Interplexiform cell in cat retina: Identification by uptake of γ-[3H]-aminobutyric acid and serial reconstruction (autoradiography, ultrastructure)

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ABSTRACT
After intravital injection of γ-[3H]-aminobutyric acid (GABA), 20% of the neurons at the outer margin of the inner plexiform layer were intensely labeled. Reconstructions of these neurons from serial electron microscope autoradiograms showed that they are interplexiform cells, which synapse on bipolar processes in the outer plexiform layer and on amacrine and bipolar processes in the inner plexiform layer. By reconstructing adjacent retinal neurons from serial thin sections, one can identify their intrinsic morphology and patterns of synaptic contact in enough detail to classify them reliably (1). Because the ultimate goal of such an undertaking is to understand how the neurons operate in a network, it would be helpful to label specific classes by means that at least hint at their function. Previous workers (2-4) have shown by autoradiography that certain transmitter substances are selectively accumulated in the amacrine layer of cat retina, but they were unable to determine from single sections which particular classes were labeled. By combining such autoradiographic labeling with the reconstruction approach, we have identified several morphologically distinct classes of neurons that show selective transport uptake (5). Here we focus on one of these, the interplexiform cell (6), whose cell body lies in the inner nuclear layer. Its processes extend into both the outer and inner plexiform layers, where they are both presynaptic and postsynaptic to other neurons. The cell is thus unique among retinal neurons in having the potential to feed information centrifugally from the inner plexiform layer back to the outer plexiform layer, where the photoreceptors establish the first synapse of the retinal pathway. Though described almost 90 years ago by Ramon y Cajal (7), this cell type has only recently attracted attention as its potential for centrifugal action has been appreciated in the goldfish retina (8). In the goldfish, where more monkeys contains dopamine and is estimated by fluorescence microscopy to constitute about 5-10% of the cells in the amacrine layer, the cell is clearly bipolar, being presynaptic to horizontal and bipolar neurons in the outer plexiform layer and to amacrine cells in the inner plexiform layer (8, 9).

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RESULTS
Labeled neurons were sought exclusively at the inner margins of the inner nuclear layers (Fig. 1a). About 2% of the cells there were heavily labeled (34±9 background) and 30% were more moderately labeled (9±9 background). Silver grains were distributed in the outer plexiform layer and throughout the inner plexiform layer with slightly greater concentration at its superficial and deep margins. In the ganglion cell layer there was an intense concentration of silver grains over glial cell bodies and processes wrapping the axons of ganglion cells. Müller cells and their processes were moderately labeled (Fig. 1a).

The intensely labeled neurons had a paler cytoplasm than those labeled more moderately, but we could not determine from examining single sections whether there were two distinct cell classes or to which of the many classes of amacrine neurons (6, 7) the labeled neurons might belong. Labeled cells were, therefore, reconstructed to determine more fully their morphology and connections. The moderately labeled neurons turned out to belong to a subclass of conventional amacrine and are described elsewhere (5). The heavily labeled neurons (Fig. 2) resembled interplexiform neurons described from Golgi material (8, 10, 11), sending presynaptic processes into both outer and inner plexiform layers.

Fine (0.3-0.4 m) processes labeled with silver grains were traced from interplexiform cell bodies into the outer plexiform layer along the process axis. Some of these processes were too narrow to resolve, but they were generally found in the outer plexiform layer of the retina.
Fig. 1. Electron microscope autoradiograms showing localization of [3H]GABA. (a) Heavily labeled, pale neuron (IP) is an interplexiform cell. Arrow points to stub of a process traced in serial sections to the outer plexiform layer. GA, moderately labeled GABA amacrine (darker cytoplasm); RB, bipolar; M, Müller cell (moderately labeled); IPL, inner plexiform layer. (b) Interplexiform process (IP) making synaptic contact with process of rod bipolar (RB) in the outer plexiform layer. R, synaptic ribbon in rod spherule. c, d Higher magnification of synaptic cleft (brackets in c). Arrow points to dense line within the cleft. e Interplexiform process contacting rod bipolar in the inner plexiform layer. f-g Interplexiform process contacting cone bipolar (CB) in the inner plexiform layer. All processes in b–g were identified by reconstruction. Scales a, 5 μm; b, d, f, g, 0.3 μm; c, 0.03 μm.

Layer to the level of the photoreceptor synaptic terminals (Fig. 1b). The label was an aid in tracing; fine processes, which in normal material would have been lost when sectioned parallel to their membranes, could often be recognized from one section to the next because they were marked by silver grains. Accumulations of synaptic vesicles were observed at various intervals, sometimes in the varicosities that were spaced irregularly along the processes.

The synaptic contacts made by interplexiform processes in both inner and outer plexiform layers were symmetrical (Figs. 1c–e). There was a pronounced widening of the synaptic cleft to 15 nm and an accumulation within the cleft of electron-dense material that formed a fine line parallel to and midway between the presynaptic and postsynaptic membranes (Fig. 1c). In the outer plexiform layer we found, in agreement with Kolb and West (11), interplexiform processes presynaptic to the dark cell bodies and processes of rod bipolar (Fig. 1d) and less frequently to the pale, tubule-filled processes of cone bipolar. There was no evidence of interplexiform contacts onto horizontal cells nor, though it has been reported (11), of contacts onto other interplexiform processes.

Labeled processes were traced from interplexiform cell

Fig. 2. Partial reconstruction from serial electron microscope autoradiograms of interplexiform cells labeled with [3H]GABA. Interplexiform cells presynaptic to rod bipolar (●), cone bipolar (○), and amacrine (●). Interplexiform cells postsynaptic to amacrine. OPL, outer plexiform layer; IPL, inner plexiform layer; INL, inner nuclear layer.
bodies into the inner plexiform layer through about one-third of its thickness (Fig. 2). In three instances interplexiform processes were found to be postsynaptic to small, unlabeled processes, which, lacking synaptic ribbons, were presumably amacrine. Which class of amacrine could not be determined, but they were not GABA-accumulating amacrine (5) because they lacked label. In nine instances interplexiform processes were presynaptic to processes of rod and cone bipolars (Fig. 1 d and e), to amacrine processes, and, in one instance, to an amacrine cell body. Rod bipolar processes were identified by tracing them to dark cell bodies in the inner nuclear layer; cone bipolar processes were identified by tracing them to their endings in the outer half of the inner plexiform layer (14). The postsynaptic amacrine cell body was dark with a stout central process and resembled a type that accumulates enogenous glycine (5).

The contribution of interplexiform cells to the neuronal mosaic in the inner nuclear layer was determined by plotting the distribution of heavily labeled cell bodies from light microscopy of a series of tangential, 1-μm sections through the inner nuclear layer. In this material, chosen from a region about 1 mm above the area centralis, there were about 50 interplexiform cells per mm², but the distribution was not uniform (Fig. 3). In some regions the cells were spaced fairly regularly, at the average 47-μm apart, but in some cases they were as close as 15 μm. The processes of neighboring interplexiform cells in such regions must overlap extensively, because Golgi impregnations show their dendritic field diameters to range between 100 and 250 μm. There were, however, small patches (roughly 100 × 200 μm) devoid of intensely labeled cell bodies. The probability that these patches result from a random distribution of interplexiform cells is less than 0.001, tested by the χ² method, comparing the goodness of fit of the actual distribution to an ideal random distribution with the same mean cell density (15). Because the patches were observed in material from two different experiments, we doubt that their origin is artificial. These empty patches were large enough that the concentration of interplexiform processes in their central regions must be quite low. Possibly they represent the dendritic territory of some other class of retinal neuron whose circuitry requires that the influence of the interplexiform cell be excluded. The significance of these patches might prove to be a clue to the function of the interplexiform cell, which at present is rather mysterious (16).

Interplexiform cells in the cat retina, at least those that accumulate GABA, constitute about 2% of neurons in the amacrine layer, the same order of magnitude as in fish and primates. Except for circumscribed patches, their postsynaptic processes in outer and inner plexiform layers must thoroughly overlap so that most of the retina is subject to their influence. That the interplexiform cell powerfully accumulates GABA suggests that it might be the transmitter. The suggestion does not seem far fetched because the retina contains substantial endogenous GABA and glutamic acid decarboxylase, the enzyme that synthesizes GABA (17). Glutamic acid decarboxylase has been identified by immunocytochemistry in synaptic terminals of the rat (18) and rabbit (19) inner plexiform layer, and histochemical GABA and application of its antagonists, picrotoxin and bicuculline, affect the responses of retinal ganglion cells in cat (20) and other species (21, 22). If GABA is indeed the transmitter of the cat interplexiform cells, it should be possible in the future to demonstrate the presence of GABA receptors postsynaptic to the interplexiform processes (23). GABA may turn out to be the transmitter not only for the interplexiform cell but also for the amacrine which show selective, though less intense, uptake (5). This would complicate the interpretation.

**Fig. 3.** Distribution in amacrine layer of neurons that were intensely labeled by [3H]GABA (interplexiform cells). Reconstructed from serial, light microtome, tangential, 1-μm sections through one inner nuclear layer. Arrow indicates location of boundary between layers. Dashed line indicates approximate position of inner plexiform layer. Arrowheads point to boundary of outer plexiform layer. Arrow over GABA-labeled amacrine cell points to adjacent, unlabeled amacrine cell. Photograph is from cat retina, animals 2.2 mm above area centralis. Photograph is 100 μm wide.
of studies that use iontophoresis of GABA or intracerebral application of its analogs in the cat retina (20) because GABA would then be acting simultaneously at many different points in the retinal circuitry. It is curious that what appears to be the same cell type in different animals can have different transmitter (GABA in cat vs. dopamine in fish and New World monkey), but it is noteworthy that the synaptic actions of both substances may be similar in some respects (16, 33, 32, 24). The reason for the possible difference as well as the role of the interplexiform cell in retinal function may be clearer when the micrometre neurones that form the counter for the interplexiform cell are more fully known.

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