Synapses of Horizontal Cells in Rabbit and Cat Retinas

Abstract. *Horizontal cells in the retinas of cats and rabbits are morphologically similar; in both species, two types can be distinguished in Golgi-stained material. Horizontal cells and their processes are readily recognized in electron micrographs, and many of the horizontal cell processes appear to make synaptic contacts with dendrites and somata of bipolar cells, and probably with other horizontal cells. The synapses of the horizontal cell appear similar to chemical synaptic contacts described throughout the nervous system. With the finding of synaptic contacts, it seems clear that retinal horizontal cells should be classified as neurons.*

Of the cells in the vertebrate retina, the horizontal cells have proved the most difficult to interpret. First, no synaptic contacts of the horizontal cells have been identified by electron microscopy, although it has been shown that fine processes of horizontal cells penetrate into the terminals of the receptor cell and end adjacent to the synaptic ribbons in the terminals (*1*). Second, in fish and other vertebrates, horizontal cells are said to have many of the morphological characteristics of glial cells (*2, 3*). Thus, the suggestion has been made that horizontal cells are not neurons, but are specialized glial cells that play some regulatory role in retinal neuronal transmission (*3, 4*).

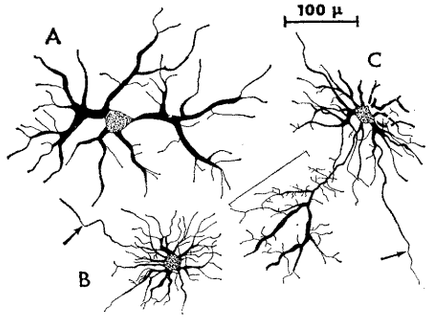

Fig. 1. Camera lucida drawing of horizontal cells from sections of flat-mounts of rabbit retinas. The retina was fixed and stained by Colonnier's modification of the Golgi-Kopsch method (*11*). (A) Large type of horizontal cell; (B) small type of cell with axon-like process (arrow); and (C) small type cell with axon-like process (arrow) and the unusual feature of one small process expanding distally into additional large processes. Cell C was cut on a slight angle with the knife passing close to the cell body. Thus, the processes on one side of the cell were cut and appear short in the drawing.

A recent electron-microscopic study of the primate retina failed to find any synaptic contacts in the outer plexiform layer except those made by the receptor terminals (*5*). However, the processes of horizontal cells in the primate are very fine, and positive identification of them proved difficult. In rabbit and cat retinas, many of the horizontal cells are very large and have stout processes that are easily identifiable in electron micrographs. Examination of these cells is worthwhile, especially since recent physiological evidence strongly suggests that directional-sensitive responses in the rabbit retina are mediated by inhibition spreading laterally in the outer plexiform layer, presumably via horizontal cells (*6*).

Cajal described two types of horizontal cells in mammalian retinas (*7*). These were distinguished primarily by the position and shape of the cell body. The outer horizontal cells are flattened, and the cell body is found in the lowermost portion of the outer plexiform layer; while the inner horizontal cells have a rounded cell body located in the outermost row of cells in the inner nuclear layer. In the primate, Polyak described a single type of horizontal cell, which appeared to correspond to Cajal's inner horizontal cell (*8*).

Horizontal cells appear very similar in the cat and rabbit retinas, and we can distinguish two types of cells in Golgi-stained material (Figs. 1 and 2, A and B). The larger cells (A) probably correspond to the outer horizontal cells described by Cajal and those recently described by Gallego in the cat retina (*3*). Their thick, flattened processes extend over an area up to 300 to 500 μ in diameter. The cell body is likewise flattened and resides in the innermost part of the outer plexiform layer (Fig. 3). There is no obvious axon arising from these cells; all their processes appear morphologically identical. The smaller cells probably correspond to Cajal's inner horizontal cells. Their processes are thinner, covering an area 100 to 200 μ in diameter, while the cell body resides in the inner nuclear layer. Often a thin process, similar to an axon, (Fig. 1, arrow) is seen running a considerable distance from the cell, but we have not been able to trace these processes to their terminations.

The above description applies to the horizontal cells that are most easily distinguished. However, many cells are intermediate to the foregoing types and are impossible to classify with confidence. Figure 1C shows one small cell with an additional unusual feature of having one thin process expanding into thick processes a short distance from the cell body (bracket). This cell also has a very thin process similar to an axon (arrow). Thin processes which expand into an arborization of thicker processes, but which are not connected to a stained cell body, have also been seen. These arborizations have been interpreted to be the terminations of the "axon-like" processes (*7*).

Light microscopy of Golgi-stained retinas also reveals that the large, proximal processes of the horizontal cells lie along the innermost portion of the outer plexiform layer. Thinner processes ascend toward the receptor terminals, and the finest branches end in the vicinity of the receptor terminals.

In electron micrographs, horizontal cells and their processes are readily recognizable (Fig. 3). Except immediately adjacent to the nucleus, the cells do not contain much endoplasmic reticulum, nor many ribosomes. The processes contain a few, scattered mitochondria; an occasional profile of smooth endoplasmic reticulum; a few, scattered neurofilaments; but little else. The large size of many of these processes, coupled with their characteristic cytology, allows identification of horizontal cell processes even when not attached to a cell body (Fig. 3).

Occasionally, morphologic specializations characteristic of chemical synapses described throughout the vertebrate nervous system are seen in the large processes of horizontal cells (Fig. 4). These consist of numerous synaptic vesicles clustered close to the membrane of the presumed presynaptic (horizontal cell) side of the contact, associated with some degree of membrane thickening and densification adjacent to the cluster of synaptic vesi-

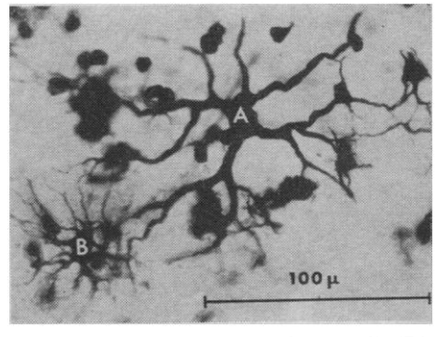

Fig. 2. Photomicrograph of a small (B) and a large (A) horizontal cell in the cat retina. The retina was fixed and stained by Colonnier's modification of the Golgi-Kopsch method (*11*).

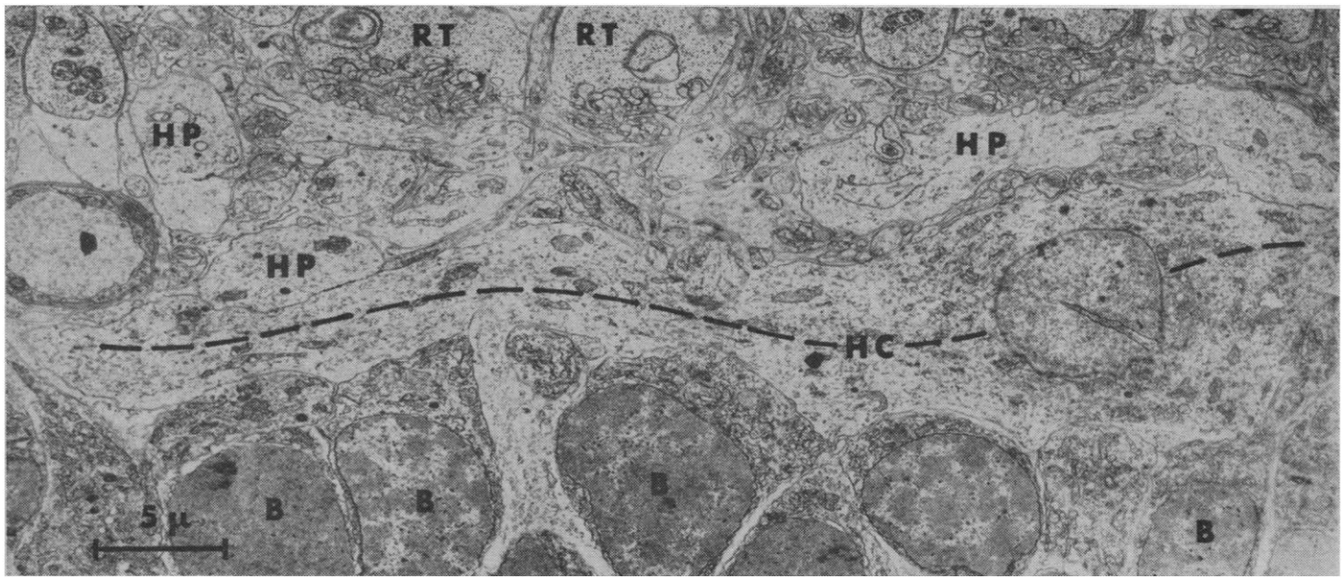

Fig. 3. A low-power electron micrograph showing a large horizontal cell (*HC*) in the innermost portion of the outer plexiform layer in the cat retina. Many processes of the horizontal cell (*HP*) can be recognized in the outer plexiform layer by their large size and characteristic cytology. The retina was fixed with osmium tetroxide buffered with veronal acetate and stained with lead hydroxide (*5*). *RT*, receptor terminals; *B*, bipolar cells (× 38 00).

cles on both the pre- and postsynaptic membranes. The majority of these contacts are with smaller processes. Occasionally the postsynaptic processes have been positively identified as dendrites of a bipolar cell, when it has been possible to follow them to a bipolar cell soma.

In the cat, small processes, presumably from horizontal cells, have also been seen making synaptic contacts with the somata of bipolar cells. Also, synaptic contacts in which a few scattered synaptic vesicles were seen on the postsynaptic side have been observed in the outer plexiform layer of the rabbit and cat. This suggests that there may also be synaptic contacts between horizontal cells. In summary, we have found morphologic evidence for synaptic contacts of horizontal cells with dendrites and somata of bipolar cells, and probably with other horizontal cells.

In fish and monkey the fine, terminal processes of the horizontal cells usually end as the lateral elements underlying the synaptic ribbon in the receptor terminals (*1*). In electron micrographs we have not as yet been able to follow horizontal cell processes into the receptor terminals in the cat and rabbit retina. However, the synapses in the terminals of both the rods and cones in rabbit and cat are very similar in appearance to those of the primate (*5*). Thus, it seems likely that processes of the horizontal cell in the cat and rabbit also penetrate into the receptor terminals and possibly receive input from them.

Whether both types of horizontal cells in cat and rabbit make similar synaptic contacts is not known. We have not been able to distinguish two types of horizontal cells with the electron microscope. In the primate, which appears to

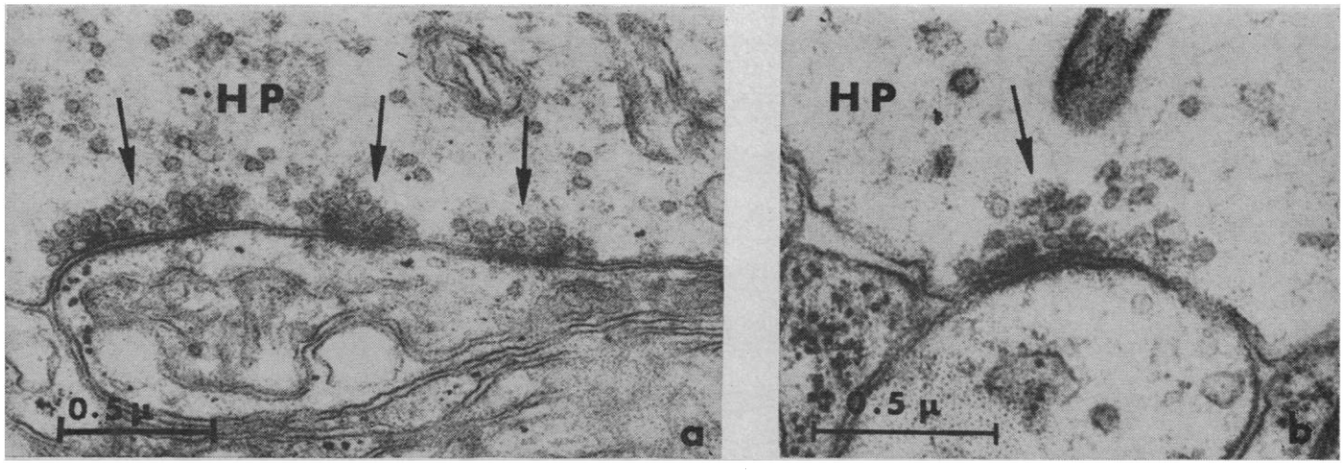

Fig. 4. Electron micrographs of synaptic contacts in processes of horizontal cells (*HP*). These contacts (arrows) are characterized by aggregations of synaptic vesicles clustered close to the presynaptic membrane and by intensely staining membranes on both the pre- and postsynaptic sides of the contact. (a) Cat retina (× 40,000); (b) rabbit retina (× 50,000). Both tissues were fixed in O_8O_4 (*5*).

have only small horizontal cells, we have not yet observed synaptic contacts in the outer plexiform layer (*9*).

The processes of the horizontal cell which we can identify in the rabbit and cat retinas are neither clearly axonic nor dendritic in appearance. It also seems likely that the same processes of the horizontal cells are postsynaptic to the receptors (*1*) and presynaptic to the bipolars and other horizontal cells. Thus, the processes of horizontal cells may be quite similar to those of the amacrine cells in the inner plexiform layer, which appear both pre- and postsynaptic along their length (*5*). Processes of the amacrine cell, like those of the horizontal cell, contain few cytoplasmic organelles, and thus usually appear quite "empty" except near sites of synaptic contact.

With the identification of synaptic contacts, it seems clear that horizontal cells should be classified as neurons. It is true that they may sometimes not be classical neurons possessing clearly differentiated dendritic and axonic terminations. Rather they appear similar to the axon-less amacrine cells of the inner plexiform layer. Recently the axon-less cells in the olfactory bulb, the granule cells, have been examined by electron microscopy, and their processes appear similar to those of the horizontal and amacrine cells of the retina (*10*). Thus, interneurons of this type, with processes that both receive and transmit stimuli, appear to be a feature of afferent pathways, and probably play an important role in the lateral interactions known to occur in afferent systems (*6, 10*).

JOHN E. DOWLING
Wilmer Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

JOEL E. BROWN
DIANE MAJOR
Department of Biology and *Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge 02138*

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Polyteny: A Source of Cryptic Speciation among Copepods

Abstract. *A large form of the copepod* Pseudocalanus *is found in two warm, semi-landlocked fiords in arctic Canada, together with a similar but smaller form attributable to the widespread* P. minutus. *The large form has the same chromosome number as* P. minutus, *but has larger chromosomes and a higher nuclear DNA content. There are suggestions in the literature that other similar polytenic and cryptic species occur among copepods.*

Students of zooplankton have traditionally made numerous length measurements to follow seasonal changes and, when possible, to distinguish succeeding generations. Seasonal and local variations in size within species are phenotypic responses to temperature, although size-temperature relations differ, presumably genetically, between widely separated populations (*1*). A number of authors have described and discussed bimodal or polymodal size distributions in species of copepods from the same plankton samples. Sometimes these are attributable to overlap of generations, but in recent years some size forms have been given specific status on the basis of slight, size-independent, morphological criteria.

An example of such a size form (a large one) has been described from Ogac Lake, the warm, semi-landlocked head of a fiord in Baffin Island, at 62°52′N by 67°21′W (*1*). The eggs and bodies of this large form of the copepod *Pseudocalanus* are about three times the volume of those of a coexisting small form, which is attributable to the widespread *P. minutus* (Krøyer). This large form was also shown to develop more slowly in nature. Otherwise the large and the small forms seem morphologically indistinguishable. The large form has since been abundantly found in the semi-landlocked head of Winton Bay on the east coast of Baffin Island, at 63°24′N by 64°39′W. No intermediates have been found, and the large form appears to be a reproductively isolated sibling or cryptic species (*2*).

It was assumed that the large form would prove to be polyploid (*1*), but collections made from Ogac Lake and elsewhere (1965) indicated that the large form, like *P. minutus*, has the usual chromosome complement for calanoid copepods ($n = 16$). The chromosomes, however, are conspicuously larger (Fig. 1). It was therefore of interest to determine whether the larger chromosomes contained more DNA.

Cytophotometric analysis was used to determine DNA content of interphase nuclei in embryos of these two forms from Ogac Lake. The embryos were removed from routine plankton samples, which were fixed and stored in formaldehyde-seawater (5 : 95). The Feul-

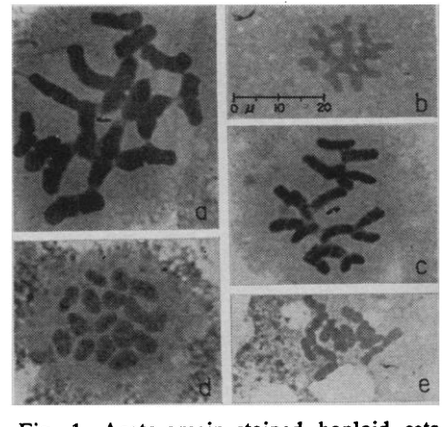

Fig. 1. Aceto-orcein–stained haploid sets of metaphase chromosomes from fresh, undivided eggs of (a) the large form of *Pseudocalanus* from Ogac Lake, and of *P. minutus* from (b) Ogac Lake, (c) Frobisher, N.W.T., (d) Millport, Scotland, and (e) Halifax, N.S. All to same scale (in b).