

# Ultrastructural Evidence That Horizontal Cell Axon Terminals Are Presynaptic in the Human Retina

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## ABSTRACT

The organization of the rod spherule and of the horizontal cell axon terminals within the invagination of the rod spherule in the human retina was examined in serial sections by electron microscopy. Twenty-one rod spherules were reconstructed in this study. Axon terminal processes of type I horizontal cells consistently make one or two small punctate synapses onto each rod spherule within the invagination. In addition, these axon terminal processes make distinct synapses upon rod bipolar dendrites outside the spherule before both processes enter the invagination.

This is the first positive description of a synapse from a horizontal cell axon terminal process onto a photoreceptor terminal and the first identification of a synapse from a horizontal cell to a rod bipolar cell in the mammalian outer plexiform layer. We speculate that the axon terminal-to-rod synapse is responsible for feedback while the synapse upon the rod bipolar cell is feed-forward and serves to expand the receptive field of the rod bipolar cell beyond its dendritic field. Alternatively, the latter may contribute to a center-surround organization of the rod bipolar's receptive field.

**Key words:** synapse, bipolar cell, retinal circuitry, rod photoreceptors, electron microscopy

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In the primate retina there are two morphologically distinct types of horizontal cells (Kolb et al., '80; Gallego, '86; Boycott et al., '87). The type I horizontal cell was first described by Polyak ('41), and is characterized by having stout dendrites that contact only cones and a single, long (2 mm) axon ending in an elaborate axon terminal arborization bearing terminals that contact only rods. These dendritic terminals form the lateral elements of the rod synapse (Kolb, '70). The type II horizontal cell, on the other hand, contacts only cones with both its slim overlapping dendritic branches and its short (100–300  $\mu\text{m}$ ), curved axon (Kolb et al., '80; Gallego, '86). Both of these horizontal cell types were identified recently in Golgi preparations of human retina (Fisher et al., '86).

Vertebrate rod spherules contain one or two presynaptic ribbons, a large complement of synaptic vesicles, and several invaginated processes coming from horizontal cells and rod bipolar dendrites (Sjöstrand, '58; Missotten, '65; Stell, '65, '67; Dowling and Boycott, '66). Primate rod spherules have a single synaptic invagination that contains horizontal cell processes ending laterally and deeply within it and rod bipolar dendrites terminating centrally and less deeply

(Missotten, '65; Kolb, '70). Human rod spherules have been described as having two or three lateral postsynaptic processes and between two and five central ones (Missotten, '65). Previous to this study no synaptic specializations have been reported within the synaptic invagination of the rod spherule.

Although it is acknowledged from physiological evidence that horizontal cells are responsible for surround antagonism to bipolar cell receptive fields in the vertebrate retina, it is unresolved whether this lateral inhibition takes place via direct synapses between horizontal cells and bipolar cells in the outer plexiform layer (OPL) (Werblin and Dowling, '69; Kaneko, '70; Naka and Nye, '70; Naka, '82) or whether the surround inhibition arises from a feedback loop through the photoreceptors themselves (Baylor et al., '71; Toyoda and Kujiraoka, '82). Morphological evidence for either of these synaptic pathways is scant for the vertebrate retina in general and until now nonexistent for the human retina in particular. In a few species, chemical synapses are observed from horizontal cell processes to bipolar cell den-

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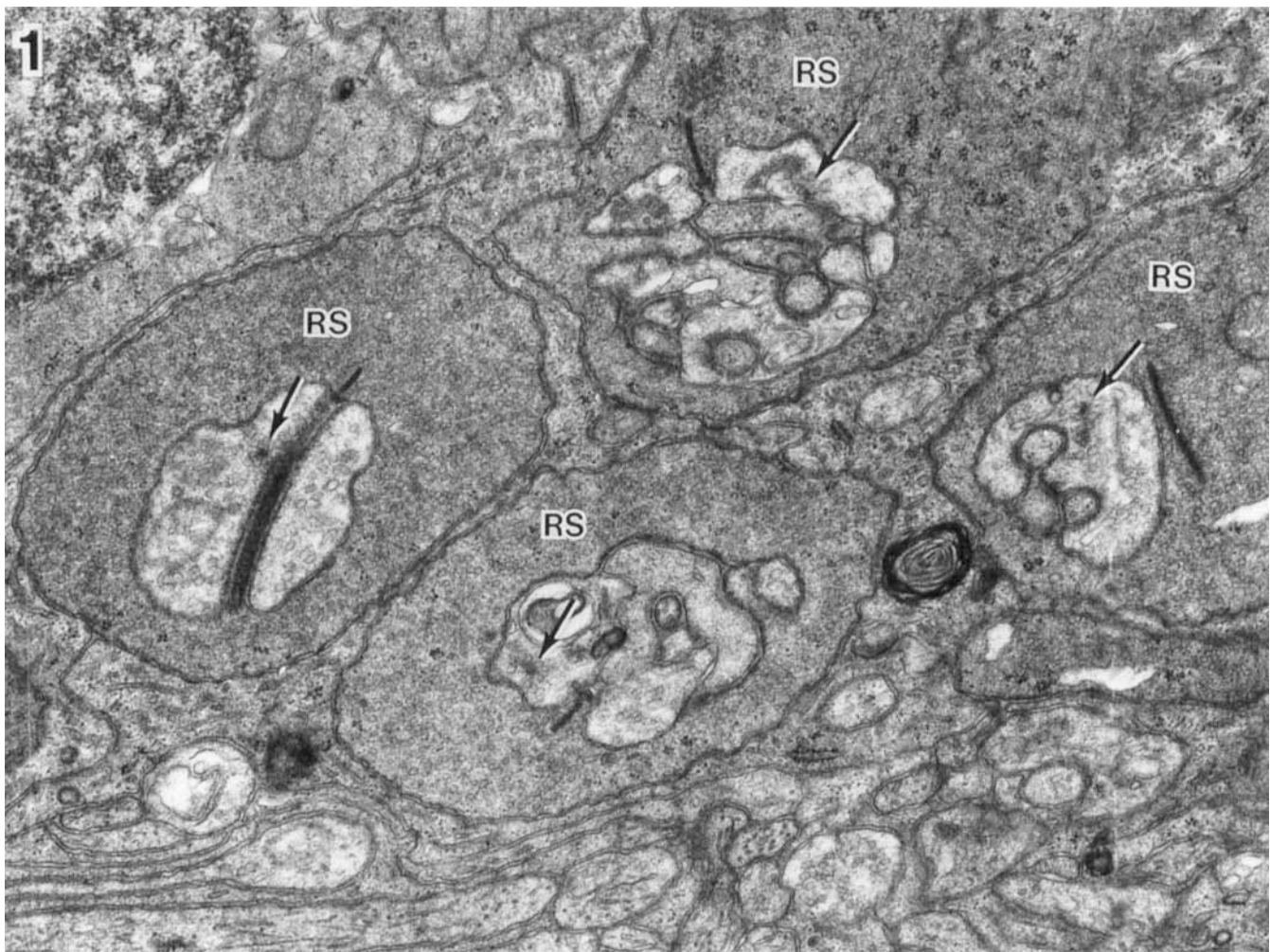


Fig. 1. A low-power electron micrograph showing four rod spherules each with a postsynaptic process containing a cluster of vesicles and one of the presynaptic dense bodies (arrows).  $\times 20,000$ .

#### Abbreviations

CP	Extensions of cone pedicle cytoplasm
H	Processes of the type I horizontal cell axon terminal
HC	Type I horizontal cell
I	Interplexiform cell process
RA	Axon of rod photoreceptor
RB	Rod bipolar cell dendrites or cell body
RS	Rod spherule

drites in the OPL neuropil (catfish: Sakai and Naka, '83, '86; mudpuppy and frog: Dowling, '70; salamander: Lasansky, '73; turtle: Kolb and Jones, '84; mouse: Olney, '68; rabbit: Fisher and Boycott, '74). In even fewer species is there evidence of a synapse between horizontal cell processes and photoreceptors within their synaptic invaginations. In cyprinid fish retina, for example, digitations or spinules on the horizontal cell lateral elements in the cone synaptic invaginations vary in size with light and dark adaptation (Raynauld et al., '79; Wagner, '80; Djamgoz et al., '83; Weiler and Wagner, '84). These authors propose that the spinules represent the site of inhibitory feedback from horizontal cells to cones in these retinas. Stronger evidence for feedback synapses has been presented by Sakai and Naka ('83, '86), who show synapses between horizontal cell dendrites and cone telodendria in catfish retina. To date, no morphological evidence exists for such synapses

from horizontal cell structures onto photoreceptors in a mammalian retina and in no vertebrate retina has a feedback synapse been observed between a horizontal cell process and a rod spherule.

In this report we provide the first evidence in a mammalian retina, the human retina, for a morphologically distinct chemical synapse between horizontal cell axon terminals and the rod spherule within the rod spherule invagination itself. In addition, we have observed the same horizontal cell axon terminals to be presynaptic to rod bipolar dendrites in the outer plexiform layer just before both processes enter the rod spherule synaptic invagination.

#### MATERIALS AND METHODS

The materials and methods for this study are identical to those published previously (Linberg and Fisher, '86). In brief, thin (ca. 80 nm thick) sections from the periphery of adult human retina fixed conventionally for electron microscopy were stained with uranyl acetate and lead citrate before examination. One series of 60 and another of 1,100 consecutive thin sections from the same tissue block were mounted on Formvar-coated slot grids and used for the reconstruction of 21 rod spherules. After the initial study of sections cut parallel to the long axis of the photoreceptors, a piece of tissue was reoriented at right angles and

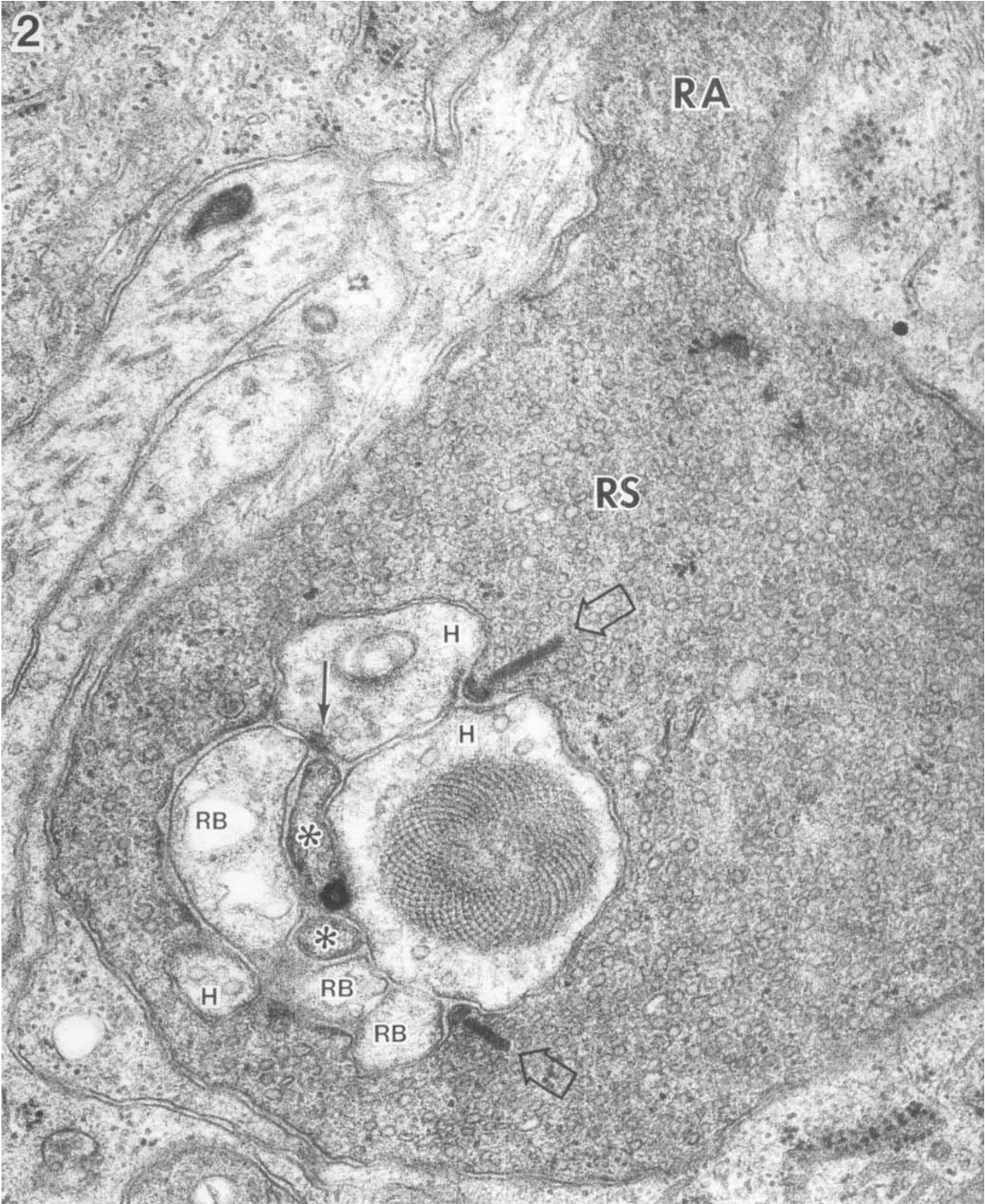


Fig. 2. Electron micrograph of a single rod spherule with its synaptic invagination. Profiles marked with an \* are projections of the rod spherule cytoplasm. The large crystalloid structure in one of the horizontal cell processes is a "cylinder organelle" and is found only in horizontal cell processes. Note that all of the horizontal cell axon terminal processes contain small vesicles and one of them shows an accumulation of vesicles adjacent to a region of membrane densification (solid arrow). Open arrows = synaptic ribbons.  $\times 66,000$ .

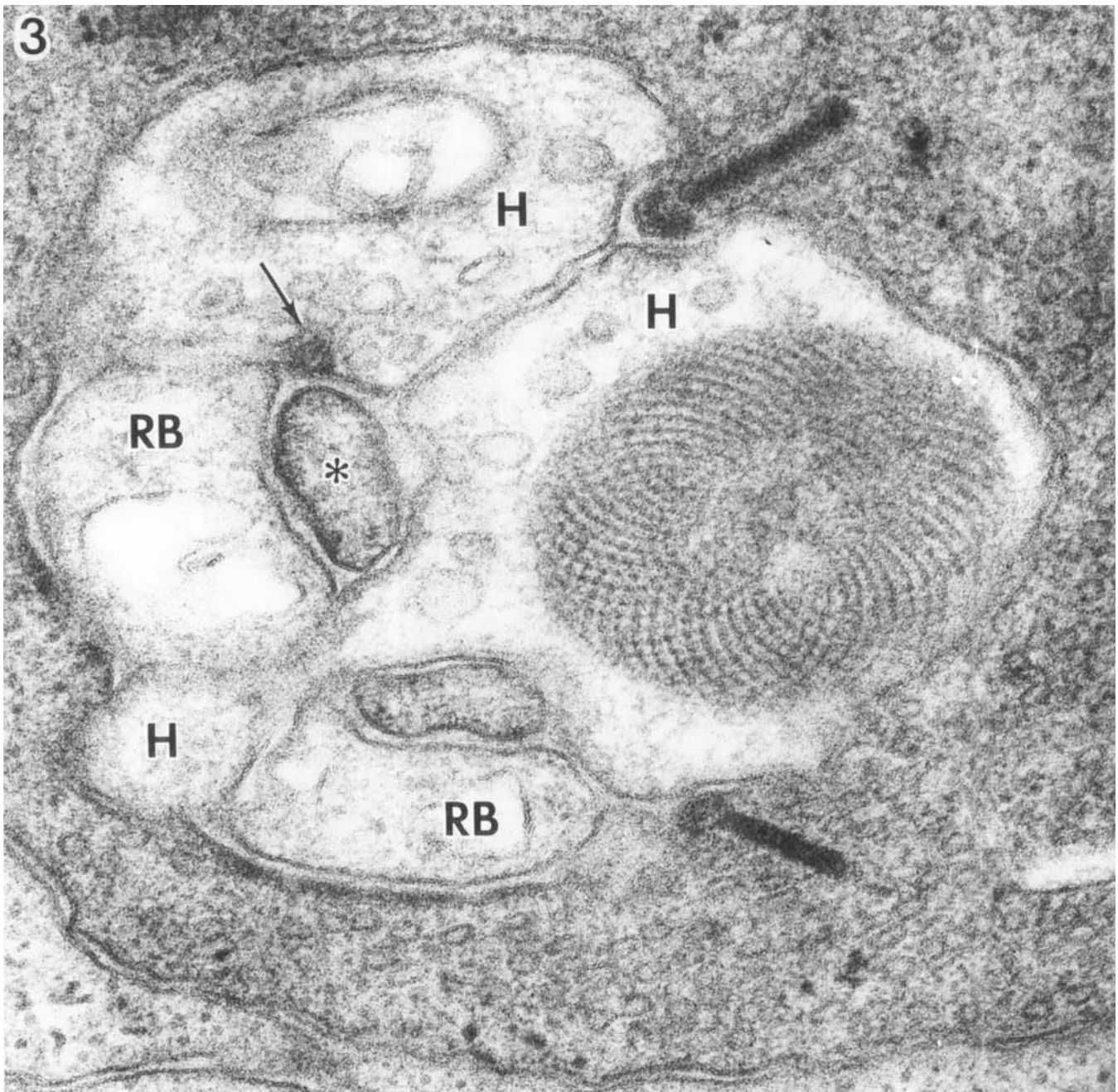


Fig. 3. An electron micrograph of the synaptic invagination of the same rod spherule shown in Figure 2 in an adjacent section and at a higher magnification. In this plane of section the synaptic densification and cluster of vesicles (arrow) in the horizontal cell process are more clearly defined. One of the projections of the rod spherule cytoplasm (\*) is postsynaptic to the horizontal cell synapse at the arrow.  $\times 130,000$ .

sectioned through the region of the photoreceptor terminals and the OPL. A second retina was also examined to confirm the observations. Tilting of the sections was accomplished with the aid of a Philips CM10 transmission electron microscope equipped with a eucentric goniometer stage.

## RESULTS

### Synapses within the rod spherule invagination

Examination of thin sections by electron microscopy reveals small, synapselike structures, each consisting of a

punctate dense body surrounded by vesicles, in at least one of the horizontal cell processes (lateral elements) invaginating almost every rod spherule (Fig. 1). A detailed study of the rod spherules in various planes of section shows the dense structures to be closely applied to the membrane of the lateral element and associated with a cluster of vesicles. The membrane of the horizontal cell process directly beneath the cluster of vesicles is usually slightly thickened. An example of one of these synapses is shown in two consecutive sections through a rod spherule in Figures 2 and 3.



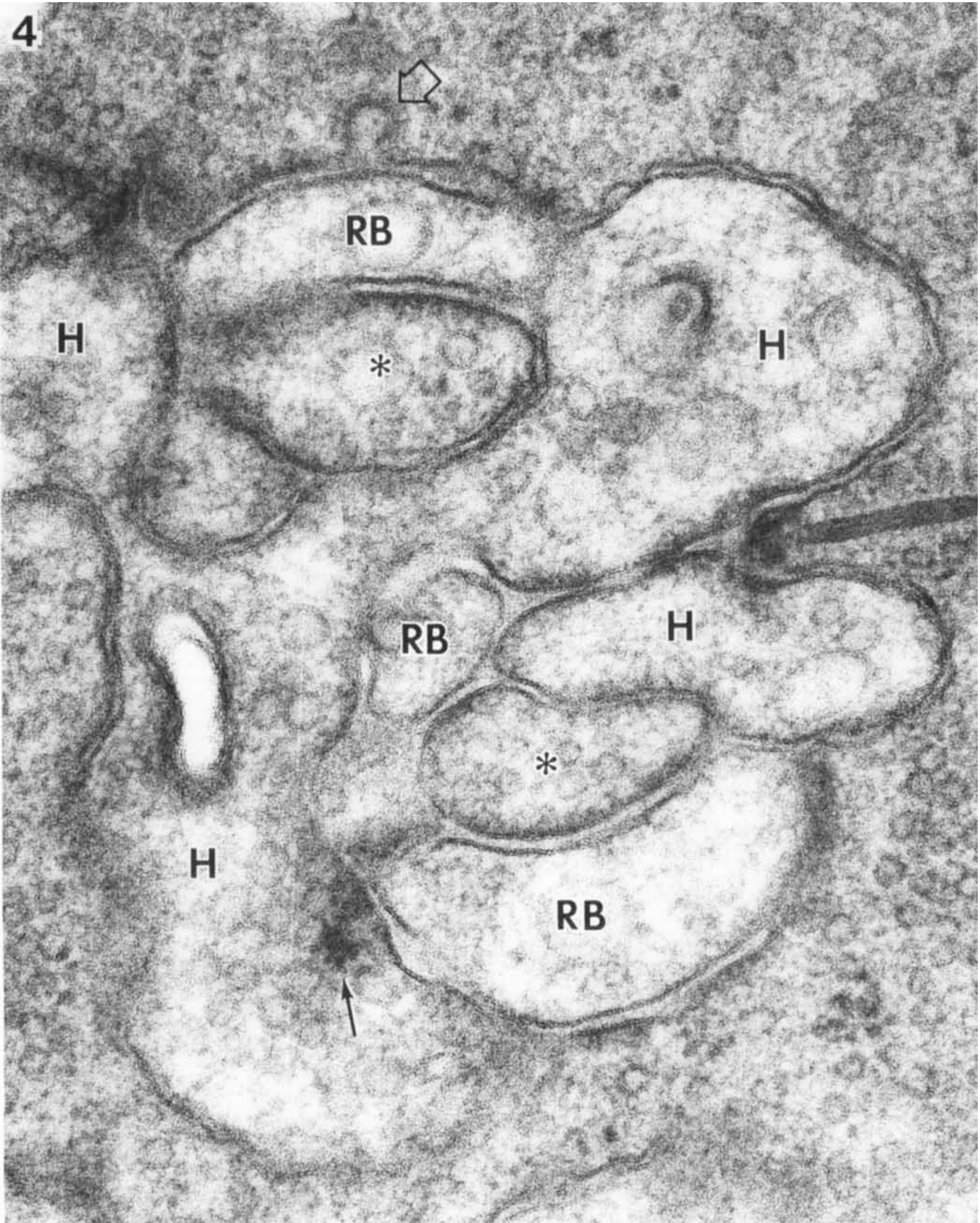


Fig. 4. Another example of a horizontal cell synapse (arrow) within a rod spherule synaptic invagination. In this case the horizontal cell process is large and multilobed and contains several synaptic vesicles. In this plane of section the post-synaptic process is just grazed. The open arrow indicates a coated vesicle still continuous with the rod spherule membrane. \* = projections of rod spherule cytoplasm.  $\times 128,000$ .

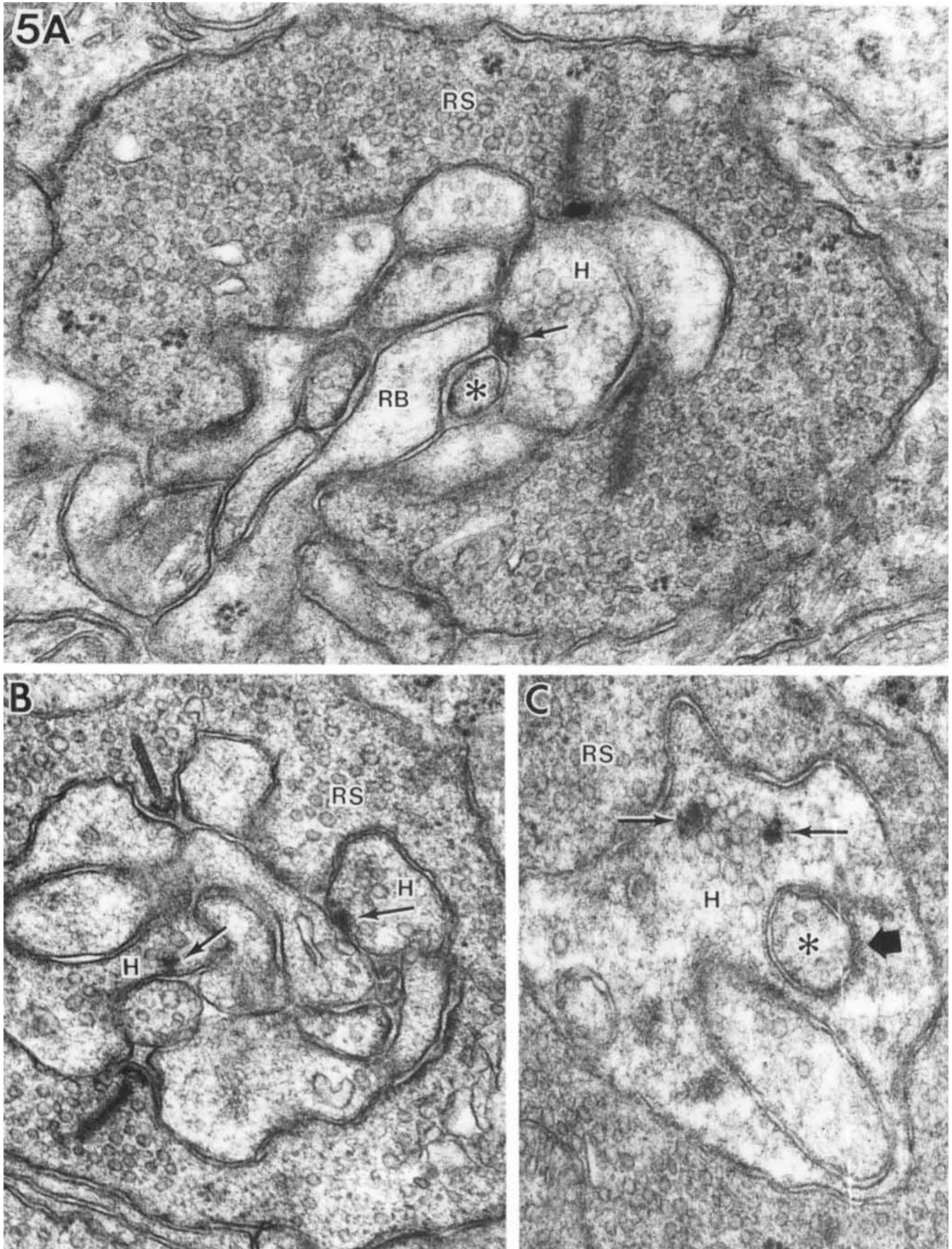


Figure 5

Figure 2 shows the overall structure of one rod spherule and its synaptic invagination (the "opening" or hilus, Misstotten, '65, of the invagination is not in this plane). Several processes are postsynaptic to the photoreceptor ribbon. Three of them contain numerous small vesicles and another an elaborate crystalloid structure known as the "cylinder organelle" (Craft et al., '75). Such crystalloids occur in horizontal cell processes in 10–20% of the rod spherules examined in this study. Of the remaining processes, two are particularly electron dense (\* in Figs. 2, 3) and by serial section analysis are seen to be projections of the rod cytoplasm; the other three have lightly staining cytoplasm and a few swollen vacuoles. One of the vesicle-containing processes also has a small patch of electron-dense material (arrow, Fig. 2) closely apposed to its membrane. In the next section (Fig. 3), this dense body projects further into the cytoplasm and is surrounded by a small number of vesicles. Most of the vesicles surrounding the dense bodies are slightly elongated in shape and measure about 60 nm along one axis and 40 nm along the other. These structural features are all diagnostic of conventional chemical synapses.

Because these putative synapses are small and punctate, it is rare to find examples in which all of the structural features occur in a single plane of section. Other presumed horizontal cell axon terminal synapses, each structurally similar to the example in Figures 2 and 3, are shown in Figures 4, 5A, and 5B. Figure 5C shows a plane of section cut *en face* to a region containing two presynaptic dense bodies encircled by vesicles. As many as four of these dense structures may compose one synaptic site. Figure 6A and B shows another example of the synaptic structures in two planes of tilt in the electron microscope. In Figure 6A, the aggregate of vesicles is clear and associated with a region of distinct electron-dense material. In Figure 6B the cluster of vesicles is less distinct but the patch of electron-dense material is clearly associated with the cell membrane. In this plane of tilt the two cell membranes are defined and parallel at the presumed site of synaptic contact.

#### Identification of pre- and postsynaptic processes

Identification of the processes containing the presynaptic structures required an analysis of serial sections. Invaginating horizontal cell lateral elements exit through the rod spherule hilus and eventually merge with axonal processes of the type I horizontal cell that run laterally in the OPL. In addition, the presence of a cylinder organelle identifies a horizontal cell axon terminal process; these crystalloids are found frequently in lateral elements containing the presynaptic structures (see Figs. 2, 3).

Rod bipolar dendrites exit the hilus of the rod spherule and descend through the OPL to their respective cell bodies in the inner nuclear layer (INL) (Fig. 7). Within the rod spherule invagination, rod bipolar dendrites appear as electron-lucent profiles lacking organelles. Outside the invagi-

Fig. 5. Three examples of horizontal cell synapses within the rod spherule synaptic invagination. A: Both the cluster of vesicles and the presynaptic densification appear in this plane of section (arrow). \* = a projection of the rod spherule cytoplasm. B: Two presynaptic densifications with a few associated vesicles (arrows) are present in two horizontal cell processes. The postsynaptic process on the left is part of the rod spherule. C: One of the horizontal cell synapses sectioned *en face*. Two presynaptic densifications (arrows) are surrounded by vesicles. The thick arrow indicates a dense undercoating of the horizontal cell plasma membrane adjacent to a profile of rod spherule cytoplasm (\*) projecting into the synaptic invagination. A =  $\times 69,000$ . B =  $\times 72,000$ . C =  $\times 64,000$ .

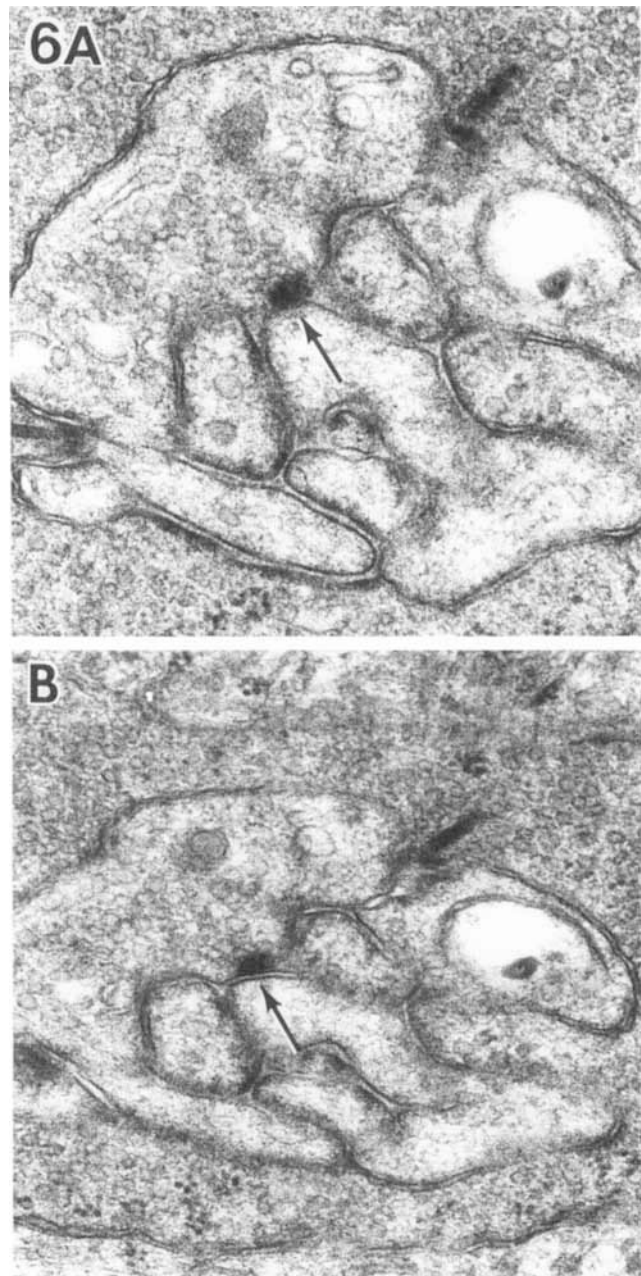


Fig. 6. A horizontal cell synapse within the rod spherule invagination in two planes of tilt. In A a cluster of vesicles is seen associated with a distinct region of densification (arrow), but the cell membranes are not discernible. Tilting of the section by 30° (B) brings the two cell membranes into parallel alignment (arrow) and shows that the densification in A is closely related to one of those membranes as well as the cluster of vesicles. A,B =  $\times 56,000$ .

nation, they are easily identified by their dense cytoplasm and by cisternae of smooth endoplasmic reticulum that closely appose their plasma membrane in a configuration known as the "helical organelle" (Misstotten, '65; Rodieck, '73; and see Fig. 7). Within the OPL the rod bipolar dendrites also contain microtubules, mitochondria, and scattered free ribosomes.



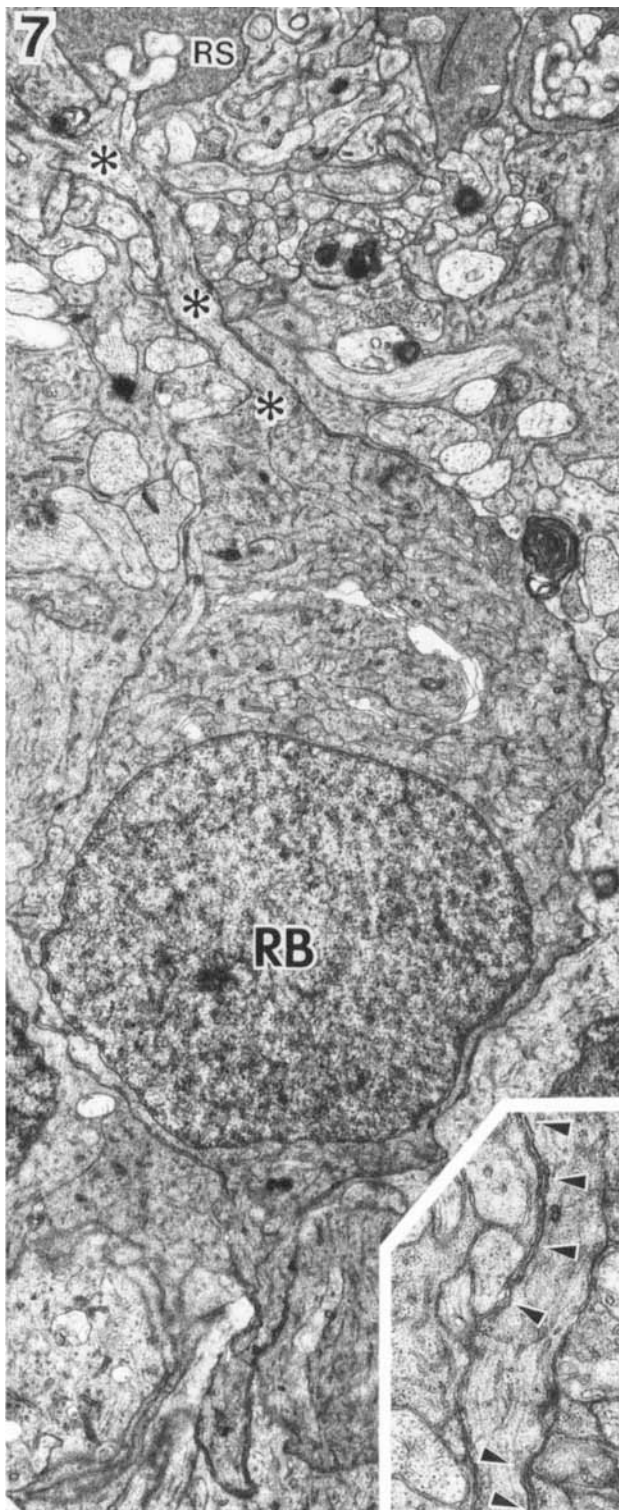


Fig. 7. An electron micrograph of a rod bipolar cell with one of its dendrites (\*) extending into the synaptic invagination of a rod spherule. The inset is a higher magnification of the dendrite just above the cell body and shows that it contains cisternae of smooth endoplasmic reticulum (arrowheads) lining its membrane in a configuration known as the helical organelle. Since only processes of the rod bipolar contain this specialized SER, it can be used to identify them in single thin sections.  $\times 8,600$ . Inset =  $\times 17,000$ .

The identification of certain postsynaptic processes as part of a rod spherule is sometimes obvious, as in Figure 8. In other instances they appear as isolated profiles within the invagination, and only after serial reconstruction do they prove to be fingerlike extensions of the rod spherule cytoplasm (see Figs. 2–4). In some instances these extensions end bluntly; in others they are continuous, spanning the invagination from one side to the other. Once the extensions of rod spherule cytoplasm within the invagination were characterized morphologically by serial section analysis, their typical electron density (see Figs. 2, 3) helped identify them in single sections. Because there is no distinctive densification of the postsynaptic membrane at the synaptic sites, it was sometimes difficult to tell which of two processes was in fact postsynaptic (see, for example, Fig. 5A). Whenever two processes lie opposite one of the presynaptic sites, one was always identified as an extension of the rod spherule and the other as a rod bipolar dendrite. Thus our evidence indicates that the spherule is probably always postsynaptic at these sites but does not exclude the possibility that rod bipolars are postsynaptic as well.

### Synapses within the OPL

The axon terminals of the type I horizontal cells are also presynaptic at the outer border of the OPL near the layer of rod spherules. At this location the synapses appear as conventional chemical synapses and tend to occur in processes abundant in organelles (Fig. 9). These synapses are characterized by a cluster of vesicles, one or more presynaptic dense structures, pre- and postsynaptic membrane densifications, and a slight expansion of the extracellular space between the synaptic membranes.

### Identification of pre- and postsynaptic processes

The axon terminal profiles that are presynaptic in the OPL are noticeably different from the interplexiform cell (IPC) processes (Linberg and Fisher, '86) that are also presynaptic within the OPL (Fig. 9A). In contrast to the IPC processes, the axon terminal processes have a more electron-dense appearance, contain more organelles, and have their synaptic vesicles clustered at the synaptic site (Fig. 9B). Several of these processes have been traced in serial sections into the rod synaptic invagination (Fig. 9C), where they were found to have the characteristic ultrastructure of the horizontal cell endings (including the "cylinder organelle"). The postsynaptic processes have been identified as rod bipolar dendrites by the presence of the "helical organelle" (see Figs. 7, 9B) and by tracing them into the rod spherule invagination where they become the central elements below the synaptic ribbon, a configuration characteristic to bipolar cell dendrites. The serial section analysis showed that the horizontal cell axon terminal processes expand in diameter where they are presynaptic and appear to enwrap the rod bipolar dendrite on either side of a synapse.

### Serial reconstruction of rod spherules

Figure 10 shows selected tracings of a single rod spherule reconstructed from serial sections cut vertically through the retina. Table 1 shows data collected from 21 reconstructed rod spherules. The spherule shown in Figure 10 is innervated by a single branch of a type I horizontal cell axon terminal (dark stippling), which, once within the rod spherule invagination, branches into lobes; when viewed in any single section, it thus appears to form multiple profiles



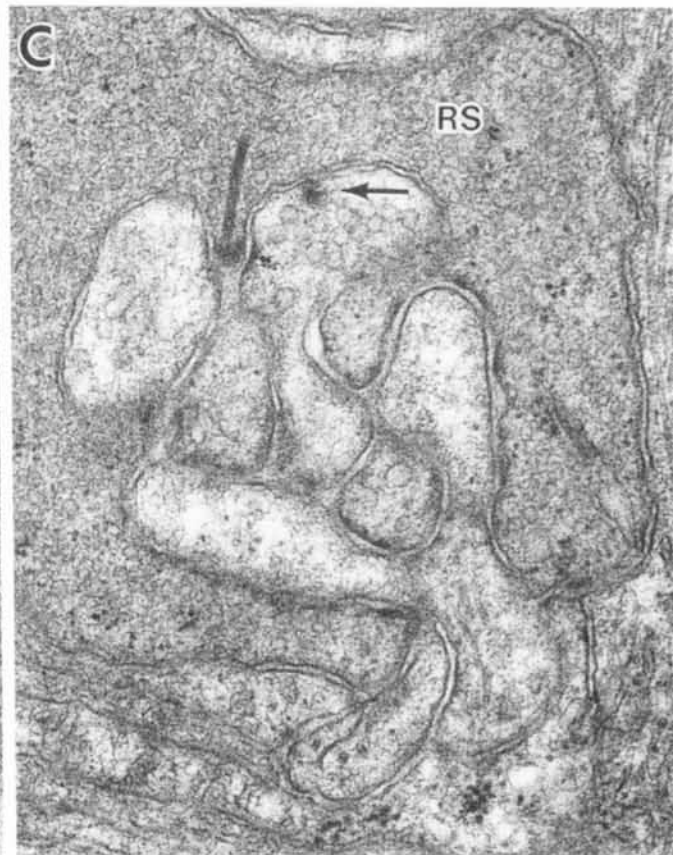
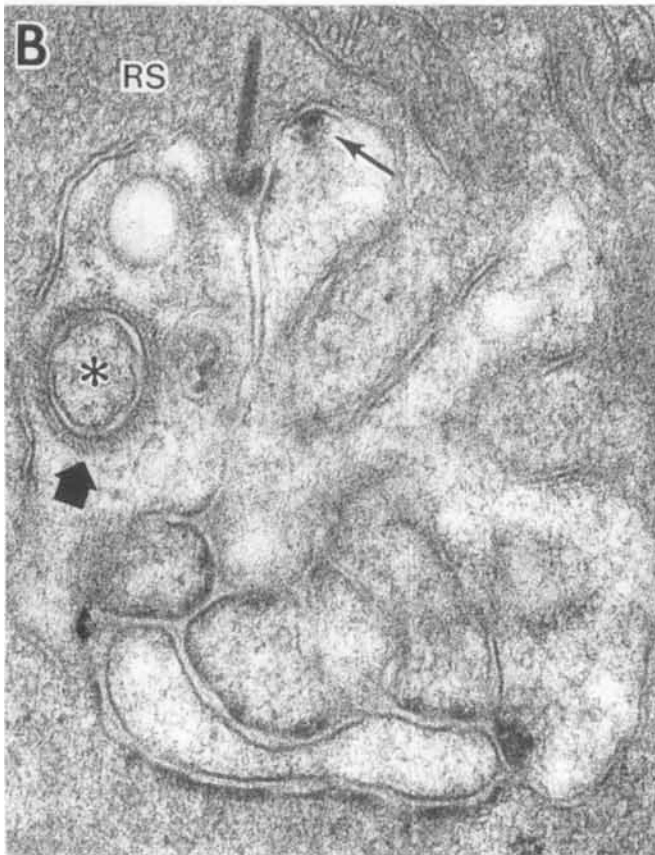
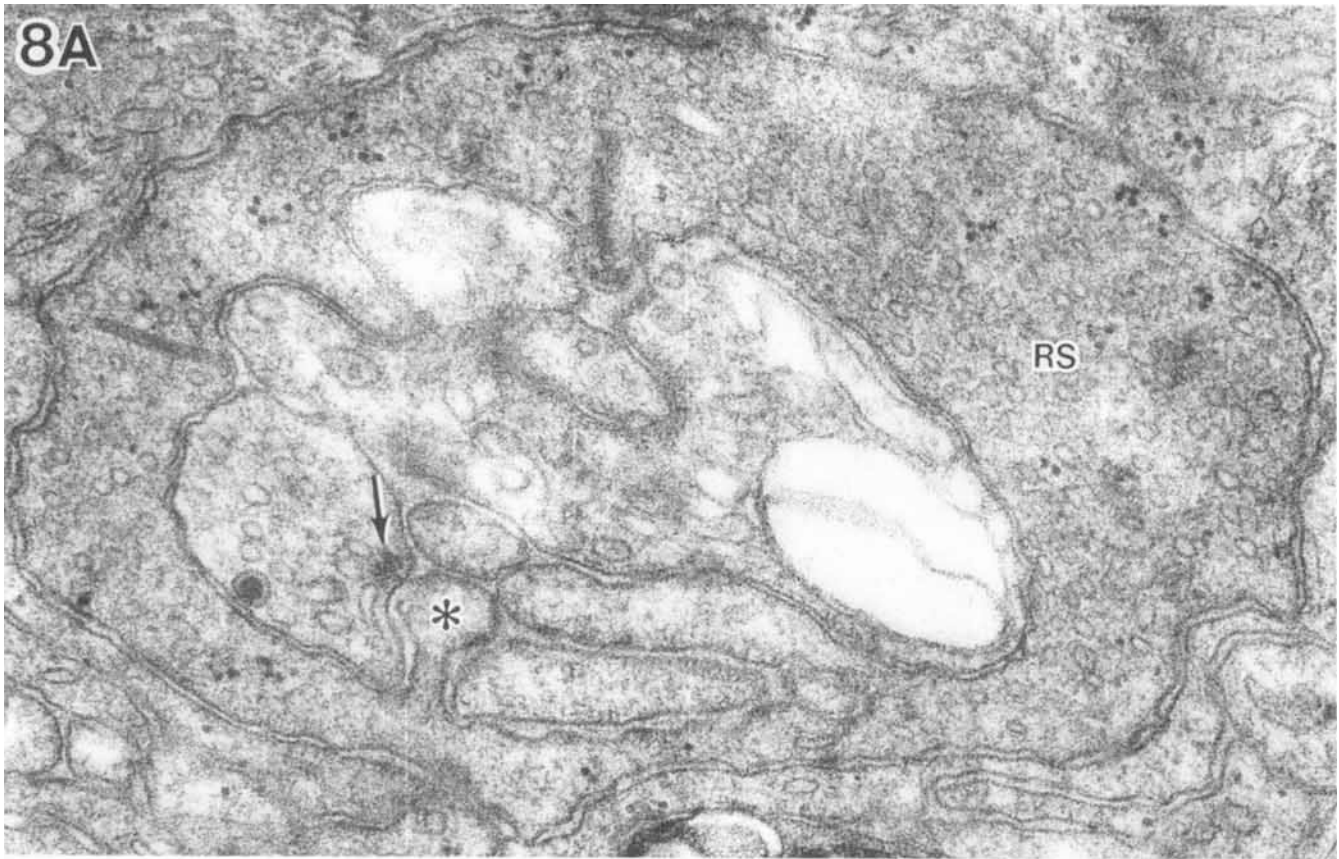


Fig. 8. Three examples of the horizontal cell synapses in which the rod spherule is clearly the postsynaptic element. A: The synapse (arrow) has both the presynaptic densification and a cluster of vesicles in this plane; the postsynaptic process (\*) is an expansion of a narrow stalk of rod spherule

cytoplasm that extends into the invagination. B,C: In these examples, the main body of the rod spherule is postsynaptic (arrow). In B, the thick arrow indicates a dense undercoating on the horizontal cell plasma membrane. A =  $\times 68,000$ . B =  $\times 50,000$ . C =  $\times 51,000$ .

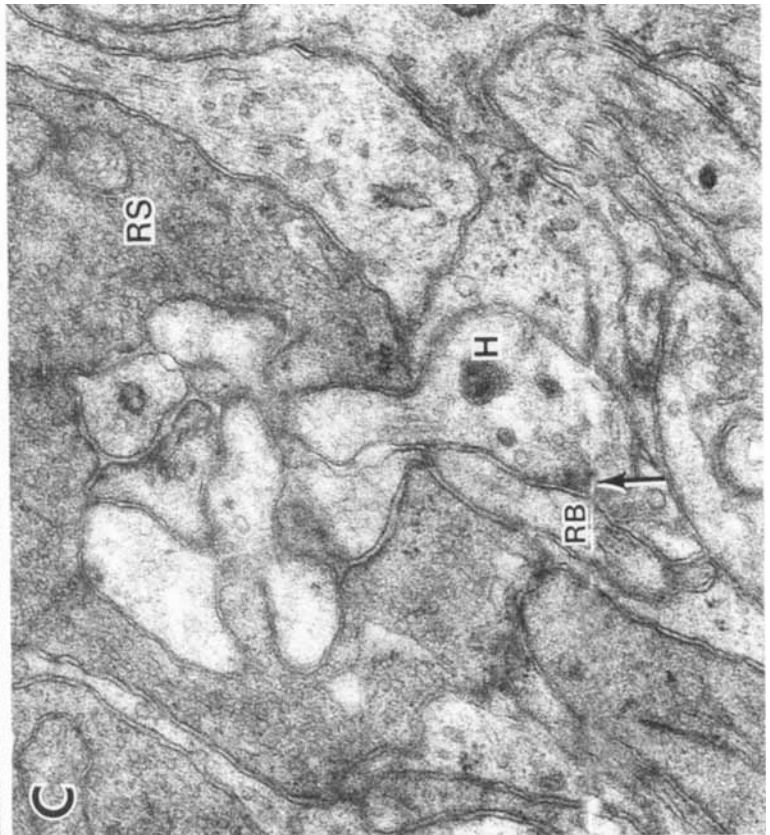
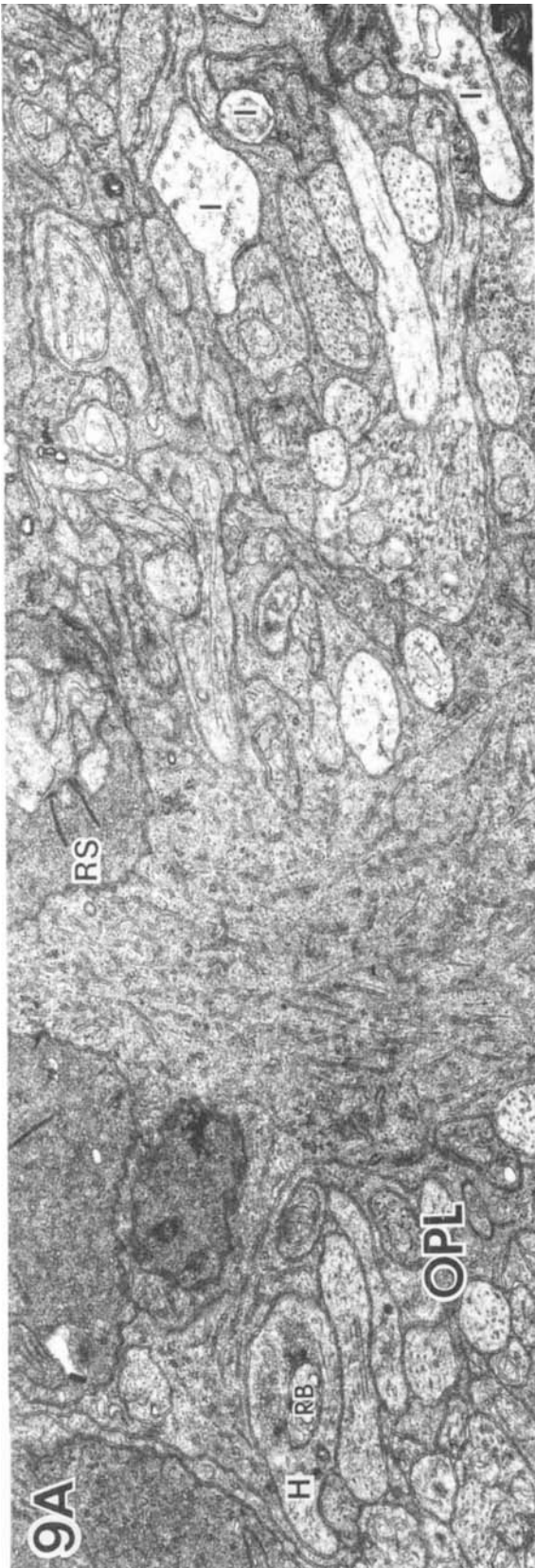


TABLE 1. Quantitative Data Collected From the Serial Reconstruction of 21 Rod Spherules

Spherule No.	No. ribbons in spherule	No. bipolar cell dendrites within spherule	No. HC processes within spherule	No. synapses by HC processes onto RS	Associated synapse in neuropil?	Cone contact onto RS?
1 <sup>2</sup>	2	?	?	?	?	?
2	2	2	2	2	+	+
3 <sup>2</sup>	2	2	2	3	+	+
4	1	2	2	2 (or more)	+	?
5	2	2	2	3	+	+
6	2	1	1	3	+	+
7 <sup>2</sup>	2	1	1	1 (or more)	+	+
8	2	2	2	1	?	+
9	1	1	1	1	+	+
10	2	2	2	3	?	+
11	2	2	2	2	+	?
12	2	2	2	2	+	?
13	2	1	1	1	?	+
14	2	1	1	2	+	+
15	1	1	1	0	?	+
16	2	2	2	0	?	+
17	2	2	2	3	-	?
18	2	1	1	2	+	?
19 <sup>2</sup>	2	1	1	2 (or more)	?	?
20 <sup>2</sup>	2	2	2	2 (or more)	?	+
21 <sup>2</sup>	2	2?	2?	?	?	?

<sup>1</sup>In only one case (21) was it not possible to determine if what appeared to be two separate ribbons were indeed independent structures. The number of bipolar dendrites and type I horizontal cell axon terminals (HC) refers to the number of processes that enter the synaptic invagination; it was not determined if they arose from the same or separate cells. The cone contact onto a rod spherule (RS) refers to gap junctions made by extensions of the cone pedicle that contact the RS.

<sup>2</sup>Incomplete serial tracing through synaptic invagination.

postsynaptic to the photoreceptor ribbons. Two different rod bipolar dendrites (light stippling and cross-hatching) enter the invagination. We were not able to determine if these are branches of a single bipolar dendritic tree or if they are from two different cells, but we assume the latter since Kolb ('70) concludes that bipolar cells in primates do not contribute more than one dendrite to a given rod spherule. The clear profiles drawn within the invagination (Fig. 10D-J) are projections of the rod spherule cytoplasm. The horizontal cell process forms two synaptic contacts within the invagination (Fig. 10C,E), both having the rod spherule as their postsynaptic element. A third synapse is made between the horizontal cell process and a projection of the rod spherule, just as the horizontal cell process enters the hilus. The cluster of vesicles in Figure 10E shows the presynaptic side of this synapse; the projection of the rod spherule that meets the horizontal cell process at the hilus in Figure 10F is the postsynaptic side. Just beneath the rod spherule in the OPL, the axon terminal is presynaptic to one (cross-hatched) of the rod bipolar dendritic branches (Fig. 10H,I).

We observed some variability in the number of bipolar and horizontal cell processes that enter an invagination, in the number of ribbons in a rod spherule, and in the number of synapses between the type I axon terminals and the rod spherules (Table 1). Fourteen of the 21 rod spherules had two clearly separate synaptic ribbons; the remainder had only one. In 18 of the spherules, two separate bipolar pro-

cesses entered the invagination, while two separate horizontal cell processes passed through the hilus in 12 spherules. All but two of the spherules had clearly defined synapses within their invagination and 11 of them had a synapse between the horizontal cell axon terminal and a rod bipolar dendrite nearby. Only in one of the reconstructions were we unsuccessful in finding a synapse in the OPL; in the remaining nine, the synapse was poorly defined, usually due to the plane of section or to imperfections in the sections.

Nearly all rod spherules were seen to make contact with extensions of nearby cone pedicles (Fig. 11A,B, Table 1). In transverse sections (cut at right angles to the long axis of the photoreceptors), it is clear that all rod spherules make this contact. Presumably, these are gap junctions similar to those linking the rod and cone systems in other mammalian retinas (Raviola and Gilula, '73; Kolb '77; Smith et al., '86).

### DISCUSSION

The elaborate axonal arborizations of the "short axon horizontal cells" (Gallego, '86) found in mammalian retina may be unique structures in the nervous system. There is evidence that this terminal system is electrically isolated from its cell body and may thus function as if it were a separate cell (Nelson et al., '75; Kolb et al., '80; Nelson et al., '85). A variety of anatomical and physiological studies have shown that the terminal arborizations receive input from rod photoreceptors where they form one of the postsynaptic elements within the synaptic invagination of the rod spherules (Sjöstrand, '58; Missotten, '65; Kolb, '70; Kolb and Nelson, '83; Gallego, '86). Until this study, though, virtually nothing was known about the synaptic output of these terminals. We have presented morphological evidence suggesting that these terminals are presynaptic to both the rod spherule itself and to rod bipolar cells, consistent with the hypothesis that they are involved with the processing of information within the rod pathway (Kolb and Nelson, '84).

Fig. 9. A: A low-power electron micrograph showing the outer plexiform layer (OPL). This micrograph shows the difference between the appearance of type I horizontal cell axon terminals and interplexiform cell processes that are both presynaptic in the OPL. The process marked "H" is presynaptic to a rod bipolar dendrite. B,C: In B, the small cisternae of smooth endoplasmic reticulum forming the "helical organelle" (arrowheads) identify the postsynaptic process as the dendrite of a rod bipolar. Examples of the axon terminal synapses (arrows) within the OPL. C shows an extension of the presynaptic (horizontal cell) process entering the synaptic invagination of a rod spherule. A =  $\times 15,000$ . B =  $\times 48,000$ . C =  $\times 32,000$ .



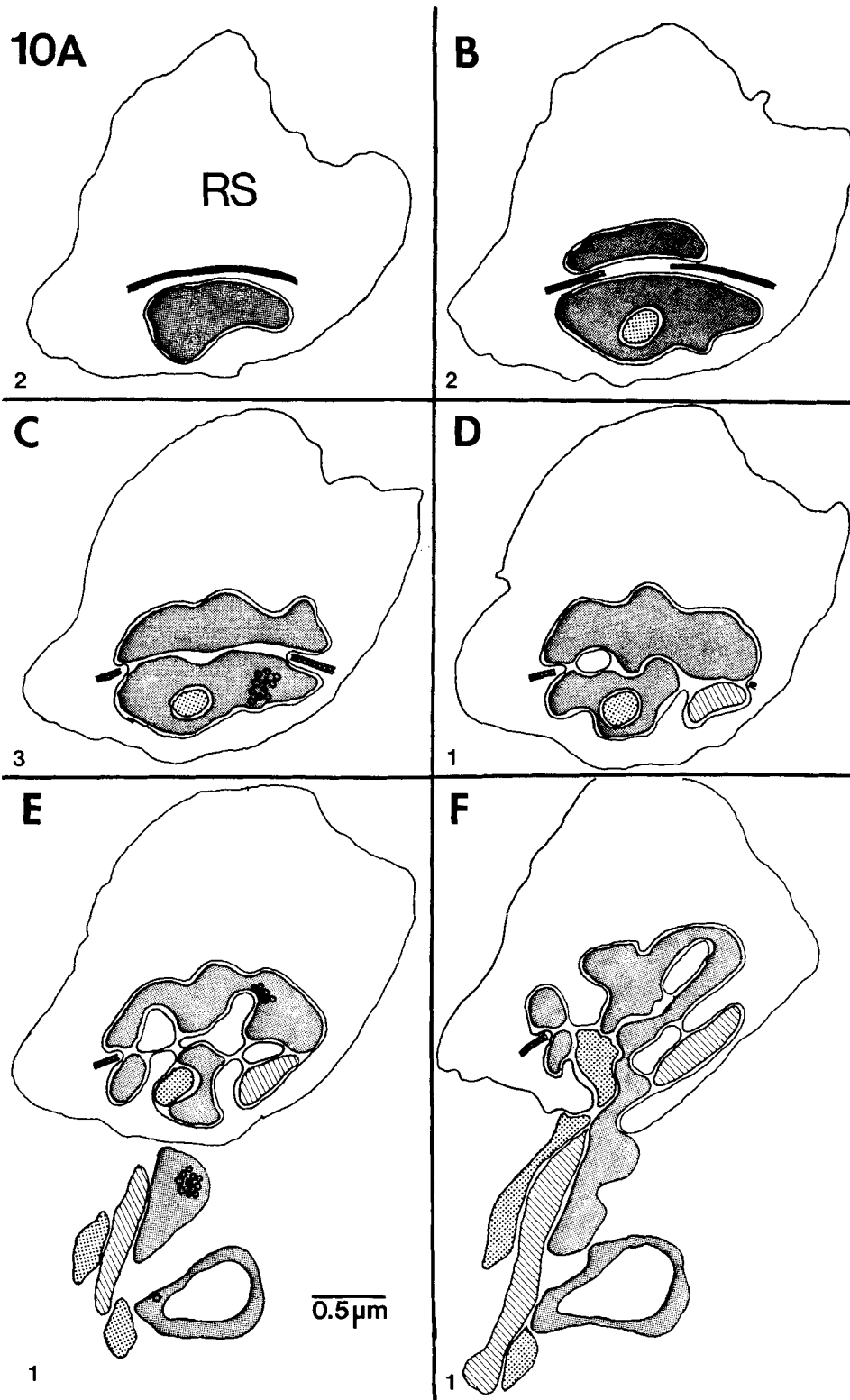


Fig. 10. Selected tracings from serial micrographs through a rod spherule. The small number in the lower left corner of each drawing indicates its relative position within the series. The first micrograph was arbitrarily labeled as "zero" so that A = two sections beyond zero, B = two sections beyond A, etc. The densely stippled process is a branch off of a type I

horizontal cell axon terminal, and it is presynaptic to the rod spherule (RS) at three locations as indicated by the cluster of vesicles in C and E. The lighter-stippled and cross-hatched processes are rod bipolar dendrites. The cross-hatched process is postsynaptic to the axon terminal in I. See text for further details.

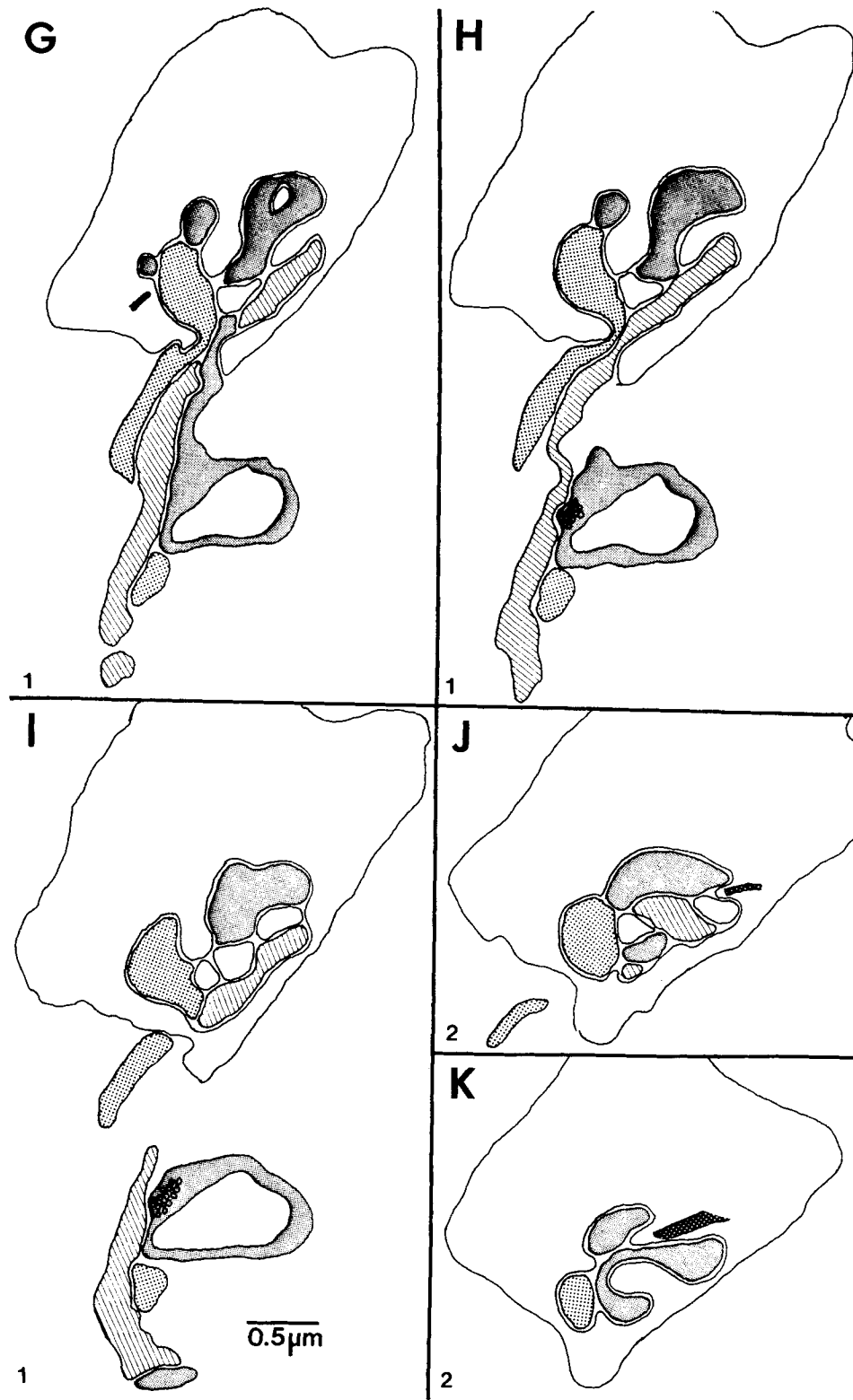


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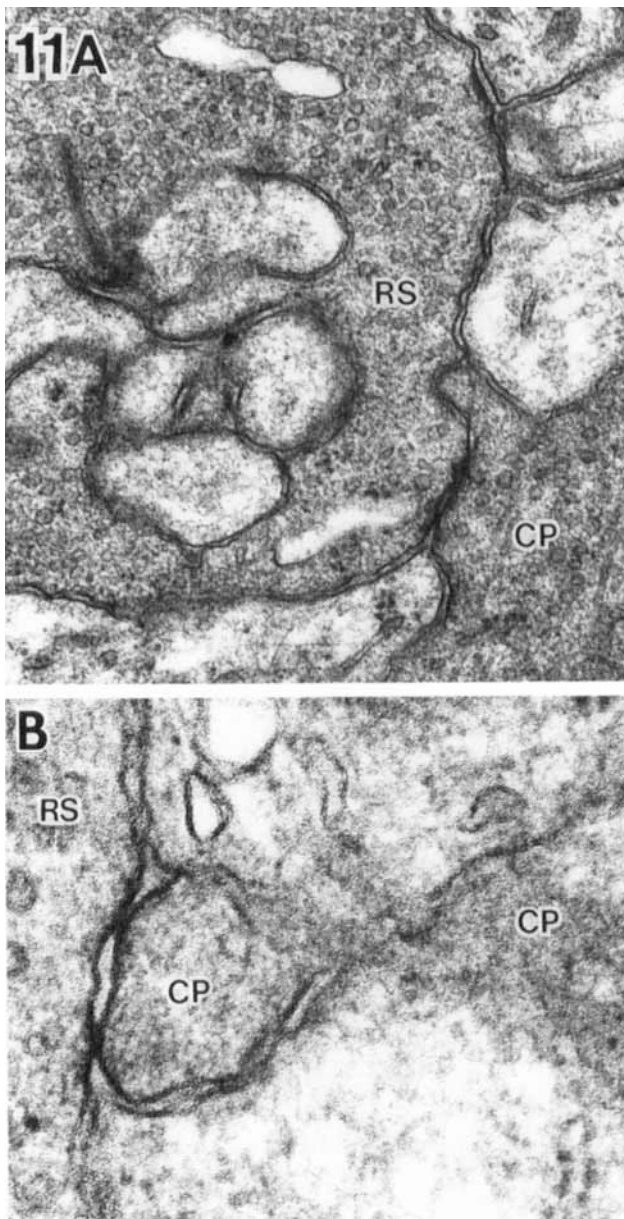


Fig. 11. Two examples of junctions between a rod spherule and an extension of the cone pedicle cytoplasm in the human retina. These junctions usually appear as two punctate regions of close membrane apposition as in B. A =  $\times 55,000$ , B =  $\times 129,000$ .

Although many physiological studies in nonmammalian retinas have shown that horizontal cells provide synaptic feedback to cone photoreceptors (Baylor et al., '71; for reviews see Piccolino, '86; and Gallego, '86), the only morphological evidence for the chemical synapse responsible is in the catfish retina, where the cone horizontal cells have been seen to synapse with the telodendria of the cone photoreceptors (Sakai and Naka, '86). There are still no physiological reports of such feedback in mammals although it is assumed. For rods of any vertebrate retina, the demonstration of some type of physiological feedback is even more limited. The only study suggesting this is by Normann and

Pochobrodsky ('76) in the toad, *Bufo*. Indeed, the lack of morphological correlates for feedback synapses between horizontal cells and photoreceptors in general, and the fact that transmitter release from goldfish cone horizontal cells is not calcium mediated, led Yazulla and Kleinschmidt ('83) to suggest that signal transmission at these synapses may be nonvesicular and therefore not characterized by the usual morphological features of a chemical synapse (see also Boycott et al., '87). Nonetheless, our electron microscopic study of the human retina shows that definite contacts exist between the horizontal cell axon terminal processes and rod spherules with the salient features of chemical synapses located appropriately to mediate such feedback to rod photoreceptors. We have not seen these contacts between horizontal cell dendrites and cones even though they were well preserved within the rod spherule invagination. It is possible that additional studies using different fixation protocols may reveal them eventually, although freeze-fracture (Raviola and Gilula, '75; Schaeffer et al., '82) used in a variety of species has also failed to demonstrate such synapses. Since the original studies of Missotten ('65) on human retina, electron micrographs in many different publications on different species have shown vesicles similar in size and morphology to synaptic vesicles in horizontal cell processes within photoreceptor synaptic invaginations (for example, Dowling and Boycott, '66; Fisher and Boycott, '74; Brandon and Lam, '83). The latter authors also observed a region of dense undercoating on the horizontal cell plasma membrane opposing the synaptic ribbon of the rod spherules in the rat retina. They do not propose this as a site of chemical transmission, but rather as the region of synaptic vesicle recycling in the horizontal cell terminal. We have observed similar dense undercoatings in the horizontal cell processes in this study (examples occur in Figs. 5C and 8B), and they are indeed separate from the sites of presumed synaptic contact between the horizontal cell and the rod spherule.

The type I horizontal cell of primate retina is similar in morphology and connectivity to the B-type (Fisher and Boycott, '74; Kolb, '74) or "short axon horizontal cell" (Gallego, '86; Boycott et al., '87) of mammalian retinas. In all cases, the dendrites of these cells become part of the synaptic triad in the cone pedicle invaginations, while the axon terminal is a complex arborization whose terminal branches enter the single synaptic invagination found in the rod spherule (Wässle et al., '78). While there is mixing of rod and cone signals in the cells of the cat retina, this apparently does not occur by the transmission of signals along the axon because the axons do not generate action potentials and are not capable of supporting electrotonic conduction over the distance separating the cell body and terminal (0.3–1 mm) (Nelson et al., '75; Kolb et al., '80; Ohtsuka, '83; Nelson et al., '85). Thus, the terminal and cell bodies of the type I cell are almost certainly electrically isolated, with the terminal arborization participating only in signal processing for the rod system.

In the cone pathway, the horizontal cells are presumed to mediate antagonistic surround responses in the bipolar cell's receptive field. It is not clear, however, whether this function applies to the rod system. Nelson and Kolb ('83) reported that rod bipolars in the cat retina hyperpolarize over their entire receptive field and show no surround effect. Dacheux and Raviola ('86) report, however, that in the rabbit retina at least some rod bipolars depolarize and show evidence of a concentric type of receptive field. If human



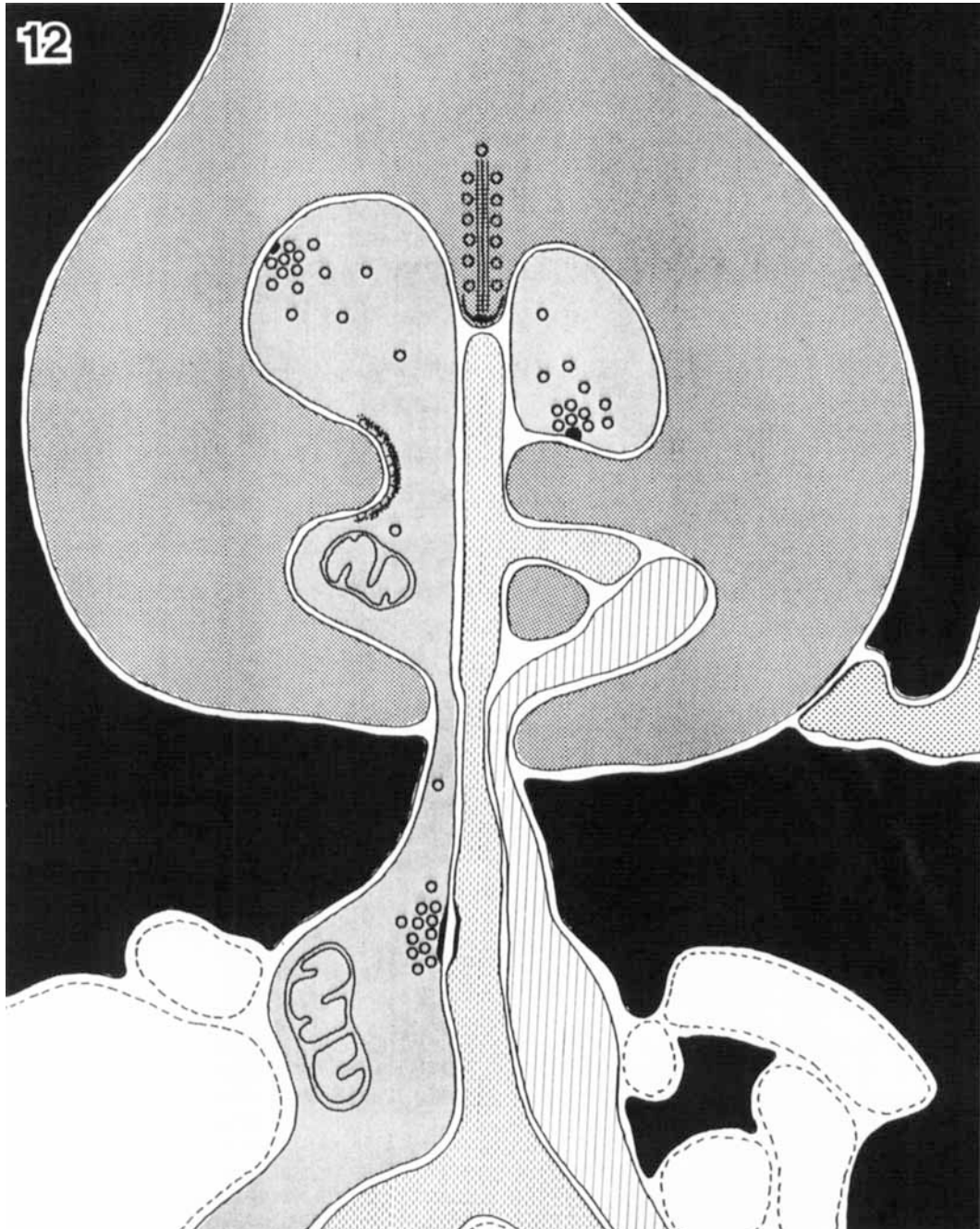


Fig. 12. A summary diagram showing the structure and organization of the synapses identified in this study of the human retina. The axon terminals of the type I horizontal cell contain vesicles of the appropriate size to be synaptic vesicles. These vesicles are clustered at certain sites which also have densifications of the membrane associated with them. In some cases, the postsynaptic element is the cytoplasm of the rod spherule, while in others (within the outer plexiform layer) it is a rod bipolar dendrite. We

have found that in the spherules we reconstructed, rod bipolar dendrites could sometimes assume the "lateral position" among the postsynaptic elements although they never invaginate as deeply as do the horizontal cell axon terminals. The horizontal cell axon terminal processes also had areas of membrane densification not associated with a cluster of vesicles and these presumably are the site of synaptic vesicle recycling as identified by Brandon and Lam ('83).

rod bipolars have concentrically organized receptive fields, then the synapses described here between horizontal cell axon terminals and rod bipolar dendrites may mediate this organization. Both types of synapses demonstrated in this study are also located in a position to mediate an unex-

plained property of mammalian rod bipolars: these cells have a receptive field which is considerably larger than their dendritic field (Kolb and Nelson, '83, '84). In the retina of the toad, the rod photoreceptors are connected by electrical junctions serving to integrate signals over many

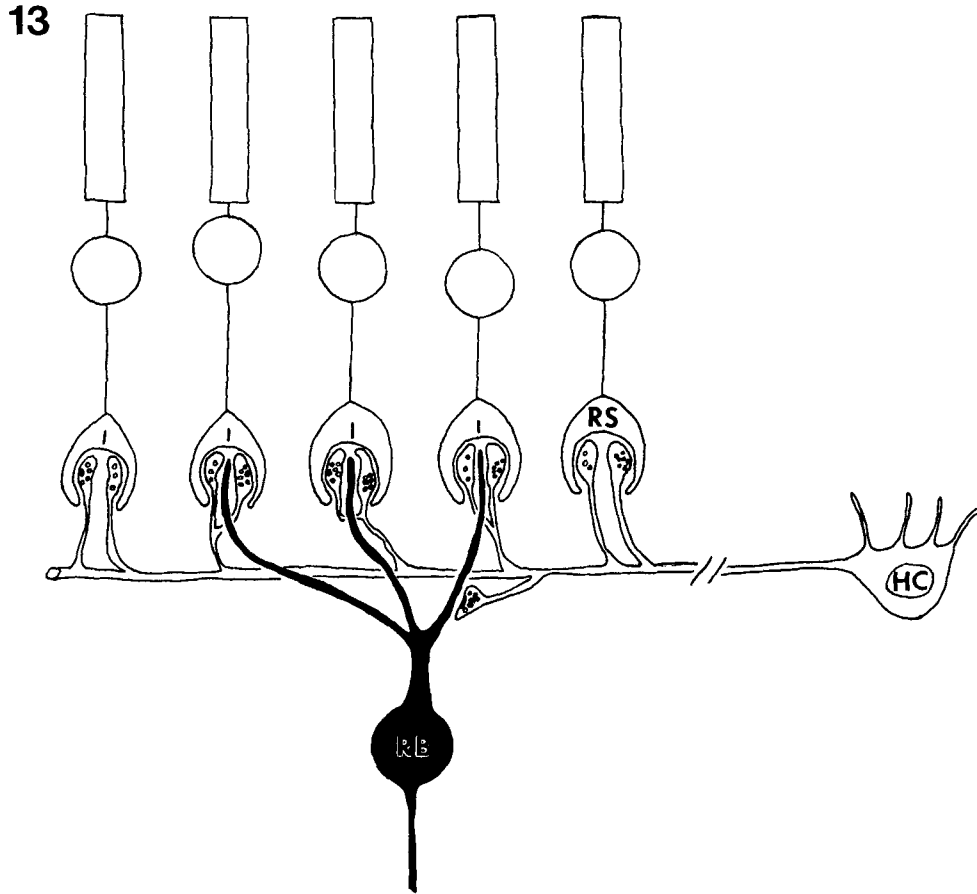


Fig. 13. A summary diagram showing the synaptic connections and circuitry identified in this study. The axons of the type I horizontal cell contact the synaptic terminals of rod photoreceptors where they are both postsynaptic and presynaptic in what is presumably a feedback arrangement. The horizontal cell axon terminals are also presynaptic to rod bipolar dendrites in the outer plexiform layer. Because these axon terminals are electrically isolated from their cell body, they may provide a circuit for altering the rod bipolar cell's receptive field. The dendrites of the type I horizontal cell contact only cone photoreceptors.

rods (Fain et al., '76); mammalian rods are, however, not linked by such electrical (gap) junctions. It has been suggested that the axon terminal of the B-type horizontal cell in the cat retina may mediate this response by some type of feed-forward mechanism (Kolb and Nelson, '84). In the cat, a rod bipolar cell contacts about 15 rods (Kolb and Nelson, '83) while the axon terminal of the B-type horizontal cell has been estimated to contact 3,000–4,000 rods (Wässle et al., '78). Available evidence suggests that a primate rod bipolar receives input from about 33 rod photoreceptors (Kolb, '70), while it has been estimated that the type I horizontal cell axon terminal integrates signals from 350 to 500 rods (Gallego, '76). Hence the formation of synapses onto rod bipolars within the field of the axon terminal may provide the synaptic circuitry necessary for expansion of the rod bipolar receptive field.

We found the majority of rod spherules to contain two independent synaptic ribbons, while an earlier study simply reported them to contain "one or more" (Missotten, '65). In the cat retina, rod spherules are reported to contain two synaptic ribbons (Boycott and Kolb, '73). The structure of the rod synapse as described in this study varies slightly from that described in the human retina by Missotten ('65),

but it is important to recognize that the fixation and tissue-processing protocols in the two studies differ significantly. The earlier studies did not identify the fairly large number of processes within the synaptic invagination that are projections of the rod spherule itself; if these were counted as postsynaptic processes then that number would be inflated. We consistently found one or two bipolar dendrites and an equal number of horizontal cell processes within the invaginations while Missotten reported that the former could be as high as five and the latter limited to two or three. There is, of course, the possibility that the number of postsynaptic processes varies with distance from the fovea, and this value is not available for either study.

The projections of rod spherule cytoplasm into the synaptic invagination are significant structures because they are often the recipient of the feedback synaptic input from the horizontal cell processes. Brandon and Lam ('83) identified similar protrusions of rod spherule cytoplasm in the rat retina and showed that they were much more prominent in dark-adapted than light-adapted eyes. Whatever the role of these projections of the rod spherule cytoplasm, it seems likely they play a role in the physiology of the rod synaptic terminal.

This study has provided evidence for a new synaptic circuit in the outer plexiform layer of the primate retina, which is summarized in Figures 12 and 13. In this circuit, the axon terminals of the horizontal cells receive input from the rod photoreceptors and reciprocate this input to the same rod terminal as a feedback synapse; they also send it to dendrites of the rod bipolar cell as a feed-forward synapse. This circuit may function to alter the receptive field properties of the rod bipolar cells in the human retina.

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