The Ventral Photoreceptor Cells of *Limulus*

I. *The microanatomy*

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ABSTRACT The ventral photoreceptor cells of *Limulus polyphemus* resemble the retinular cells of the lateral eyes both in electrical behavior and in morphology. Because of the great size of the ventral photoreceptor cells they are easy to impale with glass capillary micropipettes. Their location along the length of the ventral eye nerve makes them easy to dissect out and fix for electron microscopy. Each cell has a large, ellipsoidal soma that tapers into an axon whose length depends upon the distance of the cell from the brain. The cell body contains a rich variety of cytoplasmic organelles with an especially abundant endoplasmic reticulum. The most prominent structural feature is the microvillous rhabdomere, a highly modified infolding of the plasmalemma. The microvilli are tightly packed together within the rhabdomere, and quintuple-layered junctions are encountered wherever microvillar membranes touch each other. Glial cells cover the surface of the photoreceptor cell and send long, sheet-like projections of their cytoplasm into the cell body of the photoreceptor cell. Some of these projections penetrate the rhabdomere deep within the cell and form quintuple-layered junctions with the microvilli. Junctions between glial cells and the photoreceptor cell and between adjacent glial cells are rarely encountered elsewhere, indicating that there is an open pathway between the intermicrovillous space and the extracellular medium. The axon has a normal morphology but it is electrically inexcitable.

INTRODUCTION

Physiologists have studied the lateral eyes of *Limulus polyphemus* with great intensity and success. Although it is debatable whether this ancient and amenable crab can see at all, the animal possesses several other structures exquisitely sensitive to light. The "lateral olfactory nerves" have been shown physiologically to be among these light receptors (Millecchia, Bradbury, and Mauro, 1966). We have found that the lateral olfactory nerves also have morphological characteristics common to many other photoreceptors, particularly the retinular cells in the lateral eye of *Limulus*.

Patten (1893) gave the lateral olfactory nerves their name. He believed that

289

during the development of the *Limulus* embryo its ventral eye underwent an extraordinary transformation into an "olfactory organ." According to his interpretation, the visual cells of the ventral eye metamorphosed into "large pear-shaped ganglion cells" and were found in "small ommatidial-like clusters" along the lengths of the two lateral nerves connecting the olfactory organ to the brain. Hanström (1926 *a, b*) recognized the ventral eye in the young *Limulus* but was unsure about its fate in the adult. Johansson (1933) completely dismissed the concept of the structures being an eye at any time and instead proposed that they were an olfactory organ from the beginning of development. Demoll (1914) on the other hand never mentioned olfaction as a possible function for the structures and he referred to the lateral nerves as "the nerves of the rudimentary ventral eye." On the basis of the electrophysiology and the fine structure of the nerves, it is clear that Demoll was correct in his terminology. The ventral eye in the young *Limulus* undergoes great changes in its location and structure as the animal grows up. Some photoreceptor cells remain behind in the brain, others are distributed along the lengths of the nerves, and some are found beneath the modified patch of cuticle that marks the beginning of the nerves. Nevertheless, the photoreceptor cells retain their ability to transduce light. In spite of the longitudinal distortion of the structures in the adult, we believe it is still appropriate to call these photoreceptor cell bodies and their axons, in their entirety, the *ventral eye*.

MATERIALS AND METHODS

The ventral eye nerve and the anterior portion of the protocerebrum were dissected out of various sizes and both sexes of the horseshoe crab, *Limulus polyphemus*. Tissues were fixed overnight at room temperature with 2% formaldehyde–1% glutaraldehyde buffered with 0.1 M phosphate, pH 7.4, to which 3% sodium chloride and 4% sucrose had been added (Fahrenbach, personal communication). The tissues were rinsed for several hours in a solution of plain buffer and 8% sucrose. The tissues were then postfixed for 2 hr in a solution of 1% OsO_4 and 4% sucrose, dehydrated in a graded series of ethyl alcohols, and embedded in Epon 812 according to standard procedures. Thick sections were cut with glass knives, stained with methylene blue, and photographed on a Zeiss Ultraphot II. Thin sections were cut with a diamond knife, picked up on naked copper grids, and stained with a combination of uranyl and lead salts. Electron micrographs were taken at initial magnifications of 1000-35000 on a Hitachi HU 11B electron microscope and photographically enlarged.

RESULTS

The ventral eye of *Limulus polyphemus*, in young animals, is a paired structure and each member of the pair is surrounded by as mass of pigment cells (Hanström, 1926 *b*). The location of the eye is easily seen (Fig. 1) since the cuticle of young animals is relatively transparent. As the young *Limulus* grows up and its brain and ventral eye separate from each other, some of the

photoreceptor cell bodies of the ventral eye remain behind in the brain, while others become distributed along the ventral nerves. This appears to be a random process since the number of photoreceptor cells and their location along the nerves vary from nerve to nerve and from animal to animal.

In the full-grown *Limulus*, the photoreceptor cells are located at the be-

FIGURE 1. Light micrograph of the ventral surface of a live baby *Limulus*. At this stage of development the cuticle is still relatively transparent. The paired ventral eyes are surrounded by pigment and are easily seen (arrows). The eyes are directly in front of the animal's mouth and immediately over the ventral surface of the protocerebrum. The small, black spots are brine shrimp eggs. × 11.
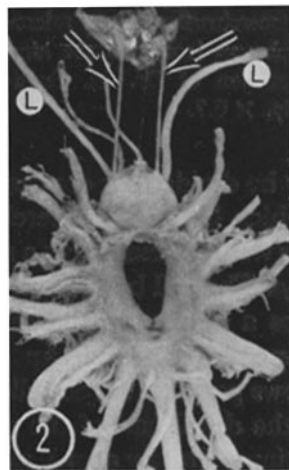

FIGURE 2. Light micrograph of a dissection of an adult *Limulus* brain showing portions of the lateral eye nerves (*L*), the ventral eye nerves (arrows), and the median olfactory nerve. The ventral eye nerves and the median olfactory nerve run from the protocerebrum to the patch of cuticle that formerly lay over the protocerebrum (Fig. 1). In *Limulus*, the brain surrounds the esophagus. The brain and nerves have not been desheathed. × 2.

ginning of the nerves under a thickened patch of cuticle and at the termination of the nerves in the protocerebrum. Single photoreceptor cells or groups of two or three are found along the lengths of the nerves (Fig. 3). Each nerve is surrounded for most of its length by a tubular blood vessel that is continuous with the brain and thus sheaths the nerve (Fig. 2). The ventral nerves of the full-grown female *Limulus* are often 2-3 cm long.
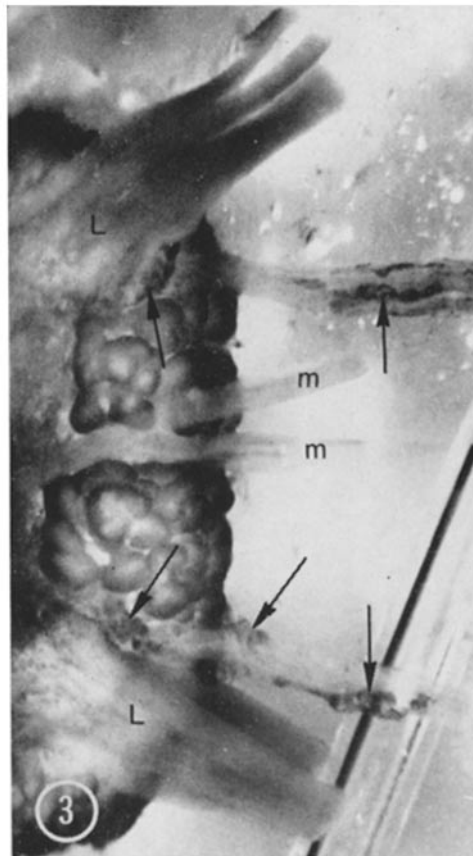

FIGURE 3. Light micrograph of a dissection of a portion of an adult *Limulus* brain that has been desheathed and stained with toluidine blue. The stumps of the lateral eye nerve (*L*) are apparent as well as those of the median eye nerves (*m*). The arrows indicate ventral photoreceptor cells along the ventral eye nerve and in the protocerebrum. The micrograph was lent with the kind permission of Dr. M. Snodderly. × 6.7.

Each photoreceptor cell of the ventral eye may be divided for convenience into two parts. First, there is a large cell body which has approximately the shape of a prolate spheroid. The largest cell bodies we have seen have a major axis of about 200 μ and a minor axis of about 60 μ. The cell body tapers into the second part of the cell, the axon, which has a maximum diameter of approximately 20 μ. The length of the axon depends, of course, upon the distance of the cell body from the brain. Fig. 4 shows part of a single cell body and longitudinal sections of several axons near the distal end of the nerve. Fig. 5 shows parts of two cell bodies and cross-sections of many axons, all part

of the ventral eye nerve as it travels a short distance under the brain sheath and close to the protocerebrum.

Fig. 6 is an electron micrograph at low magnification of part of a relatively
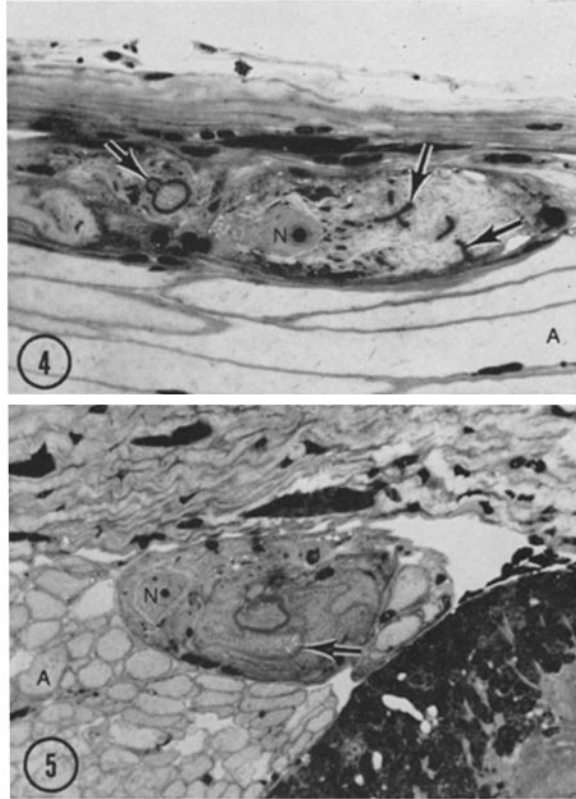

FIGURE 4. Light micrograph showing a ventral photoreceptor cell and several axons (A) in longitudinal section near the distal end of the ventral eye nerve. The nucleus of the cell (N) has a large, densely staining nucleolus. Several portions of the rhabdomere (arrows) are also visible. Above the cell is part of the blood vessel that also serves as a ventral nerve sheath. × 600.

FIGURE 5. Light micrograph showing portions of two ventral photoreceptor cells and several axons in transverse section in the short segment of ventral eye nerve that runs underneath the brain sheath. Only one photoreceptor cell nucleus with its prominent nucleolus is visible in the section. Both cells appear to share the rhabdomere indicated by the arrow. In the lower right corner of the figure is a portion of one of the corpora pedunculata. × 600.

small photoreceptor cell. Most of the cytoplasmic components found in much larger cells are represented here, although the positions of the components as shown here are not necessarily typical. In our experience, it is almost impossible to assign these components typical positions. The highly schematic drawing in Fig. 7 also represents part of a single, relatively small photoreceptor cell

and some of the adjacent glial cells. A segment has been removed from the cell body in an attempt to show diagrammatically a few cf the relationships between the organelles. The drawing is a crude approximation of the rich variety found in these cells.

The nucleus of the photoreceptor cell is large with a maximum diameter of
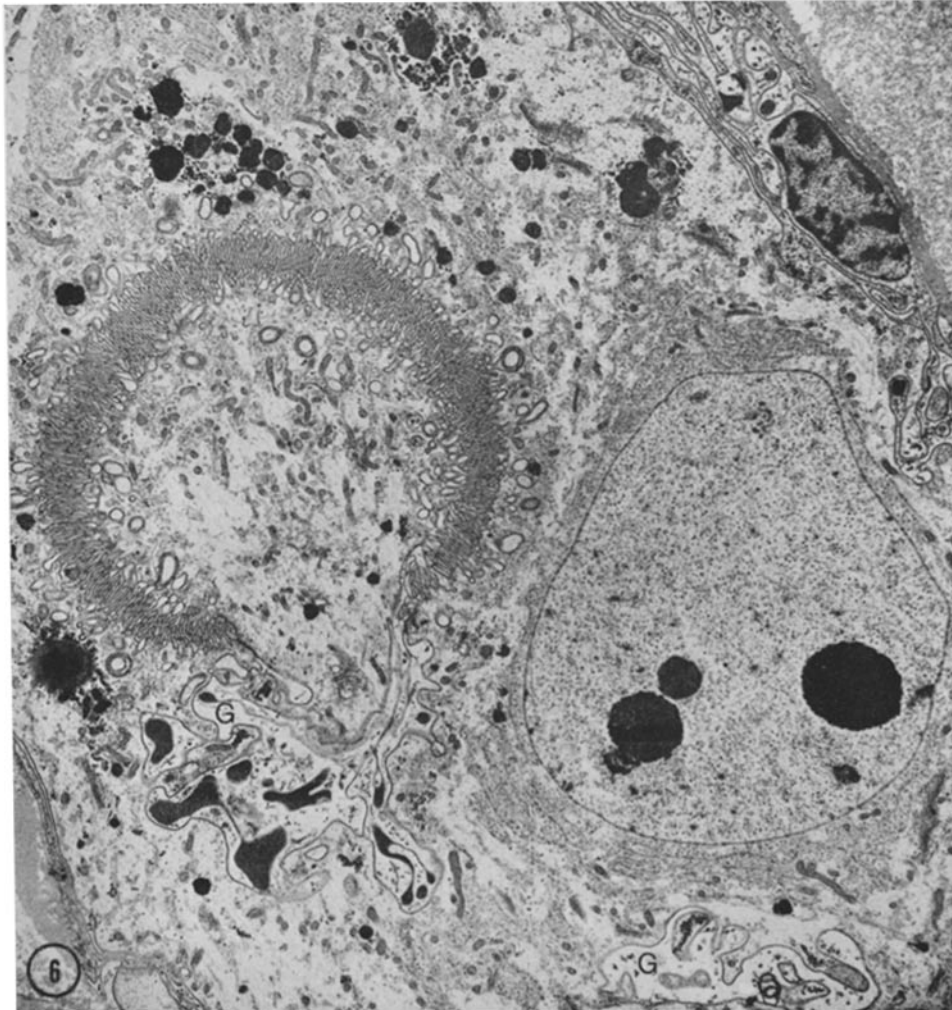

FIGURE 6. Electron micrograph showing a portion of a relatively small ventral photo-receptor cell. There is a large nucleus the nucleoplasm of which, aside from the three densely staining nucleoli, is finely granular and evenly distributed. The cytoplasm has a rich variety of organelles. A portion of the rhabdomere is shown as are portions of several sheet-like infoldings of glial cells (G). A glial nucleus is seen in the upper right corner. The photoreceptor cell–glial cell complex is surrounded by a basal lamina which intervenes between the complex and the blood. × 5500.

about 25 μ. Except for several nucleoli that stain intensely, the nucleoplasm is finely granular and evenly distributed. At higher magnification, cross-sections through the nuclear envelope reveal a large number of nuclear pores (Fig. 8). The structure of the pores is complicated by the presence of thin fibers, approximately 300 A long, that extend straight out from both sides of the pore rim into the nucleoplasm and the cytoplasm.
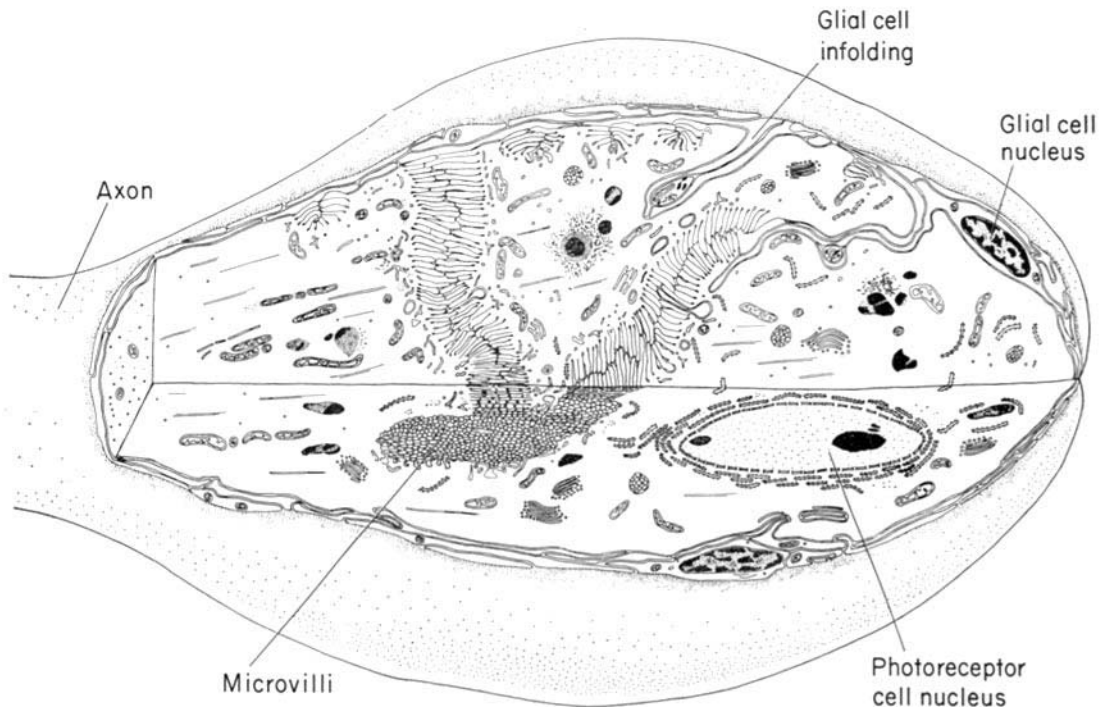

FIGURE 7. A highly schematic representation of a single, relatively small ventral photoreceptor cell and a few of its glial cells. A segment of the cell body has been removed in order to give a rough approximation of a few of the possible relationships between the organelles. The drawing does little justice to the rich variety found within these cells.

The endoplasmic reticulum of the cell is abundant and displays great variety in its form and location. As shown in Figs. 6 and 8, a shell of rough-surfaced endoplasmic reticulum several cisternae thick is often observed surrounding the nucleus. Individual cisternae of rough reticulum are distributed throughout the cytoplasm of the cell body. In a similar fashion, smooth-surfaced endoplasmic reticulum is scattered throughout the cytoplasm, although there is usually a concentration of vesicles and anastomosing tubules just at the base of the rhabdomere. As shown in Fig. 9, some of these are stacked in a well-ordered array. Cisternae of both types of reticulum are some-

times found next to the plasmalemma in places where glial cells send processes into the photoreceptor cell (Fig. 8) and where neurosecretory axons terminate (Fig. 15). While the arrangements and locations mentioned here occur with some regularity, great variations are found within a given cell body as well as from cell to cell (Fig. 11).
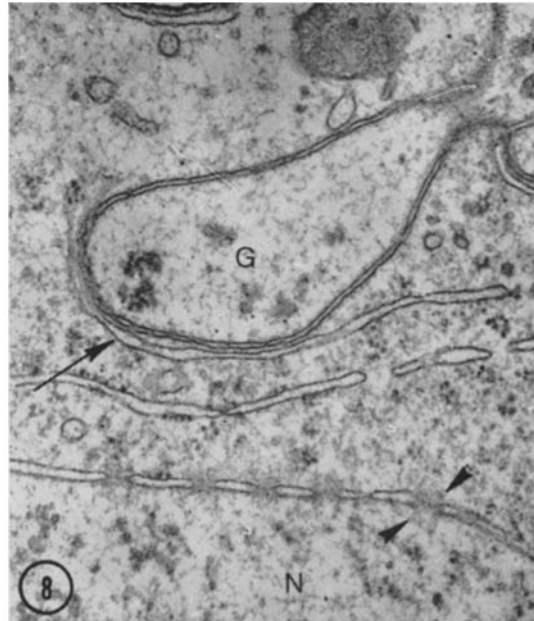

FIGURE 8.   Electron micrograph showing a portion of the nucleus of a ventral photo-receptor cell and the adjacent cytoplasm. The nuclear envelope has many nuclear pores, a few of which are visible here. The structure of the pores is complicated by fibers that extend a short distance into both the cytoplasm and the nucleoplasm around the pore rim (arrowheads). A shell of endoplasmic reticulum often surrounds the nucleus. One cisterna of the shell bends around an infolding of glial cytoplasm and forms a subsurface cisterna (arrow). This cisterna is almost free of ribosomes unlike the other cisternae in the shell. × 42,000.

The variability of the endoplasmic reticulum is repeated in the location and concentration of other cell organelles. Golgi complexes, pools of glycogen particles, lipid droplets, autophagic vacuoles, multivesicular bodies, micro-filaments, microtubules, and mitochondria are distributed throughout the cell body with little apparent pattern. One exception to the lack of order is found in the cytoplasm connecting some parts of the rhabdomere with the axon. In these regions, microfilaments, microtubules, and mitochondria are arranged with a polarity that resembles the axoplasm (Fig. 10). However, the cytoplasm near other parts of the rhabdomere in the same cell can have an entirely different combination and arrangement of organelles.

The rhabdomere is the distinguishing structural feature cf the cell bcdy. The rhabdomere is a highly modified microvillous infolding of the plasma-lemma and it is encountered along portions of the perimeter of the cell body as well as deep within. We have not been able to determine whether the rhabdo-mere as a whole represents infoldings of the plasmalemma at several different and unconnected places on the surface of the cell or whether it is joined to the
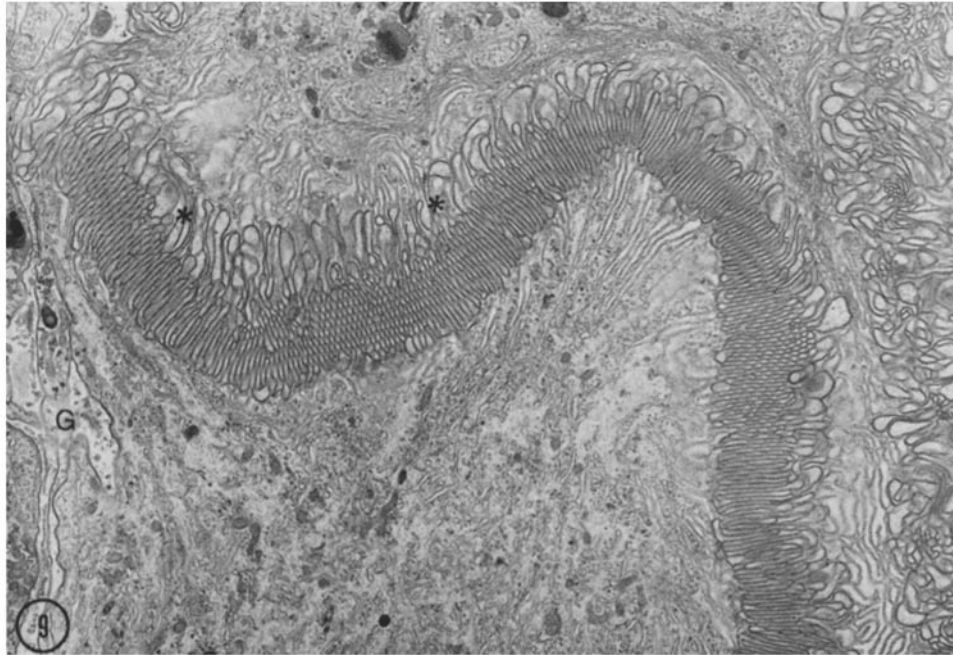

FIGURE 9. Electron micrograph showing a portion of the rhabdomere of a relatively large ventral photoreceptor cell deep within the cell body. Two layers of microvilli appear to interdigitate with each other. One of them is well-ordered and many parallel cisternae of subrhabdomeric reticulum underlie it; the other, marked with asterisks, appears disarranged. A sheet of glial cytoplasm is seen communicating with the rhab-domere at the left of the figure (*G*). × 6000.

surface along a single, convoluted line; we believe the latter possibility is the more likely one. Determining the exact pattern of derivation of the rhabdo-mere is difficult because the organelle has no characteristic geometry. Within a given cell, the rhabdomere may have the form of a relatively flat sheet in one place and then grade into a cup or globe somewhere else (Fig. 6). A few parts of the rhabdomere consist of a single layer of microvilli with their tips abutting against a smooth portion of the receptor cell or glial cell plasmalemma. This arrangement usually occurs at the periphery of the cell. Within the cell body, the predominant arrangement consists of two layers of microvilli interdigitat-ing with each other to varying degrees. In Fig. 6, the interdigitation of the two
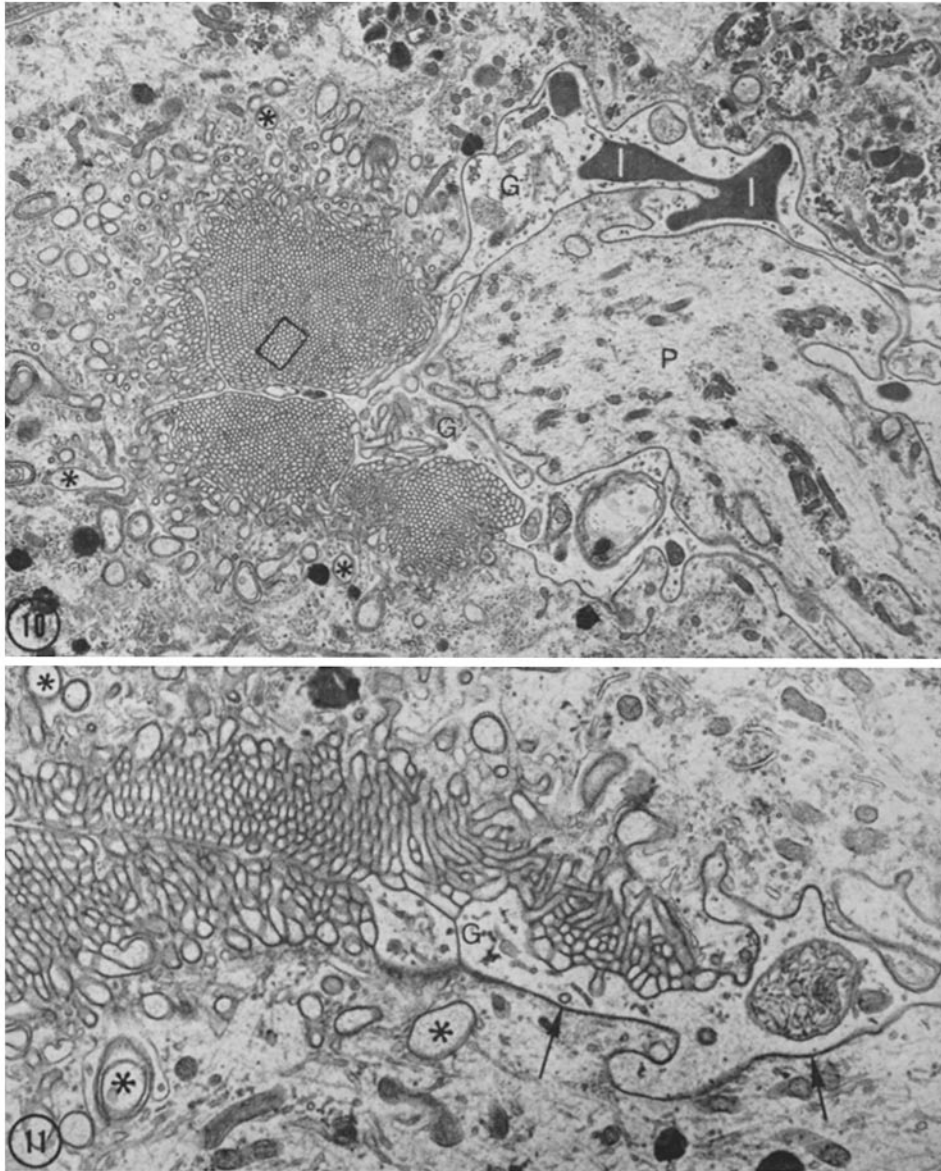
FIGURE 10. Electron micrograph of the ventral photoreceptor cell showing a portion of the rhabdomere deep within the cell body. The figure also shows the close inter-relation of the rhabdomere and infoldings of the glial cells (G). Asterisks mark a few of the many tubular prolongations of the glial cytoplasm that weave around in the vicinity of this portion of the rhabdomere. One part of the figure (box) is shown at higher magnification in Fig. 12. The cytoplasm (P) of the photoreceptor cell at the right of the figure resembles the axon in the parallel alignment of its microtubules, microfila-ments, and mitochondria (P) (I, hemocyanin). × 7700.

FIGURE 11. Electron micrograph showing close association of glial cells with the

layers is relatively complete. The situation in Fig. 9 is more difficult to interpret. We believe there are two interdigitating layers; however, the basal region of one of them is comparatively disordered (asterisks). The regions of microvilli that appear disarranged in this way are usually encountered in relatively large photoreceptor cells and are often found side by side with stretches of well-ordered microvilli.

Figs. 12 and 13 show portions of the rhabdomere at high magnification with many of the microvilli cut in cross-section. The membrane of each microvillus has a "unit" or trilaminar appearance and a thickness of approximately 75 A. A careful examination of the places where microvilli touch each other reveals that the outer, dense layers of their membranes are fused or at least very closely apposed. These regions of close apposition with three dark layers and two lighter ones, or quintuple-layered junctions (Lasansky, 1967), are approximately 150 A thick.

In well-ordered regions of the rhabdomere, the microvilli are packed together more or less in a hexagonal array. Most of the intermicrovillous space is formed by this array and has the shape of long slender prisms. The space appears to be continuous throughout the rhabdomere via the ring at the base of each microvillus and via the space at the microvillous tips. Since it is difficult to calculate precisely the extent of the rhabdomere in a ventral photoreceptor cell, it is equally difficult to estimate the volume of the intermicrovillous space; all that can be said is that both are considerable.

As shown in Figs. 10–13, the rhabdomere is closely associated with glial cells even when the microvilli are deep within the photoreceptor cell body. The glial cells send many long, thin sheets of their cytoplasm into the cell body and several of these projections are involved with the rhabdomere (Figs. 10–12). The sheets are often double-layered and occasionally there is a cleft between them filled with a dense, homogeneous substance (I, Fig. 10) that is hemocyanin (Fahrenbach, personal communication). A careful examination of the places where glial membranes are in contact with microvillous membranes reveals quintuple-layered junctions; however, the regions of close apposition are extensive only at the places where the long glial projections reach the rhabdomere deep within the photoreceptor cell. In Figs. 10 and 12, it is clear that the glial cell actually penetrates the rhabdomere. In addition, serial sections have established that tubular ramifications of the glial projections

rhabdomere deep within the body of the ventral photoreceptor cell. Arrows indicate places where normal intercellular gaps occur between the plasmalemma of the two cell types. Asterisks mark a few of the many tubular prolongations of the glial cytoplasm that weave around in the vicinity of this portion of the rhabdomere. In areas of contact between the glial cell and the microvilli, there are quintuple-layered junctions (Fig. 12). Although there is abundant subrhabdomeric endoplasmic reticulum, it is not so well-arranged as that seen in Fig. 9. × 22,000.
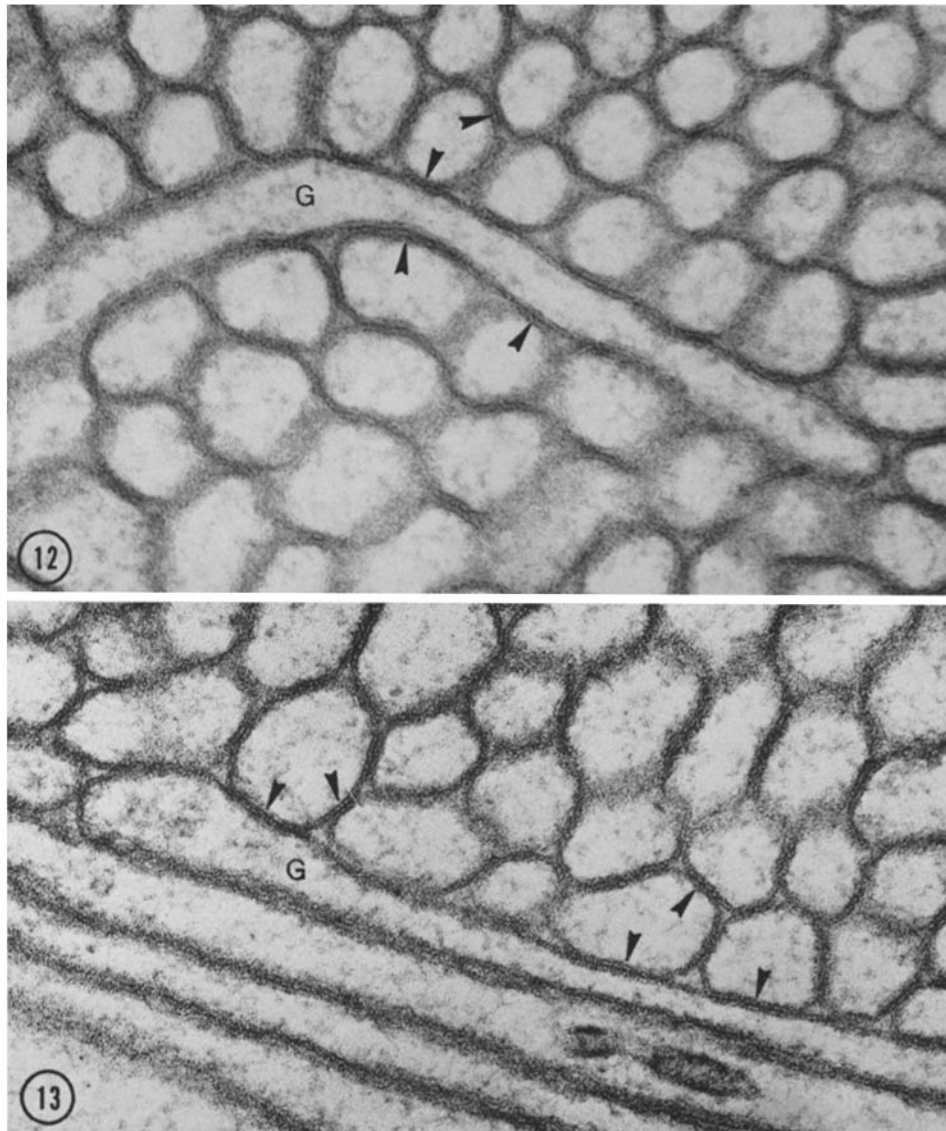
FIGURE 12.   A portion of Fig. 10 (box) seen at higher magnification. The figure shows the relationship of a projection of glial cytoplasm (G) to the microvilli in the rhabdomere. There are numerous quintuple-layered junctions (arrowheads) between the glial plasmalemma and the microvilli as well as between adjacent microvilli. × 135,000.

FIGURE 13.   Electron micrograph showing portions of glial lamellae (G) and part of the rhabdomere at the periphery of a ventral photoreceptor cell. As in Fig. 12, there are numerous quintuple-layered junctions (arrowheads). The figure is atypical in that there are junctions between almost all the microvilli and the adjacent glial plasma-lemma. At the periphery of the cell such junctions occur intermittently along the length of each microvillus. × 120,000.

weave about in the immediate vicinity of some portions of the rhabdomere (asterisks, Fig. 10 and 11).

Glial-microvillous junctions at the periphery of the cell are less extensive and occur at intervals along the lengths of the microvilli. Fig. 13, the result
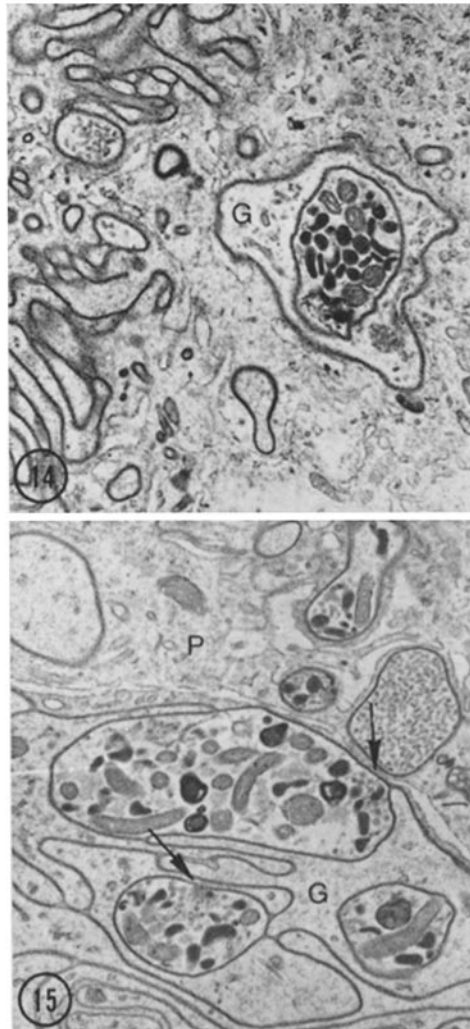
Figures 14 and 15. Electron micrographs showing sections through neurosecretory axons. In Fig. 14, the axon is accompanied by a sleeve of glial cytoplasm (G), and is well within the cell body. × 14,000. In Fig. 15, the axons are at the periphery of the cell body and several of them are in direct contact with the photoreceptor cell (P). In Fig. 15, two synapses are visible (arrows). × 16,000.

of a long search, is unusual in that it includes most of these intermittent junctions in the plane of the section. Except when in proximity to the rhabdomere, the plasmalemmas of the glial and photoreceptor cells are separated by a space of 70–100 A. We have observed places away from the rhabdomere where the two plasmalemmas are apposed but they are few in number, very short in length, and display no regularity in their arrangement.

Occasionally, neurosecretory axons are seen deep within the cell body, as well as at the periphery. When they are within the cell body, the neurosecretory axons are usually accompanied by a sleeve of glial cytoplasm as seen in Fig. 14. The axons contain small mitochondria and small irregularly shaped granules that stain intensely. Fig. 15 shows a rare instance of neurosecretory
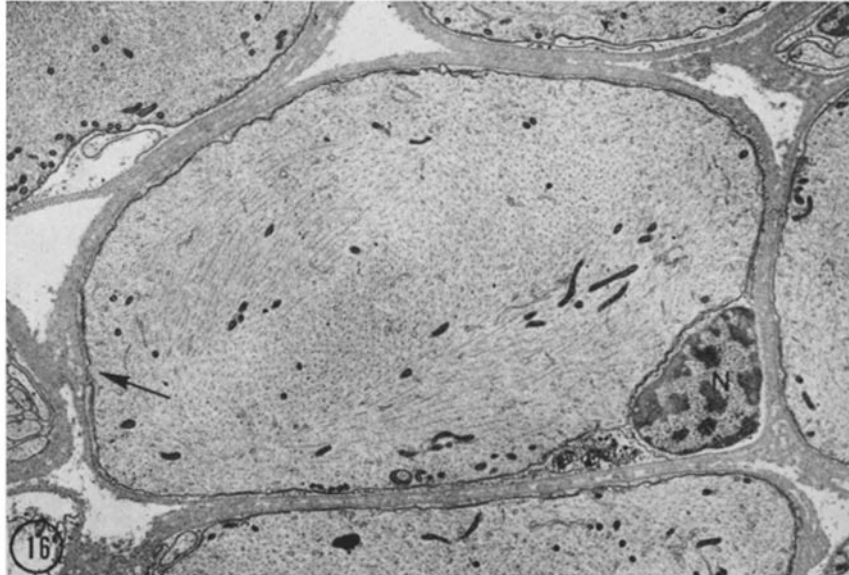

FIGURE 16. Electron micrograph showing portions of several axons of ventral photoreceptor cells in the part of the ventral eye nerve that runs a short distance into the brain. The axoplasm is characterized by large numbers of microtubules and microfilaments and by mitochondria. There is a glial nucleus (N) in the plane of the section. The glial covering of the axon is usually a single layer thick and is occasionally discontinuous (arrow). One of these discontinuities is seen at higher magnification in Fig. 17. Axons of much smaller diameter are seen in the upper right corner of the figure and just to the left of the glial gap indicated by the arrow. × 6000.

axons without glial sleeves. There also appear to be two synapses (Scharrer, 1968) in the plane of the section.

Glial cells cover the entire surface of the photoreceptor cell body and in some places the glial covering may be as many as 10 layers thick. Elsewhere, only a single glial layer intervenes between the cell body and the hemocoel. We have never seen any places on a cell body without at least a single layer of glial covering. Quintuple-layered junctions between adjacent glial plasmalemmas are observed infrequently and with absolutely no regularity. As routine it is possible in single sections to trace a route along the space between the photoreceptor cell and glial cell through the interglial space to the outside without meeting a single quintuple-layered junction.

Several glial cells are associated with the body of a single photoreceptor cell. The glial nuclei are relatively small and the nucleoplasm has regions of coarse granules and densely staining chromatin. Glial cytoplasm contains mitochondria, Golgi vesicles, scattered glycogen particles, and various elements of endoplasmic reticulum although in concentrations below that of the photoreceptor cell. The relative "transparency" of the glial cytoplasm may be artifactual but it provides a means, supplemental to serial sections, for differentiating glial cytoplasm from that of the photoreceptor cell.
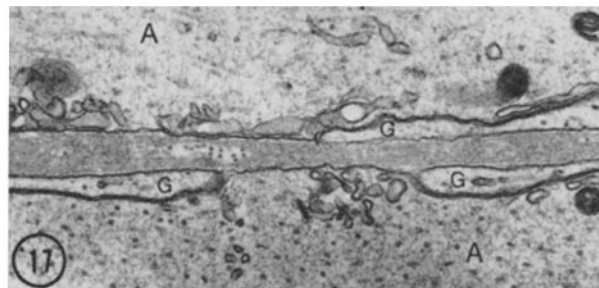

FIGURE 17. Electron micrograph showing two discontinuities in the glial covering (G) of the axon. The figure shows small portions of two axons (A) lying close to each other and both of them are free of glial covering. There are a few elements of smooth-surfaced endoplasmic reticulum that appear to be associated with these areas. × 15,000.

As the body of the photoreceptor cell attenuates into the axon, much of the rich variety of the cytoplasm is lost. What remains are mitochondria, microfilaments, and microtubules, all arranged more or less parallel to each other. Fig. 16 shows the cross-section of an axon close to its termination in the brain. In addition to the organelles already mentioned, scattered elements of smooth endoplasmic reticulum are also present. The glial covering of the axon is usually one layer thick (Fig. 16) and is occasionally discontinuous (Fig. 17).

A basal lamina approximately 0.5–0.7 μ thick covers the entire length of the photoreceptor cell–glial cell complex until it reaches the second optic ganglion in the brain.

DISCUSSION

The photoreceptor cells in the ventral eye of *Limulus* have a unique combination of virtues. The most important of these is their great size since it permits the relatively easy insertion of two microelectrodes for voltage-clamp studies of the membrane currents. Second, there is the convenience of the cells located along the length of the ventral eye nerve. The nerve is easily dissected out of the animal and then out of its sheath so that the photoreceptor cell–glial cell complex can be bathed directly with fluids of any desired composition. The accessibility of the cells probably accounts for their good fixation and it cer-

tainly accounts for the ease of sectioning them. Finally the photoreceptor cells in the ventral eye resemble the retinular cells in the lateral eye of Limulus both in electrical behavior and in morphology.

The fine structure of the retinular cell has been described by Lasansky (1967) and most recently by Fahrenbach (1969). Both authors emphasize the tremendous amount of endoplasmic reticulum as a striking feature of the cell. Although the ventral photoreceptor cell has a variety and quantity of endoplasmic reticulum that match the retinular cell, the arrangement of the cisternae is not as discrete and well-ordered. For instance, subsurface cisternae (Fig. 8) are found with little regularity or pattern in the ventral photoreceptor cell and long stretches of plasmalemma have no cisternae adjacent to them. In the retinular cell, subrhabdomeric cisternae underlie the origins of the microvilli in an uninterrupted series or "palisade." There is much greater variation along the same region in the ventral photoreceptor cell. In addition, most of the subrhabdomeric cisternae in the ventral photoreceptor cell are not expanded. Both conditions may be a result of the light-adapted state of the cells during their fixation and may represent the light-dispersed remnants of a well-ordered palisade seen in other arthropod eyes (Eguchi and Waterman, 1967; Horridge and Barnard, 1965). The comparative disorder of the endoplasmic reticulum is typical of the morphological contrariness of the ventral photoreceptor cell and does not set it apart from the retinular cell.

Lasansky went on to discuss the functional significance of the great amount and the regular arrangement of endoplasmic reticulum in the retinular cell and raised the possibility of its serving as an internal conduction system. He observed no continuities between cisternal membranes and the plasmalemma in retinular cells and therefore thought the possibility an unlikely one. So do we since no such continuities are seen in the ventral photoreceptor cell.

Fahrenbach (1969) suggested that the endoplasmic reticulum of the retinular cell may be involved with the production of the visual pigment and we also believe this is its most likely function. A highly developed endoplasmic reticulum is typical of a great many photoreceptor cells, so many in fact that it would be hard to list the exceptions. Two recent papers suggest that the endoplasmic reticulum in photoreceptor cells is engaged in the synthesis of cell components. Eakin and Brandenburger (1968) have followed the uptake of vitamin A in the retina of a snail and Young and Droz (1968) have followed the uptake of several amino acids in the retinal rods and cones of the frog. Both these studies show that there is fast and continuous turnover of photoreceptor membranes. Aside from transducing light, the major function of photoreceptor cells may well be production of a transducing membrane.

Ventral photoreceptor cells and retinular cells also resemble each other in the complicated and extensive relation they have with their glia. Thin sheets of glial cytoplasm or trophospongium (Holmgren, 1914) penetrate the soma

of both cell types, a relation found in other arthropod (Hess, 1958) and invertebrate (Rosenbluth, 1968) nerve cells. Fahrenbach (1965) has described the median photoreceptor of the barnacle, an arthropod eye in which glial cells and photoreceptor cell rhabdomeres are extremely close together. The association, however, is not so tight as it is in *Limulus* where Lasansky found quintuple-layered junctions between retinular microvilli and glial plasmalemmas. Such an involvement appears to be exceeded by the ventral eye in which the glial processes actually penetrate the rhabdomere deep within the cell (Figs. 10 and 11). The significance of the interpenetration of the two cell types is unknown.

The patterns of quintuple-layered junctions in the lateral eye and the ventral eye represent another area in which the fine structure of the two eyes appears to be essentially the same. In both eyes, there are junctions between almost all adjacent microvilli. Lasansky found extensive junctions between retinular cells and glial cells, especially along the lateral margins of the retinular cell and along the glial infoldings mentioned before. He also found junctions between adjacent glial cells. Fahrenbach, on the other hand, has determined that the extent of quintuple-layered junctions is closely related to the concentration of sucrose used in the fixatives. According to his results, the only sets of quintuple-layered junctions in the retinular cell that persist regardless of the sucrose concentration are those between adjacent microvilli. Quintuple-layered junctions between photoreceptor cells and glial cells in the ventral eye occur only at those places where a long glial process reaches the rhabdomere deep within the cell (Figs. 10 and 12). Glial-microvillous junctions at the periphery of the photoreceptor cell are less regular and do not occur along the entire length of the microvillus. Fig. 13 is unusual in that it includes most of these intermittent junctions in the plane of a single section. We have never found extensive junctions between photoreceptor cells and their glial cells in any other location. The same is true for junctions between adjacent glial cells. When they are found, the junctions are very short and appear to occur at random. It must be emphasized that sucrose was used in all our fixatives and may be responsible for many of these junctions.

Quintuple-layered junctions are interesting because some of them probably represent the site of electrotonic coupling between cells (Loewenstein, 1966). Smith et al. (1965) have shown that the retinular cells and eccentric cell dendrite in a lateral eye ommatidium are all in electrical continuity with each other and Lasansky has pointed out that the junctions between the microvilli in the rhabdome probably form structural pathways for the coupling. Whether or not the ventral photoreceptor cell and its glial cells are electrotonically coupled through their junctions in the rhabdomere has not been answered experimentally since a glial cell is extraordinarily hard to impale with microelectrodes.

It is reassuring that there are not extensive junctions between the ventral photoreceptor cell and its glial cells because such an arrangement would imply a high-resistance pathway (Loewenstein, 1967) between the intermicrovillous space in the rhabdomere and the outside. Experiments by Millecchia and Mauro (1969 a) in which the ventral eye nerve preparation was perfused with low-sodium seawater show an almost instantaneous abolition of the sodium-dependent light response. The speed of the reaction to low-sodium seawater implies that there is an open, low-resistance pathway from the intermicrovillous space in the rhabdomere (Figs. 12 and 13), through the space between the retinular cell and the glial cell, and then through the space between adjacent glial cells to the extracellular medium. In a sealed-off rhabdomere, the decay of the light response would presumably be much slower. The partial recovery of the light response that occurs after 5–10 min in low-sodium seawater most likely is due to the utilization of other ions; however, it may represent a "zipping up" of glial-photoreceptor junctions into a configuration like that seen in the lateral eye with high concentrations of sucrose in the fixatives.

Recent work with low-molecular weight tracers has brought into question the idea of longitudinal impermeance in transversely conducting tight junctions (Karnovsky, 1967; Revel and Karnovsky, 1967; Benedetti and Emmelot, 1968). Brightman and Reese (1969) have shown that there are several classes of junctions in the vertebrate brain. Some of these junctions are completely impermeable to tracers such as peroxidase and lanthanum while others come and go with different methods of fixation. In work that is more applicable to the ventral photoreceptor cell, Perrelet and Baumann (1969) have shown that an open pathway exists between the exterior of the ommatidium and the extracellular space around the microvilli in the rhabdome of the honeybee drone eye. In the unfixed eye, they have been able to penetrate the extracellular space to the base of the microvilli with ferritin. In the fixed eye, they have been able to penetrate the space to the tips of microvilli with lanthanum.

The rationale for quintuple-layered junctions in arthropod ommatidia has a reasonable electrophysiological basis at first glance. Presumably, the junctions are the site of electrotonic coupling between adjacent retinular cells and between retinular cells and the eccentric cell dendrite. However, the rationale breaks down in the ventral eye where many of the photoreceptor cells have their rhabdomere all to themselves and do not share it with any other photoreceptor cell. Viewed strictly from an electrophysiological point of view, such "self-coupling" seems perverse. Even in those ommatidia where junctions make functional sense, their vast expanse does not. It would seem more reasonable to have junctions only at the places in the rhabdome where retinular cell and eccentric cells or adjacent retinular cells touch each other.

The vast expanse of these junctions in arthropod photoreceptor cells probably has some functional meaning beyond electrotonic coupling.

Another similarity of the lateral eye to the ventral eye is the occurrence of neurosecretory axons. Fahrenbach (1969) has observed a relatively well-ordered system of neurosecretory axons in each ommatidium of the lateral eye. Furthermore, Baumann (personal communication) has seen neurosecretory axons in the honeybee eye, so the presence of the structures may be general among arthropod eyes. We do not understand their presence in the ventral eye where they make direct contact with the soma of photoreceptor cells that must be completely out of electrical contact with the brain.

The final and most vexing similarity of the ventral photoreceptor cell to the retinular cell is the inexcitability of its axon. Although the axon is big, there is nothing to distinguish it from any other functioning axon. In spite of the normal morphology of the axon, the action potentials we can evoke with electrical stimulation of the ventral eye nerve preparation are of low amplitude and slow velocity, with values around 100 μv and 50 cm/sec, respectively (unpublished observations). We believe this activity comes from the small axons visible at the edges of Fig. 16 although we know neither their origin nor their destination. The nerve preparation shows no propagated activity with light stimulation and the values seen with electrical stimulation are not appropriate to the size and number of photoreceptor cell axons. There is the possibility that the photoreceptor cell axons are extremely labile, or that our dissection methods are too crude.

It is interesting that Snodderly (personal communication) has recorded activity in the output tract of the second optic ganglion after shining light on a *Limulus* brain completely severed from its afferent connections. Presumably the activity is evoked by electrotonic spread from the ventral photoreceptor cells left behind in the brain during development (Figs. 3 and 5). Nevertheless, if activity of this sort is all that goes on in the mature animal, it does not explain the apparently senseless waste of photoreceptor cells strung out along the silent ventral eye nerve where they transduce light with great elegance to absolutely no purpose.

One of the most interesting properties of the ventral photoreceptor cell revealed by double electrode studies is the high specific resistance of the plasmalemma. Millecchia and Mauro (1969 *a*) have calculated values of 50,000-100,000 Ωcm^2. Such values are one to two orders of magnitude higher than those for most nerve and muscle cells.

Millecchia and Mauro (1969 *b*) have proposed that within the plasmalemma of ventral photoreceptor cells there are two, physically distinct regions. One region is insensitive to light and has a conductance that is time- and voltage-dependent. Potassium ion is the predominant current species flowing through this dark conductance. In fact, the high specific resistance of the

plasmalemma is a measure of the dark conductance and therefore this conductance is unusually low. The other region of the plasmalemma is made up of small discrete areas. The conductance of these discrete areas is time- and voltage-dependent; however, it also depends on light intensity. Sodium ion is the predominant current species flowing through this light conductance. In combination, these two conductances give a system with great sensitivity and amplification. Very small amounts of current flowing through the light-sensitive conductance, for instance, will cause great changes in the potential difference across the membrane. Because the vast area of receptor membrane has such high specific resistance, the cell is able to keep track of remote changes in conductance, even though the amount of current involved is small. The model is appealing since it helps to explain how the ventral photoreceptor cells can be so large and yet so sensitive.

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