are in most cases hyperpolarized, or turned off, by light. It is less clear where the horizontal cell sends its output: in some species such as turtles we know that they feed information back to receptors; in other species they make synapses with the dendrites of bipolar cells and doubtless feed into them; in primates we do not have either type of information. In summary, horizontal cells get their input from receptors; their output is still uncertain, but is either back to receptors, or to bipolar cells, or to both.

The relatively wide retinal area over which receptors supply horizontal cells suggests that the receptive fields of horizontal cells should be large, and they are. They are about the size of the entire receptive fields of bipolar cells or ganglion cells, center plus surround. They are uniform, giving hyperpolarization wherever you stimulate, and more hyperpolarization the larger the spot. Much evidence points to the horizontal cells as being responsible for the receptive-field surrounds of the bipolar cells—indeed they are the only plausible candidates, being the only cells that connect to receptors over so wide an expanse. When horizontal cells connect directly to bipolars, the synapses to on-bipolars would have to be excitatory (for the effect of light in the surround to be inhibitory) and those to off-bipolars, inhibitory. If the influence is by way of the receptors, the synapses would have to be inhibitory.

To sum this up: Bipolar cells have center-surround receptive fields. The center is supplied by *direct* input from a small group of receptors; the surround arises from an *indirect* path stemming from a wider expanse of receptors that feed into horizontal cells, which probably feed into the bipolars. The indirect path could also be the result of the horizontal cells feeding back and inhibiting the receptors.

AMACRINE CELLS

These cells come in an astonishing variety of shapes and use an impressive number of neurotransmitters. There may be well over twenty different types. They all have in common, first, their location, with their cell bodies in the middle retinal layer and their processes in the synaptic zone between that layer and the ganglion cell layer; second, their connections, linking bipolar cells and retinal ganglion cells and thus forming an alternative, indirect route between them; and, finally, their lack of axons, compensated for by the ability of their dendrites to end presynaptically on other cells.

Amacrine cells seem to have several different functions, many of them unknown: one type of amacrine seems to play a part in specific responses to moving objects found in retinas of frogs and rabbits; another type is interposed in the path that links ganglion cells to those bipolar cells that receive rod input. Amacrine cells are not known to be involved in the center-surround organization of ganglion-cell receptive fields, but we cannot rule out the possibility. This leaves most of the shapes unaccounted for, and it is probably fair to say, for amacrine cells in general, that our knowledge of their anatomy far outweighs our understanding of their function.

CONNECTIONS BETWEEN BIPOLAR CELLS AND GANGLION CELLS

We have seen that the main features of the ganglion-cell receptive fields are to be found already in the bipolar cells. This leaves open the question of what transformations of information occur between bipolars and ganglion cells. It is hardly likely that nothing happens, if the complexity of the synaptic layer between the middle layer and ganglion-cell layer is any indication, for here we often find convergence between bipolars and ganglion cells in the direct path, and also the intervention of amacrine cells, whose functions are not well understood.

The synapses between bipolar cells and ganglion cells are probably all excitatory, and this means that on-center bipolar cells supply on-center ganglion cells, and off-center bipolars supply off-center ganglion cells. That simplifies the circuit: we could have had on-center cells supplying off-center cells through inhibitory synapses, for example. We should be thankful for small mercies.

Until 1976, it was not known whether on-center cells and off-center cells differed in their shapes, but in that year Ralph Nelson, Helga Kolb, and Edward Famiglietti, at the National Institutes of Health in Bethesda, recorded intracellularly from cat ganglion cells, identified them as on- or off-center, and then injected a dye through the microelectrode, staining the entire dendritic tree. When they compared the dendritic branchings in the two cell types they saw a clear difference: the two sets of dendrites terminated in two distinct sublayers within the synaptic zone between the middle and ganglion-cell layers. The off-center-cell dendrites always terminated closer to the middle layer of the retina, the on-center dendrites, farther. Other work had already shown that two classes of bipolar cells, known to have different-shaped synapses with receptors, differed also in the position of their axon terminals, one set ending where the on-center ganglion-cell dendrites terminated, the other, where the off-center dendrites terminated. It thus became possible to reconstruct the entire path from receptors to ganglion cells, for both on-center and off-center systems.

One surprising result of all this was to establish that, in the direct pathway, it is the off-center system that has excitatory synapses at each stage, from receptors to bipolars and bipolars to ganglion cells. The on-center path instead has an inhibitory receptor-to-bipolar synapse.

The separation of bipolar cells and ganglion cells into on- and off-center categories must surely have perceptual correlates. Off-center cells respond in exactly the same way to dark spots as on-center cells respond to bright spots. If we find it surprising to have separate sets of cells for handling dark and light spots, it may be because we are told by physicists, rightly, that darkness is the absence of light. But dark seems very real to us, and now we seem to find that the reality has some basis in biology. Black is as real to us as white, and just as useful. The print of the page you are reading is, after all, black.

An exactly parallel situation occurs in the realm of heat and cold. In high school physics we are amazed to learn that cold is just the absence of heat because cold seems equally real—more so if you were brought up, as I was, in frigid Montreal. The vindication of our instinct comes when we learn that we have two classes of temperature receptors in our skin, one that responds to the raising of temperature, and another to lowering. So again, biologically, cold is just as real as hot.

Many sensory systems make use of opposing pairs: hot/cold, black/white,

head rotation left/head rotation right—and, as we will see in Chapter 8, yellow/blue and red/green. The reason for opposing pairs is probably related to the way in which nerves fire. In principle, one could imagine nerves with firing rates set at some high level—say, 100 impulses per second—and hence capable of firing slower or faster—down to zero or up to, say, 500—to opposite stimuli. But because impulses require metabolic energy (all the sodium that enters the nerve has to be pumped back out), probably it is more efficient for our nerve cells to be silent or to fire at low rates in the absence of a sensory stimulus, and for us to have two separate groups of cells for any given modality—one firing to less, the other to more.

THE SIGNIFICANCE OF CENTER-SURROUND FIELDS

Why should evolution go to the trouble of building up such curious entities as center-surround receptive fields? This is the same as asking what use they are to the animal. Answering such a deep question is always difficult, but we can make some reasonable guesses. The messages that the eye sends to the brain can have little to do with the absolute intensity of light shining on the retina, because the retinal ganglion cells do not respond well to changes in diffuse light. What the cell does signal is the result of a *comparison* of the amount of light hitting a certain spot on the retina with the average amount falling on the immediate surround.

We can illustrate this comparison by the following experiment. We first find an on-center cell and map out its receptive field. Then, beginning with the screen uniformly and dimly lit by a steady background light, we begin turning on and off a spot that just fills the field center, starting with the light so dim we cannot see it and gradually turning up the intensity. At a certain brightness, we begin to detect a response, and we notice that this is also the brightness at which we just begin to see the spot. If we measure both the background and the spot with a light meter, we find that the spot is about 2 percent brighter than the background. Now we repeat the procedure, but we start with the background light on the screen five times as bright. We gradually raise the intensity of the stimulating light. Again at some point we begin to detect responses, and once again, this is the brightness at which we can just see the spot of light against the new background. When we measure the stimulating light, we find that it, too, is five times as bright as previously, that is, the spot is again 2 percent brighter than the background. The conclusion is that both for us and for the cell, what counts is the relative illumination of the spot and its surround.

The cell's failure to respond well to anything but local intensity differences may seem strange, because when we look at a large, uniformly lit spot, the interior seems as vivid to us as the borders. Given its physiology, the ganglion cell reports information only from the borders of the spot; we see the interior as uniform because no ganglion cells with fields in the interior are reporting local intensity differences. The argument seems convincing enough, and yet we feel uncomfortable because, argument or no argument, the interior still looks vivid! As we encounter the same problem again and again in later chapters, we have to conclude that the nervous system often works in counter-intuitive ways. Rationally, however, we must concede that seeing the large spot by using only cells whose fields are confined to the borders—instead of tying up the entire population whose centers are distributed throughout the entire spot, borders plus interior—is the more efficient system: if you were an engineer that is probably exactly how you would design a machine. I suppose that if you did design it that way, the machine, too, would think the spot was uniformly lit.

In one way, the cell's weak responses or failure to respond to diffuse light should not come as a surprise. Anyone who has tried to take photographs without a light meter knows how bad we are at judging absolute light intensity. We are lucky if we can judge our camera setting to the nearest f-stop, a factor of *two*; to do even that, we have to use our experience, noting that the day is cloudy-bright and that we are in the open shade an hour before sunset, for example, rather than just looking. But like the ganglion cell, we are very good at spatial comparisons—judging which of two neighboring regions is brighter or darker. As we have seen, we can make this comparison when the difference is only 2 percent, just as a monkey's most sensitive retinal ganglion cells can.

This system carries another major advantage in addition to efficiency. We see most objects by reflected light, from sources such as the sun or a light bulb. Despite changes in the intensity of these sources, our visual system preserves to a remarkable degree the appearance of objects. The retinal ganglion cell works to make this possible. Consider the following example: a newspaper looks roughly the same—white paper, black letters—whether we view it in a dimly lit room or out on a beach on a sunny day. Suppose, in each of these two situations, we measure the light coming to our eyes from the white paper and from one of the black letters of the headline. In the following table you can read the figures I got just by going out into the sun in the Harvard Medical School quadrangle:

	Outdoors	Room
White paper	120	6.0
Black letter	12	0.6

The figures by themselves are perfectly plausible. The light outside is evidently twenty times as bright as the light in the room, and the black letters reflect about one-tenth the light that white paper does. But the figures, the first time you see them, are nevertheless amazing, for they tell us that the black letter outdoors sends twice as much light to our eyes as white paper under room lights. Clearly, the appearance of black and white is not a function of the amount of light an object reflects. The important thing is the amount of light *relative* to the amount reflected by surrounding objects.

A black-and-white television set, turned off, in a normally lit room, is white or greyish white. The engineer supplies electronic mechanisms for making the screen brighter but not for making it darker, and regardless of how it looks when turned off, no part of it will ever send *less* light when it is turned on. We nevertheless know very well that it is capable of giving us nice rich blacks. The blackest part of a television picture is sending to our eyes at least the same amount of light as it sends when the set is turned off. The conclusion from all this is that "black" and "white" are more than physical concepts; they are biological terms, the result of a computation done by our retina and brain on the visual scene.

As we will see in Chapter 8, the entire argument I have made here concerning black and white applies also to color. The color of an object is determined not just by the light coming from it, but also—and to just as important a degree as in the case of black and white—by the light coming from the rest of the scene. As a result, what we see becomes independent not only of the intensity of the light source, but also of its exact wavelength composition. And again, this is done in the interests of preserving the appearance of a scene despite marked changes in the intensity or spectral composition of the light source.

CONCLUSION

The output of the eye, after two or three synapses, contains information that is far more sophisticated than the punctate representation of the world encoded in the rods and cones. What is especially interesting to me is the unexpectedness of the results, as reflected in the failure of anyone before Kuffler to guess that something like center-surround receptive fields could exist or that the optic nerve would virtually ignore anything so boring as diffuse-light levels. By the same token, no one made any guesses that even closely approximated what was to come at the next levels along the path—in the brain. It is this unpredictability that makes the brain fascinating—that plus the ingenuity of its workings once we have uncovered them.