Organization of vertebrate retinas

The Jonas M. Friedenwald Memorial Lecture

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The vertebrate retina is a portion of nervous tissue that has long been of interest both to investigators of brain function and of the visual process. It is one of the most accessible parts of the central nervous system; it can be easily and precisely stimulated with patterns of light; and its output, via the optic nerve, can be readily monitored.

Recent studies employing electron microscopy and intracellular recording techniques have described the synaptic contacts in the vertebrate retina and the electrical responses of the six principal types of retinal cells. This new information, coupled with the knowledge of the retinal cells derived from light microscopy, permits us to infer probable sites of interaction and major synaptic pathways that occur within the vertebrate retina. In this paper I shall review some of this recent work and present a scheme of the synaptic organization of the vertebrate retina that suggests some of the functions of the two plexiform (synaptic) layers. I shall also discuss briefly some studies concerned with the cellular origins of the components of the electroretinogram (ERG).

Cellular organization

The vertebrate retina consists of five types of neurons and one type of glial cell. The cell bodies (perikarya) of the retina are organized into three nuclear layers; the overwhelming majority of synapses are confined to two synaptic areas, the outer and the inner plexiform layers. In each plexiform layer the processes of three cell types synaptically interact. Fig. 1 is a light micrograph of a vertebrate retina (mudpuppy) which illustrates the three cellular layers, the two plexiform layers, and the prominent glial elements (Müller cells).

Most of the information we have concerning the cell types in the vertebrate retina, and the extent and distribution of their processes, has come from the light microscopy of retinal tissue processed by the method of Golgi. The many variants of this impregnation technique have been used profitably since the late nineteenth
Fig. 1. Light micrograph of the mudpuppy retina showing the three nuclear layers, the two plexiform layers, and the prominent Müller (glial) cells (M). R, receptors; ELM, external limiting membrane; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; and ILM, inner limiting membrane. (x450.)

Fig. 2. The principal cell types found in the vertebrate retina. This drawing is based on observations of cells in the mudpuppy retina that were impregnated by the Golgi method. See text for description of cells. R, receptor; H, horizontal cell; B, bipolar cell; A, amacrine cell; G, ganglion cell; and M, Müller (glial) cell.

century and are still providing critical information.

Fig. 2 depicts schematically the principal cell types found in the vertebrate retina by the Golgi method and some of their characteristics. This drawing is based mainly on the mudpuppy, Necturus maculosus, because it is from this retina, with its large cells, that most of the physiological data I shall discuss are derived. The perikarya
in the vertebrate retina are found mostly in defined layers. The perikarya of the receptors (R) are located in the outer nuclear layer; the horizontal cells (H), along the outer margin of the inner nuclear layer. The bipolar cell perikarya (B) lie in the distal half of the inner nuclear layer; the perikarya of the amacrine cells (A), in the proximal half of the inner nuclear layer. The ganglion cell perikarya (G) are found along the inner margin of the retina. The Müller cells (M) extend vertically through the thickness of the retina; their nuclei are most often found in the middle of the inner nuclear layer.

In the outer plexiform layer, processes from the bipolar and horizontal cells extend outward to the level of the receptor terminals. Horizontal cells in most species have a much wider spread of processes in this layer than do the bipolar cells, and this has been interpreted to mean that horizontal cells mediate lateral interactions in the outer plexiform layer.1,2

The bipolar cells are the vertical pathways carrying visual information from the outer to the inner plexiform layer. In this layer processes from the bipolar, amacrine, and ganglion cells synaptically interact. The bipolar terminals in many species are often large and bulbous. The processes of some amacrine cells extend laterally for considerable distances, sometimes forming discrete strata in the inner plexiform layer.1-3 These are often the longest processes found in any vertebrate retina. The processes of other amacrine cells spread diffusely throughout the thickness of the inner plexiform layer. The dendrites of the ganglion cells may be stratified or diffuse, depending on the type of ganglion cell from which they originate. The axons of the ganglion cells run along the inner margin of the retina and through the optic disc to form the optic nerve.

As noted before, the Müller (glial) cells extend vertically through the retina. Fine processes that envelop, at least partially, all the nearby neurons extend laterally from the prominent column of glial cell cytoplasm that runs from the external to the internal limiting membranes.

Synaptic organization

Electron microscopy provides the resolution necessary to examine the synaptic contacts of the retinal cells. Fig. 3 shows survey electron micrographs of the outer plexiform layer of the cat retina and a portion of the inner plexiform layer of the human retina. Synapses cannot readily be identified at such low magnification, but some general characteristics of the fine structure of the retinal cell types and their processes are illustrated. Fig. 3, a shows that the vesicle-filled receptor terminals are aligned along the distal edge of the plexiform layer, and that, in the cat, the horizontal and bipolar cells may be easily distinguished morphologically along the outer margin of the inner plexiform layer.

In the inner plexiform layer of the human retina (Fig 3, b) the larger bipolar terminals (BT) are readily recognizable by their size, bulbous shape, content of vesicles, and occasional synaptic ribbons. Observations such as these permit correlations in many species between light and electron microscopic observations, and allow, for example, the assignment of particular synaptic contacts to specific cell types.

Although studies of Golgi-processed material by light microscopy have permitted workers to classify two or more subtypes of cell for each of the five major types of retinal neurons,1-3 it has not been possible generally to distinguish subtypes of cells by electron microscopy.4 For example, bipolar terminals in all vertebrates have similar cytoplasmic morphology and synaptic contacts. This is true for most amacrine processes, ganglion cell dendrites, and horizontal cell processes.

Synaptic morphology. Two principal types of synaptic contacts have been described by electron microscopy in vertebrate retinas; both synaptic types are found in each plexiform layer (Figs. 4 to 7). Ribbon synapses are characterized by
Fig. 3. For legend see opposite page.
a dense ribbon or bar in the presynaptic cytoplasm\textsuperscript{4-10} (Figs. 4 and 7). The ribbon is surrounded by a precisely arranged array of synaptic vesicles and is oriented at right angles to the adjacent plasma membrane. At ribbon synapses in the retina, there are always multiple, postsynaptic elements. Increased membrane density on both the pre- and postsynaptic processes is usually observed at ribbon synapses; the synaptic cleft is often wider than the extracellular space seen at nonsynaptic areas.
Fig. 5. Electron micrograph of a Golgi-impregnated bipolar cell (B) from the monkey retina. One dendrite can be followed from the cell body through the outer plexiform layer to its termination as the central element in an invagination of a cone terminal (inset). The arrow points to the synaptic ribbon overlying the invagination (x12,000); inset (x22,000).

Fine, filamentous material is occasionally observed in the synaptic cleft; this may extend into the adjacent cytoplasm of the postsynaptic elements, giving the postsynaptic membranes a "thickened" appearance (Fig. 7, a). Ribbon synapses of the outer plexiform layer are found exclusively in the receptor terminals (x10) (Fig. 4); in the inner plexiform layer, synaptic ribbons have been found only in the bipolar terminals (Fig. 7).

Conventional synaptic contacts in the retina are similar to synaptic contacts described throughout the vertebrate nervous system (Figs. 6, 7, and 8). They are characterized by a dense aggregation of synaptic vesicles clustered close to the presumed, presynaptic membrane. Some increase in membrane density is seen on both pre- and postsynaptic elements, and fine filaments can occasionally be distinguished running between pre- and postsynaptic membranes in the synaptic cleft. Only one postsynaptic element is present at these contacts. Conventional synapses are made by horizontal cell processes in the outer plexiform layer (Fig. 6) and by the amacrine cell processes in the inner plexiform layer (Figs. 7 and 8).

Bipolar cell dendrites in the outer plexiform layer and ganglion cell dendrites in the inner plexiform layer have never been observed to make a presynaptic contact. Thus, of the three types of retinal neurons contributing processes to each synaptic layer, only the processes of two cell types make synapses; the present evidence indicates that these synaptic contacts are different. That is, in the outer plexiform layer synaptic ribbon contacts are made only by receptor terminals; conventional contacts, only by horizontal cells and their processes. In the inner plexiform layer, ribbon contacts are made only by bipolar terminals; conventional contacts, mainly by amacrine cells and their processes. These findings are of considerable importance for elucidating synaptic pathways in the retina, since it is usually possible to identify an isolated process in either plexiform layer by the nature of its synaptic contact.

In the pigeon retina, synaptic contacts of the centrifugal fiber terminals have been identified in the inner plexiform layer. These synapses are of the conventional type; they are all located along the border of the inner plexiform layer with the inner nuclear layer. The centrifugal fiber synapses are made onto the proximal processes and perikarya of amacrine cells. The number of centrifugal fiber synapses seen in the pigeon retina is very low compared to the number of amacrine synapses observed throughout the inner plexiform layer.
Fig. 6. Conventional synapses (open arrows) of horizontal processes (H) in the mudpuppy retina. In both cases, the conventional synapses are made by horizontal cell processes that are themselves contacted by a ribbon synapse of a receptor terminal (filled arrow), suggesting that these horizontal cell processes are both pre- and postsynaptic elements. (x42,500.)

Synaptic pathways.

Outer plexiform layer. The majority of synapses seen at the level of the outer plexiform layer are the ribbon synapses of the receptors. In most species these synapses occur in invaginations in the receptor terminals. In the larger (often cone) terminals there may be numerous invaginations (as many as 25) (Fig. 4, a); in the smaller (rod) terminals, there may be only a single invagination (Fig. 4, b). Three or more processes extend into a single invagination and appear related to the synaptic ribbon overlying the invagination. It is generally assumed that all the processes in an invagination receive input from the receptor via the ribbon synapse.

In most species there is a precise arrangement of processes in the invaginations. Two deeply inserted processes are positioned on either side of the synaptic ribbon, while one to three processes lie centrally and more superficially. Studies on human and fish retinas have demonstrated that the deeper lateral elements are horizontal cell processes, while the central elements are bipolar cell dendrites. Recent work in which cells, stained by the Golgi method and identified by light microscopy, were serially sectioned and studied by electron microscopy, has shown unequivocally that this general arrangement holds for both rod and cone terminals in the monkey, cat, and guinea pig. Fig. 5 is an electron micrograph of a Golgi-stained bipolar cell process that can be followed from the cell body to the receptor terminal where it ends as the central element in an invagination (Fig. 5, inset).

In addition to the invaginated ribbon synapses, a superficial, flat, or basal contact has been described between the receptor terminals and certain processes (Fig. 4, a, inset). Not much synaptic specialization is seen at these contacts in most species. For example, no ribbon or cluster of synaptic vesicles is usually observed as-
Fig. 7. Ribbon synapses of bipolar terminals (filled arrows) and conventional synapses of amacrine processes (open arrows). Two postsynaptic processes are contacted at the ribbon synapses of the bipolar terminals. One of the postsynaptic processes is almost always an amacrine (A), and often the amacrine process is observed making a reciprocal (feedback) synapse back onto the bipolar terminal (b). In primates (b) the second postsynaptic element is usually a ganglion cell dendrite (G). a, chicken retina (×30,000); b, human retina (×31,000).

Associated with these contacts, which are distinguished by some membrane densification on both sides of the contact and by occasional filamentous material extending across the extracellular space. However, it has been shown that certain bipolar cells (the flat bipolars) make only this type of contact with the receptors. Also, synaptic ribbons are observed associated with the superficial contacts in the mudpuppy, and thus there is clear morphological evidence for a synaptic function of these contacts in at least one species.

In most species, conventional synaptic contacts made by horizontal cell processes have been observed (Fig. 6). These contacts are on adjacent bipolar cell dendrites or other horizontal cell processes. A horizontal cell synapse has never been observed feeding back onto a receptor terminal, and so it is assumed that horizontal cell processes mediate lateral interactions in the outer plexiform layer by acting on the bipolar cell dendrites.

Although in some species it has been shown that the majority of horizontal cell synapses are on the bipolar cell dendrites, the total number of horizontal-bipolar synapses is so low in all species so far studied, that it seems questionable whether horizontal-bipolar cell interactions are significant. Thus, it has been suggested that the un-
Fig. 8. Complex synaptic arrangements in the frog inner plexiform layer. a, At the ribbon synapse (filled arrow), the two postsynaptic elements contacted are both amacrine processes. One amacrine process ($A_1$) makes a conventional synapse (open arrow) on the other amacrine process ($A_2$), which in turn synapses (open arrow) on a ganglion cell dendrite ($G$). ($\times48,000.$) 

b, Complex serial synapse arrangement between four amacrine processes ($A_i$); synapses marked by open arrows. This micrograph illustrates well that amacrine processes may be both pre- and postsynaptic. ($\times60,000.$)
usual, invaginated synaptic complexes in the receptor terminals could allow for interactions between horizontal and bipolar cell processes in some way not understood. For example, the deeply inserted horizontal cell processes are strategically positioned in the invaginations to regulate transmitter flow from receptor to bipolar cell dendrite4'10 (Fig. 4).

Occasionally a synapse is observed in a horizontal cell process that is itself postsynaptic to a nearby receptor ribbon synapse10 (Fig. 6). Such a process, therefore, is both a pre- and a postsynaptic element. In the inner plexiform layer, amacrine cell processes in all species studied have likewise been shown to be both pre- and postsynaptic along their length4'13'14'19'20 (Figs. 7 and 8). Such types of processes would appear well suited to mediate lateral and reciprocal interactions between adjacent neurons, and it appears that the great majority of horizontal and amacrine processes in the retina are capable of functioning in such a manner. Although from light microscopy many horizontal cells are described as having both dendrites and axons;1'2 evidence from electron microscopy suggests that many, if not all, horizontal cell processes are both pre- and postsynaptic along their length.

Current views concerning synaptic interactions in the outer plexiform layer of vertebrate retinas are summarized in the upper half of Fig. 10. This summary diagram was originally drawn to illustrate synaptic contacts of the frog retina13; it now appears that it can be generalized to summarize synaptic contacts in many vertebrate retinas. Processes from bipolar and horizontal cells are postsynaptic at ribbon synapses in the invaginations of receptor terminals; processes from flat bipolars are postsynaptic at the superficial contacts on receptor bases. Horizontal cells synaptically contact bipolar cell dendrites or other horizontal cell processes (not shown). It is further supposed that interactions be-

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Fig. 9. Ratios of amacrine-to-bipolar synaptic contacts in the inner plexiform layer of various vertebrate retinas. Except where noted, the ratios were determined from synapse counts made in the more central regions of the retinas. The duplicate data for cat, rabbit, and frog (Rana pipiens) were obtained from different specimens. The animals listed on the left have a low ratio of amacrine-to-bipolar synaptic contacts; those on the right, a higher ratio of amacrine-to-bipolar contacts.20
Fig. 10. Summary diagram of the arrangements of synaptic contacts found in vertebrate retinas. In the outer plexiform layer, processes from bipolar (B) and horizontal (H) cells penetrate into invaginations in the receptor terminals (RT) and terminate near the synaptic ribbons of the receptor. The processes of flat bipolar cells (FB) make superficial contacts on the bases of some receptor terminals. Horizontal cells make conventional synaptic contacts onto bipolar dendrites and other horizontal cell processes (not shown). Since horizontal cells usually extend further laterally in the outer plexiform layer than do bipolar dendrites, distant receptors can presumably influence bipolar cells via the horizontal cells. In the inner plexiform layer, two basic synaptic pathways are suggested. Bipolar terminals may contact one ganglion cell dendrite and one amacrine process at ribbon synapses (left side of diagram) or two amacrine cell (A) processes (right side of diagram). When the latter arrangement predominates in a retina, numerous conventional synapses between amacrine processes (serial synapses) are observed, and the ganglion cells (G) are contacted mainly by amacrine processes (right side of diagram). Amacrine processes in all retinas make synapses of the conventional type back onto bipolar terminals (reciprocal synapses).

tween bipolar and horizontal cell processes could occur in the receptor invaginations. Since light microscopy shows that horizontal cell processes in most species extend much further laterally in the outer plexiform layer than do the bipolar cell dendrites, these observations suggest not only that bipolar cell dendrites are directly activated by nearby receptors, but also that they are influenced by distant receptors via the horizontal cells.

*Inner plexiform layer.* In all vertebrate retinas, the inner plexiform layer is considerably thicker than the outer plexiform layer. Many more synaptic contacts per unit area are seen, and a greater variety of synaptic contacts is observed. Differences between species are much more ap-
parent in the inner plexiform layer than in the outer plexiform layer.19, 20

The bipolar terminals contact amacrine cell processes and ganglion cell dendrites at ribbon synapses. In all species studied, two postsynaptic elements are observed associated with the synapse (Fig. 7). This synaptic arrangement has been termed a dyad.4, 13 Occasionally, three postsynaptic elements have been found at these contacts.13, 20

With regard to the postsynaptic elements of a dyad, the pairings consist of a ganglion cell dendrite and an amacrine cell process,4 or two amacrine cell processes,13 or, very rarely, two ganglion cell dendrites.20 Which of the two principal pairings predominates in a retina is species dependent.13 For example, monkey and cat retinas have the amacrine-ganglion cell pairing at about 80 per cent of their dyads (Fig. 7, b), while the frog has two amacrine cell processes at about 75 per cent of its dyads (Fig. 8, a). In the mudpuppy and rabbit, about 50 per cent of the dyads are amacrine-amacrine; the other 50 per cent are amacrine-ganglion.19

Abundant, conventional synapses of the amacrine cells are seen in the inner plexiform layer of all species (Figs. 7 and 8). Such amacrine cell synapses are observed on bipolar terminals, ganglion cell dendrites, and other amacrine cell processes.4, 13, 20

Two types of synaptic arrangements entered into by amacrine processes are unusual and deserve special comment. First, at many dyads of the bipolar terminals, the participating amacrine cell process is observed to make a nearby synapse back onto the bipolar terminal (Fig. 7, b). This arrangement has been termed a reciprocal synapse; it suggests that a local, feedback interaction may occur between bipolar terminals and amacrine processes near these ribbon synapses.4, 13, 14, 19, 20, 32

The second noteworthy synaptic arrangement in the inner plexiform layer involves mainly amacrine processes. When an amacrine process is observed to make a synapse on an adjacent amacrine process, occasionally the second amacrine process is seen to make a nearby synapse on a third element (Fig. 8). This arrangement has been termed a serial synapse and suggests the possibility of very local interactions between amacrine processes.13, 19, 20

The third element in the series may be a ganglion cell dendrite, a bipolar terminal, or yet another amacrine process.20 In species with numerous amacrine cell synapses, the serial synapses observed in a single section may be more extensive and involve as many as four consecutive synapses13, 20 (Fig. 8, b).

Observations indicating alternate synaptic pathways in the inner plexiform layer have come from comparative studies of vertebrate retinas whose ganglion cell physiology has been extensively examined.13, 20 For example, the receptive field organization of the ganglion cells in the monkey and cat has been termed “simple” in the sense that their fields can be satisfactorily mapped using static spots of light projected onto the retina. These receptive fields are organized into two concentric and antagonistic zones, such that stimulation of one zone excites or inhibits firing of the cell; stimulation of the other zone elicits the opposite response in the cell.33, 34

On the other hand, the receptive field organization of animals such as the frog and pigeon has been termed “complex” in the sense that more than simple static spots of light are needed to map adequately most of their receptive fields. That is, many receptive fields in these species respond best to spots, bars, or edges of light moving through the receptive field in a specific direction.35, 36 When stimulated with static spots of light, receptive fields of such cells respond with short, transient on- and off-bursts of impulses to stimulation anywhere in the field.

When the anatomy of these various species is compared, it is found that the retinas with simple, receptive field organization (the monkey and cat) have most of the dyad pairings consisting of one ama-
crine and one ganglion cell process, a relatively low number of amacrine synapses per unit area, and few serial synapses. On the other hand, the retinas with more complex receptive field organization (the frog and pigeon) have dyad pairings consisting mostly of two amacrine cell processes, abundant amacrine synapses per unit area, and many serial synapses. Retinas with numerous examples of both types of receptive fields (rabbit, ground squirrel or mudpuppy) show about an equal number of amacrine-ganglion cell and amacrine-amacrine pairings at the dyads, and intermediate numbers of amacrine synapses, and serial synapses per unit area.

When these species are compared with regard to the number of ribbon synapses per unit area of the inner plexiform layer, no significant differences are usually found. Thus, a convenient way to compare these species quantitatively is to determine the ratio of amacrine to bipolar synapses in the inner plexiform layer. Such data are provided in Fig. 9. The retinas with primarily simple receptive fields (including different retinal areas in the same species) have a low ratio of amacrine synapses compared to bipolar synapses; the retinas with both simple and complex receptive fields have an intermediate ratio of amacrine to bipolar synapses; and the retinas with primarily complex receptive fields have a high ratio of amacrine to bipolar synapses.

The comparative observations indicate that (1) in the retinas where the simple type of receptive field organization is predominant, bipolar terminals make numerous direct contacts with ganglion cell dendrites; in the retinas where the complex type of receptive field organization is predominant, relatively few, direct, bipolar-ganglion cell contacts occur; and (2) there are significantly more amacrine synapses and amacrine-amacrine interactions in retinas with complex receptive fields as compared with retinas with simpler receptive fields. The amacrine cells, therefore, would appear to be the cell type mediating complex interactions, such as motion detection and directional selectivity, in the inner plexiform layer. A further implication of the above data is that in the retinas with more complexly organized receptive fields, amacrine cells may be interposed between the bipolar terminals and ganglion cell dendrites. In such a pathway there may be a four-neuron chain through the retina: receptors to bipolars to amacrines to ganglion cells.

In the inner plexiform layer, therefore, two different synaptic pathways to the ganglion cells may be postulated. The first consists mainly of direct bipolar, ganglion cell connections. In the second pathway the bipolar terminals contact mainly the amacrine cells. The amacrine cell processes interact among themselves and provide the primary input into the ganglion cells. These ideas are summarized in the lower half of Fig. 10. The left-hand side of the drawing represents the simpler, inner plexiform organization: bipolars connect directly with the ganglion cell dendrites. The right-hand side represents the more complex, inner plexiform layer organization: bipolars feed into the amacrine processes and the amacrine processes synapse among themselves and onto the ganglion cell dendrites. This suggests two basic types of ganglion cells in the vertebrate retina: one which receives mainly direct input from bipolar cells and one which receives its input mainly from the amacrine cells.

**Intracellular electrical activity**

Although it has been possible to study synaptic organization anatomically in a great variety of vertebrate species, only in one animal, the mudpuppy, has it been possible, as yet, to record intracellularly from all the types of retinal cells. The mudpuppy is an amphibian with extraordinarily large perikarya; and this feature makes it advantageous for intracellular recordings. Anatomically, the mudpuppy retina resembles the vertebrate retinas that
physiologically have ganglion cells with both the simple and complex types of receptive field organization.\(^\text{19}\)

In a number of species, it has been possible to record intracellularly from one or a few of the types of neurons in the retina; these responses are in general similar to the responses obtained from the comparable cell type in the mudpuppy retina.\(^\text{39-42}\) Thus, the results obtained in the mudpuppy retina can probably be generalized to other vertebrate retinas. Fig. 11 shows responses from each of the neuronal types in the mudpuppy retina elicited with a spot of light about 100 \(\mu\) in diameter focused on the electrode, or with centered annuli of 250 \(\mu\) or 500 \(\mu\) radius. The responses were assigned to their respective cell types after intracellular staining with Niagara Sky Blue, which permitted identification of the perikaryon shape and its location in the retina by light microscopy.\(^\text{37}\)
The more distal neurons—the receptors, the horizontal cells, and the bipolar cells—respond to retinal stimulation with slow, graded potentials. Impulses have never been seen in association with these responses. The neurons in the proximal retina respond mostly with depolarizing and transient potentials, on which nerve impulses are superimposed. The absence of impulses in the distal retinal neurons is of considerable interest and may be explained by the fact that these are neurons with relatively short processes, which do not need to transmit information over long distances. Thus, electrotonic spread of slow potentials is probably sufficient for information to reach the furthest extensions of these cells.

The other finding of unusual interest is that these distal neurons respond mostly with hyperpolarizing potentials. In spike-generating neurons, hyperpolarization is associated with inhibition. Here, however, no impulses are fired by the cells, and, presumably, excitation is signaled as well by hyperpolarizing potentials as by depolarizing ones. That all vertebrate photoreceptors so far recorded hyperpolarize when excited by light would appear to be compelling evidence that excitation can be signaled by hyperpolarizing potentials in the distal retina.

With spot and annulus stimulation, it is possible to characterize the responses of each cell type and describe its receptive field organization (Fig. 11). For example, receptors respond well to spot illumination, and poorly to both small and large annuli. The small responses evoked by the annular stimulation are believed to be caused by stray light. Experiments using spot and annular stimuli together show no differences when compared with spot stimulation alone. This is in agreement with the idea that receptors have a very small receptive field and respond relatively autonomously; they are not affected by substantial surround illumination.

Horizontal cells, on the other hand, respond with large, hyperpolarizing potentials over a retinal area several hundred microns in diameter. Thus, both spot and annular stimulation evoke sizable potentials. When spots and annuli are presented together, their effects are summed. Therefore, as the anatomy suggests, horizontal cells appear to receive input from receptors over a wide field.

Two physiological types of bipolar cells have been found in the mudpuppy retina. With one type, spot stimulation evokes a sustained hyperpolarizing potential (Fig. 11). With the other type, spot illumination evokes a sustained depolarizing potential. With either cell type, annular illumination added while the central region is illuminated antagonizes or reduces the sustained hyperpolarizing potential.
potential produced by the central spot. This is shown clearly in Fig. 12 for both types of bipolar cell. Annular illumination does not drive the membrane potential back beyond the resting potential of the cell, so that to see the effects of the surround illumination, central illumination must be present. At the bipolar cell level, therefore, an antagonistic center-surround receptive field organization is observed; with appropriate stimulus conditions, potentials of opposite polarity may be obtained from the bipolar cell (Fig. 11).

Amacrine cells respond transiently to static retinal illumination regardless of the configuration of stimulus used, or where in the receptive field of the amacrine cell it is presented. The amacrine cells are the first cell type along the visual pathway to respond primarily in a transient fashion, and they often give on- and off-responses to illumination anywhere within their receptive fields. Some differences between amacrine cells in the relative sizes of the on- and off-components are observed; these differences depend on the geometry and position of stimulation used. For example, Fig. 11 illustrates a cell that gives a large on-response to central spot illumination; with annular illumination, the off-responses are enhanced, and are comparable in size to the on-responses.

Superimposed on the transient, depolarizing responses of the amacrine cells are nerve impulses. However, seldom are more than two spikes observed riding on the transient depolarizations, regardless of intensity or configuration of stimulus. Thus, it is unclear whether it is the slow potential part of the response or the spikes which is the most important component for signal transmission in the amacrine cells. Recent experiments in the dragonfly ocellus have shown that the single spike observed on the leading edge of the generator potential contributes nothing to synaptic transmission in the ocellus, because after tetrodotoxin administration, which eliminates this spike, the intracellularly recorded, postsynaptic response is unaltered.

Two types of ganglion cell response are found in the mudpuppy retina; this would appear to correlate with the two types of ganglion cells suggested from anatomical considerations. One type strongly resembles an amacrine cell, giving transient responses at both the onset and cessation of stimulation (Fig. 11). Differing amounts of on- and off-contributions may be evoked with different stimulus configurations, as is observed with the amacrine cell response. These ganglion cell responses differ from the amacrine cell responses in having nu-

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Fig. 13. Summary figure correlating the synaptic organization of the vertebrate retina with the intracellularly recorded responses of the neurons. A complete description and discussion of the figure is given in the text. R, receptors; H, horizontal cell; B, bipolar cells; A, amacrine cell; G, ganglion cells.
numerous spikes riding on the transient depolarizations, and in that the number of spikes fired appears closely related to the amount of depolarization.

The second type of ganglion cell (lowermost records, Fig. 11) has a receptive field organization which closely resembles that of the bipolar cell. With central illumination, a sustained slow potential and steady discharge of spikes are evoked in the cell. With some central illumination maintained, large annular illumination drives the membrane potential back toward the resting potential and inhibits firing in a sustained fashion.

How the various potentials and receptive field properties of the neurons may be produced by synaptic interactions in the retina is suggested in Fig. 13. This drawing correlates a wiring diagram of the synaptic pathways in the vertebrate retina (based on the connections suggested in Fig. 10) with the potentials recorded from the neurons in the mudpuppy. The experimental conditions which would give the responses illustrated in Fig. 13 are (1) a flash of light presented to the receptor on the left, while (2) a dimmer, background light continuously illuminates the right-hand receptor. (The background light on the right-hand receptor is needed to demonstrate the effect of the antagonistic synaptic interactions.)

Receptors respond relatively autonomously. There is no anatomical evidence of any feedback from horizontal cells onto the receptors, and substantial surround illumination does not reduce the receptor response in the mudpuppy. The small, hyperpolarizing receptor response, seen when adjacent receptors are illuminated (right side, Fig. 13), appears to be due to stray light.

Horizontal cells summate inputs from a wide retinal area. Latencies of horizontal cell responses closely match the latencies of bipolar cell responses, indicating that both are driven by the receptors. The anatomy suggests that bipolar dendrites and horizontal cell processes are activated together at the synaptic ribbon synapses along the bases of the receptors.

The bipolar cells are polarized strongly in a graded, sustained fashion by direct receptor-bipolar cell contacts (left side of Fig. 13). Sustained, bipolar cell polarization is antagonized by horizontal cells acting on bipolar cell dendrites (right side of Fig. 13). Anatomical evidence suggests that such horizontal, bipolar cell interaction could occur at the horizontal-bipolar cell synapses, or perhaps in the receptor terminal invaginations, or both.

Since horizontal cells usually have a greater lateral extent in the outer plexiform layer than do the bipolar cells, a center-surround receptive field organization is found in the bipolar cell response. The central response appears mediated by the direct receptor-bipolar contacts; the antagonistic surround response, by the horizontal-bipolar contacts. In the mudpuppy, the bipolar cell receptive-field center matches closely the dendritic spread of the bipolars in area, while the surround area approximates the lateral spread of the horizontal cells. The only other cell type in the mudpuppy retina spreading far enough laterally to account for the antagonistic surround in the bipolar cell response is the amacrine cell, which also synaptically contacts the bipolar cells. The amacrine cells, however, respond transiently to retinal illumination at both on and off. The surround inhibition observed in the bipolar cell response is graded and sustained, and has the approximate form of the horizontal cell response.

Amacrine cells respond transiently at both the onset and cessation of static illumination placed anywhere in the receptive field. How the sustained responses of the distal retinal cells are converted to transient responses at the level of amacrine cells is not known, but the anatomy of the bipolar-amacrine cell synaptic complex provides the basis of a suggestion. That is, the reciprocal synapses of the amacrine cell processes back onto the bipolar terminals just adjacent to the bipolar ribbon.
synapses could conceivably turn off the bipolar excitation locally, and a transient response in the amacrine could result.

The two types of ganglion cell responses found in the mudpuppy retina may be related to the primary type of input into each type of cell. For example, one type of ganglion cell has a receptive field organization quite similar to the bipolar cells (left- and right-hand sides of Fig. 13). Central illumination depolarizes the cell in a sustained fashion; surround illumination inhibits the activity of the cell in a sustained fashion. This type of ganglion cell would appear to receive most of its synaptic input directly from the bipolar terminals.

The second type of ganglion cell (center, Fig. 13) responds transiently to retinal illumination much as the amacrine cells do. This type of ganglion cell presumably receives its major synaptic input from the amacrine cells.

Evidence has recently been provided that such on- and off-type ganglion cells respond very well to motion; many may show directionally selective responses. This suggests, as does the anatomy, that the amacrine cells are the neurons in the retina responsible for mediating complex ganglion cell activity, such as motion and direction selectivity. Recent experiments showing that the bipolar and more distal neurons show no directionally selective responses provide more direct evidence that the amacrine cells must play such a role.

How amacrine cell interactions might account for directionally selective responses is yet to be determined. The amacrine responses, by being transient in nature and occurring at both the onset and cessation of illumination, seem well suited for mediating the motion-sensing responses. For example, amacrine cells respond in a similar fashion to a bright spot on a dark background or a dark spot on a light background, a feature of many motion-sensitive and directionally selective cells.

An anatomical scheme which suggests how directionally selective responses may be mediated by the amacrine cells is presented in Fig. 14. This scheme incorporates the main anatomical features of those species demonstrating motion and directional selectivity. For example, (1) the bipolar cells synapse mainly with the amacrine processes; (2) the ganglion cells receive their primary input from amacrine cells; and (3) serial synapses are arranged such that an amacrine-amacrine synapse inhibits the amacrine-ganglion cell synapse. With the serial synapses organized as in the diagram, a spot moving from left to right (null direction) will result in no firing in the ganglion cell; +, excitatory synapse; -, inhibitory synapse.

Fig. 14. A scheme of how directionally selective responses could be mediated in the inner plexiform layer by amacrine cells. This scheme incorporates the main anatomical features of those species having directionally selective ganglion cells; (1) bipolar terminals synapse mainly with amacrine processes; (2) the ganglion cells receive their primary input from amacrine cells; and (3) serial synapses are arranged such that an amacrine-amacrine synapse inhibits the amacrine-ganglion cell synapse. With the serial synapses organized as in the diagram, a spot moving from left to right (null direction) will result in no firing in the ganglion cell; +, excitatory synapse; -, inhibitory synapse.

In summary, the outer plexiform layer of the vertebrate retina appears concerned mainly with the static or spatial aspects of the illumination on the receptors. The neu-
Fig. 15. Intracellular responses recorded from the cells of the inner nuclear layer of the mudpuppy compared with the ERG. All the responses were evoked by light flashes that illuminated the entire retina evenly. The Müller (glial) cell response closely matches the b-wave in latency and waveform, especially at relatively low intensities (right side). Comparison of latencies and waveforms of neuronal and Müller cell responses is shown on the left.57

rons contributing processes to the outer plexiform layer respond with slow, graded, sustained potentials, and the neuronal interactions in the outer plexiform layer accentuate contrast in the retinal image by forming an antagonistic center-surround organization at the level of the bipolar cells.

The inner plexiform layer, on the other hand, appears concerned with the more dynamic or temporal aspects of illumination on the receptors. Amacrine cells accentuate the changes in retinal illumination, and they respond vigorously to moving stimuli.46, 49 For example, interactions in the inner plexiform layer probably account for the motion- and direction-selective responses of the ganglion cells.

The two physiological types of ganglion cells found in vertebrate retinas appear closely related to either the bipolar or amacrine cell activity, and they carry information concerning the transformations occurring at the two plexiform layers to the higher visual centers.

Origins of the electroretinogram

The electroretinogram (ERG) was among the earliest recorded biological potentials, and it has proved useful for studying retinal function for almost a century.53 Understanding the cellular origins of the
ERG components is of considerable importance, and now that intracellular responses from the retinal cells can be recorded, it is possible to investigate directly the origins of this gross retinal response. The two components of the ERG that have been of most interest are the a- and b-waves. These are the first two waves that occur after a flash of light; they are the ones that seem most related to excitation in the visual system. During the past few years, a considerable amount of evidence has accumulated linking the a-wave with receptor activity. In the mudpuppy, for example, the a-wave and the intracellularly recorded receptor response have a very similar latency, and the leading edge of the two types of response has a very similar waveform. Thus, there appears to be rather general agreement that at least the leading edge of the a-wave represents receptor activity.

The origin of the b-wave has been less clear. Much evidence has been presented that the b-wave arises more proximally in the retina than does the a-wave, and that the b-wave probably arises from cells residing in the inner nuclear layer. Some evidence has also been presented that the b-wave arises from a cell that undergoes depolarization when the retina is stimulated. For example, it has been demonstrated that the b-wave is rapidly eliminated when KCl is applied to the retina. Intracellular responses, evoked with diffuse illumination and recorded from the inner nuclear layer of the mudpuppy retina are shown in Fig. 15. The responses of four cell types are found: three of these are from neuronal elements and are similar to the responses evoked with discrete stimuli that were described above; the other response arises in the Müller (glial) cell of the retina. When these intracellularly recorded responses of the inner nuclear layer are compared with the b-wave of the ERG, it is the Müller cell response that best matches this ERG component.

The Müller cell response is always depolarizing, and consists of an initial, transient slow wave followed by a sustained component of variable amplitude that lasts as long as the light persists. At off, there is a second, transient depolarizing wave, which is generally smaller than the initial portion of the response. The resting potential of most Müller cells is substantially higher than the resting potentials of the neurons.

When the receptive field of the Müller cell response is explored with spots of light, depolarizing potentials of similar form are recorded over a relatively wide area of retina, up to 1 to 2 mm. in diameter. No suggestion of any center-surround organization is observed. No single Müller cell has processes that extend laterally over such large distances in the mudpuppy retina; but at the level of the external limiting membrane in the mudpuppy retina, glial cell processes form close, probably gap, junctions with each other (Fig. 16). Perhaps such junctions mediate electrical interactions between the Müller cells, thus explaining the large response area of these units.

Identification of this response as arising from the Müller cells was accomplished mainly by intracellular staining after recording. Fig. 17 shows three micrographs of a cell at different levels of focus, stained with a combination of dyes that permitted diffusion of stain throughout much of the cell. Dye can be followed from the internal, limiting membrane to a nucleus in the middle of the inner nuclear layer and beyond, well into the outer nuclear layer. A reconstruction of the stained cell is shown on the right. No cell type other than the Müller cell extends as far through the retina as does this stained cell, and no other cell type has such a large column of cytoplasm in the inner plexiform layer. Since the Müller cells extend vertically through the retina, it is to be expected that Müller cell responses may be recorded throughout much of the retina; this has been observed.

The over-all waveforms of the Müller cell response and the b-wave of the ERG...
Fig. 16. Electron micrographs of junctional complexes (arrows), mainly between Müller cell processes, at the level of the external limiting membrane in the mudpuppy retina. At high magnification, the junction between Müller cell processes is seen to consist of an extended, close (probably gap) apposition zone (arrows), on either side of which is a desmosome-like area; RN, receptor cell nuclei. a (x30,000); b (x60,000).

Fig. 17. A Müller cell stained after intracellular recording. Three focuses of the cell are shown on the left; a reconstruction of the cell is on the right. Stain can be seen along the internal limiting membrane, in a large column of cytoplasm in the inner plexiform layer, in a nucleus in the middle of the inner nuclear layer (INL), and in a process extending into the outer nuclear layer (ONL) (arrow). Only the Müller cells extend this far vertically through the vertebrate retina; GC, ganglion cells.
are remarkably similar, particularly at relatively low stimulus intensities (Fig. 15). As expected, no a-wave is observed in the Müller cell response since the a-wave originates in the receptors. Also the oscillatory potentials on the leading edge of the b-wave are absent from the Müller cell response. However, the latency of the Müller cell response and the b-wave are very close over a wide range of intensities, and the intensity-response relations agree over a stimulus range of about 5 to 6 log units. Both types of electrical activity also show a similar, positive off-response at the termination of the stimulus.

No other intracellular response in the inner nuclear layer matches the b-wave so closely (Fig. 15). For example, (1) the horizontal cell response is of the wrong polarity; (2) the latencies of both the bipolar and horizontal cell responses are much shorter than the latencies of the Müller cell response or the b-wave; and (3) the intensity-response curves for the amacrine and bipolar cell responses are much steeper than the intensity response curves for the Müller cell response and b-wave. Considerable evidence has accumulated from studies on several species that ganglion cell potentials do not contribute significantly to the ERG, while the receptors appear responsible for the a-wave of the ERG. It thus appears that the b-wave of the ERG is best explained as arising mainly from the Müller (glial) cells of the retina.

If the above hypothesis is correct, we are left with two important questions that need consideration. The first is how the light response in the Müller cell is generated, considering that no synaptic-type contacts are observed in any retina be-

![Graph](image-url)
between the neurons and the Müller cells. We have no positive evidence on this question, but recent observations made on glial cells elsewhere in nervous systems may have a bearing on this point. Glial cells generally, like the Müller cells, have high resting membrane potentials. Glial cells also appear considerably more sensitive to K⁺ at physiological concentrations than are neurons. When K⁺ in the extracellular space increases, as a result of directly applied K⁺ or neuronal activity, K⁺ moves into the glial cell, depolarizing it.

A similar mechanism might be proposed to explain the depolarization of the Müller cell. For example, K⁺ released as a result of neuronal activity in the retina, perhaps in the outer plexiform or inner nuclear layer, might depolarize the Müller cell locally. Since the Müller cell extends vertically through the retina, this local depolarization could initiate a vertical flow of current through the retina. Such a current could be recorded, with extracellular electrodes, as the b-wave of the ERG.

The second question to be considered is this: if the b-wave originates from the Müller cells, how good a measure of retinal excitability and sensitivity is it? Recent experiments in the rod retina of the skate compared the adaptation properties of the b-wave of the ERG with the responses of individual ganglion cells. Diffuse light was used to elicit the b-wave; spots of light, just smaller than receptive-field centers, elicited the ganglion cell responses. The results were unequivocal in showing that, although the absolute sensitivity of the b-wave is about 1 log unit less than the absolute sensitivity of the ganglion cell response, both types of response show identical adaptation properties.

Fig. 18 (inset) shows the increment, or contrast, thresholds over an adapting range of more than 6 log units. Over most of the adapting range there is a linear relation

![Graph](http://tvst.arvojournals.org/)
between log background intensity and log relative threshold. The relation observed here is similar to results that have been obtained in various species in which increment thresholds have been tested with a variety of methods.61, 64

Fig. 18 also shows the course of dark adaptation for both the b-wave and ganglion cell response after flash adaptation that bleached about 80 per cent of the rhodopsin. Initially, after the flash, the retina is totally insensitive to any intensity of test light applied to the eye. After electrical activity reappears, thresholds for both types of response rapidly fall; so that by 20 minutes after the flash, thresholds are within 3 log units of absolute sensitivity. Thereafter there is a very slow recovery of threshold that requires an additional 80 to 100 minutes.

In other visual systems, fast and slow components of dark adaptation have been observed after a light adaptation period that bleaches away a significant fraction of the visual pigment.65 The slow component of dark adaptation has been linked to the slow regeneration of visual pigment in the eye.64, 65 Fig. 19 shows that an almost exact correspondence exists between the slow fall of threshold observed in both the b-wave and ganglion cell response in the skate eye and the regeneration of rhodopsin. This is a linear relation between the concentration of rhodopsin and log relative threshold.

It would appear in conclusion that the b-wave of the ERG provides a good measure of retinal excitability, even though it does not arise directly from the retinal neurons. It should continue to be a useful tool to measure visual excitability and sensitivity, especially of the distal retina.

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