The Architecture of Functional Neural Circuits in the Vertebrate Retina

The Proctor Lecture

Helga Kolb

Understanding the organization of the vertebrate retina has been the goal of many talented visual scientists during the past 30 years. With Cajal's (1892) anatomic descriptions of the cell types that constitute the retina in a number of vertebrate species, and with an early understanding of the role of visual purple in photochemistry in combination with psychophysical studies of adaptation and color vision, we had in the sixties the rudiments of an understanding of how the retina might be organized and functioning. To go further, though, we were beginning to need detailed information of neural circuits that underlay these functions. It was the advent of electron microscopy, microelectrode recording techniques, and pharmacology that then allowed us an era of very rapid advancement. The purpose of this presentation is to summarize these recent advances and to describe our present understanding, based primarily on anatomic investigations, of the underlying architecture of four important functional circuits in the vertebrate retina.

The retina is formed during development from an outpouching of the brain that becomes encased in the protective and nourishing eyeball layers behind the focusing structures of the pupil and lens (see refs. 1 and 3 for a review). Light rays forming images of the outside world directly impinge upon this piece of the brain known as the retina, where they stimulate first the sensory rods and cones and then a cascade of neurons to create an output pattern of neural activity in the ganglion cells. All vertebrate retinas are similarly organized into three layers of neurons and two areas of neuropil. Each vertebrate species has some kind of topographic specialization of the retina unique to the species, such as a fovea or a visual streak, predominance of rod or cone photoreceptors, or presence or absence of cone types specific for certain wavelength of stimulation, but the basic cell layers and neuropils are constant. In a vertical section of the human retina as shown in Figure 1, we can see that the retina is a highly complex, layered structure containing millions of closely packed nerve cells. The retina is actually organized in reverse from a way that might be intuitive. The photosensitive elements are not the first structures to be hit by the image. Instead, light rays have to pass through the whole thickness of the retina before stimulating the rods and cones, and, in contrast, the output message to the brain can only exit the retina from the most superficial cell layer containing the ganglion cells.

A naive understanding of the retina emphasizes only the sensory photoreceptors and the ganglion cells; however, it is evident on deeper probing that there are many interneurons packed into the central nuclear layer here (Fig. 1) and the neuropil layers, particularly the inner plexiform layer (IPL), are thick and composed of a tangle of interconnecting neural profiles. The first area of neuropil is the outer plexiform layer (OPL), where connections between rod and cones and vertically running bipolar cells and horizontally oriented horizontal cells occur. The second neuropil of the retina, the IPL, functions as a relay station for the vertical information-carrying nerve cells, the bipolar cells, to connect to ganglion cells. Moreover, a multitude of different varieties of horizontally and vertically directed amacrine cells somehow interact in further networks to influence and integrate the ganglion cell signals (Fig. 1). It is only at the culmination of all this neural processing that the message concerning the visual image is transmitted to the brain along the optic nerve.

The central question for many of us concerns what kinds of interaction are going on between the cells of the layers of the retina before the visual message is sent to the brain. Clearly, the retina is more than just photoreceptors and ganglion cells, and we need to understand what circuits of neurons are interposed between the photoreceptors and the optic nerve and how they are organized for functional roles.
TECHNIQUES THAT HAVE BEEN USED TO UNDERSTAND NEURAL PATHWAYS IN THE RETINA

In 1892, the great Spanish anatomist Ramon y Cajal first described many of the different nerve cells that make up these neural layers of the retina and contribute processes to the synaptic neuropils. He made beautiful drawings and descriptions of the different nerve cells in a variety of vertebrate retinas, after staining with a technique invented by Italian anatomist, Camillo Golgi. The Golgi silver staining technique had the advantage over other silver techniques in staining individual nerve cells in isolation from surrounding nerve cells. Also, it stains the complete cell throughout its finest dendritic and axonal processes. Cajal was the first to state that bipolar cells of the retina were involved in rod or cone channels of information through...
the vertical pathways and that horizontal and amacr ine cells were involved in lateral interactions. He introduced the idea that there were specific connections between specific cell types in the plexiform layers by virtue of stratification patterns of axons and dendrites in the neuropils.

Following in the footsteps of Cajal and Steven Polyak, who worked on the primate retina, more recent Golgi studies have described the different nerve cell types in retinas that are receiving attention from physiologists and where intracellular recordings can be made. 

Cajal's and Polyak's images and classifications of cell types were comprehensive and were often done on retinas that were good experimental models, too; however, they were made from views of vertically sectioned retina. Unfortunately, vertical sections can only give us a limited view of stained neurons because their complete dendritic trees are usually wider than section thicknesses. We need to understand full dendritic tree dimensions and details of dendritic morphology that could indicate synaptic interactions in networks of neurons. This is where wholemount Golgi staining techniques, developed in several laboratories at about the same time, were a great improvement. Some of the cell types seen by Golgi staining in wholemounted cat retina are illustrated in Figure 2. It is obvious that such views give us complete information on dendritic tree expanse and allow us to classify cells with greater ease. For example, cells A1, A2, A11, and A8 have much smaller dendritic trees than cell A17 (Fig. 2). In addition, such wholemount views provide information on characteristics of branching frequency and dendritic field coverage that can be measured either subjectively or objectively with computer-aided microscopy. For example, the ganglion cells labeled alpha and beta in Figure 2 have more bushy branching patterns than the cell labeled G19.

An overview of the whole cell, as is best seen in wholemounts, has been particularly helpful in revealing the differences between three important ganglion cell types of the human retina that are involved with spatial and color vision, namely, the midget ganglion cells, the small parasol ganglion cells, and the large parasol ganglion cells. It is only when the these three cell types are Golgi stained close together in the same area of retina that we see that midget and small parasol cells are two distinct types. Figure 3 shows clearly that the midget ganglion cell has an 18 μm diameter dendritic tree compared with the small parasol, which is 35 μm in diameter. In contrast, the large parasol cell, lying close by the two previous cells, has an 85 μm dendritic tree span (Fig. 3). Other authors have, since Polyak's time, confused the midget and small parasol cell types and lumped them together as "midget" ganglion cells. However, the latter classification scheme is too broad a generalization that does not consider functional morphology, a topic we shall return to later in this paper.

A very important aspect of neural architecture in the retina is the arrangement of the neurons' dendrites and axons in a stratified manner. This is best appreciated by through-focusing on stained cells in wholemount preparations or by viewing stained cells in vertical sections. This aspect of neuronal morphology, which had been so emphasized by Cajal, was unfortunately neglected in some of the following decades. It was only when the significance of stratification involving midget bipolar cell axons and midget ganglion cell dendrites in monkey retina was appreciated (which we shall discuss later in this paper) that we began to look at all retinas, particularly in the IPL, for stratification patterns for all neurons again. Thus, we saw that ganglion cells in the cat retina had specific stratification levels to put their dendrites into contact with particular stratified bipolar cell axons and amacrine cell dendrites (Fig. 4). These particular ganglion cells (Fig. 4), now known as beta cell types, come as paramorphic pairs. One variety branches only in distal IPL (area labeled "a"), whereas the other restricts its branches to proximal IPL (area labeled "b"), thereby allowing only synaptic interactions between the beta a type and the flat bipolar and between beta b types and the invagination bipolar (Fig. 4).

From an understanding of the different nerve cell types that make up the retina, plus an understanding of where their dendritic trees lie in relationship to each other by virtue of stratification in the IPL, we can begin to make guesses as to which cells could interact. However, seeing stained cell processes lying close together does not necessarily mean that they make synaptic contacts. The only method for determining synaptic interaction unequivocally is to study the neuropils of the retina by electron microscopy (EM).

EM has revealed differences between many of the neural elements of the retina on cytological criteria. Thus, we have been able to distinguish rod photoreceptors from cone photoreceptors by differences in outer segment morphology and differences in structure of their synaptic endings in the OPL. Rod spherules can be distinguished by their content of a single synaptic ribbon and a single complex of invaginated profiles where they communicate with bipolar and horizontal cells. Cone pedicles, on the other hand, clearly have larger synaptic endings that contain many synaptic ribbons and invaginated profiles of second-order neurons.

In the IPL where bipolar cells contact ganglion and amacrine cells, we learned, originally from Missotten's and Dowling's and Boycott's elegant studies that bipolar cells can be recognized by the presence of ribbons at their synapses (Fig. 5, rod BP, cone BP), and amacrine cells can be recognized by the pres-
FIGURE 2. Camera lucida drawings of Golgi-impregnated neurons of the cat retina as seen in wholemount views. A1 and A2 are narrow-field amacrine cells, and A7/AII and A8 are bistratified narrow-field types. A17, by contrast is a wide-field diffuse amacrine cell. α, β, and δ (G19) are ganglion cells types. Small numbers or a/b refer to the strata or sublamina, respectively, of the IPL in which the cell’s dendrites ramify. Scale bar, 25 μm.
FIGURE 3. Light micrographs of each of the three major ganglion cell types of the human retina as impregnated by Golgi procedures. (a) The large parasol cell (lpgc) is in the diffuse cone system and has a large-diameter dendritic tree. (b) Midget ganglion cell (mgc) is a single cone/midget bipolar-connected ganglion cell with a very small dendritic tree. (c) The small parasol cell (spgc) has an intermediate dendritic tree diameter and is in the diffuse cone system. Scale bar, 25 μm.

FIGURE 4. Camera lucida drawings of Golgi-impregnated neurons in the cat retina as seen in vertical section to show stratification of axons and dendrites in the IPL. βa ganglion cell has dendrites branching in distal IPL (sublamina a) to overlap the axon branches of FB bipolar cell. βb ganglion cells has lower branching dendrites in proximal IPL (sublamina b) to contact axon branches of IB bipolar cell. Bipolar cell RB ends in proximal IPL (sublamina b) overlapping (small arrows) the lower strata of dendrites of a narrow-field bistratified amacrine cell AII. Scale bar, 25 μm.
FIGURE 5. Electron micrograph of neural profiles in the IPL. Rod bipolar (rod BP) and cone bipolar (cone BP) axon terminals make synapses at synaptic ribbons (r) to two postsynaptic profiles which are either amacrine (A) or ganglion cell (G) dendrites. Frequently, amacrine processes make a feedback or reciprocal synapse to the bipolar cell (arrowhead). All amacrine cells communicate with cone bipolar cell axons by means of gap junctions (open arrows). Scale bar, 0.5 μm.

ence of clusters of synaptic vesicles at a dense membrane specialization in conventional synapses (Fig. 5, A17). We also found that certain amacrine cells and, in particular, the one labeled A11 in Figure 5, communicates by electrical synapses, morphologically described as gap junctions, with neighboring neuronal profiles (in this case A11 engages in a gap junction, open arrows, with a cone bipolar axon, Fig. 5, cone BP). Interpreting these EM images thus allows us to recognize the various general classes of neurons such as bipolar cell, amacrine cell, and ganglion cell and their synapses. However, they do not tell us exactly what types of bipolar, amacrine, or ganglion cells are involved in these interactions.

A better method for resolving this quandary is to examine Golgi-stained neurons by EM. This technique was first used in the goldfish retina by Stell(8) to determine photoreceptor contacts of Golgi-stained bipolar and horizontal cells in the OPL.(9,11,13,19,27-32) In the primate retina using the same technique, we learned that rod bipolar cells contacted only rod photoreceptors and that cone bipolar cells contacted only cones (both Cajal and Polyak had thought it possible that some bipolar cell types in mammalian and primate retinas might be mixed, contacting both rods and cones, as in nonmammalian retinas). Furthermore, we saw that rod bipolar dendrites penetrated the rod spherule to end only at synaptic ribbons in central invaginating positions in mammalian retina, whereas cone bipolar cell dendrites made synaptic contacts with cones in one of two ways.

As shown in Figure 6, EM of Golgi-impregnated midget bipolar cell types in monkey retina indicated that one variety, called invaginating midget bipolar, made central invaginating contacts to get their dendrites close to the synaptic ribbons in the cone triads (Figs. 6a, 6b, imb). The other variety, called flat midget bipolar (Figs. 6a, 6c, fmb), had dendritic terminals ending at basal junctions on the surface of the cone pedicle away from the ribbon. Another important finding, confirming Stell's and Missotten's studies,(8,24,26) was that horizontal cells made synaptic contacts with rods and cones as lateral elements at the synaptic ribbon triads in monkey retina (Fig. 6a, H).

It turns out that this arrangement of horizontal cell dendrites on either side of the ribbon synapse in photoreceptors, plus the bipolar cell dendrites forming the central or basal junction position, is very important. A small local circuit is formed here that influences the flow of information throughout the whole retina (Fig. 7). We know that the photoreceptor neurotransmitter, thought to be glutamate, is released in the dark in the vertebrate retina1,30,34 (for reviews). When a light stimulates the photoreceptor, transmitter release ceases and the photoreceptor responds with a
FIGURE 6. (a) Electron micrograph of synaptic contacts (known as triads) in a cone pedicle of monkey retina. Second-order neurons contact cones as invaginating central elements (imb) at basal junctions (fmb, arrows) or as lateral elements (H) to the synaptic ribbon. (b) A single dendritic terminal of the imb (Golgi-stained profile) projects into the central position of the ribbon triad. (c) Two Golgi-stained fmb terminals end on the cone pedicle surface at basal junctions (arrows) on either side of the central invaginating dendrite at the ribbon triad. Scale bar, 0.5 μm.

slow, sustained hyperpolarizing response (Fig. 7b), but the postsynaptic bipolar cells respond with either slow, sustained hyperpolarization (Fig. 7c) or slow, sustained depolarization (Fig. 7d) of the membrane. The horizontal cells respond, too, with slow, sustained hyperpolarizations (S-potentials), but a lateral inhibitory feedback synapse from the horizontal cell to the photoreceptor is also thought to occur. The feedback signal consists of summed information from a network of horizontal cells connected over a wide spatial area of the OPL. Thus, this large spatial area influences both the photoreceptor and the bipolar cell to include an inhibitory response coming from a surround region of retina. This local circuit provides the bipolar cell with a spatially antagonistic receptive field in a center-surround organization.

A similar local circuit at the bipolar to amacrine or ganglion cell synapse occurs in the IPL. In Figure 5, we see the manner in which rod and cone bipolars are presynaptic at ribbon synapses to two postsynaptic elements. One of the postsynaptic amacrine cell profiles in each case makes a feedback (reciprocal) synapse to the bipolar cell (Fig. 5, arrowheads) in the same strategic position that the horizontal cell dendrite does at the photoreceptor ribbon synapse. Some researchers interpret this to indicate a possible similarity in function. Thus, it has been suggested that another surround mechanism coming from the amacrine cells could be added at this synapse in the IPL.

A real understanding of even small local circuits, such as those illustrated in Figures 5 and 7, are the result of large-scale serial section analyses. By making tracings of serial electron micrographs, we can reconstruct portions of retinal cells or even the complete dendritic tree of a cell when it is small enough, to study all the synaptic input and output relationships. One of our earliest reconstructions in the cat retina was of an All amacrine cell. This small-field amacrine cell type could be reconstructed from its cell body through its primary dendrites and into the finest dendritic terminals. All amacrine cells have a bistratified morphology (Figs. 4 and 8, All), with their distal fine dendrites...
FIGURE 7. (a) Electron micrograph of the ribbon triad in a cone pedicle in the monkey retina. (b) The cone itself gives a hyperpolarizing slow potential response to a light flash. (c) The bipolar that invaginates to the cone ribbon gives a depolarizing slow potential response to a light flash. (d) The bipolar that contacts the cone at a basal junction gives a hyperpolarizing slow potential response to a light flash. H, horizontal cell dendrites; imb, invaginating midget bipolar cell; fmb, flat midget bipolar cell; curved arrows, possible sites of horizontal cell feed back synapses. Scale bar, 0.5 μm.

postsynaptic to rod bipolar cell axons (rb) and their proximal lobular appendages making chemical synapses to ganglion cell dendrites (Fig. 8, synapses to GCa). Gap junctions join the larger AII dendrites with cone bipolar axons (Fig. 8, large black spots, gj to cb). By means of the latter contact, rod-driven information passes through the AII channel to the cone bipolar (cb), and thence to the ganglion cell that the cone bipolar synapses upon (Fig. 8, GCB). All amacrine cells are now recognized as being pivotal components of the rod system pathways in all mammalian retinas thus far studied, including human retina.64,47-51

Another technique that we made use of during the last decade is anatomic investigation of intracellularly recorded and horseradish peroxidase-stained (HRP) neurons. This technique, which was first used in the retina by Lasansky,52 allows us to determine physiological responses of a neuron to light stimulation, and then study the synaptic relationships of the same electron-dense marked neuron by EM. It has been used with great success in turtle, fish, rabbit, cat, and even in monkey retina53 (for review). Figure 9 shows the morphology and synaptology of a small ganglion cell in the cat retina, known as a beta cell, that has been stained with intracellular iontophoresis of HRP. Its appearance by light microscopy in a vertical thick section is shown (Fig. 9a). The cell's light response consists of a burst of spikes at light OFF: it is thus an OFF-center physiological type54 (Fig. 9a).

When the stained beta cell is sectioned for EM, the
FIGURE 8. Drawing of a three-dimensional reconstruction from serial electron micrographs of an AH amacrine cell and its synaptic relationships with rod (rb) and cone bipolar cells (cb) and ganglion cells (GCa, GCb) in the IPL of the mammalian retina. gj, gap junction. See text for further details.

electron-dense HRP deposit throughout the cell allows it to be readily picked out and its synaptic input studied. In this case, synaptic input comes from cone bipolar cells (Fig. 9b), the AH amacrine cell (Fig. 9c), and some other amacrine cell types that we call A8 and A2 or A3 (Figs. 9d, 9e). It was possible to reconstruct the complete dendritic tree of this relatively small beta ganglion cell (Fig. 9a), enabling us to evaluate the total number and distribution of bipolar and amacrine cells impinging on it. We calculated that this small, central beta cell in cat retina received about 1000 synapses contributed by 12 to 14 bipolar cells, 7 to 10 AH amacrine cells, and 28 to 41 other amacrine cell types.

Based on what we have learned from using the techniques just described, we are now in a position to understand the architecture of several functional circuits in the vertebrate retina. The remainder of this paper will summarize what we currently consider to be the organization of (1) circuitry of rod pathways underlying night vision, (2) circuits mediating successive contrast otherwise known as ON and OFF pathways, (3) circuits mediating simultaneous contrast or center-surround organization and, finally, (4) circuitry of red and green color-opponent pathways in human retina.

CIRCUITS OF ROD PATHWAYS UNDERLYING NIGHT VISION

EM studies of bipolar cell connections with photoreceptors in monkey and cat retinas revealed that rod pathways through the retina were differently wired than were cone pathways. Rod pathways involve huge convergences and divergences of cell contacts. They start with many rods converging information to the rod bipolar cell in the OPL. Rod responses are carried to the next stage of processing in the IPL, where divergence occurs, to several different amacrine cell types, the most important of which are the AH and the A17 cells. Rod bipolar cells do not contact ganglion cells directly. Instead, they contact amacrine cells, which spread out the rod information before converging on ganglion cells. Two other cell types are also influential upon the rod pathways. These are the dopaminergic amacrine and the GABAergic...
FIGURE 9. Electron microscopy of the synapses upon an HRP and intracellularly recorded \( \beta a \) ganglion cell in the cat retina. (a) Camera lucida drawing of the HRP ganglion cell in vertical section. The cell's dendrites branch in sublamina a. The cell's light response is a depolarization and burst of spikes at the "off" of the light (black bar). Scale bar, 30 \( \mu m \). (b) Cone bipolar input (CB) recognizable by the synaptic ribbon, occurs to the HRP-stained beta cell dendrite. All amacrine cells make a typical punctate synapse (arrows) upon the HRP-stained dendrite. (d) A dark A8 amacrine cell profile and (e) paler A2–A3 profiles also make synapses upon the HRP-stained ganglion cell (arrow). Scale bar, 0.5 \( \mu m \).
gic interplexiform cell. They may modulate and feed back and forth rod signals to further amplify and reset levels of dark adaptation between the input in the OPL and output of the system in the IPL.

The huge convergence of the rod system has recently been calculated by Sterling and coauthors and Kolb and Nelson. Thus, about 1500 rods have input to a single small-field ON-beta ganglion cell via 100 rod bipolars, five All amacrine cells, and four cone bipolar axons. In the case of a large-field OFF-alpha ganglion cell, 75,000 rods drive 5000 rod bipolars and 250 All amacrine cells before converging on the ganglion cell. In terms of divergence of the rod system, we know that a single rod photoreceptor passes input from the All amacrine cell lobular appendages to two ON-beta cells. So it is that divergent and then convergent circuitry provides pooling and amplification of the rod signal in very low light levels to allow our visual system to be sensitive to a single quantum of light.

Combining the physiological responses we now understand for the neurons of the rod system in the cat retina, with their anatomic connections, we can construct a summary diagram (Fig. 10). Signals from hyperpolarizing rod photoreceptors depolarize rod bipolar cells (Fig. 10, rb, physiological response to the left). Rod bipolar cells synapse upon two depolarizing amacrine cells, the wide-field A17 (Fig. 10, A17, response to the left) and the small, bistriated All amacrine cells (Fig. 10, AII, response to the right). Rod signals reach OFF-center ganglion cells (Fig. 10, a-type beta GC) via chemical synaptic input from the All amacrine cell lobular appendages (Fig. 10, boxed region a). ON-center ganglion cells (Fig. 10, b-type beta GC) receive rod signals, via All amacrine cell electrical synapses at gap junctions to cone bipolar cells (Fig. 10, ibc, boxed region b). The latter cone bipolars stimulate the ON-center ganglion cells by excitatory chemical synapses. Dopaminergic amacrine cells (Fig. 10, DA) influence the rod pathways by chemical synapses in the IPL upon All and A17 amacrine cells and via an internuncial GABA-ergic interplexiform cell, which in turn synapses upon rod bipolar cells in the OPL (Fig. 10, IPC).

CIRCUITS MEDIATING SUCCESSIVE CONTRAST (ON AND OFF PATHWAYS)

Cone pathways in mammals and humans run as two parallel streams of information directly from the cone photoreceptor to the ganglion cell through the straight pipeline, the cone bipolar cell. What is the reason for two parallel channels for the cone system when the rod system had only one? The answer is that this organization allows one channel to provide information to the ganglion cell concerning brighter-than-background stimuli (the ON-center channel) and the other, darker-than-background stimuli (the OFF-center channel). This is the basis of successive contrast in visual perception.

The anatomic substrate for the origins of these two important ON-center and OFF-center channels in the bipolar cell was discovered to be the types of synaptic contacts midget cone bipolars made with cone pedicles in the monkey retina. Thus, an EM study of the Golgi-impregnated midget bipolar cells had described one type of midget bipolar cell making invaginating contacts and another making basal contacts with the cone pedicle (summarized in Fig. 6). Rod bipolar dendrites were known to make only the invaginating type of contact with rod spherules. It is now thought that in the monkey retina, postsynaptic midget bipolar cells respond to light just like the photoreceptor with a slow hyperpolarization when the bipolar dendrite ends at a basal junction. In contrast, the other type of midget bipolar cell, that invaginates to the ribbon synapse, responds to light with an inverted sign compared with the cone. It gives a slow depolarizing response. Thus, the nature of the postsynaptic membrane channels on the cone bipolar cell dendrite is the important designator of the response sign the bipolar will have. The hyperpolarizing bipolar types are the start of OFF-center channels, and the depolarizing types are the start of ON-center channels through the retina.

The same difference of contacts with cones has been found for flat and invaginating diffuse cone bipolar cell in the cat retina, too. In other words, it is not just a trait of the midget bipolar system in primates. At the level of information transfer between cone bipolar cells and ganglion cells in the IPL, only excitatory channels are present. Therefore, the type of signal transmitted by the ganglion cell, as either ON- or OFF-center, is essentially determined by the nature of the cone bipolar cells contacting it.

To keep the ON and OFF channels separate through the ganglion cells to the brain, the IPL is essentially divided into two functionally discrete sublaminae, called a (the two strata of the IPL below the amacrine cell bodies) and b (the other three strata of the IPL stretching to the ganglion cell bodies) (Fig. 4). Interactions are only allowed between basal-contacting cone bipolars and one set of ganglion cells in sublamina a (Fig. 4, FB and βa ganglion cells), whereas...
invaginating-contacting cone bipolar cells can only interact with another set of ganglion cells branching in sublamina b (see Fig. 4, IB and βh ganglion cells). Gouras was the first to suggest that this specificity of bipolar-to-ganglion cell contacts underlay ON-center and OFF-center midget ganglion cell responses in monkey. Later, Nelson conclusively proved this hypothesis by means of intracellular recording and marking experiments in ganglion cells of cat. Figure 11 shows the intracellular recordings from two different ganglion cells of Nelson and coauthors' study. One cell proves to be ON-center, giving a burst of spikes riding on a depolarization of the membrane as soon as the light flash goes on (Fig. 11). In contrast, the other cell is OFF-center, giving a hyperpolarization to the membrane when the light flash is on but a burst of spikes riding on the depolarization when the light flash is over (Fig. 11). The ON-center ganglion cell has dendrites restricted to branching in sublamina b. The OFF-center cell has dendrites reaching higher to branch only in sublamina a (Fig. 11).

A summary of the manner in which the cone pathways carry the message concerning brightness and darkness through the retina in the cat is shown in Figure 12. Cones hyperpolarize to light, but two bipolar channels, one carried by a depolarizing bipolar (Fig. 12, ibc and light response) and the other by a hyperpolarizing bipolar (Fig. 12, fbc and light response), split the original cone signal into lightness or ON-center and darkness or OFF-center. These bipolar responses are transmitted directly to ganglion cells architecturally separated to the different sublaminae of the IPL, resulting in one channel of ganglion cells with dendrites in proximal IPL (sublamina b) becoming ON-center and the other type with dendrites only in distal IPL (sublamina a) becoming OFF-center (Fig. 12).

CIRCUITS MEDIATING SIMULTANEOUS CONTRAST (CENTER-SURROUND RECEPTIVE FIELDS)

Information concerning the overall brightness or darkness of the image is of primary importance for visual sensation, but putting them in simultaneous contrast to each other greatly improves the resolution of the
FIGURE 11. Camera lucida drawings of two ganglion cells that have been intracellularly recorded and stained. The ganglion cell (GC) to the left gives an ON-center burst of spikes to a light flash, and its dendrites branch in sublamina b of the IPL. The ganglion cell (GC) to the right gives an OFF-center response to light, and its dendrites branch in sublamina a. Scale bar, 35 μm and 10 mV.

FIGURE 12. Summary diagram of the neurons and their physiological responses to light for the diffuse cone pathways responsible for successive contrast (ON and OFF) pathways in the mammalian retina. ibc, invaginating cone bipolar cell; fbc, flat cone bipolar cell; a-type and b-type GC; OFF-center and ON-center ganglion cells, respectively. See text for full description.
Simultaneous contrast is achieved by lateral inhibition where a dark boundary inhibits a light area or vice versa. In the retina, lateral inhibition creates a concentric (center-surround) arrangement of the cell’s receptive field. High acuity and color channels particularly need to have a center-surround organization.

It is thought that horizontal cells at the OPL provide, through a mechanism of lateral inhibition, a surround arranged around the receptive field center of the bipolar cell response. The wiring responsible appears to start at the small, local circuit we saw in the cone triads at the ribbon synapses (Fig. 7). Thus, the negative feedback synapse of the horizontal cell to the cone photoreceptor at the ribbon triad synapse (Fig. 7, curved arrows) allows the larger receptive field of the coupled horizontal cell network to provide a surround to the narrow-field central cone response. This concentric organization is then transmitted to the bipolar cells making contact with the cone. Figure 13 summarizes the architecture for center surround organization. The center pathway is created by the cone to bipolar-to-ganglion cell channel, whereas the injection of horizontal cell information provides an antagonistic surround to the center: an OFF-surround for the ON-center channel (Fig. 13, horizontal cell, and ibc, lefthand pathway) and an ON-surround for the OFF-center channel (Fig. 13, horizontal cell, and fbc, righthand pathway). In cat retina, surround responses are not as strong a component of bipolar cell receptive field as they are in cold-blooded vertebrate bipolars. However, surround responses are strong in cat ganglion cells. Thus, it has recently been suggested that an additional surround is constructed by certain amacrine cells in the IPL in this and other species.

**CIRCUITS UNDERLYING RED AND GREEN COLOR OPPONENCY IN THE HUMAN RETINA**

Color vision is based on the occurrence of at least three different types of cone photoreceptor in the retina, each with a different sensitivity of its visual pigment to different parts of the spectrum. Thus, there are blue-sensitive, green-sensitive, and red-sensitive cones. It appears certain, then, that three types of cone are the start of color-specific channels in the primate retina.
From the time of Polyak, we have known that primate retinas have a unique set of neurons devoted to single cone, high-acuity, and color-opponent channels not found in species with poor color vision, such as the cat. The diffuse cone channel bipolar and ganglion cells are, of course, also present in primate retinas. The latter ganglion cells are the small and large parasol ganglion cells mentioned earlier in this paper (Fig. 3). The exclusive primate midget bipolar and midget ganglion cells have such tiny dendritic trees that they fit single cone pedicles, in the case of midget bipolar cells, and single midget bipolar axons in the case of midget ganglion cells. Because these midget channels are related to a single cone in the foveal region of the primate retina, and the individual cones are of specific wavelength-prefering types, we know that a single color preference is carried by each midget system neuron throughout the retina.

The organization of color channels in foveal ganglion cells of the monkey retina was described by Gouras in 1968. The receptive fields of midget ganglion cells fell into two basic types. They were ON- or OFF-center responding and could be stimulated most strongly by either red or green light. Thus, midget ganglion cells were red ON or OFF or green ON or OFF types. Ganglion cells sensitive to blue were also seen, but they appeared to be different and had larger receptive field characteristics. The red and green midget ganglion cells demonstrated sensitivity in their surround to the complementary color of their centers. This made them color opponent. Thus, red center ganglion cells could be red ON-center/green OFF-surround or red OFF-center/green ON-surround. The same organization prevailed for green center ganglion cells. Blue center ganglion cells appeared to have both larger receptive fields and coextensive yellow surrounds. Blue-carrying channels do not seem, therefore, to be midget channels.

We think that red and green channels are organized through the retina in the manner shown in Figure 14. A red cone would contact two red midget bipolars and through them, two red midget ganglion cells. For example, a red ON-center midget bipolar would contact a single red cone at invaginating synapses in the OPL, and a single red ON-center midget ganglion cell in sublamina b of the IPL. The same red cone would also be contacted by a single red OFF-center midget bipolar at basal junctions, and the bipolar would, in turn, contact a single red OFF-center midget ganglion cell in sublamina a of the IPL (Fig. 14). Thus, red ON- and red OFF-center receptive field ganglion cell types are generated (Fig. 14, red + center and orange − center, left). A green cone would be connected in a similar manner to two green midget bipolars (ON and OFF types) and two midget ganglion cells, which would also be ON- and OFF-center receptive field types (Fig. 14, dark green + center and light green − center, right). Horizontal cells and small-field amacrine cells are thought to provide the spatially and spectrally antagonist surround response to the four types of receptive field centers (Fig. 14, green and red annuli around the centers).

Two of the three types of horizontal cell could make green surrounds to red center bipolars and red surrounds to green center bipolar cells (Fig. 14, HII and III). In addition, red surrounds for a green center ganglion cell might be provided by an amacrine that collects information from a cluster of red-driven midget bipolar cells to pass to a green-driven midget bipolar/midget ganglion cell synaptic complex (Fig. 14, red and orange a). Of course, the opposite of a green-driven amacrine cell providing green surrounds to red center bipolar and ganglion cells could also occur.

ARCHITECTURE OF FUNCTIONAL PATHWAYS IN THE HUMAN RETINA

During the past 25 years, we have had an explosion in our knowledge concerning the architecture of many of the functional pathways underlying processing of the visual image in the retina. Most of the new knowledge comes from anatomic and electrophysiological investigation of animal models, particularly from investigations of the cat and monkey retinas. Anatomic investigations of human retina have progressed more slowly for obvious reasons, but these investigations have consistently shown that the neurons composing the human retina are so similar in morphology and architecture that we are safe in assuming that they fit into the same circuits we have elucidated in the cat or monkey retina. The culmination of the present knowledge concerning cat and monkey retinal organization as applied to the human retina is shown in the final color schematic of this paper (Fig. 15).

This figure depicts a vertical section of human retina containing the cell types and major pathways that have been discussed in this paper. At night, dim light information flows from rods to several amacrines, chief among them the small-field bistratified AIIIs and the wide-field A17s. OFF-center ganglion cells are driven by direct synaptic input from A17 cells and ON-center ganglion cells by indirect gap-junction mediated AII to cone bipolar inputs (Fig. 15, AII to di to ON-center g). In daylight, diffuse cone pathways consist of cones contacting two straight-through channels of bipolar cells (di and df) to ON-center and OFF-center ganglion cells. ON- and OFF-center responses of the ganglion cells are determined by specific cone bipolar contacts with cone pedicles, and separation of synaptic interactions between bipolars and ganglion cells to the correct sublamina of the
FIGURE 14. Summary diagram of the neurons and their receptive fields that are involved in red and green color opponency in the human retina. Lighter colored neurons are ON-center carrying channels and darker colored neurons are OFF-center carrying channels. See text for a full description. HI, HII, and HIlll, three horizontal cell types; mb, midget bipolar cells; a, small-field amacrine cells; mgc, midget ganglion cells.

FIGURE 15. Summary diagram of a schematic human retina with the neurons of the four major pathways described in this paper shown: (1) night vision [red] pathways, (2) successive contrast [ON and OFF] pathways, (3) simultaneous contrast [center-surround] pathways, and (4) red and green color-opponent and spatially opponent pathways. Blue pathway is also shown. c, cones; r, rod; HC, horizontal cell; rb, rod bipolar cell; bb, blue-cone bipolar cell; fm, flat midget bipolar cell; im, invaginating midget bipolar cell; di, diffuse invaginating cone bipolar cell; df, diffuse flat cone bipolar cell; A17, A17 amacrine cell (rod system); AII, AII bistratified rod amacrine cell; A8, A8 amacrine cell, rod and cone system amacrine cell; sfa, small field amacrine cell; wfa, wide-field amacrine cell; IPC, interplexiform cell; mg, midget ganglion cell; SPg, small parasol ganglion cell; LPg, large parasol ganglion cell. ON and OFF, example ON-center and OFF-center ganglion cells of the small parasol types. See text for a full description.

IPL. Spatially opponent surrounds to the ganglion cell responses are provided by horizontal cells (HC) in the OPL and possibly by amacrine cells (for example, A8) in the IPL. Color-opponent pathways for red and green are via two the midget bipolar cells (im and fm) and their specific connections with ON- and OFF-center midget ganglion cells (mg). Chromatically opponent surrounds could be provided at the OPL by horizontal cells (HC) and at the IPL by small field amacrine cell circuits (sfa). Wide-field amacrine cells (wfa) and interplexiform cells (IPC) are some of the many other interneurons in the retina probably responsible for other integrations, such as coding movement in the periphery and for resetting the retinal pathways in different states of adaptation.

More pathways than can be described in the present paper are being discovered at a rapid rate. But so far we only understand some of the simplest pathways and in very simple terms. Undoubtedly, many more details will be resolved in the future, and the day is not too far off when we will understand how this myriad of neural circuitry in the retina codes all the information presented in the visual scene.

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References


86. Maxwell JC. Experiments on colour, perceived by the eye, with remarks on colour blindness. Edinb Trans. 1855.
92. Boycott BB, Wüssel H. Morphological classification of


