Principles and Practice of Clinical Electrophysiology of Vision

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PART II

Principles of Retinal Cell Biology

5

The Neural Organization of the Human Retina

Helga Kolb

The human retina is a marvelously constructed piece of neural tissue consisting of millions of neurons arranged within an intricate, layered architecture. Exquisitely sensitive photoreceptors are capable of responding to individual photons of light and interpreting this message for transmission to second- and third-order arrays of neurons organized into pathways specific for particular stimuli in our visual world. Yet, the whole retina is a thin sheet of tissue (less than a quarter of a millimeter thick) lining the back of the eye and composed of these millions of highly organized neurons and circuits and a complement of glial cells to provide architectural stability and the physiological milieu essentially for the functioning of the neurons. Running throughout this delicate neural network is the life-giving support system of blood vessels and capillaries, while many metabolic needs are supplied by the retinal pigment epithelium.

A view into an enucleated eye with the anterior part of the eye removed and the eyecup cut in half and flattened to an open position is shown in Figure 5–1.[86] This is a right eye with the longitudinal cut passing vertically through the optic nerve head. The optic nerve head (papilla, disc) lies in the center of the cut and is a circular to oval white area measuring about 2 × 1.5 mm across. From the center of the optic disc radiate the major blood vessels of the retina. Approximately 17 degrees (4.5 to 5 mm) or 2½ disc diameters to the left of the disc can be seen the slightly oval shaped, blood vessel–free spot, the fovea, the center of the macula area familiar to ophthalmologists. A circular field of approximately 6 mm around the fovea is considered the central retina, while beyond this is peripheral retina stretching to the ora serrata, 21 mm from the center of the optic disc (i.e., the total retina is a circular disc of approximately 42-mm diameter) (see Polyak,[72] Van Buren,[86] and Kolb, unpublished observations).

If the thin sheet of retina is now fixed and embedded for histological examination and very thin radial sections are made at various positions in the retina, the layered architecture and the different neural components are revealed (Fig 5–2,A and B). In the foveal area (2 mm from the foveal pit, Fig 5–2,A) the retina is considerably thicker than retina from the periphery (Fig 5–2,B). This is due to the greater packing density of photoreceptors, particularly cones and their associated second- and third-order neurons. In the foveal retina the cones are closely spaced and the rods, fewer in number, seem to be inserted between the cones. The outer nuclear layer (ONL), composed of the cell bodies of the rods and cones, is about the same thickness in the foveal and peripheral retina, but in the periphery the rod cell bodies outnumber the cone cell bodies, while the reverse is true for the foveal area. The cones have oblique axons displacing their cell bodies from their synaptic pedicles in the outer plexiform layer (OPL), and these oblique axons with accompanying Müller

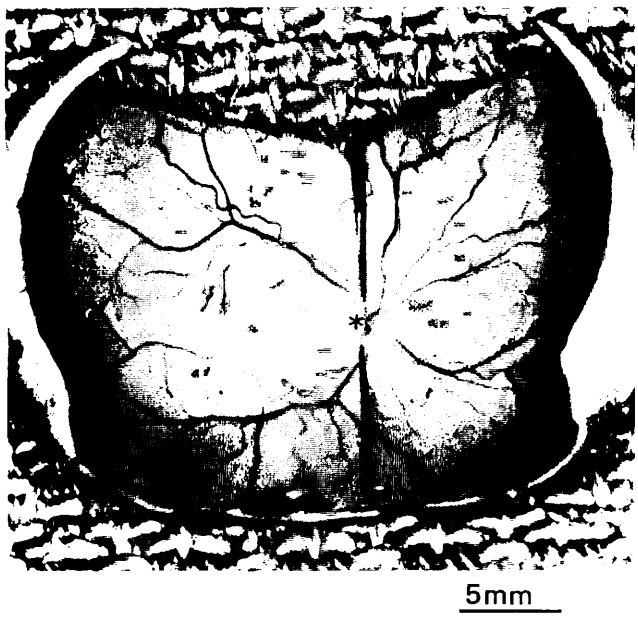

FIG 5–1.
A normal human right eye injected with formalin fixative 2 hours postmortem and opened as a flat display of the posterior pole. The superior and inferior retinas have been somewhat trimmed away. The vertical cut of the posterior pole is through the optic nerve at the *asterisk*. The fovea is the small avascular spot to the left of the optic nerve position (scale bar, 5 mm). (From Van Buren JM: *The Retinal Ganglion Cell Layer.* Springfield, Ill, Charles C Thomas Publishers, 1963. Used by permission.)

cell processes form a pale-staining, fibrous-looking area known as the Henle fiber layer (Fig 5–2,A). The Henle fiber layer is completely lacking in the peripheral retina (see Fig 5–2,B). In the OPL of all areas of the retina are found the synaptic terminals of the photoreceptors, called cone pedicles and rod spherules, which interact with second-order neurons, bipolar cells, and horizontal cells. The OPL is a layer of synaptically interactive processes sometimes also called the outer synaptic layer of the retina.

The inner nuclear layer (INL) is again composed of cell bodies, this time of second- and third-order neurons, i.e., bipolar cells, horizontal cells (in the row closest to the OPL), and amacrine cells (in the row closest to the inner plexiform layer [IPL]). The INL is thicker, i.e., composed of more cell bodies in the foveal area of the retina (Fig 5–2,A), than the peripheral retina (Fig 5–2,B) due to a greater density of cone-connecting second-order neurons (cone bipolar cells) and smaller-field and more closely spaced horizontal cells and amacrine cells concerned with the cone pathways. Cone-connected circuits of neurons are less convergent in that fewer cones impinge on second-order neurons than do rods in rod-connected pathways. Thus, in the area of retina where cone density is highest, i.e., approaching and in the fovea, the numbers of second- and third-order neurons are consequently higher for dealing with the lesser-convergent cone-driven circuits.

A remarkable difference between foveal and peripheral retina can be seen in the relative thicknesses of the IPL, ganglion cell layer (GCL), and nerve fiber layer (NFL) (Fig 5–2,A and B). This is again due to the greater numbers and packing density of ganglion cells that are needed for the cone pathways in the cone-dominant foveal retina as compared with the rod-dominant peripheral retina. The greater number of ganglion cells means more synaptic interaction in a thicker IPL and greater numbers of ganglion cell axons coursing to the optic nerve in the NFL in foveal as compared with the peripheral retina (Fig 5–2,A and B). The IPL is the second synaptic layer of the retina where the second-order neurons, bipolar cells, have synaptic connections with third-order neurons, amacrine and ganglion cells. This neuropil of interacting dendrites and axons is also known as the inner synaptic layer.

The outer and inner limiting membranes (OLM and ILM, Fig 5–2,A and B) are formed by junctions between Müller cell processes at the level of the photoreceptor cell inner segments and the vitreal apposition of Müller cell endfeet and associated basement membrane constituents, respectively. The OLM forms a barrier between the neural retina proper and the subretinal space, into which the inner and outer segments of the photoreceptors project to be in close association with the pigment epithelial layer behind the retina. The ILM is the inner surface of the retina that borders the vitreous humor and thereby forms a relative diffusion barrier between the neural retina and vitreous.

Throughout the retina the major blood vessels of the retinal vasculature supply the capillaries that run into the neural tissue from the NFL to the OPL (Fig 5–2,A and B). Occasionally capillaries are even seen as high as in the ONL. Nutrients from the vasculature of the choriocapillaris behind the pigment epithelium layer supply the delicate photoreceptor layer (Fig 5–2,B).

The center of the fovea is known as the foveal pit (foveola) (Polyak[72]) and is a highly specialized region of the retina different again from the foveal and peripheral retina. A radial section of the foveola measures less than a quarter of a millimeter (200 μm) across and is shown in Figure 5–3,A. It is an area where rods are excluded and cone photorecep-

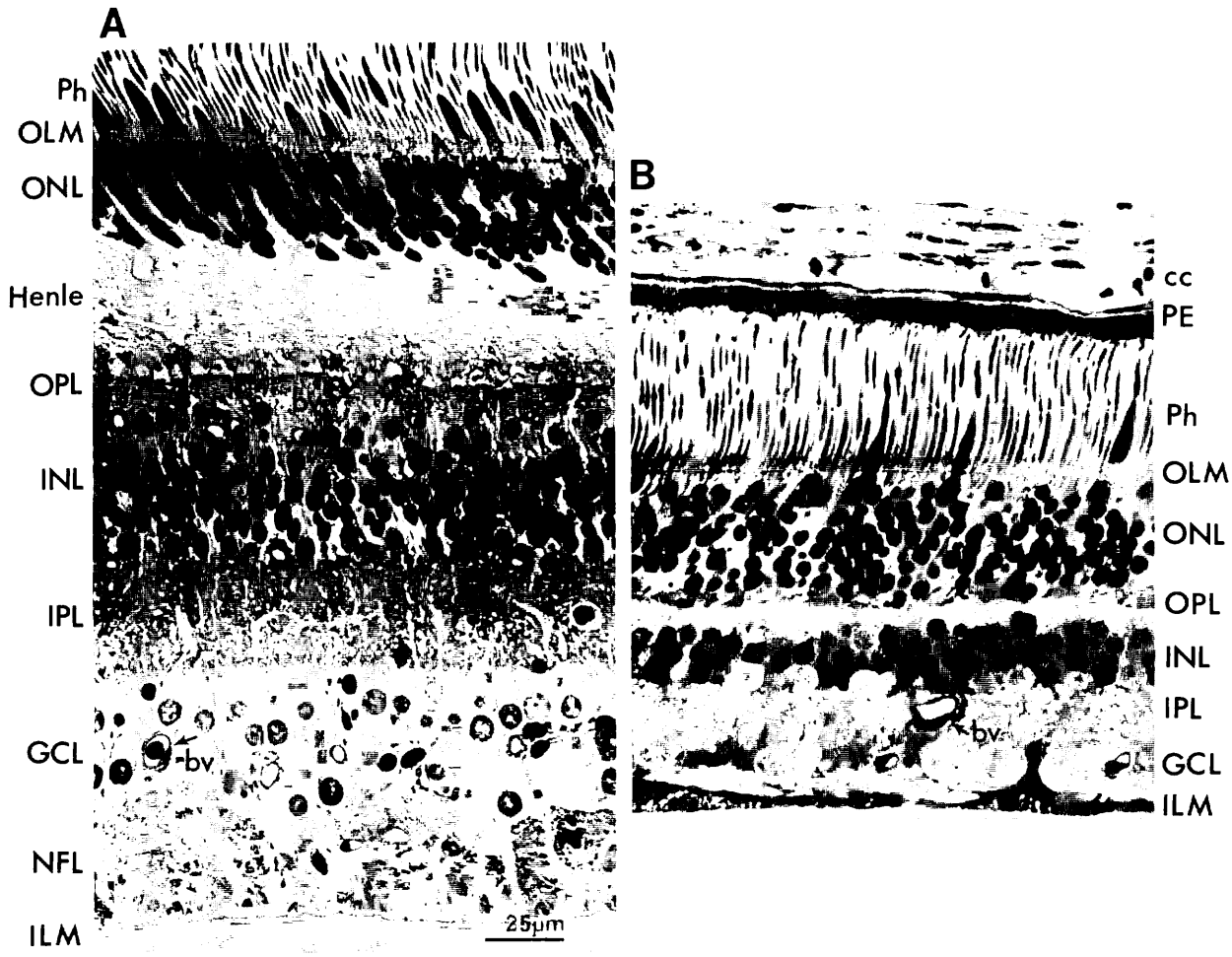

FIG 5–2.
A, radial section of the human retina approximately 2 mm from the foveal center. **B,** radial section of peripheral human retina. See the text for descriptions. Abbreviations for most figures (*cc* = choroidocapillaris; *PE* = pigment epithelium; *Ph* = photoreceptors; *OLM* = outer limiting membrane; *ONL* = outer nuclear layer; *Henle* = Henle fiber layer; *OPL* = outer plexiform layer; *INL* = inner nuclear layer; *IPL* = inner plexiform layer; *GCL* = ganglion cell layer; *NFL* = nerve fibre layer; *ILM* = inner limiting membrane; *bv* = blood vessel) (scale bar for **A** and **B**, 25 μm).

tors are concentrated at maximum density and arranged at their most efficient packing density of a hexagonal mosaic. This is more clearly seen in the tangential section through the foveal cone inner segments illustrated in Figure 5–3,B. Below this central 200-μm-diameter central foveal pit the other layers of the retina are displaced concentrically, leaving only the thinnest sheet of tissue consisting of cone cells and some of their cell bodies (Fig 5–3,A). Radially distorted but complete layering of the retina then appears gradually along the foveal slope (right and left sides of the section, Fig 5–3,A) until the rim of the fovea is made up of the displaced second- and third-order neurons related to the central cones. Here the ganglion cells are piled into a six-layered ganglion cell layer, thus making this area, called the foveal rim or parafovea,[72] the thickest portion of the entire retina.

The whole foveal area, including foveal pit, foveal slope, parafovea, and perifovea, is considered the macula of the human eye. Familiar to ophthalmologists is a yellow pigmentation to the macular area known as the macula lutea. This pigmentation is the reflection from a yellow screening pigment, the xanthophyll lutein,[76] present in the cone axons of the Henle fiber layer. The macula lutea is thought to act as a short-wavelength (ultraviolet [UV] to blue) filter, additional to that provided by the lens.[76] Because the fovea is the most important part of the retina for human vision, protective mechanisms for

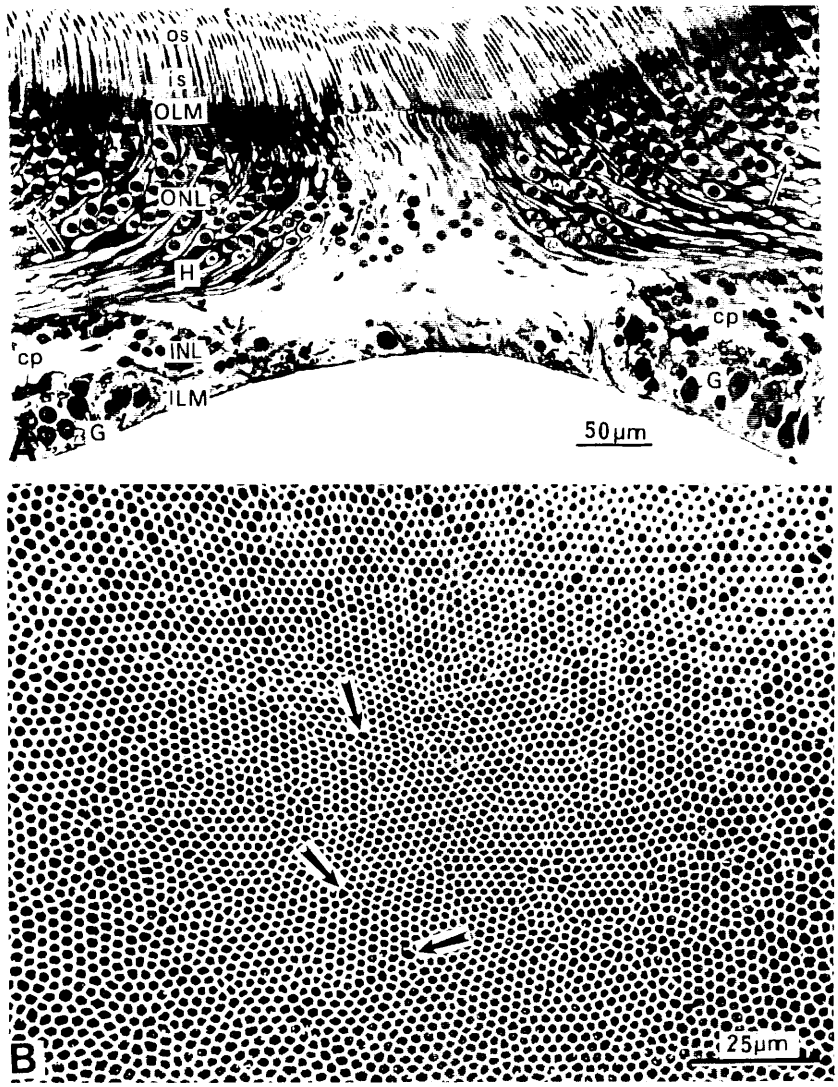

FIG 5–3.
A, radial section through the human foveal center. The outer and inner segments *(os, is)* of the very slim foveal cones are all that occur in the very central region. Some scattered cone cell bodies are dispersed among the pale-appearing Müller cell material. Capillaries are *(cp)*, inner nuclear layer cells *(INL)*, and ganglion cells *(G)* appear on the foveal slope (*H* = Henle fibers; *double arrows* point out the first rod nuclei (scale bar, 50 μm). **B**, human fovea, tangential section cut through the level of the inner segments of the cones. The hexagonal arrangement of the cone mosaic can be seen. Some cones with larger inner-segment diameters *(arrows)* break up the regular hexagonal array into small subunits (scale bar, 25 μm). (**A**, from Yamada E: *Arch Ophthalmol* 1969; 82:151–159. Used by permission. **B**, From Ahnelt PK, Kolb H, Pflug R: *J Comp Neurol* 1987; 255:18–34. Used by permission.)

avoiding light toxicity and especially UV irradiation damage are thought essential.

PHOTORECEPTORS

Three types of cone photoreceptor and a single type of rod photoreceptor are present in the normal human retina. In sections of retina prepared for light microscopy (see Fig 5–2,A and B) the rods and cones can be distinguished rather easily. Cones are robust conical-shaped structures that have their cell body situated immediately below the OLM, and their inner and outer segments protrude into the subretinal space (see Fig 5–2,B). In the foveal retina where the cones are densely packed, their cell bodies are of necessity also layered in oblique strands (Fig 5–2,A) below the OLM. Rods, on the other hand, are very slim structures, with their cell bodies staggered in the several layers of the ONL. Their thin inner and outer segments are found in the subretinal space. Apical processes from the pigment epithelium envelop the outer-segment portions of both rods and cones but are not clearly seen in histological sections such as illustrated in Figure 2,A and B.

The higher magnification afforded by an electron microscope allows better resolution of rod and cone photoreceptor anatomy. In Figure 5–4,A ultrathin sections show portions of rods (Fig 5–4,A) and a cone (Fig 5–4,B) from a rhesus retina. Human photoreceptors look exactly the same as those of monkeys. The inner segments of rods are thinner than those of the cones except in the fovea. Central foveal cones have inner and outer segments of 1.5-μm diameter. In humans, peripheral retinal rod inner segments are 2 μm in diameter, and the cone about 6

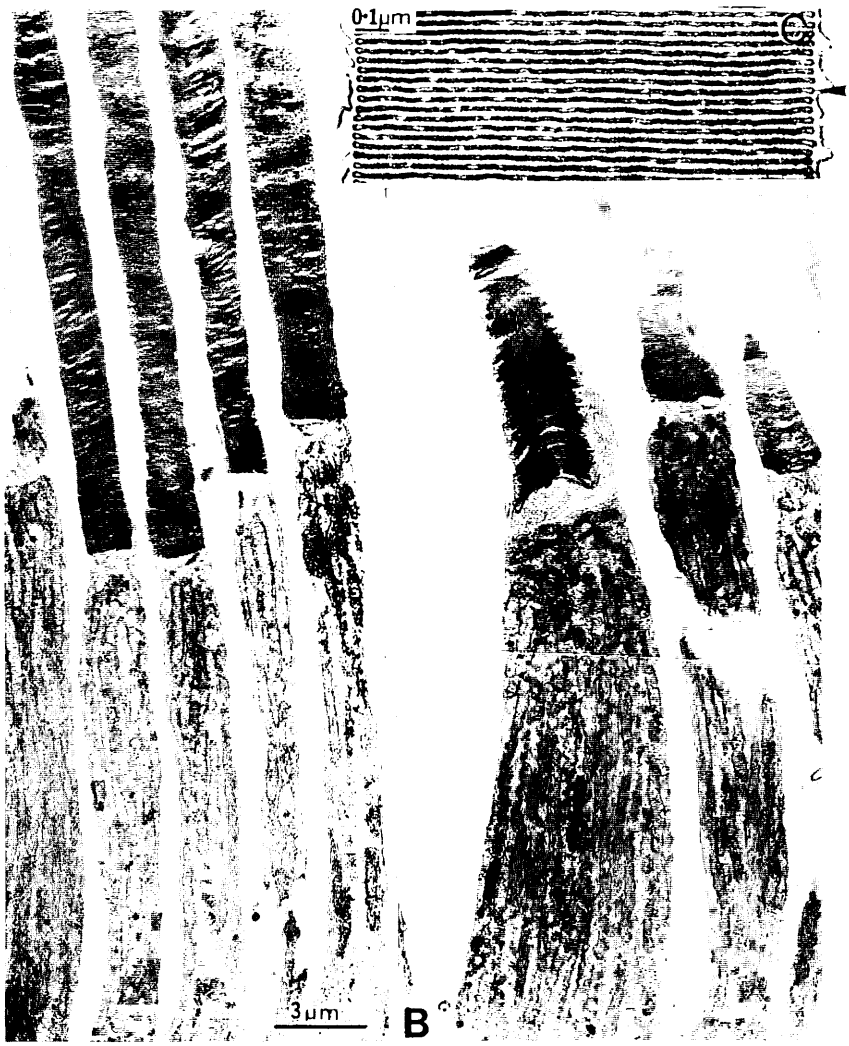

FIG 5–4.
A, electron micrograph of rods of a rhesus monkey retina. **B**, electron micrograph of a cone in the rhesus monkey retina. Both **A** and **B** have the same scale bar of 3 μm. **C**, high-magnification electron micrograph of a portion of a rod outer segment to show the freely floating discs *(arrowhead)* discontinuous with the plasma membrane (scale bar, 0.1 μm).

μm^2 (Fig 5–4,A and B). The inner-segment regions of both rods and cones are filled with elongated mitochondria that are the energy supply of the cell. At the scleral side of the inner segment, a thin cilium gives rise to the outer segment, and from the cilium radiates a long structure known as the rootlet (neither cilium nor rootlet is seen on Fig 5–4,A and B). The outer segment arises from the cilium[81] and is a structure filled entirely with membranous material organized into discs of folded double membrane in which are embedded the light-sensitive visual pigments (Fig 5–4,C). In rods most discs are isolated from the outer plasma membrane and lie like a stack of pennies piled one atop another. The outer segment grows from its base. The most distal of discs are daily shed and engulfed by the apical processes of the pigment epithelium. These packets are called phagosomes in the pigment epithelial cells.

Cone outer segments differ from rod outer segments in several respects. First, they are shorter and conical with a wider base and tapered shape as compared with rod outer segments. Second, cone discs are connected to the plasma membrane throughout the extent of the outer segment and thus are open to extracellular space. Apical processes of the pigment epithelium also phagocytize chunks of cone outer segments, but at a different time in the diurnal cycle as compared with rods (i.e., at light's offset compared with light onset).[6, 47, 82, 91, 92]

The job of the photoreceptor cell in the retina is to catch quanta of light in the photoreceptive membranes of the outer segment and pass a message concerning numbers of quanta of light to the next stage of integration and processing at the OPL. Because the spectral absorbance of cone pigments differ, the relative intensity of the cone signals indicates the wavelength composition of the incident light, and this information ultimately leads to the

sensation of color. The information-transmitting end of the cone cell is known as the pedicle, and that of the rod cell is known as the spherule. Pedicles are large, conical, flat endfeet (8 to 10 μm in diameter) of the cone axon that lie on more or less the same plane at the outer edge of the OPL, while the rod spherules are small, round enlargements of the axon (3 to 5 μm in diameter), or even extensions of the cell body of the rod, and terminate stacked between and above the cone pedicles. Electron microcrographs of a cone pedicle and a rod spherule are shown in Figure 5–5,A and B, respectively. Both synaptic endings are filled with synaptic vesicles, and a dense structure known as the synaptic ribbon points to a group of invaginated processes from bipolar and horizontal cells. In each cone pedicle there are approximately 19 of these ribbons that are associated with 19 triads of invaginated processes.[1] The triad of invaginated second-order processes consists typically of a central element, which is a dendritic terminal of an invaginating bipolar cell, and two lateral elements, which are dendritic terminals of horizontal cells. In addition, other varieties of bipolar cells have dendrites that make synaptic contacts on the undersurface of the cone pedicle at basal junctions (Fig 5–5,A, arrows).[18, 37, 61] Rod spherules have only one long synaptic ribbon associated with two lateral elements that are horizontal cell axon terminals and two central invaginating dendrites of rod bipolar cells.[18, 37, 61] There are no basal junctions on rod spherules.

In submammalian species intracellular recordings from the cones have indicated that they receive a feedback inhibitory message from horizontal cells.[5] To date, in no mammalian *cone* has morphological evidence for this feedback synapse been established. Clearly, lateral elements, i.e., horizontal cell dendrites, have vesicles within them (see Fig 5–5,A), but these cannot be seen to be directed in a cluster, typical of a synapse, at the membrane adjoining the cone pedicle. However, the axon terminals of the horizontal cell that end as lateral elements in the *rod* spherules can occasionally be seen to have small punctate synapses directed at the rod (Fig 5–5,B, arrows).[50] This is probably a feedback synapse from the horizontal cell axon terminal to the rod.

There also appears to be a pathway for cone-cone crosstalk and also cone-rod interaction in the human retina. Cone pedicles have small projections from their sides or bases that pass to neighboring rod spherules and cone pedicles. Where these projections, called telodendria, meet, a specialized junction forms that is known to be typical of electrical

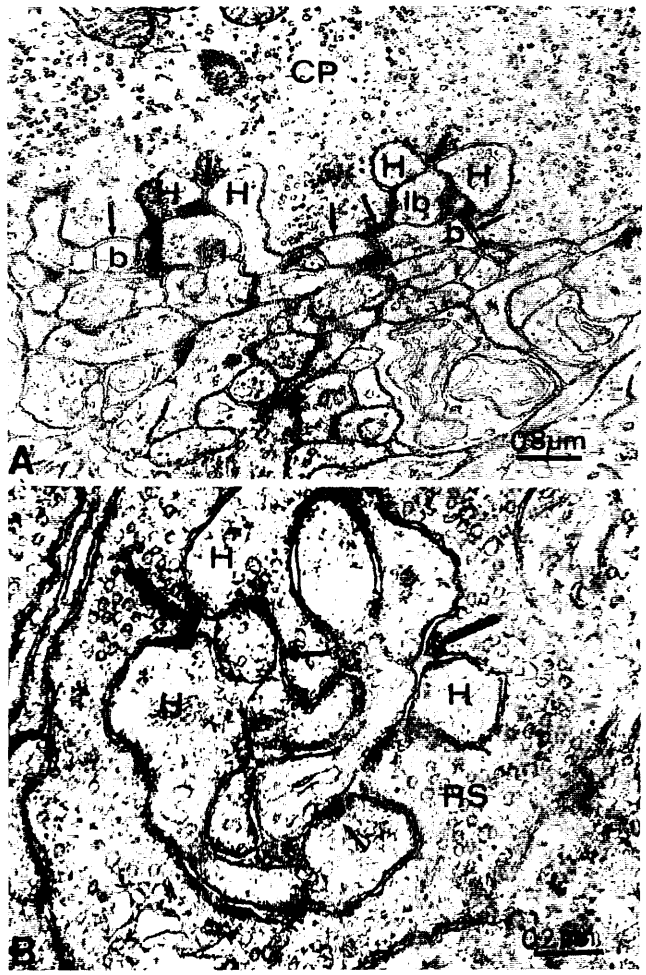

FIG 5–5.
A, electron micrograph of a portion of a cone pedicle in the OPL of the human retina. Cone pedicles *(CP)* make synapses upon horizontal cells *(H)* and invaginating bipolar cells *(ib)* at ribbon synapses pointing to triads of processes. Other bipolar cell dendrites *(b)* end at basal junctions *(arrows)* with the cone pedicle (scale bar, 0.8 μm). **B**, electron micrograph of a rod spherule in the human retina. Two synaptic ribbons point to large lateral elements of horizontal cell axon terminals *(H)*. Small punctate synapses are made by the H profiles *(arrows)* back onto the rod spherule *(RS)* (scale bar, 0.2 μm). (From Linberg KA, Fisher SK: *J Comp Neurol* 1988; 268:281–297. Used by permission.)

synaptic transmission. These minute gap junctions are illustrated in Figure 5–6,A, which shows a cone-to-cone gap junction, and in Figure 5–6,B, which shows a cone pedicle-to-rod spherule junction.[69] As many as three to five gap junctions occur on a single rod spherule, and a single cone pedicle can have as many as ten contacts to neighboring rods. Pedicles of short-wavelength cones do not have as many telo-

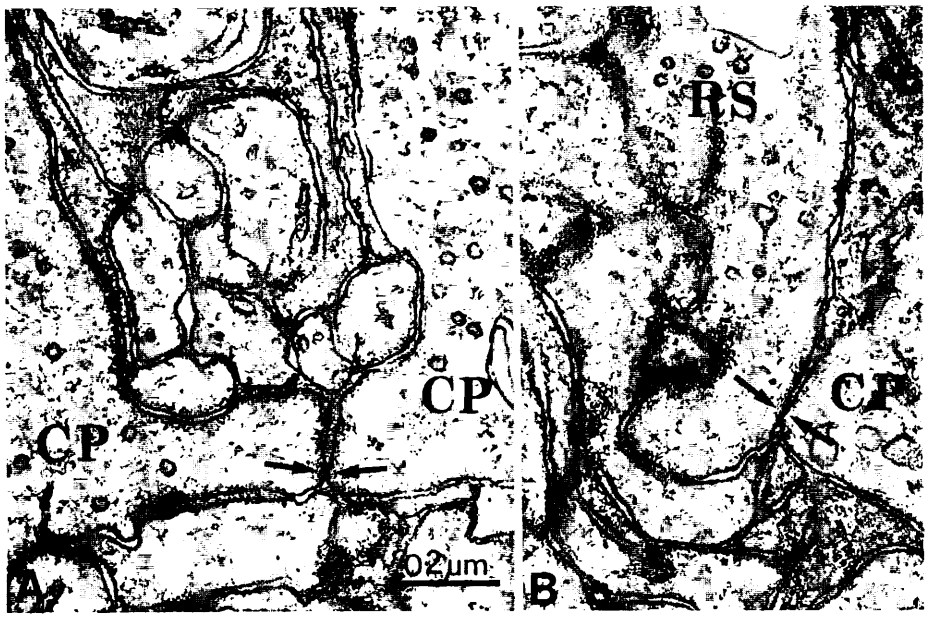

FIG 5–6.
A, a small gap junction *(arrows)* occurs between two apposing telodendria of neighboring cone pedicles *(CP).* **B,** small gap junction between a cone pedicle *(CP)* and a rod spherule *(RS)* (scale bar, 0.2 μm for both **A** and **B**). (From Nelson R, Lynn T, Dickinson-Nelson A, et al: Spectral mechanisms in cat horizontal cells, in Gallego A, Gouras G (eds): *Neurocircuitry of the Retina: A Cajal Memorial.* New York, Elsevier Science Publishing Co, Inc, 1985, pp 109–121. Used by permission.)

dendrial gap junctions with either neighboring rods or cones,[1] and thus, this cone type remains relatively isolated in the cone mosaic, and, as we shall see later, remains isolated to the ganglion cell level too due to connections with a specific "blue cone bipolar cell."

The cones are of three different types according to the structure of the visual pigment residing in the disc membranes of their outer segments. Thus long-, medium-, and short-wavelength cones have been demonstrated to exist in human retina by microspectrophotometry and psychophysical experiments: they are known to absorb maximally at 558, 531, and 420 nm, respectively.[29] Until recently the three cone types were thought to be identical in appearance and to be revealed only by specialized histochemical techniques,[52] dye uptake studies,[16] or the application of antibodies specific for visual pigments.[85]

Now careful morphological studies have enabled us to distinguish the short-wavelength cone from the medium- and long-wavelength cones in the human retina.[2] The short-wavelength cones have longer inner segments that project further into the subretinal space than is the case for longer-wavelength cones. Their inner-segment diameters do not vary much across the entire retina; thus they are fatter in the foveal area but thinner in the peripheral retina than are longer-wavelength cones. The short-wavelength cones also have a smaller and morphologically different pedicle than do the two types of longer-wavelength cones.[1] Furthermore, throughout the retina, the short-wavelength cones have a different distribution and do not fit into the regular hexagonal mosaic of cones typical of the other two types. This is illustrated in Figure 5–3,B, where the tangential section of the foveal cone mosaic is seen to consist of hexagonal groupings distorted in many places by a larger-diameter cone (arrows) breaking up the perfect mosaic into many irregular subunits. The larger-diameter cones are short-wavelength cones. These have their lowest density in the foveal pit at 3% to 5% of the cones' population, reach a maximum density of 15% on the foveal slope (1 degree from the foveal pit), and then form an even 8% of the total population elsewhere in the retina.[2] Analogous information concerning relative distributions of the medium- and long-wavelength cones in the human retina is not yet available from histological studies. In the monkey retina, however, according to Marc and Sperling,[52] about 33% of cones throughout the retina are of the long-wavelength type. The medium-wavelength cones are most frequent in the fovea, where they form 64%, and vary between 52% and 59% elsewhere.

OUTER PLEXIFORM LAYER

A certain amount of integration of the visual message goes on at the first synaptic layer of the retina, the OPL. Here cone pedicles and rod spherules have synapses upon various different bipolar cell types and three different horizontal cell types. In addition, as mentioned above, the cones also pass electrical messages from one to another, and rod messages are passed into cone pedicles via this same telodendrial pathway.[65] Two further synaptic interactions that occur at the OPL concern feedback to cones within the layer from horizontal cells and feedback from the IPL to bipolar cells via an interplexiform cell (see a later section for details).

The morphology of individual neurons has been discovered over the years principally by using a specific neural stain named after a famous early Italian neuroanatomist, Camillo Golgi.[28] This staining method was used most extensively and with extraordinary success by the great Spanish anatomist Ramón y Cajal,[13] and it is his work that forms the basis of neuroanatomy for the vertebrate nervous system in general. Stephen Polyak was the man most responsible for applying the Golgi stain to primate retinas, especially in monkeys and chimpanzees, and his 1941 book concerning the organization of the primate retina and visual system was a landmark.[72] Today, we continue to use the Golgi method in research on monkey and human retinas (see Boycott and Dowling[7]; Kolb[37]; Mariani[53, 54] and Kolb, Fisher, and Linberg, unpublished data), and we have identified more significant cell types than in Polyak's original description. In addition, we can elucidate more complete neural circuitry by electron microscopy, histochemical staining, immunocytochemical staining, and electrophysiological single-cell recording and staining. All the descriptions of cells and circuits that follow in this chapter result from a combination of these techniques, but the morphological data from Golgi staining are always the basis.

In human retina nine different bipolar cell types are revealed by Golgi staining. The six most common of these are illustrated in Figure 5–7. A single morphological type of bipolar cell connects exclu-

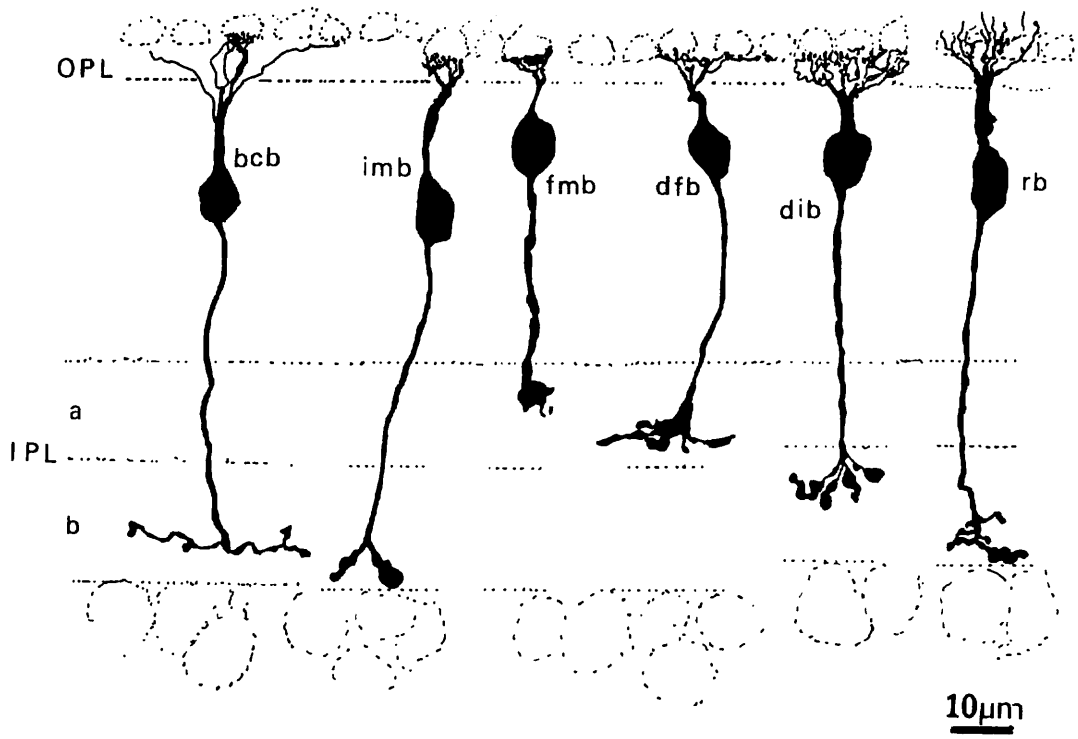

FIG 5–7.
Camera lucida drawings of Golgi-stained bipolar cells of the human retina as seen in vertical views (*bcb* = blue cone bipolar; *imb* = invaginating midget bipolar; *fmb* = flat midget bipolar; *dfb* = diffuse flat bipolar; *dib* = diffuse invaginating bipolar; *rb* = rod bipolar). The OPL and IPL are indicated, and the IPL is subdivided into sublamina a *(a)* and sublamina b *(b)* (scale bar, 10 μm).

sively to rods. It (rb, Fig 5–7) is typically a stout bipolar with a cell body situated in the middle to high INL. A tuft of dendrites enters the OPL and runs up to different levels between cone pedicles to reach the stacked rod spherules. Each dendritic terminal ends as the central invaginating dendrite of a rod spherule (see Fig 5–5,B).[37] In the central retina the tuft of dendrites is small (15 μm across) and 15 to 20 rods are contacted. In the peripheral retina the dendritic tuft is 30 μm across and contacts 40 to 50 rods.

Eight different types of cone bipolar cells are present in the human retina. Five of them are concerned with converging information from many cones. Three cone bipolars, not illustrated, are rarely stained by Golgi techniques. The dendrites spread over 70 to 100 μm, so they can be classified as *wide field* or giant. Each connects with as many as 15 to 20 cones.[54] Little is known concerning the wide-field cells, and we will not deal with them any further here. Common cone bipolar cells of the human retina are *diffuse flat* and *diffuse invaginating* types (Fig 5–7, dfb and dib). As suggested by their name, both of these collect information from several cones (5 to 7 in the central retina and 12 to 14 in the peripheral retina). The diffuse flat cone bipolar cells contact cones at wide-cleft basal junctions, while diffuse invaginating cone bipolar cells contact cones at invaginating ribbon, narrow-cleft synapses (see Fig 5–5,A for the morphology of cone pedicle-to-bipolar cell synapses).

Two further types of cone bipolar cells are in a one-to-one relationship with a single cone, moreover, with the same cone. Thus, cones of the fovea output to *two midget* bipolar cells and, of course, still also output to the two types of diffuse bipolar cells just described. The two types of midget bipolar cells differ in the details of their contact with the cone pedicle. The invaginating midget bipolar cell (imb, Fig 5–7) connects with the cone pedicle as central invaginating dendrites at narrow-cleft, ribbon synapses as shown in Figure 5–8,A and B, while the flat midget bipolar (fmb, Fig 5–7) contacts the cone pedicle by means of semi-invaginating, wide-cleft basal junctions, often two contacts on either side of the central invaginating dendrite from the other midget bipolar cell (Fig 5–8,C and D). Finally, a cone bipolar cell that is thought to be specific for the short-wavelength cones has been described in monkey[53] and human retina (Kolb, unpublished observations). This blue cone bipolar (Fig 5–7, bcb) typically contacts one cone heavily by several dendrites converging on that particular cone pedicle as central elements at the ribbons. Two or more other wispy

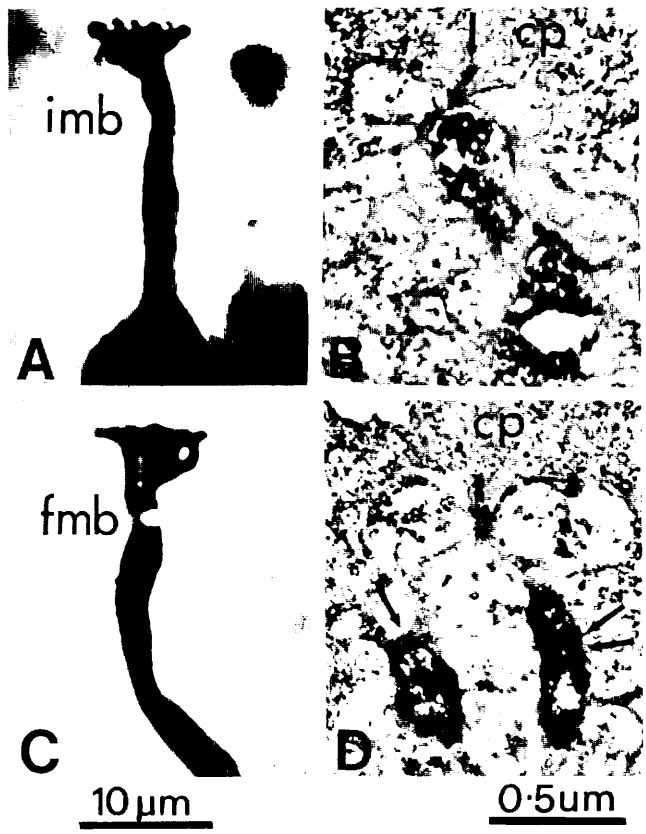

FIG 5–8.
A, light micrograph of the dendritic top of a Golgi-stained invaginating midget bipolar cell *(imb)* with fingerlike projections. **B**, electron micrograph of a single fingerlike projection of the imb (densely stained profile) into the triad of the cone pedicle *(cp)* ribbon synapse. **C**, light micrograph of the blunt flat top of a Golgi-stained flat midget bipolar cell *(fmb)*. **D**, electron micrograph of two stained fmb terminals that end on the cone pedicle surface *(arrows)* on either side of the central invaginating dendrite at the ribbon synapse (scale bars, 10 μm for **A** and **C**, 0.5 μm for **B** and **D**). (Adapted from Kolb H: *Philos Trans R Soc Lond [Biol]* 1970; 258:261–283.)

dendrites contact either another cone pedicle or end blindly in the OPL.

We have recently been able to distinguish three types of horizontal cells in the human retina.[51] Drawings of Golgi-stained examples of these three types are illustrated in Figure 5–9. HI is the classic horizontal cell of primate retina that was first described by Polyak.[72] It is a small-field cell (15-μm-diameter dendritic tree in the fovea, 80 to 100 μm in the periphery) with stout dendrites. In the fovea there are 7 distinct clusters of round or donut-shaped terminals contacting cones as lateral elements of the ribbon synapses, and in the peripheral

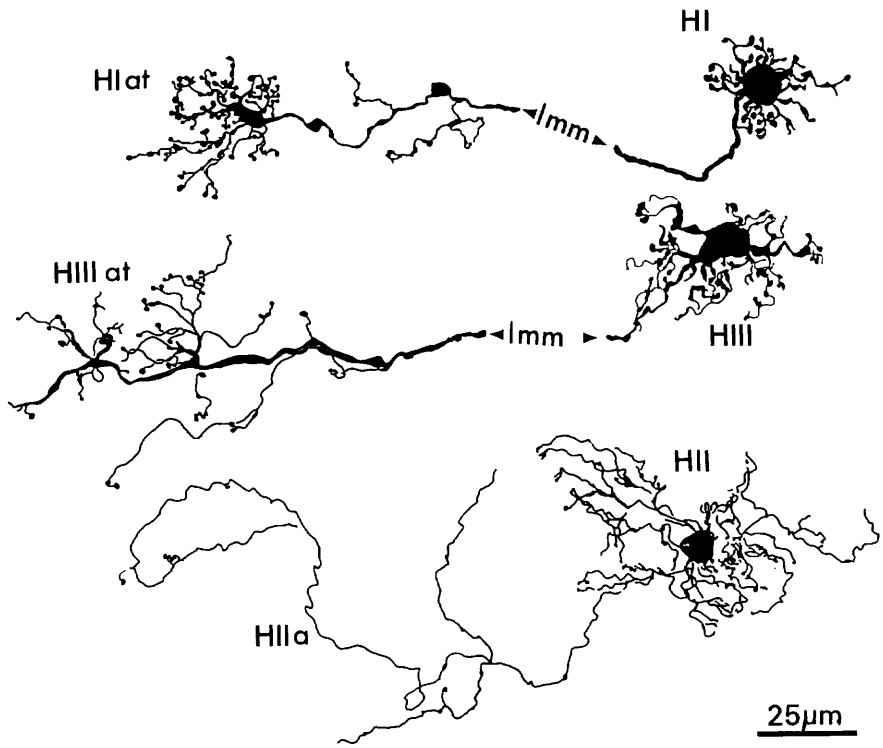

FIG 5–9.
Camera lucida drawings of Golgi-stained horizontal cells in the human retina. *HI*, *HII*, and *HIII* have different morphologies and different axon terminals *(HIat, HIIIat, HIIa)*. See the text for details (scale bar, 25 μm).

retina there are as many as 18 clusters. The HI cell has a single thick axon that passes laterally in the OPL to terminate more than 1 mm away in a thickened axon terminal stalk bearing a fan-shaped profusion of lollipop-like terminals, each of which ends in a rod spherule as a lateral element of the ribbon synapse.[37] HIII cells are similar in appearance to HI cells, but everywhere in the retina are one third bigger in dendritic tree size. The typical appearance, best seen in the peripheral retina, is asymmetrical in shape: one or two dendrites are much longer than the others. The clusters of terminals contact cones in the same manner as the HI cell terminals and, because of their bigger field size, contact more cone pedicles (9 to 12 in the foveal retina, 20 to 25 in the peripheral retina). The axon of the HIII cell is larger field and more irregular in shape than the HIs (Fig 5–9). The contacts of the HIII axon terminal have not yet been investigated, although they are thought to relate primarily with rods like the HI cells'. HII cells are more spidery and intricate in dendritic field characteristics than are either of the other types.[43] Their terminals are not clearly seen as clusters approaching cone pedicles. HII cells also bear an axon, but this is quite different from that of the other two horizontal cell types. The HII axon is short (200 to 400 μm) and curled instead of straight and has contacts to cone pedicles by means of small wispy terminals (Fig 5–9).

Recent findings from electron microscopic studies of Golgi-stained horizontal cells of the human retina suggest that the connections are related to retinal chromatic function. Thus HIs primarily contact medium- and long-wavelength cones but also have a small number of contacts to any short-wavelength cones in the dendritic field. HII cells specifically contact short-wavelength cones, direct major dendrites to these cones in their dendritic field, and contact lesser numbers of terminals of other types of non–short-wavelength cone. The HII cell's axon contacts short-wavelength cones specifically. HIII cells have large dendritic terminals in medium- and long-wavelength cones and completely avoid short-wavelength cones in their dendritic tree.[39]

INNER PLEXIFORM LAYER

The axonal endings of bipolar cells bring information from the OPL to the neuropil of the IPL. Here

bipolar cells "talk" to different varieties of functionally specialized amacrine cells and to dendrites of the various ganglion cells. The neuropil is a confusing network of interconnecting profiles that, to be understood, has to be investigated at the high magnification afforded by the electron microscope, using knowledge gained from Golgi staining for morphology and from intracellular electrophysiology for the function of individual cells in the network.

A view of a small part of the neuropil of the IPL is shown in the electron micrograph of Figure 5–10. Bipolar cell axon terminals are vesicle-filled profiles containing irregular, long mitochondria and neurotubules. Their synapses are typified by a small synaptic ribbon pointing into a wedge with two post synaptic profiles at the apex (arrowheads in CB profiles, Fig 5–10). Amacrine cell dendrites are also vesicle filled but have round mitochondria and sometimes neurofilaments as well as neurotubules. Amacrine profiles vary between having very small cross sections and appearing like thin straight tubes, to being larger dendritic varicosities. Typically amacrine cells synapse upon other profiles (bipolar axons, amacrine cells or ganglion cell dendrites) in the enlarged varicosities at what is known as a conventional synapse consisting of synaptic vesicles clustered at a premembrane and postmembrane density (small arrows, Fig 5–10). Ganglion cell dendrites (G, Fig 5–10) are recognizable as profiles lacking synaptic vesicles but filled with ribosomes, tubules, and

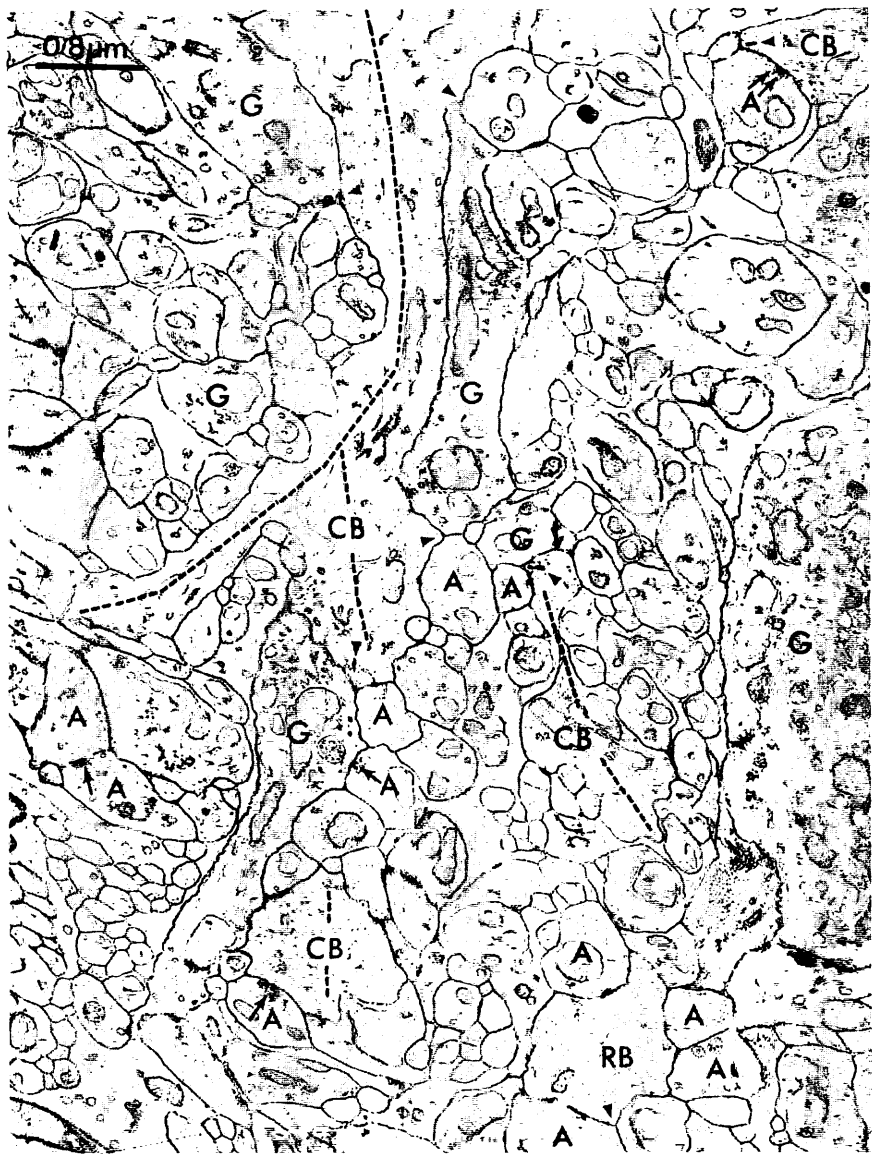

FIG 5–10.
Electron micrograph of a small portion of the neuropil in the human foveal IPL. A cone bipolar axon terminal *(CB, dotted lines)* is seen as it runs through the center of the field. Ganglion cell dendrites *(G)* and amacrine cell profiles *(A)* are in various synaptic relationships with one another and the cone bipolar and a rod bipolar terminal *(RB). Arrowheads* point to synapses, both ribbon and conventional. *Double arrows* point to a reciprocal synapse. See the text for more details (scale bar, 0.8 μm).

filaments. In Figure 5–10 ganglion cell dendrites are seen to be postsynaptic to bipolar ribbon synapses and amacrine conventional synapses. Bipolar cells are also postsynaptic to amacrine synapses often at what is known as a reciprocal synapse, close to the site of its ribbon output (double arrows, Fig 5–10).

It is clear that there are many different kinds of amacrine cell and ganglion cell branching in the IPL of the human retina. From Golgi staining studies we know that there are at least 25 different amacrine cell types in the human retina. The amacrine cells are classified into different types on morphological characteristics of dendritic tree size such as small (cells 1 to 10, Fig 5–11), medium (cells 10 and 11, Fig 5–11), and large (cells 12 to 15, Fig 5–11) branching characteristics (i.e., tufted, varicose, linear, beaded, and radiate, Fig 5–11) and, most importantly, on the stratification of their dendrites in the IPL. The neuropil of the IPL was arbitrarily divided into five strata by Cajal,[13] and since then this descriptive stratification scheme has been adhered to. Thus amacrine cells that stratify in strata S1 and S2 (cell 1, Fig 5–11) will be different types from cells that stratify in S4 and S5 (cells 4 and 5, Fig 5–11). Some amacrine cells have dendrites ramifying diffusely through all the strata of the IPL (cells 11 and 14, Fig 5–11) while others are very strictly limited to only one stratum (monostratified) (e.g., cell 13, Fig 5–11).

By using similar morphological criteria, the ganglion cells of the human retina can be distinguished into 20 or more different types, a few of which are shown here (Fig 5–12). Both amacrine and ganglion cells increase in dendritic tree span with eccentricity from the fovea. The very smallest dendritic fields for

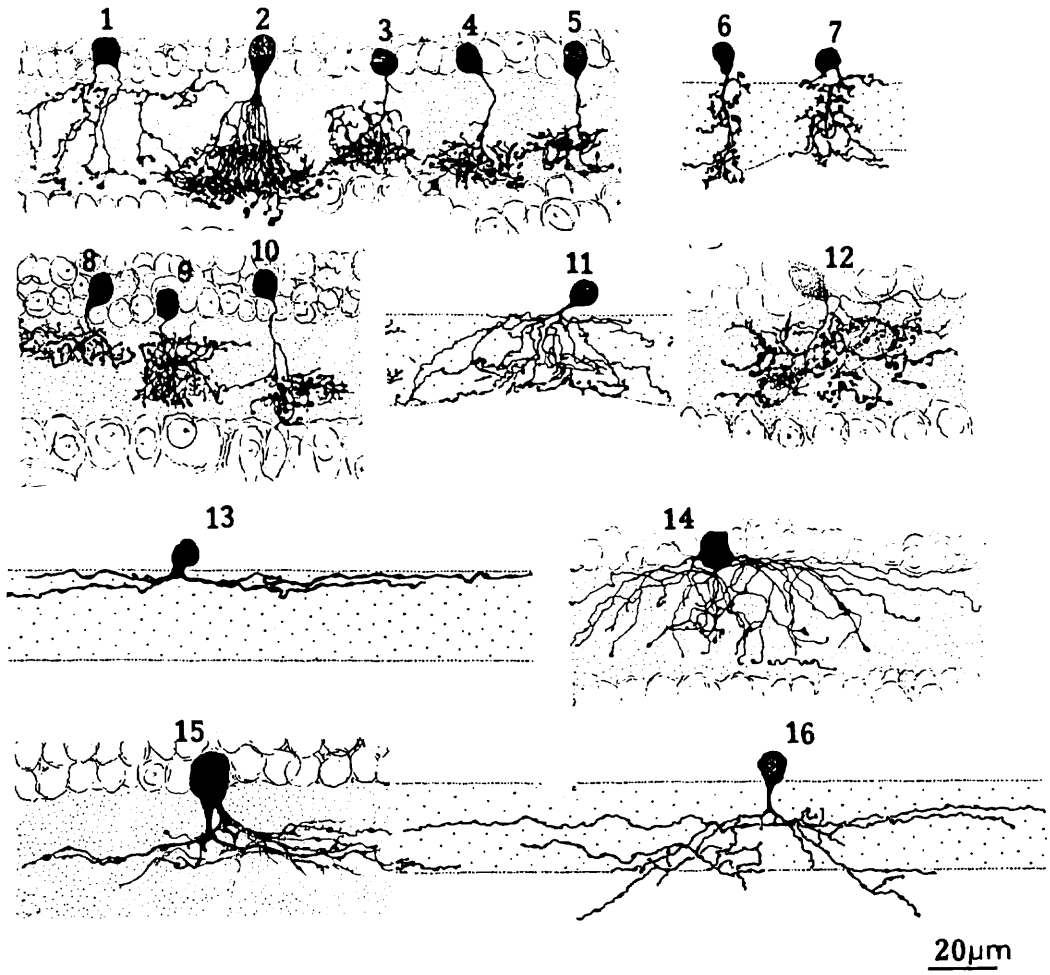

FIG 5–11.
Drawings of Golgi-stained amacrine cells in vertical view in the monkey retina. See the text for more details of the different cell types. An approximate scale representing 20 μm has been added to the original drawings. (From Polyak SL: *The Retina.* Chicago, University of Chicago Press, 1941. Used by permission.)

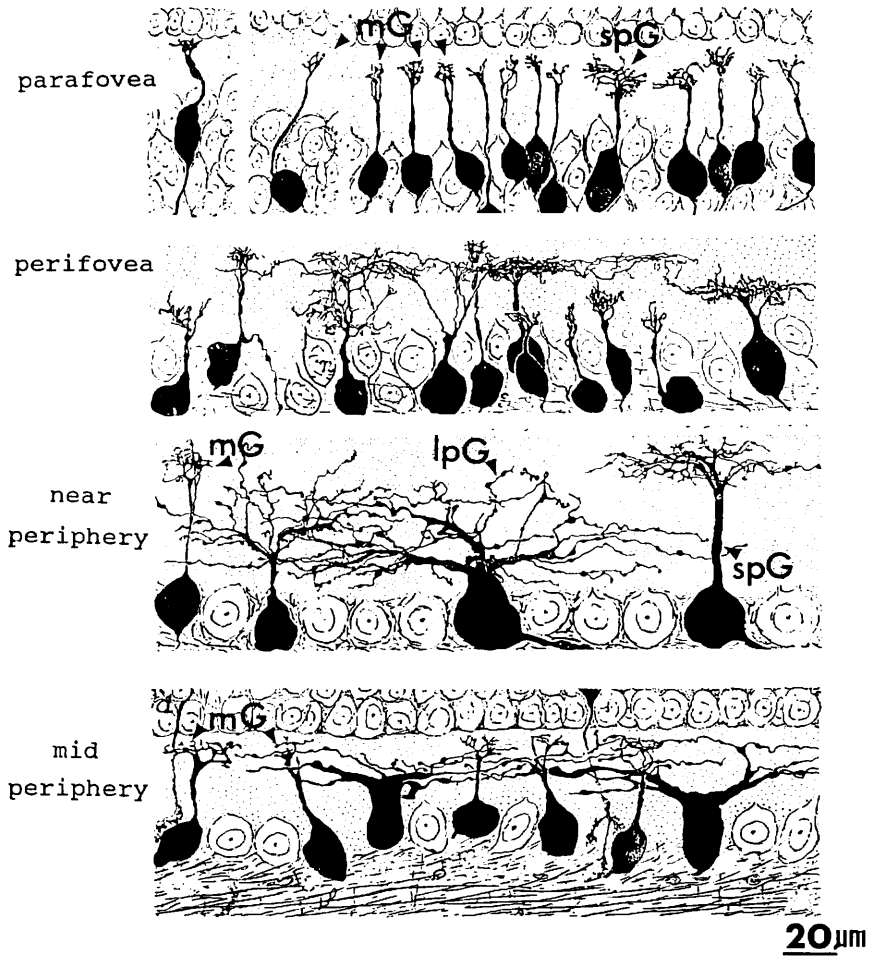

FIG 5–12.
Drawings of the most common Golgi-stained ganglion cell types in the monkey retina. Cells from different parts of the retina from the parafovea to the midperiphery are illustrated (*mG* = midget ganglion cells; *spG* = small parasol ganglion cells; *lpG* = large parasol ganglion cells). A 20-µm scale bar has been added to the original drawings. (From Polyak SL: *The Retina.* Chicago, University of Chicago Press, 1941, Used by permission.)

all cells types are found in the cells of the fovea. For this reason any classification of amacrine and ganglion cells has to always take eccentricity from the fovea into account, and a comparison must be made to cells in similar areas of the retina. In the ganglion cell classification, the three most common ganglion cell types of the human retina are the large parasol, small parasol, and midget ganglion cells (Fig 5–12), all so named by Polyak.[72] Other types that are found in lesser numbers are small and large diffuse ganglion cells, bistratified ganglion cells, and various monostratified large-field cells with different stratification levels in the IPL.

One may ask why amacrine and ganglion cells should be classified on the stratification level of their dendrites, particularly if they look the same in other respects such as field size, cell body size, and dendritic morphology. The reason has become clear over the years from intracellular recording studies of ganglion and bipolar cells in the cat retina,[66, 68] a topic we shall consider in greater detail in a later section. Suffice it for the moment to state that it is the bipolar cell-to-ganglion cell synaptic connections that are the determining factors. The bipolar cell types, described in the previous section on the outer plexiform layer, have already been noted to make different types of synapses with cone pedicles. We can see from the drawing of the most common bipolar types (Fig 5–7) that these cells can also be differentiated by their axon terminations at different depths of the IPL. Flat midget bipolar and diffuse flat bipolar cells have axons ending in strata S1 and S2 of the IPL, which together form a sublamina known as sublamina a.[24] Blue cone bipolar, invaginating midget bipolar, diffuse invaginating bipolar, and rod bipolar cells all have axons ending in one or more of the lower three strata S3, S4, and S5 of the IPL, otherwise known as sublamina b (see Fig 5–7).[24] By virtue of their different termination levels in the IPL, specific bipolar cells will connect only with ganglion cells and amacrine cells that have dendrites ramifying in the same stratum or sublamina.

GLIAL CELLS OF THE RETINA

Three basic types of glial cell are found in the human retina, Müller cells, astroglia, and microglia. All were described for the retina by Cajal about 100 years ago.[13]

Müller cells are the basic architectural supporting structures of the retina and stretch radially across the retina to form the outer and inner limiting membranes, respectively. A complete interpretation of the shape of a Müller cell is best seen after Golgi staining as in Figure 5–13. Müller cell bodies sit in the INL and project irregularly thick and thin processes in either direction to the OLM and the ILM. Müller cell processes insinuate themselves between cell bodies of the neurons in the nuclear layers and envelop groups of neural processes in the plexiform layers. In fact, retinal neural processes are only allowed direct contact, without enveloping Müller cell processes, at their synapses. The Müller cell-to-Müller cell junctions that form the OLM (Fig 5–13) are sturdy desmosomes with associated gap junctions. Other desmosomes form between Müller cells and photoreceptor inner segments[60]: desmosomes are architectural attachment points, while gap junctions are specialized membrane associations that allow the passage of small molecules and ions. The surface of the Müller cell facing the pigment epithelium and subretinal space is expanded by many projections of the Müller cell membrane that are known as apical villi. The ILM (Fig 5–13), on the other hand, is formed by the flattened endfeet of the Müller cell. The endfeet are also expanded in surface area by membrane convolutions of the Müller cell, but no specialized junctions are seen here. The surface of the Müller cell membrane facing the vitreous is covered with a mucopolysaccharide material and thus forms a true basement membrane. Recently, Müller cells have received much attention because of their roles in maintaining the constancy of retinal extracellular fluid and in generating electroretinograms (ERGs).

FIG 5–13.
A drawing and a light micrograph of radial views of Müller cells in the monkey retina. See the text for details (scale bar, 15 μm). (Drawing from Polyak SL: *The Retina.* Chicago, University of Chicago Press, 1941. Used by permission.)

Astrocytes have their cell body and processes restricted to the NFL of the retina. In Golgi staining they look like a fibrous tangle aligned along the ganglion cell axons coursing through the NFL (Fig 5–14,A). In distribution, astrocytes reach their peak on the optic nerve head and have a fairly uniform decline in density in radiating rings from the nerve head. They are not present in the avascular fovea. Thus astrocytes are also associated with the blood vessels that run in the NFL. The function of astrocytes enveloping ganglion cell axons and the relationship to blood vessels of the NFL is poorly understood (see Schnitzer[79] for the most recent information on astrocytes), but because they are known to contain abundant glycogen, they may form a nutritive service in addition to being a possible ionic homeostatic base similar to that of the Müller cells.

The third glial cell type is supposedly of mesodermal origin and thus strictly speaking not glial as the astrocytes and Müller cells are. Microglial cells are ubiquitous in the human retina and are found in every layer of the retina. In Golgi-stained retina material they look like strange irregular forms sometimes mistaken for nerve cells if they lie in a nuclear layer with a single orientation of their processes (Fig 5–14,B). Microglial cells appear to be blood-borne cells that arrive in the retinal neural tissue from the blood vessels and capillaries.[8, 27] They perform some sort of macrophagic function on degenerating retinal neurons.

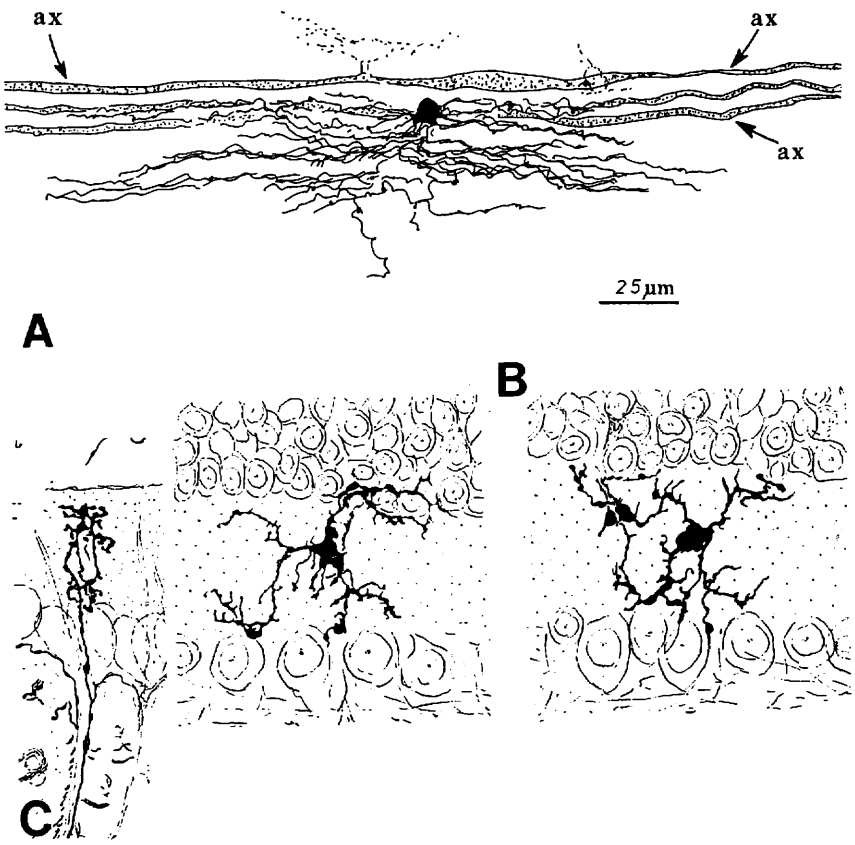

FIG 5–14.
A, camera lucida drawing of a horseradish peroxidase (HRP)-stained astrocyte in the cat retina. The fibrous processes of the astrocyte are arranged along the axon bundles *(ax)* in the NFL. **B**, Drawings of two microglial cells stained by the Golgi method. They both lie in the IPL. **C**, Golgi-stained centrifugal fiber emerging from the GCL to end in the distal IPL of the monkey retina (scale bar for **A**, **B**, and **C**, 25 μm). (**B** and **C** from Polyak SL: *The Retina.* Chicago, University of Chicago Press, 1941. Used by permission.)

CIRCUITRY FOR ROD SIGNALS THROUGH THE RETINA

Despite the concentration of cones and cone pathways in the fovea that serve color vision and high-acuity visual tasks, the human retina is actually a rod-dominated retina. That is, rod photoreceptors outnumber cone photoreceptors by orders of magnitude, and the consequent second- and third-order neurons recruited for processing scotopic vision outnumber the cone pathways neurons everywhere but in the central fovea. Rods peak in density in a ring approximately 5 mm (18 degrees) from the center of the fovea (Osterberg[71] [human], see Fig 5–19; Mariani et al.[56] [rhesus monkey]), as do the rod-specific neurons. Most of what we know concerning the wiring of the rod pathways has been learned from anatomical and electrophysiological experiments performed on cat central retina material. But, an examination of human retina with Golgi staining and electron microscopy indicates that all the same cell types and the same synaptology as in the cat retina exist for the rod pathways in the monkey and human retina too.

As was pointed out in a previous section, only one bipolar cell type has been found to make connections with the rod photoreceptors, and there is a great deal of convergence of rods onto their bipolar cell in the OPL (see the section on OPL). Rod bipolar cells send axons to the IPL where they terminate in the most vitreal portion as a rather narrow axon terminal (see Fig 5–7). The output of rod bipolar cells is *not* to ganglion cell dendrites, however.[38, 41] Instead, the rod bipolar synapses upon various amacrine cell types. This allows for both divergence of the rod signal and collection (convergence) of signals from many rods and rod bipolars, by means of these amacrine cells, before synaptic output to ganglion cells. Figure 5–15 is a summary diagram of the main cell types involved in the circuitry for rod signals

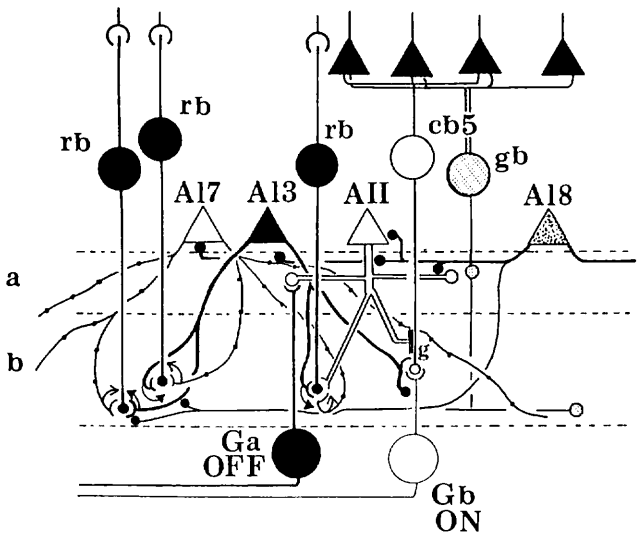

FIG 5–15.
Wiring diagram of the neurons involved in the rod pathways through the retina. The diagram is based on findings in the cat retina but is thought to be directly applicable to the human retina. See the text for descriptions (*rb* = rod bipolar cell; *cb5* = diffuse cone bipolar cell of sublamina b; *gb*, giant bistratified cone bipolar cell with axon branching in sublaminas a and b; *AII* = bistratified narrow-field rod amacrine; *A13* = rod/cone amacrine; *A17* = rod amacrine; *A18* = rod/cone amacrine known to be dopaminergic; *GaOFF* = off-center ganglion cell; *GbON* = on-center ganglion cell; *a* = sublamina a; *b* = sublamina b; *g* = gap junction; *curved arrows*, reciprocal synapses. (Adapted from Kolb H, Nelson R: Functional neurocircuitry of amacrine cells in the cat retina, in Gallego A, Gouras G (eds): *Neurocircuitry of the Retina: A Cajal Memorial.* New York, Elsevier Science Publishing Co, Inc, 1985, pp 215–232.

through the retina. Rod bipolars synapse directly upon A17 (wide-field amacrine, cell 14, Fig 5–11), A13 (medium-field amacrine, cell 11, Fig 5–11), and AII (bistratified narrow-field amacrine, cells 6 and 7, Fig 5–11).

The AII amacrine is a fascinating, small-field cell type that has a seminal role in the rod pathways and in linking the rod and cone pathways so that the rod signals can also use the cone bipolar pathways to ganglion cells. The AII amacrine cells receive input from rod bipolar axons and pass information to a specific cone bipolar type called cb5 in the cat but equivalent to the diffuse invaginating cone bipolar of the human (dib, Fig 5–7) in sublamina b of the IPL. This synapse with the cone bipolar cell is not a chemical synapse but an electrical synapse, i.e., a gap junction (g, Fig 5–15). In sublamina a, the AII amacrine makes chemical synapses upon ganglion cell types that branch only in this sublamina.

We know from intracellular recordings of cat retinas that ganglion cells with dendrites in sublamina a respond to light with a hyperpolarizing or off-center response and that ganglion cells with dendrites in sublamina b respond with a depolarizing or on-center response.[66] Thus the AII amacrine cell, which we know responds to light with a depolarizing response (on-center response),[64] makes a sign-inverting synapse upon the center-hyperpolarizing type of ganglion cell to contribute rod signals to the center response of the receptive field of that ganglion cell. The AII uses the gap junction with the cb5-type cone bipolar to pass center-depolarizing excitatory input to the center-depolarizing ganglion cell with which the cone bipolar has synapses (Fig 5–15). By this division of function within the small dendritic field of the AII amacrine cell, rod signals can be ensured of reaching both on- and off-center types of ganglion cells.

A17 (Fig 5–15) receives synaptic input from approximately 1,000 rod bipolar cells but does not appear to have direct synaptic output upon other amacrine or ganglion cells; instead it merely interconnects the rod bipolar cells by reciprocal synapses (curved arrows, Fig 5–15). The A17 thus has a completely different receptive field area over which it collects rod signals as compared with the narrow-field AII amacrine cell. Presumably the A17 is an integrating unit that helps set a sensitivity level over a very large field of rod bipolar cells.[67]

Two other amacrine cells, A13 and A18 (Fig 5–15), appear to be involved in the rod pathways, perhaps to a lesser extent than AII and A17, because they are also linked to cone bipolar cells. A13 is a medium-field, diffusely branching amacrine cell (see cell 11, Fig 5–11) that receives synaptic input from rod bipolar cells and cb5 bipolar cells in sublamina b of the IPL (Fig 5–15). It makes reciprocal synapses upon both bipolar cell types. This amacrine cell then synapses upon ganglion cells of sublamina b that are of the center-depolarizing kind (on-center). Because we know that A13 responds to light with a sustained center-hyperpolarizing response,[44] it is likely that it makes sign-inverting synapses upon the ganglion cell and may again be, in some way, involved with the formation of the on-center response of the ganglion cell, probably by disinhibition.

A18 is a wide-field amacrine with extensive dendritic branching in the stratum next to the amacrine cell bodies, i.e., S1 of sublamina a (cell 13, Fig 5–11, Fig 5–15). Extremely thin dendrites of A18, not depicted on the drawing of Figure 5–11, surround cell bodies and dendrites of amacrine cells AII and

A17.[73] The A18 thereby synapses in profusion upon the two major amacrines of the rod pathways. In addition, the A18 receives many synapses from other amacrine cells and some few synapses from a cone bipolar cell type, as yet not positively identified, but possibly the type known as a giant bistratified cone bipolar cell.[33] Recently it has also been discovered that deep axon-like processes of the A18 amacrine cell that pass in S5 of sublamina b make synapses directly upon AII amacrine dendritics close to rod bipolar axon terminals.[88] A18 has not yet been studied by intracellular recordings, but it will be extremely interesting to discover, in the future, how this cross talk between cone bipolar cells and rod amacrine cells influences the rod signal pathways.

CIRCUITRY FOR CONE SIGNALS THROUGH THE RETINA

The circuitry whereby cone signals pass through the retina to the ganglion cells is rather different from that of the rod pathways. The first difference is at the OPL. The cones synapse upon *various* cone bipolar types rather than on a single type like the rod system. Thus at the OPL a choice of pathways is already installed for the cone system. As we have already mentioned (see the section on the OPL), cone bipolars come in varieties distinguished by the size of their dendritic field (midget, diffuse, and large diffuse [giant]) (see Fig 5–7) and by their different types of synaptic contact with the cone pedicles, i.e., invaginating ribbon synapses, semi-invaginating basal junctions, or non–ribbon-related basal junctions (see the section on the OPL). The nature of the postsynaptic receptors on the cone bipolar dendrite determines whether the cone bipolar cell will respond to light with center hyperpolarization or center depolarization. Thus for cat central retina, we know that the cone bipolar types that have postsynaptic receptors on the dendrites that make central ribbon contacts or narrow-cleft, semi-invaginated contacts will be center-depolarizing types (cb5, Fig 5–16,A), while cone bipolar cells that make wide-cleft basal junctions, ribbon or non–ribbon related, will be center-hyperpolarizing types (cb2 and cb6 of Fig 5–16).[68]

In the human retina the common cone bipolar cells illustrated in Figure 5–7 are, like the cat, classified not only by the nature of their synapses with cone pedicles but also according to which sublamina of the IPL their axons terminate in. Thus, some of the cone bipolar types send axons to sublamina a (fmb and dfb, Fig 5–7) and others to sublamina b (imb and dib, Fig 5–7) of the IPL. We expect that, like the cat, human cone bipolar cells with axons in sublamina a will connect to off-center (center-hyperpolarizing) ganglion cells and bipolar cells with axons in sublamina b will connect to on-center (center-depolarizing) ganglion cells. The second major difference in the circuitry of the cone pathways from that of the rod pathways is, then, that cone bipolar cells make direct synapses with ganglion cell dendrites without the need for intermediate amacrine cell circuitry as in the rod pathway. The cone pathways are therefore both more direct and more narrow field and convergent than the rod pathways. Fewer cones converge onto cone bipolars than do rods to rod bipolar cells, and correspondingly only a relatively small number of cone bipolar cells converge onto their ganglion cells. The ultimate in low convergence ratio is found in the midget system in the human and primate retina, which we shall deal with separately in another section.

The connectivity of cone bipolar cells with ganglion cells is direct and at the same time forms the underlying architecture of the on-center and off-center ganglion cell responses. Although we do not have direct evidence from intracellular recordings in human or monkey retina of the sign of the light responses of the bipolar and ganglion cells, we can assume that these cells function very similarly to those in the cat retina, which has been studied by these methods.[66, 68] Thus, Figure 5–16,A shows the basic cone bipolar-to-ganglion cell connections in the mammalian IPL. Two different cone bipolar types connect to off-center and to on-center ganglion cells in sublamina a and b respectively. It is known that cb5 cone bipolars transmit on-center (center-depolarizing) responses and that cb6 transmits off-center (center-hyperpolarizing) responses to on-center ganglion cells. A push-pull model of excitation and disinhibition to the center response of the ganglion cell receptive field has been proposed for these two opposing response-type bipolar cells.[84] Similarly a push-pull model is proposed for the input of cb1 and cb2 to the off-center ganglion cell (Fig 5–16,A). Cb2 is known to respond with a center-hyperpolarizing response in the cat retina[68] and thus transmits this center response by a straight excitation to the ganglion cell with which it is synapsing.

Amacrine cells are also involved in the cone system pathways, probably adding both excitation and inhibition to the ganglion cell receptive field center when they are small-field amacrines. A4 (Fig 5–16,B) is a small-field, hyperpolarizing amacrine

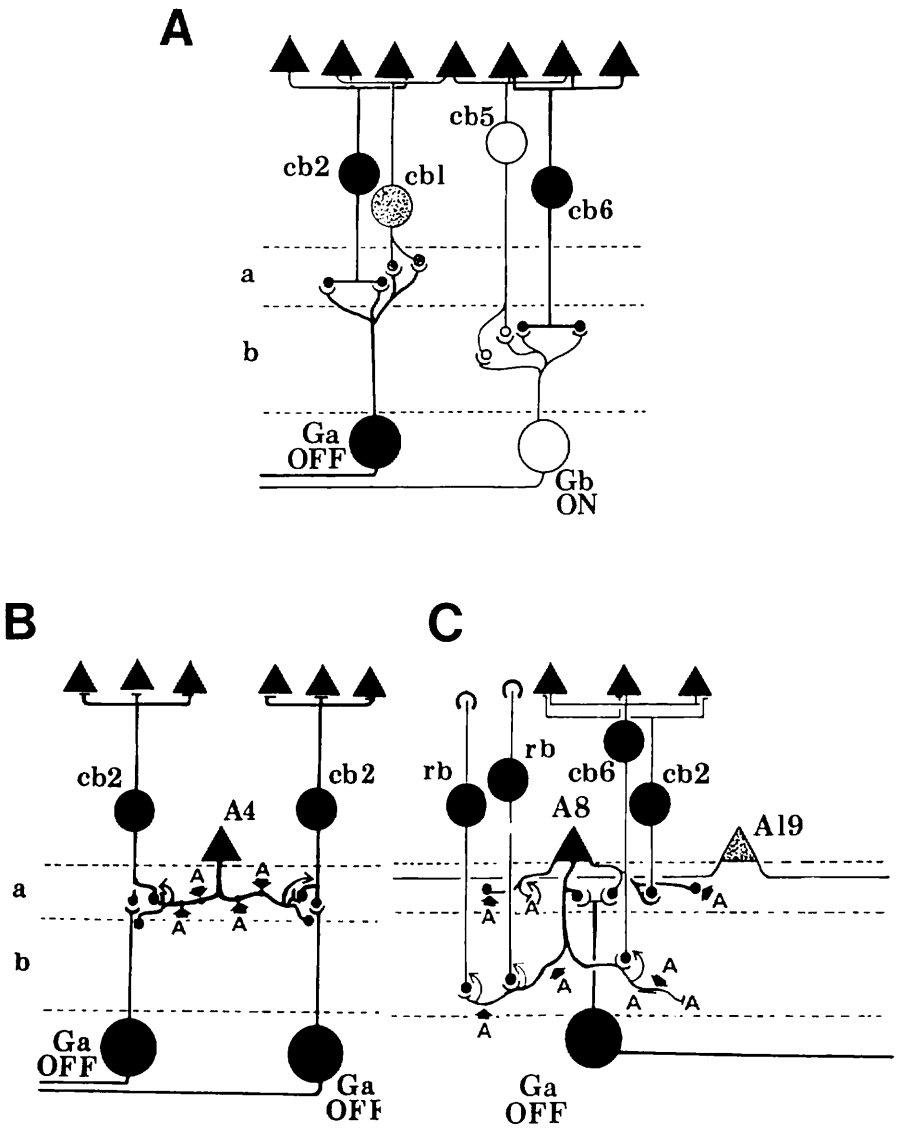

FIG 5–16.

A, wiring diagram of the parallel on-center and off-center pathways of the cone system in the cat retina (thought to be identical in the human retina). **B**, wiring diagram of the involvement of A4, a small-field amacrine cell in the cone pathways. **C**, wiring diagram of the involvement of the small, bistratified cone amacrine cell A8 and the large-field monostratified cell A19 in the cone pathways (*cbl* = cone bipolar of sublamina a; *cb2* = cone bipolar cell of sublamina a, which is known to be center hyperpolarizing; *cb5* = cone bipolar of sublamina b, which is known to be center depolarizing; *cb6* = cone bipolar of sublamina b, which is known to be center hyperpolarizing; *GaOFF* = off-center ganglion cell; *GbON* = on-center ganglion cell; *a* = sublamina a; *b* = sublamina b; *A, arrows*, unknown amacrine input; *curved arrows*, reciprocal synapses. (From Kolb H, Nelson R: Functional neurocircuitry of amacrine cells in the cat retina, in Gallego A, Gouras G (eds): *Neurocircuitry of the Retina: A Cajal Memorial.* New York, Elsevier Science Publishing Co, Inc, 1985, pp 215–232. Used by permission.)

cell that probably simultaneously modifies the ganglion cell center and the bipolar terminal of input at a reciprocal synapse. A8, on the other hand, is involved in both the cone and rod pathways (Fig 5–16,C). Intracellular recordings of such a small-field bistratified cell indicate that A8 is the cone pathway equivalent of AII for the rods in many ways.[44] Thus it receives rod bipolar input and cb6 (a center-hyperpolarizing cone bipolar) input in sublamina b, makes reciprocal synapses to these bipolars, and then has synaptic output only in sublamina a to off-center ganglion cells (Fig 5–16,C). In sublamina a, A8 also receives synaptic input from cb2, i.e., hyperpolarizing cone bipolar input, and syn-

apses upon other amacrine cells, among them an on-off amacrine cell known as A19 (Fig 5–16,C). It is therefore thought to influence the off-component of both the ganglion cell and the A19 amacrine, again probably adding more to the center response than the surround response of both. As we shall see in a later section, A4 and A8 are thought to use the same inhibitory neurotransmitter; thus their influence upon their ganglion cell of contact has to be through an inhibitory interaction, although this is not yet well understood.

MIDGET PATHWAYS IN THE FOVEA

The specialized cone pathways of the central fovea are designed to have the least convergence and the greatest resolution capabilities of the visual system. This is accomplished by making the connections as "private" as possible and narrowed to a one-to-one relationship in the so-called midget pathways. The midget pathways consist of midget bipolar cells and midget ganglion cells. Each of the latter projects to a different individual parvocellular layer cell of the lateral geniculate nucleus. Because the high-acuity midget pathways also are organized into on-center and off-center channels (like the diffuse cone pathways) every cone of the fovea must be connected to two midget bipolars, one for an on center and the other for an off center, which in turn connect with on-center and off-center midget ganglion cells.

As can be seen in the Golgi-stained examples of midget bipolar cells in Figure 5–7 (also see Fig 5–17, summary diagram of the organization of the foveal retina in the human), two types of midget bipolar cells are distinguishable in the human retina—by the type of synapse made with the cone pedicle (see Fig 5–8) and by the sublamina in the IPL in which they branch. Thus, invaginating midget bipolar cells (imb, Fig 5–7) connect with cone pedicles at narrow-cleft ribbon synapses (see Fig 5–8,A and B) and have axon terminals connecting with ganglion cells in sublamina b of the IPL. Flat midget bipolar cells connect to cone pedicles at wide-cleft, semi-invaginating basal junctions (Fig 5–8, B and C) and have axon terminals connecting with ganglion cells in sublamina a of the IPL.[37] This distinction was not made until electron microscopy was available,[37] but Polyak had already described two types of midget ganglion cell in the primate retina in 1941. He noted from vertical sections of Golgi-stained monkey retina (see Fig 5–12) that the midget ganglion cells in particular (although it is also true of the small and large parasol ganglion cells), had dendrites branching at one of two levels in the IPL: close to the ganglion cell bodies (the area we now know as sublamina b) or in the neuropil close to the amacrine cell bodies (the area we now know as sublamina a).[72] With the knowledge we now have from cat retinas[66] that ganglion cells branching in sublamina a will be off center and those branching in sublamina b will be on center we can postulate that midget ganglion cells branching close to the amacrine cells will be off-center and those branching close to the ganglion cell layer will be on-center midget ganglion cells.

The connections between the midget bipolar and the midget ganglion cells have always been thought to be "private," i.e., one to one. Because of the similar size and branching level of the axon terminal of the bipolar and the dendritic arbor of the midget ganglion cell it has been assumed that they overlap and synapse with no room for convergence from more than one bipolar axon per ganglion cell (Fig 5–17, summary diagram). This has actually only just been positively proved to be the case. A recent electron microscope study of serially sectioned midget ganglion cells has shown that the small 7-μm-diameter dendritic tree of midget ganglion cells of the fovea is completely covered and synapsed upon by a single axon terminal of the relevant midget bipolar cell; all the ribbon synapses (75 to 100) of the midget bipolar axon terminal are directed at the midget ganglion cell dendrites.[40] The midget bipolar is completely involved with a single midget ganglion cell, and the midget ganglion cell is almost completely invested by its bipolar cell that there is only room for one or two ribbon synapse from another bipolar. We can now say unequivocably that the midget pathways of the human fovea are organized in the following manner: one cone to two midget bipolar cells (on- and off-center types) to two midget ganglion cells (on- and off-center types) (Fig 5–17, right-hand side). Three millimeters beyond the fovea, in the near periphery, the midget bipolar cells become two- and three-headed, connecting to two and three cones respectively. The midget ganglion cells are found at least in the near and midperipheral retina (see Fig 5–12, near and midperiphery), and their dendritic trees remain the same dimension as the two- and three-headed midget bipolar cell axons. So it is probable, although not yet conclusively proved, that the one-to-one relationship between midget bipolar and midget ganglion cells is retained across much of the retina. In the fovea where a single cone connects with its midget system, the pathway will of

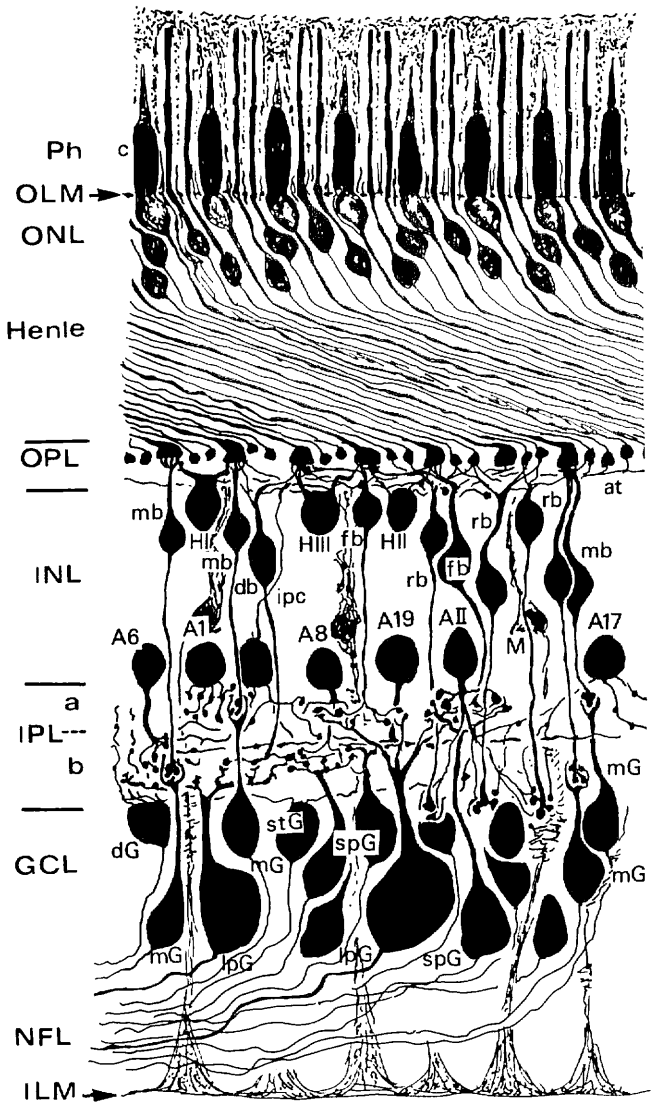

FIG 5–17.
Summary drawing of the neural connections in the human fovea. Abbreviations for the major layers of the retina are as in Figure 2,**A** and **B** (additional abbreviations: *c* = cone, *r* = rod; *mb* = midget bipolar cell; *rb* = rod bipolar; *db* = diffuse invaginating cone bipolar; *fb* = diffuse flat cone bipolar; *HI, HII,* and *HIII,* horizontal cells; *at* = HI and HIII horizontal cell axon terminals; *M* = Müller cells; *A1, A6, A8, AII, A17,* and *A19,* varieties of amacrine cell (see the text for roles); *ipc* = interplexiform cell; *dG* = diffuse ganglion cell; *mG* = midget ganglion cell; *stG* = stratified ganglion cell; *spG* = small parasol ganglion cell; *lpG* = large parasol ganglion cell.

necessity be carrying single-color information in its receptive field center. Beyond the fovea where the midget pathway is two and three channeled due to connection with two or three cones it remains to be seen whether these will be the same or a mixture of chromatic types.

Midget ganglion cells of the monkey retina (presumably also in human retina) are thought to respond to light with an opponent chromatic organization.[29, 30] That is, midget ganglion cells, when recorded electrophysiologically, have the smallest receptive fields and are organized as red on or off center/green off or on surround or vice versa. Pathways involving blue/yellow opponency appear to behave differently and may not be using midget ganglion cells. The color of the center response in the midget ganglion cells is clearly coming from the midget bipolar's connection with a single-color cone type (either a long-wavelength cone or a medium-wavelength cone), but the opponent color for the surround results from more complex circuitry. The horizontal cells are thought to contribute in some way to the surround response of bipolar cells in submammalian species,[17] but this is not so clearly established for mammalian and primate retinas. The role of amacrine cells may be more important in the formation of surrounds at ganglion cells in these animals. Suffice it to say that in terms of color opponent surrounds for midget ganglion cells, the best candidates for being the basis of such circuits are small-field amacrine cells such as illustrated in Figure 5–11 (cells 4, 5, 6, and 8) and Figure 5–17 (A1 and A6).[42]

SPECIALIZED CIRCUITS

We know from single-cell electrophysiology that certain lower vertebrate retinas are capable of processing rather complex messages concerning orientation, directionality, movement, and color, and that this information is already encoded at the ganglion cell level. In primates and humans the retina and visual system are specialized for foveal vision. High acuity and fine discriminations of color are the most important aspects of vision, as shown by the disproportionately large areas of the brain nuclei that are devoted to the fovea. Correspondingly, the processing of complex stimuli typically found in the retinas of lower vertebrates is found in higher visual centers rather than the retina. But remnants of the specialized circuitry, typical of lower primates, are still found, particularly in the peripheral retina. Pupil constriction and eye movements also rely on information from neural circuits in the retina.[46]

Much interest has focused on the retinal circuitry underlying motion and directional selectivity at the ganglion cells. These types of ganglion cells are predominant in animals that have retinas with visual streak specializations, i.e., turtle, rabbit, and ground

squirrel. The underlying circuit appears to involve a specific type of amacrine cell that uses acetylcholine (ACh) as its neurotransmitter, bipolar cells, and another amacrine known use γ-aminobutyric acid as its neurotransmitter (GABAergic).[23, 36, 58] All of the components of the circuitry, i.e., the ACh amacrine, the GABA amacrine, and the cone bipolars, are present in the human retina, so it is likely that directional selective circuits similar to but probably less emphasized, also occur in human extrafoveal retina although their importance and extent is less than in lower animals.

Retinal circuitry underlying motion detection is not yet well understood in any vertebrate retina. Certainly the very largest ganglion cells of the cat retina (known as Y cells physiologically[22] and alpha cells morphologically[9]) are involved in this activity. The ganglion cells of the human retina most likely to be the equivalent of the alpha/Y cells of the cat are the very largest parasol ganglion cells of human far peripheral retina.[48, 80] The underlying mechanism for fast movement detection is thought to reside in the amacrine-dominated input to these large ganglion cells. Many subunits, i.e., bipolar-to-amacrine cell subcircuits, feed into the ganglion cells' dendritic field in an organization that is not yet well understood. It is possible that the on-off amacrine called A19 (see the section on cone pathways) in the cat retina, which is also present in human retina (Fig 5–17), interconnects groups of cone bipolar cells and amacrine cells across the retina. The field of influence of A19 cells may well be rather large, and their function may be to pass information very fast across relatively long distances. This would be eminently possible due to the fact that A19 amacrine cells are connected by gap (electrical) junctions in a type of lateral syncytium across the retina.[44]

The pathways for transmitting information from the short-wavelength cones to ganglion cells appears to be different from the midget pathways for the medium- and long-wavelength cones. As we have discussed above, the latter two chromatic pathways are via midget bipolar and midget ganglion cells. In contrast, the short-wavelength cone does not connect through a typical midget bipolar/ganglion cell chain. Instead, a special blue cone bipolar cell connects to two or three short-wavelength cones (see Fig 5–7, bcb) and carries this information to the S5 sublayer of sublamina b in the IPL.[53] The blue cone bipolar has an extremely widespread axon terminal that contrasts greatly with the small club-shaped constricted terminals of the midget bipolars. It is not known exactly which ganglion cell type might be postsynaptic to the wide-field blue cone bipolar, but it is evidently of a wide-field type with branches in S5 of the IPL. In fact, electrophysiological investigation of monkey retinal ganglion cells indicates that blue/yellow opponency is carried primarily by a blue on-center ganglion cell type with a much larger receptive field center than is typical of the red/green or green/red opponent midget ganglion cells.[29]

FEEDBACK LOOPS IN THE RETINA

At every level of the retina there are reciprocal or feedback loops in the circuitry so that certain neurons can interact laterally within the same layer, vertically from one layer to the other, and indeed from the brain to the retina. The intralayer feedback loops are typically provided by neurons that use inhibitory neurotransmitters such as GABA, and they have a function in sharpening the image by adding antagonistic concentricity to receptive fields of the neurons; however, the feedback loops between the layers or from the brain are less clear in function. The latter loops tend to use neuromodulators as their transmitters and thereby have a more generalized effect on groups of neurons or on the state of excitability of the neuron chains (adaptation, for example).

As mentioned in the section on photoreceptors, in submammalian retinas the cones receive an antagonistic reciprocal feedback message from horizontal cells[5] that serves to provide a restricted concentric receptive field for the individual cones. Morphological evidence for the feedback synapse from horizontal cell dendrites to the cone pedicle has only been demonstrated in the catfish retina so far.[77] Feedback synapses have been difficult to demonstrate either electrophysiologically or morphologically in the mammalian cones and, of course, have not been seen in human cones at all. However, the rod axon terminals of HI horizontal cells that end in the rod spherules are seen to make small punctate chemical synapses upon both the rod spherule (see Fig 5–5,B) and the rod bipolar cell dendrite (not illustrated) in the human retina.[50]

In the IPL, many amacrine cell types probably provide feedback information to bipolar axon terminals. One we known something about is the reciprocal synapses from the wide-field rod amacrine A17 upon the rod bipolar axon terminal (see the section on the rod system). It is intriguing that this IPL input-output synaptology between the rod bipolar and A17 amacrine parallels the input-output of the rod and horizontal cell at the OPL and suggests some

necessity of the rod system, in the mammalian retina at least, to be in repetitive feedback loops all through the retina. We have also noted that A8 and A4 of the cone system (see the section on cone pathways) are also involved in feedback loops with cone bipolar cells.

A neuron in the retina of just about every species studied, including human, links the two plexiform layers by receiving synaptic input in the IPL and having synaptic output upon neurons of the OPL. It is known as the interplexiform cell (Fig 5–18,A). First described by Gallego[26] in the cat retina, it has been extensively studied in the goldfish retina by Dowling and colleages (see Dowling[17]) and now also in the cat and the human retina.[45, 49] The synaptic connections of the cells are very similar in the cat and in humans (Fig 5–18). The cell branches diffusely in the IPL, and there its dendrites receive input from amacrine cells of unknown type and make synapses upon rod and cone bipolar axons.[45, 63]

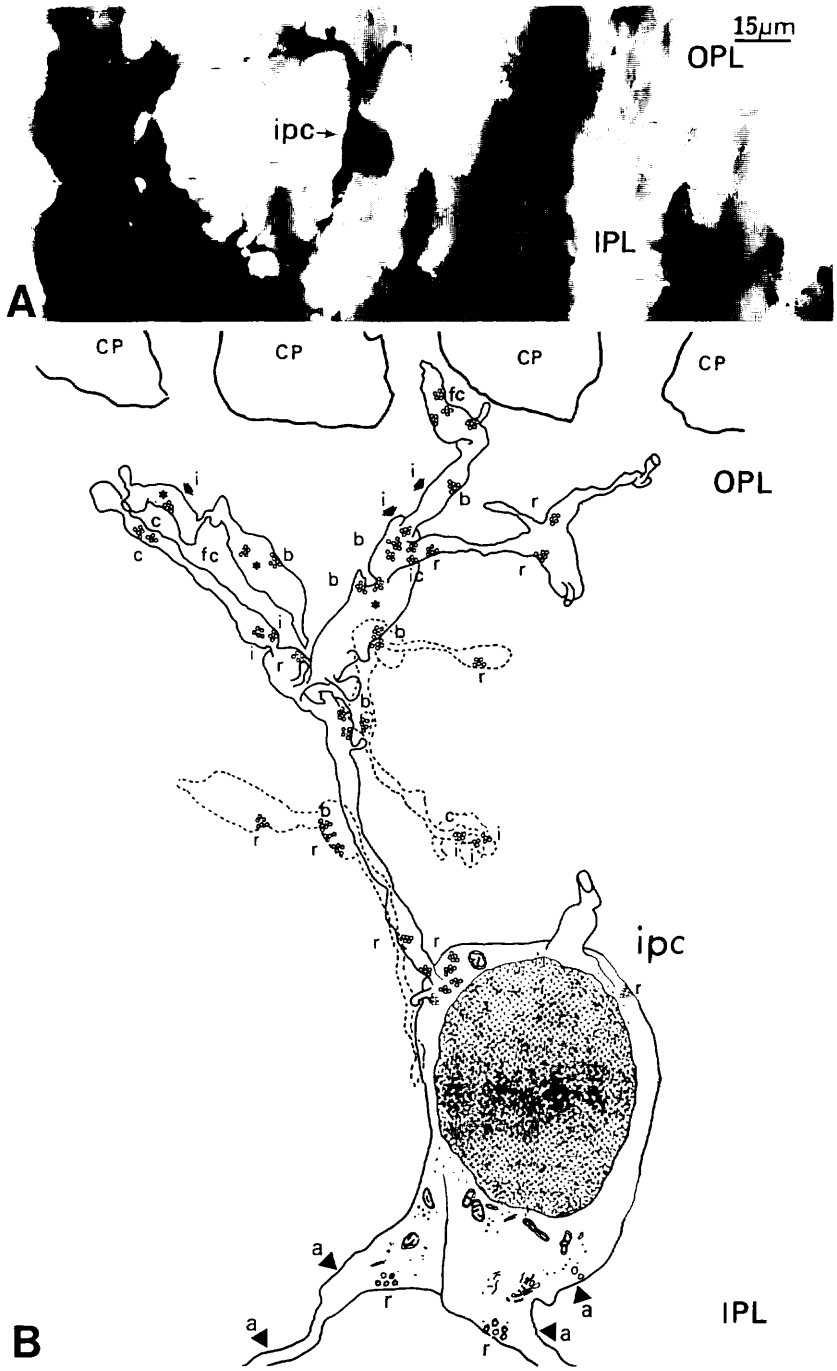

FIG 5–18.
A, light micrograph of a radial view of a Golgi-stained interplexiform cell in the cat retina. An identical cell type occurs in the human retina. The cell body sits in the INL and emits a stout apical process that passes up to the OPL and there ramifies and branches. The dendrites given off the cell body to the IPL branch diffusely through all the strata and sublayers of the IPL (scale bar, 15 μm). **B,** reconstruction from electron microscope serial sections of an interplexiform cell as seen in the cat retina. The apical process passing to the OPL makes numerous synapses (clusters of vesicles) upon cone *(c)* and rod bipolar *(r)* cell bodies and dendrites in the INL and the OPL (*CP* = cone pedicles; *ic* = invaginating cone bipolar; *fb* = flat cone bipolar; *i* = other interplexiform cell profiles. The dendrites in the IPL are presynpatic to rod bipolar axons *(r)* and postsynaptic to numerous amacrine profiles *(a, large arrowhead).* (**B,** adapted from, and **A,** from Kolb H, West RW: *J Neurocytol* 1977; 6:155–170. Used by permission.)

Throughout the INL and in the OPL the interplexiform cell makes numerous conventional synapses upon rod and cone bipolar cell bodies and dendrites (Fig 5–18,B). In both the cat and human, the interplexiform cell has processes that touch cone pedicles but make rather unspecialized junctions at this site that we have been reluctant to call synapses. The interplexiform cell, just described in the human and cat, is known to be GABAergic. This differs considerably from the interplexiform cell of the fish retina where it is known to be dopaminergic.[21] In the fish retina the dopaminergic interplexiform cell is known to have synapses in the OPL primarily upon horizontal cells. Thus it seems that the fish interplexiform cell has a rather different role to play in the retina as compared with the GABAergic interplexiform cell of the human.

In both human and cat retinas there exists a dopaminergic amacrine cell, called A18, that is involved in the rod system pathways of the IPL (see Fig 5–15). However, a minority of these A18 cells have processes ascending from their cell bodies or from their plexus of dendrites in the IPL that pass up to the OPL. In the INL and OPL these dendrites have been proposed to be presynaptic to horizontal cell processes.[25] Further investigation is needed to fully elucidate the synaptic connections and function of these dopaminergic "interplexiform" processes.

Centrifugal fibers arising in brain nuclei and passing back to the retina have been noted in many vertebrates, but they are particularly well developed in the avian retina.[13, 59, 70] A few centrifugal fibers were described in the monkey retina by Polyak[72] and later by Honrubia and Elliott.[34] The centrifugal fibers in monkeys (illustrated in Fig 5–14,C, from Polyak) have varicose, bulbous terminals that end in the IPL close to amacrine cell bodies. Little else is known concerning them. In the fish retina we now know that centrifugal fibers use the molluscan cardioexcitatory peptide FMRFamide and luteinizing hormone–releasing hormone as neurotransmitters[83] and synapse directly upon cell bodies of the dopamine interplexiform cell.[93] It will be interesting to see whether future work finds any link between the rare centrifugal fibers of the human and the dopamine amacrine cell system.

NEUROTRANSMITTERS IN THE RETINA

Today much research on the retina focuses on neurotransmitters. New techniques using autoradiography, immunology, and molecular biology are developing specific stains for neurochemicals, their synthesizing enzymes, or the nucleic acids manufacturing these chemicals so that the cells containing each transmitter can be marked. Cells stained with antibodies to the neurotransmitters that are conjugated to horseradish peroxidase are particularly spectacular. They are stained to their finest dendrites and so can be readily classified with their Golgi-stained equivalents. Furthermore, the whole population of neurotransmitter-specific neurons are stained, so one can understand their topographical organization into mosaics across the entire retina. Not too much immunocytochemistry has been performed on the human retina yet, but the consistency of cell types staining across species boundaries suggests that most, with a few exceptions, of the neurotransmitters, neuromodulators, and neuropeptides discovered in nonhuman retinas will also be present in the human retina (Table 5–1).

This chapter is not the place to go into lengthy details of all the different neurotransmitter candidates and cell types that have been discovered so far in the vertebrate retina. We refer the interested reader to a recent review article on this subject by Ehinger and Dowling.[20] Tables 5–2 and 5–3 included here are taken from that review and form a good summary list of the present status of this field.

Of the more regular neurotransmitter candidates found to have excitatory synaptic effects in other parts of the nervous system, glutamate, aspartate, and ACh are found in the mammalian retina, including monkeys and humans. Glutamate is the strongest candidate for being a neurotransmitter of the photoreceptors, and some evidence exists for aspartate being specific for rods.[62] Glutamate is also thought to be the transmitter for some bipolar cells and some ganglion cells. ACh is apparently restricted to an amacrine cell type known as the "starburst amacrine"[23, 58] that is involved in directional selective circuitry upon ganglion cells (see the section on special circuits). This cell is also found in the human retina.[35] It is possible that certain ganglion cells also use ACh.

The classic inhibitory neurotransmitter GABA occurs in the retina in many different varieties of amacrine cells (A2, A10, A13, A17, A19, interplexiform cell, Fig 5–17),[74] and a class of horizontal cell in most vertebrate retinas. A type of bipolar cell is thought to be glycinergic in mammalian retinas including monkeys, and the narrow-field amacrines of the rod and cone pathways (AII and A4, A8, Fig 5–17) are the strongest amacrine cell candidates for using glycine in neurotransmission.[31]

The neuromodulator dopamine is found in a type of amacrine cell called A18 that is involved in the

TABLE 5–1.

Facts and Figures Concerning the Human Retina*

Size of the retina
42 mm from ora to ora across the horizontal meridian (Van Buren[86]; Kolb, unpublished measurements)
Size of the optic nerve head or disc
2 × 1.5 mm
Degrees and distance in micrometers
One degree of visual angle is equal to 288 μm on the retina without correction for shrinkage[19]
Foveal position
17 degrees temporal to the disc
Cross diameter of the macula
3 mm of intense pigmentation surrounded by 1-mm-wide zone of less pigmentation[72]
Cross diameter of the central fovea from foveal rim to foveal rim
1.5 mm,[72] 1.2–1.5 mm (Ahnelt and Kolb, unpublished data)
Cross diameter of the central rod free area
400–600 μm,[72] 750 μm,[32] 570 μm,[90]
250 μm^2
Vertical thickness of the fovea from ILM to ELM
In the foveal pit, 150 μm[90]; foveal rim, 400 μm
Central region of the fovea where there are no cone pedicles
250 μm,[90] 200 μm,[32] 300 μm^3
Length of foveal axons (Henle fibers)
150–300 μm^3
Age when the fovea is fully developed
Not before 4 years of age[32]
Highest density of cones (cones per square millimeter) at the center of the fovea (50 × 50 μm)
147,000,[71] 178,000–238,000,[2] 96,900–281,000 (mean of 161,900)[15]; see Figures 5–19 and 5–20.
Total number of cones in the retina
6,400,000[71]
Total number of rods in the retina
110,000,000 to 125,000,000[71]
Rod distribution
Rods peak in density 18 degrees from the center of the fovea in a ring around the fovea at 150,000–160,000 rods/mm^2. No rods in the central 200 μm. Average, 80,000–100,000 rods/mm^2 (see Fig 5–19)
Number of axons in the optic nerve
564,776–1,140,030,[12] 800,000–1,000,000,[72] 1,200,000[4, 75]
Number of cones to ganglion cells in the fovea
1 cone to 2 ganglion cells out to about 2.2 degrees[78]

TABLE 5–2.

Neurotransmitters and Neuromodulators in the Retina*

Transmitter/Modulator†	Retinal Cells
Acetylcholine	Amacrines
Adrenaline (?)	Amacrines
Aspartate (?)	Photoreceptors, bipolars
Dopamine	Amacrines, interplexiform cells
GABA	Amacrines, horizontals, and interplexiform cells
Glutamate	Photoreceptors, bipolars, ganglions
Glycine	Amacrines, bipolars
Histamine (?)	(?)
5-Hydroxytryptamine (serotonin, indoleamine)	Amacrines, bipolars
Noradrenaline (?)	Amacrines
Melatonin (?)	Photoreceptors (?)

*Adapted from Ehinger B, Dowling JE: Retinal neurocircuitry and transmission, in Bjorklund A, Hokfelt T, Swanson LW (eds): *Handbook of Chemical Neuroanatomy*, vol 5, *Integrated Systems of the CNS*, part 1. Amsterdam, Elsevier Science Publishers, 1987, pp 389–446.
†Note that not all substances appear in the same species. (?) denotes less well documented evidence for this neurotransmitter.

rod system in monkeys and humans (see the section on rod pathways and Fig 5–15). It may also have some interplexiform cell–like function (see the above section on feedback circuits). There is an indoleamine-accumulating amacrine cell (5-hydroxytryptamine or serotonin) in the rabbit retina that has been equated with the A17 cell in the cat.[57] However, indoleamine is not thought to be a transmitter in the mammalian retina, and its coexistence with GABA, which is also said to be in the A17 cell and which may be the releasable transmitter, has to be considered.[20] A few cold-blooded vertebrates have a bistratified amacrine cell type and a bipolar type that stains for serotonin. Noradrenaline may be present in a small-field amacrine cell type in the monkey retina.[55]

The neuropeptides listed in Table 5–3 have been seen in a number of vertebrate retinas but only neuropeptide Y, somatostatin, and substance P have been seen in monkey or human retinas so far.[10] Ev-

TABLE 5–3.

Neuropeptides in the Retina

Neuropeptide*	Retinal Cell
Atrial natriuretic peptide	Amacrines
Bombesin	Amacrines, ganglions
Cholecystokinin (CCK)	Amacrines, ganglions
Calcitonin gene–related peptide (CGRP) (?)	Amacrines
Enkephalins	Amacrines, ganglions
FMRFamide	Centrifugal fibers
Glucagon	Amacrines
Luteinizing hormone–releasing hormone (LHRH)	Centrifugal fibers
Neurotensin	Amacrines
Neuropeptide Y (NPY)	Amacrines, ganglions
Peptide HI (PHI) (?)	Amacrines
Somatostatin	Amacrines
Substance P	Amacrines, ganglions
Gastrin-releasing peptide (GRP) (?)	Amacrines
Thyrotrophin-releasing hormone (TRH)	(?)
Vasoactive intestinal peptide (VIP)	Amacrines

*Neuropeptides shown to be present in some retinas. Not all are seen in every species. (?) denotes weak evidence for these neuropeptides.

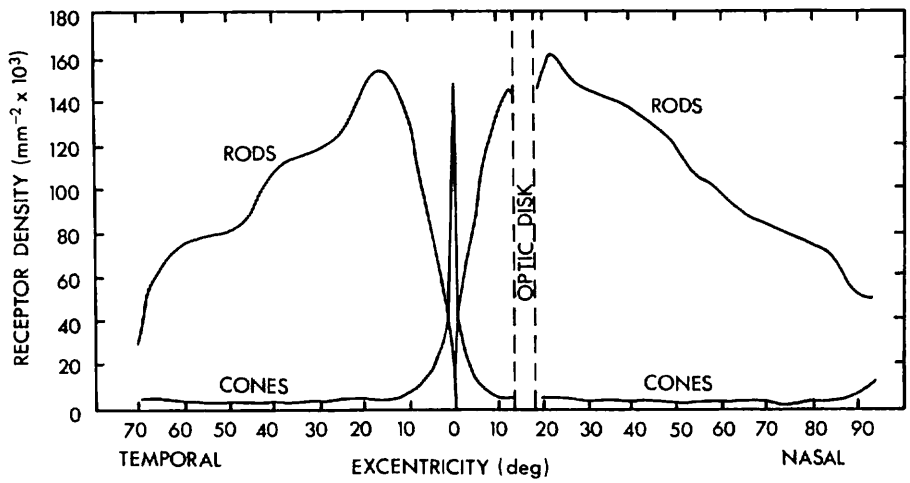

FIG 5–19.
Graph showing the cone and rod photoreceptor density along the horizontal meridian of the human retina. (From Østerberg G: *Acta Ophthalmol Suppl* 1935; 6:1–103. Used by permission.)

ery variety of amacrine cell in every retina studied appears to have a different neuropeptide, and colocalization with a regular neurotransmitter or neuromodulator is usually the case. A ganglion cell type in the rabbit retina has definitely been proved to contain substance P, almost certainly colocalized with a more standard neurotransmitter too.[11] In fact, we are presently hearing of good morphological evidence for colocalization of two classic excitatory and inhibitory neurotransmitters, namely, ACh and GABA, in one and the same amacrine cell type in the mammalian retina.[14, 57, 87] Similarly, dopamine

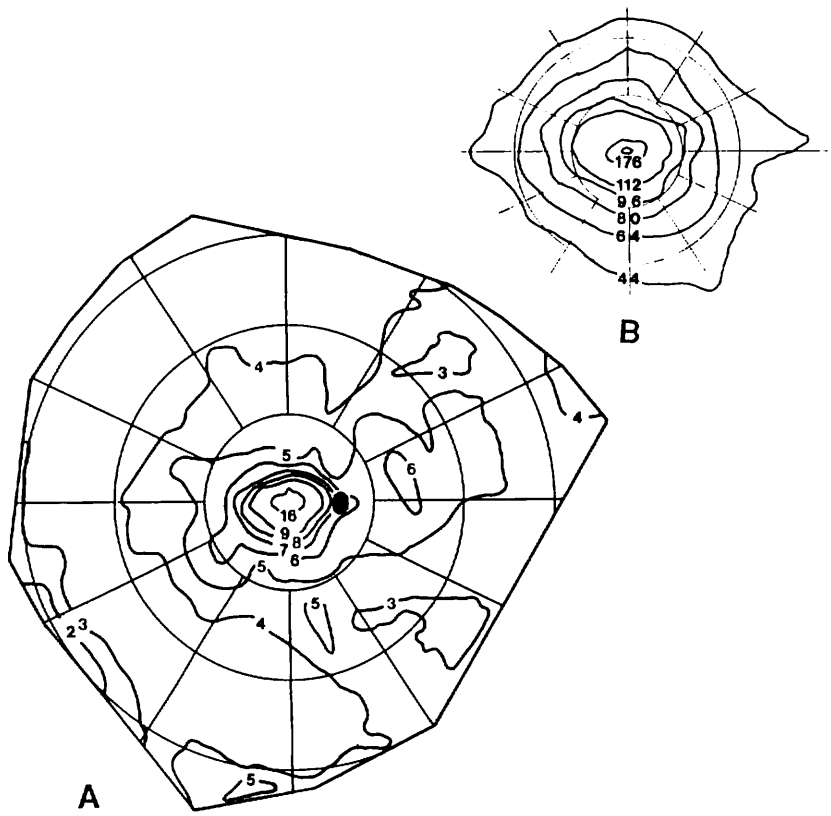

FIG 5–20.
Isodensity maps of the cone photoreceptor density in the whole human retina **(A)** and in the foveal area **(B).** Numbers are expressed as cones × 10,000/mm^2 in **A** and cones × 1,000/mm^2 in **B.** (From Curcio CA, Sloan KR, Packer O, et al: *Science* 1987; 236:579–582. Used by permission.)

and serotonin have been found to coexist in in the same amacrine cells in cat retinas.[89] Whether this plethora of neurotransmitters in the same neurons is going to be a generalized finding for the human retina, and what this means in terms of creating different functions in the neural networks of the retina, remains to be seen.

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