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An Ultrastructural Study of Interplexiform Cell Synapses in the Human Retina

KENNETH A. LINBERG AND STEVEN K. FISHER
Department of Biological Sciences & Institute of Environmental Stress, Neuroscience Research Program, University of California, Santa Barbara, Santa Barbara, California 93106

ABSTRACT

Using serial sections and electron microscopy, we have found several morphological types of synapses within the outer plexiform layer (OPL) of the human retina. The most conspicuous of these is described in this paper. They have a unique morphology and form synapses with rod and cone bipolar cells in the OPL and onto bipolar and amacrine cell bodies in the inner nuclear layer (INL). Because they occur in processes that extend across the INL, we believe these synapses are made by interplexiform cells (IPCs). These same processes also contact cone pedicles with specialized cell junctions like those made between cones and flat bipolars. These junctions have densification of both cell membranes and widening of the extracellular cleft, but no accumulation of synaptic vesicles. Similar-appearing processes in the inner plexiform layer are thought to belong to IPCs but their contacts were less completely identified. Possible circuitry for these IPCs is described and the possibility that there are different classes of IPCs in the human retina is discussed.

The OPL forms in the posterior retina during the tenth fetal week. Our observations suggest that different types of synapses including those of the IPCs are present in this layer from the time of its first appearance.

Key words: electron microscopy, serial sections, retinal development, retinal synapses

Although the retina represents one of the best-characterized parts of the vertebrate nervous system, there has been little data published on the anatomical organization of the human retina, especially the outer plexiform layer (OPL). The OPL contains the synaptic terminals of the photoreceptor cells and a neuropil consisting of processes from second-order neurons. Until a recent publication by Frederick *et al.* ('82), it was thought that the neuropil of the human OPL did not contain any synapses (Dowling and Boycott, '66). By using autoradiography, Frederick *et al.* ('82) identified presumed dopaminergic nerve terminals in the human OPL that were presynaptic to horizontal cell processes. They showed these processes to ascend to the OPL from cell bodies in the amacrine cell layer of the inner nuclear layer (INL). Retinal neurons with this morphology are known as interplexiform cells (IPCs). These cells were once thought to be rare, with only a few odd cells described in Golgi-impregnated tissue. More recent studies have shown them probably to be present in all vertebrate retinas (Boycott *et al.*, '75; Kolb and West, '77; Oyster and Takahashi, '77; Dowling, '79), indicating that they are an important component of the retinal synaptic circuitry. The cebus monkey is the only other primate in which IPCs have been described in any detail and thus neither these cells nor their synaptic connections are well-characterized in the primate retina.

This study was undertaken to determine, as precisely as possible, the origin of synaptic contacts we identified in the OPL of adult and developing human retinas. Because these contacts were only presynaptic to bipolar cells they were clearly different from those described by Frederick *et al.* ('82). There were potentially two candidates for the origin of the processes forming these synapses: horizontal cells or a second class of IPCs. We show here that the synapses occur in processes that ascend to the OPL from the inner plexiform layer (IPL) and are, thus, most likely from IPCs. Our data also show that these processes form synaptic circuits similar to those of IPCs in other species, except that they also appear to form contacts with cone pedicles.

In addition, we describe briefly synapses in the OPL neuropil of the 10–11 week human fetal retina. At this stage, the appearance of the OPL synapses coincides with the

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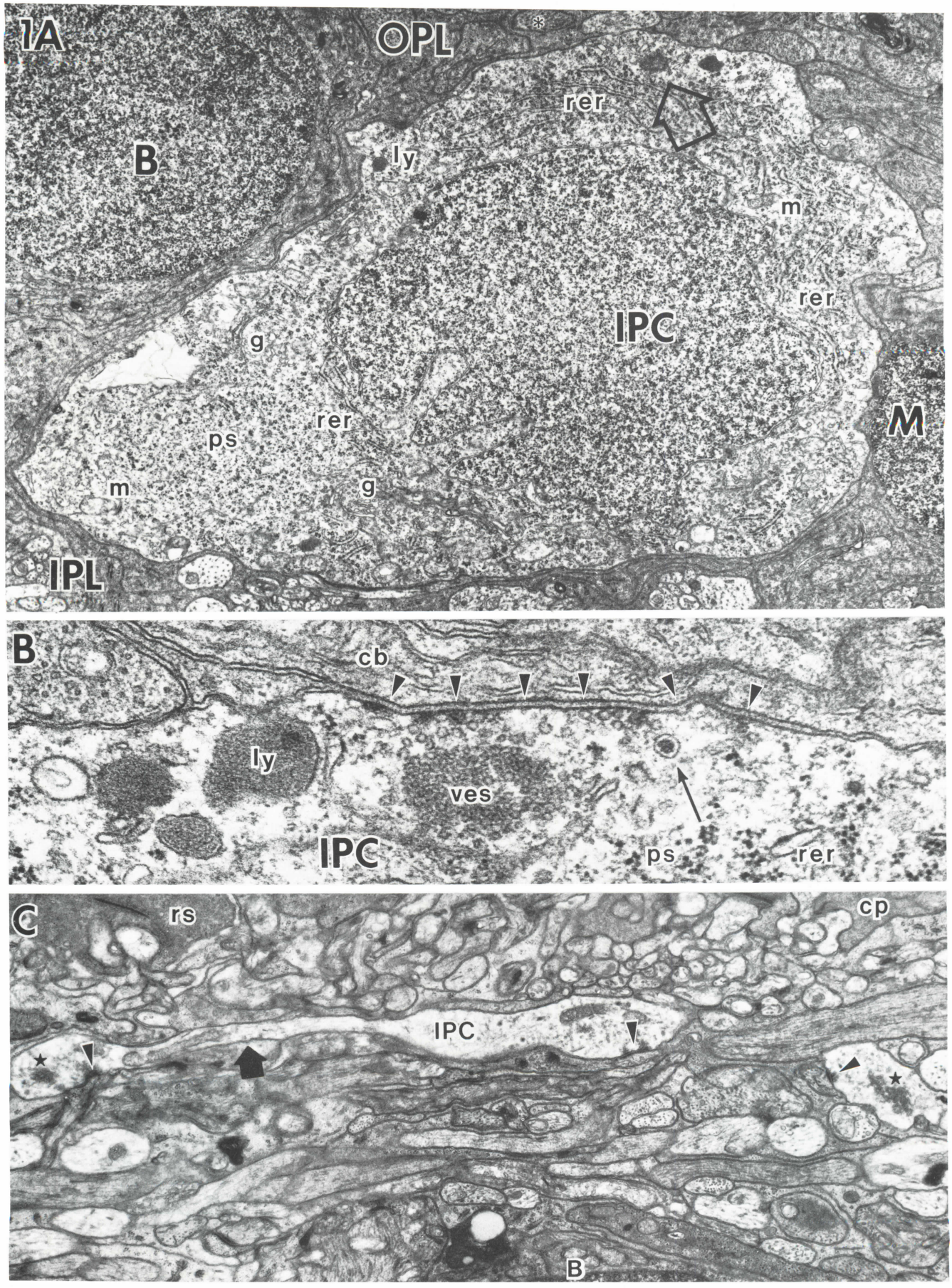
Address reprint requests to Steven K. Fisher, Department of Biological Sciences, University of California, Santa Barbara, CA 93106.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initiation of synaptogenesis in cones and the first appearance of the OPL.

MATERIALS AND METHODS

Adult retinas were obtained from two men aged 21 and 36 whose eyes were enucleated due to retinoblastoma. Nonaffected areas of the retinas were fixed by immersion in fresh Karnovsky fixative (3% glutaraldehyde, 3% paraformaldehyde) buffered with 0.2 M sodium cacodylate, postfixed in 2% OsO_4 in veronal acetate, dehydrated in a graded ethanol-water series, and embedded in Araldite 502. Although the same initial results were obtained from both eyes, the retina of the 36 year-old was better preserved and hence was used for our serial section analysis and for the illustrations shown here. Over 1,100 serial thin sections (ca. 80 nm thick), mounted on Formvar-coated slot grids, were used to reconstruct parts of several cells.

Fetal human retinas were fixed by immersion in 2.5% glutaraldehyde buffered by 0.067 M sodium cacodylate, postfixed in 2% OsO_4 in veronal acetate, dehydrated in a graded ethanol-water series, and embedded in Araldite 6005. Sections were taken from the posterior pole of each of three stages studied: 55 mm CR (10 weeks), 61 mm (10.5 weeks), and 145 mm (17 weeks) (CR = crown-rump length; Corliss, '76).

All sections were stained conventionally with uranyl acetate and lead citrate and were examined in a Siemens 1A, 101, or JEOL-100U electron microscope.

In this study we never reconstructed a whole IPC, though portions of many were studied and their relative positions noted in the sequence of serial thin sections. The area sampled is about 1.5 mm wide by 90 μm thick. The latter dimension is much less than the supposed dendritic spread of this cell type in all other species for which such data are available.

RESULTS

The most frequently encountered morphological type of synaptic contact within the OPL is made by processes that cross the INL, usually terminating in the OPL. One of the first synapses of this type was found to be made by a large cell body lying on the border of the INL and IPL, extending across the INL to the OPL (Fig. 1A, B). The most characteristic feature of this type of synapse is the large, tightly-clustered group of vesicles located at some distance from the actual synaptic site (Fig. 1B) and lying in electron-lucent cytoplasm. Usually this cluster is linked to the synaptic site by a thin stream of vesicles extending to the region of membrane densification. Other examples are shown in Figures 7A, 10C, and 10D. At these synapses the normal extracellular space widens from 8 to 14 nm. Pre- and postsynaptic membranes are symmetrically electron-dense. Conical-shaped dense projections extend from the presynaptic membrane into the region where vesicles cluster near the synapse (Figs. 1B, 2A, 5). The vesicles surrounding the presynaptic densifications and those in the large central cluster are about 40 nm in diameter. When a process is presynaptic to two or more postsynaptic elements in the near vicinity, the central vesicular cluster often appears to span the synaptic sites (an example is shown in Fig. 10C). In the OPL the processes bearing these presynaptic structures are usually very thin (0.3μm in diameter, on average), but they periodically dilate to 1 μm or more (Fig. 1C). Synapses always occur at the dilations, while mitochondria and 80-nm dense-core vesicles often occur within them as well (other examples of these specialized dilations are shown in Figs 6A and 10B).

We consistently identified bipolar dendrites as the postsynaptic elements to these synapses in the OPL and INL. The processes of invaginating cone bipolar cells could be easily identified by a characteristic postsynaptic plate lying parallel to and about 50–60 nm from the postsynaptic membrane (Figs 2B, 7A, 10C). In cross section the plate has a nodular appearance and is about 45 nm thick. Rod bipolars, however, were identified by the presence of the helical organelle (see below) in their dendrites and axons.

Processes with the same pale cytoplasm and bearing presynaptic structures of identical morphology also occur in the IPL (Fig. 6A–D), most frequently in the outermost (scleral) stratum of that layer.

The cells that make the somatodendritic synapses or synapses within the OPL with the characteristic morphology described above have large somata in the INL bordering the IPL; sometimes they are large enough to span the width of the INL (Fig 1A). Ultrastructurally these cells resemble amacrine cells with their most prominent features being numerous ribosomes, stacks of rough endoplasmic reticulum, and a lobed nucleus (Fig. 1A). Microtubules occur in both the somata and processes of these cells (examples in Figs. 2C, 6A, 10B). Large 2–4 μm-diameter processes extend laterally from the base of the cells (Fig. 2C) and then branch in the outermost stratum of the IPL. Sometimes branches from these basal dendrites ascend into the INL where they

Fig. 1. A. A presumed interplexiform cell body (IPC) spans the inner nuclear layer (note: OPL, IPL). Its nucleus is lobed and the nuclear chromatin appears homogeneous. The cell's cytoplasm contains Golgi bodies, mitochondria, lysosomes, polysomes, and cytoskeletal components. Cisternae of rough endoplasmic reticulum (rer) contour the nucleus. The apex of the cell contains a cluster of synaptic vesicles (arrow). The synapse is somatodendritic. The bipolar cell process indicated by "*" is postsynaptic just out of this plane of section (see Fig. 1B). B. A few sections serial to that in Figure 1A, the synapse with characteristic morphology appears. An extensive and widened synaptic cleft (arrowheads) defines the synapse between the cell body (IPC) and the cone bipolar dendrite (cb). Note the dense core vesicle (arrow), lysosome, polysomes, and "rer" in the apical IPC cytoplasm. C. An IPC process in the mid-OPL. Three synapses are seen (arrowheads), one each in three dilations of the process that also contain mitochondria, dense-core vesicles, and clusters of light-core vesicles (stars). These dilations are linked by thin (0.2–0.3 μm) connections (thick arrow). A = x13,000, B = x80,000, C = x11,000.

Abbreviations

A, a	Amacrine cell
B, b	Bipolar cell
C	Cone nucleus
CB, cb	Cone bipolar cell
cp, CP	Cone pedicle
FB	Flat bipolar cell
g	Golgi apparatus
HC	Horizontal cell
IB, ib	Invaginating cone bipolar cell
INL	Inner nuclear layer
IPC	Interplexiform cell or presumed interplexiform cell
IPL	Inner plexiform layer
k	Kolmer's organelle
ly	Lysosome
m	Mitochondrion
M	Müller cell
mt	Microtubule
OPL	Outer plexiform layer
ps	polysomes
psp	Postsynaptic plate
r	Synaptic ribbon
RB, rb	Rod bipolar cell
rer	Rough endoplasmic reticulum
rs	Rod spherule
ves	Vesicles

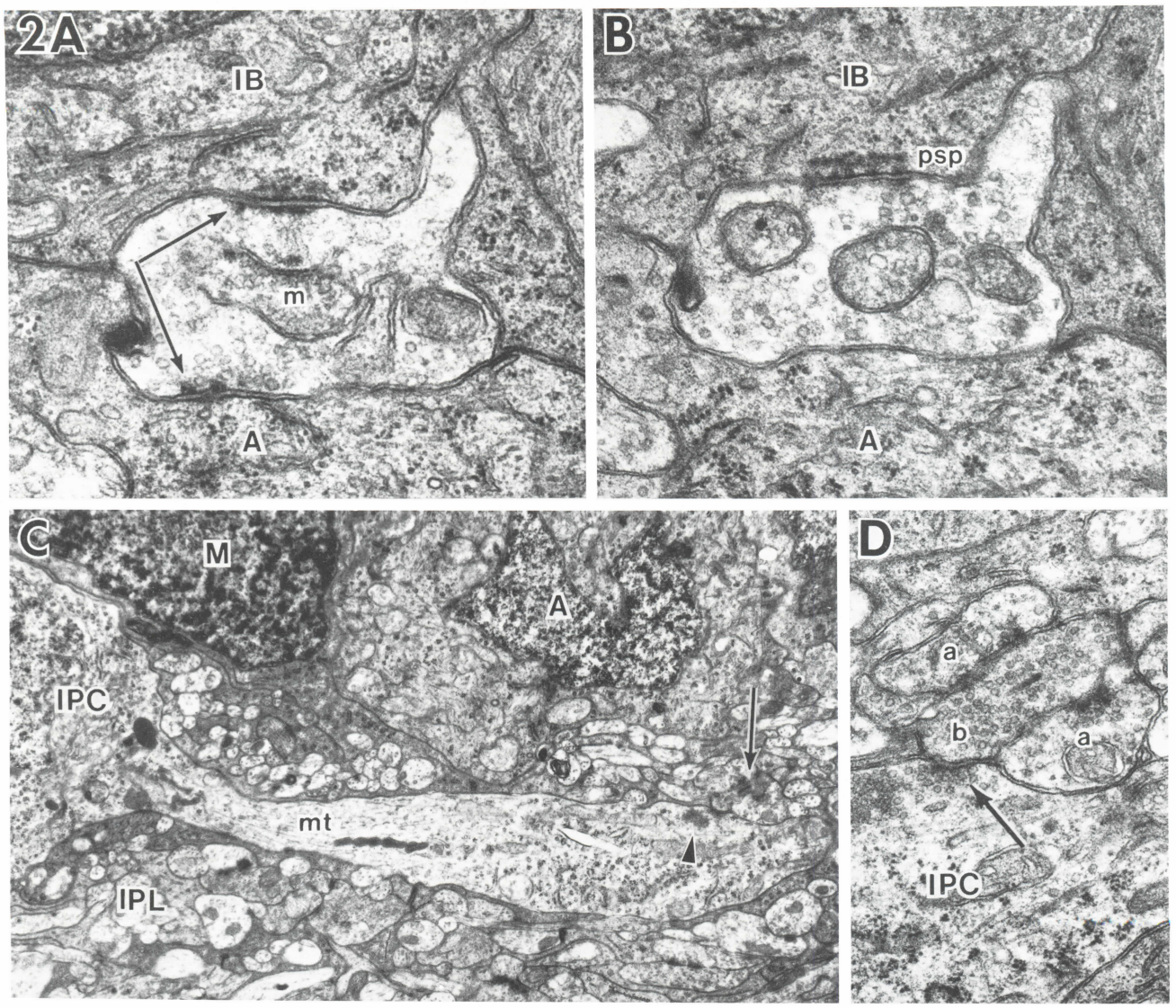


Fig. 2. A. In the mid-INL, a dilated process is presynaptic (arrows) to both invaginating cone bipolar and amacrine cell perikarya. The process contains many light core vesicles in addition to mitochondria. Note widened synaptic clefts and pre- and postsynaptic membrane densifications. Dense projections extend from the presynaptic membrane at the amacrine synapse. B. Two sections from 2A. The synapse with the amacrine cell is no longer in the plane of section, while the synapse with the invaginating cone bipolar is accompanied by a prominent postsynaptic plate. C. A large IPC process extends basally from its perikaryon into the outermost stratum of the IPL. One of the characteristic vesicular clusters (arrowhead) lies near a synapse with a bipolar cell process (arrow) containing a synaptic ribbon. D. Seen at higher magnification and a few sections serial to Figure 2C, the IPC basal process is presynaptic (arrow) to the bipolar cell process that is also postsynaptic to two amacrine cell processes. The vesicular cluster in the IPC is still evident. A = x41,000, B = x41,000, C = x8,000, D = x32,000.

synapse onto bipolar and amacrine cell bodies (Figs. 2A, B) and then terminate (see Fig. 12).

In our series we found several electron-lucent processes that cross the INL and are presynaptic in the OPL. Because of their pale cytoplasm, these processes stand out distinctly against the darker-appearing bipolar, Müller, and horizontal cells (Fig. 3). In general, all of the light-staining processes are remarkable for their lack of organelles and identifiable ground substance (see examples in Figs. 1C, 5C, 6B, 10C), containing a few scattered microtubules and vesicles but little else. In the OPL they extend laterally along the innermost (vitreal) border of that layer. The processes do not seem to branch extensively in the OPL but rather to give rise to small appendages that ascend toward the photoreceptor terminals. In single thin sections these appendages have the appearance of a "plexus" of profiles making numerous synapses and nearly always occurring beneath cone pedicles (Fig. 4). Along the length of the main process in the OPL there are the frequent synaptic dilations referred to above (Figs. 1C, 10B). All of the *en passant* synapses occur at these dilations and they probably account for the beaded appearance of IPC processes described in Golgi and histoflourescence studies (Boycott *et al.*, '75; Kolb and West, '77; Ehinger *et al.*, '69). Coated pits occur all along the length of the thin connectives, as well as at the synaptic dilations (see examples in Fig. 10B, F).

Müller cell processes are usually closely associated with the light-staining processes. In the plexiform layers, the

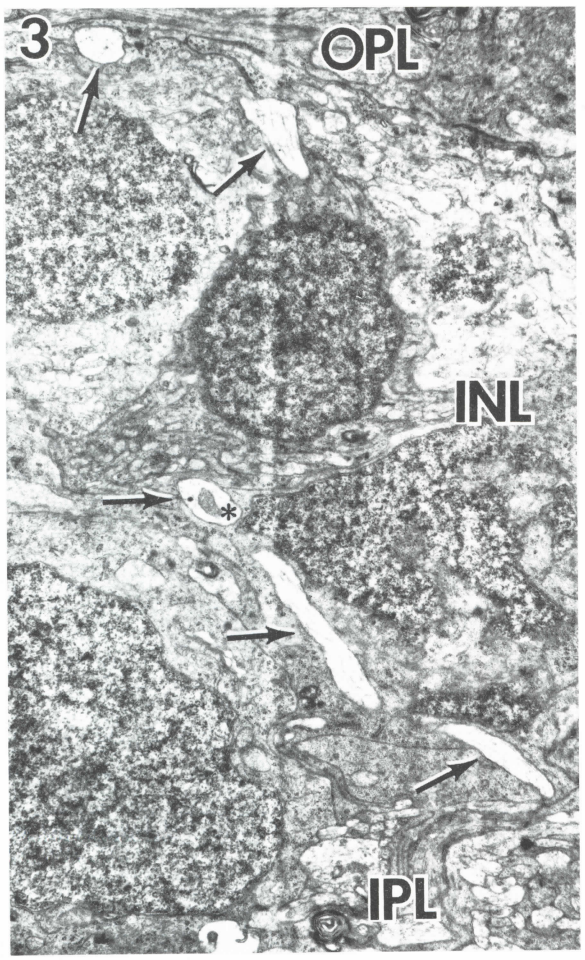


Fig. 3. Five profiles of one of the light-staining ascending processes (arrows) are seen in this single section. Portions of the process are shown at the borders of the IPL and OPL and within the INL. x7,300.

thinnest of these processes may be almost completely wrapped by Müller cell cytoplasm. Such ensheathment drops away at synaptic sites. In the OPL and INL, the light-staining processes sometimes directly appose the processes or cell bodies of horizontal cells but we have not found evidence for either gap or synaptic junctions between them.

Synaptic Connections

The identity of processes postsynaptic to the electron-lucent processes was determined in serial sections by tracing them either to their termination at the photoreceptor terminals or back to their cell body.

In the OPL, bipolar dendrites are the exclusive synaptic target of the processes we have studied. Although we have identified synapses onto both rod and cone bipolars, the invaginating cone bipolar cells are their most frequent postsynaptic element. These are easily identified by the presence of the postsynaptic plate. Indeed, the postsynaptic process in Figure 7A was confirmed to be an invaginating cone bipolar by tracing it to its termination as the central element of a cone pedicle triad (Fig. 7B–D). Flat cone bipolar dendrites were also identified as postsynaptic to these processes by tracing them to flat contacts against a cone pedicle. Rod bipolar dendrites were distinguished from those of cone bipolars by a combination of more electron-dense cytoplasm and a characteristic arrangement of small tubules of smooth endoplasmic reticulum that underlies the plasma membrane and is known as the "helical organelle" (Fig. 4). This structure was originally reported to occur in bipolar axons and somata (Missotten, '65) but our data show it also to be present in rod bipolar dendrites. Other postsynaptic cells were identified as most likely cone bipolars in the serial sections but since they lacked the postsynaptic plate and they were not traced all the way to a flat contact, their specific identity remains inconclusive.

Within the OPL, synaptic interactions between light-staining processes and bipolar cells fall into two classes. They can make a single *en passant* synapse onto a bipolar dendrite (Figs. 7A, 10A, C, E) or cell body. These synapses occur mainly along the vitreal edge of the OPL. Most of the synapses, however, occur in clusters beneath cone pedicles (Fig. 4; also, see Figs. 8, 9).

Appendages making these synapses within the OPL can also directly appose the membrane of a cone pedicle where they form specialized junctions characterized by a widening of the extracellular space and densification of both cell membranes (Fig. 5A–C), but without an accumulation of synaptic vesicles. Often an electron-lucent process will form both a junction with a cone pedicle and a synapse with a cone bipolar dendrite (Fig. 5A–C).

In the IPL these light-staining processes are less distinctive because of the frequency of other equally pale processes from amacrine and ganglion cells. As in the OPL, it is the combination of the characteristic synaptic morphology and the pale cytoplasm that identifies these specific processes (Fig. 6A–D). Presynaptic structures occur most frequently in dilations of the pale processes and have the characteristic cluster of vesicles remote from the synaptic site. So far, we have determined that these processes are usually pre- and postsynaptic to amacrine cells (Fig. 6A–D), and occasionally presynaptic to bipolar processes (Fig 2D). Since we found no evidence that they are presynaptic to processes with the helical organelle (rod bipolars), their synaptic output in the IPL is probably mainly onto amacrines and, perhaps, cone bipolars. Also, we found no evidence of synapses with ganglion cell dendrites. As in the OPL, the pale processes can extend for long distances in the INL without branching but making *en passant* synapses at synaptic dilations (Fig. 6A). We have not yet traced any of these processes to their termination in the IPL.

Serial reconstruction of ascending processes

Figures 8 and 9 show reconstructions of two of these light-staining processes. Figure 8 is a process that was traced through 415 sections within the OPL. Although in all, seven such processes have been traced across the INL in serial sections; the one most completely reconstructed is shown in Figure 9.

The process in Figure 8 made 15 synapses over the length we followed it in the OPL (approximately 45 μm). Some are *en passant* within the OPL but one major cluster occurs beneath a cone pedicle (in the center of Fig. 8). The process forms three of the specialized junctions with one cone pedicle (above the cluster of synapses). Cone bipolars are postsynaptic at 12 of the 15 sites.

The most unusual process reconstructed is shown in Figure 9. We followed it through about 85 μm and it crossed the INL twice, forming numerous synapses within the OPL.

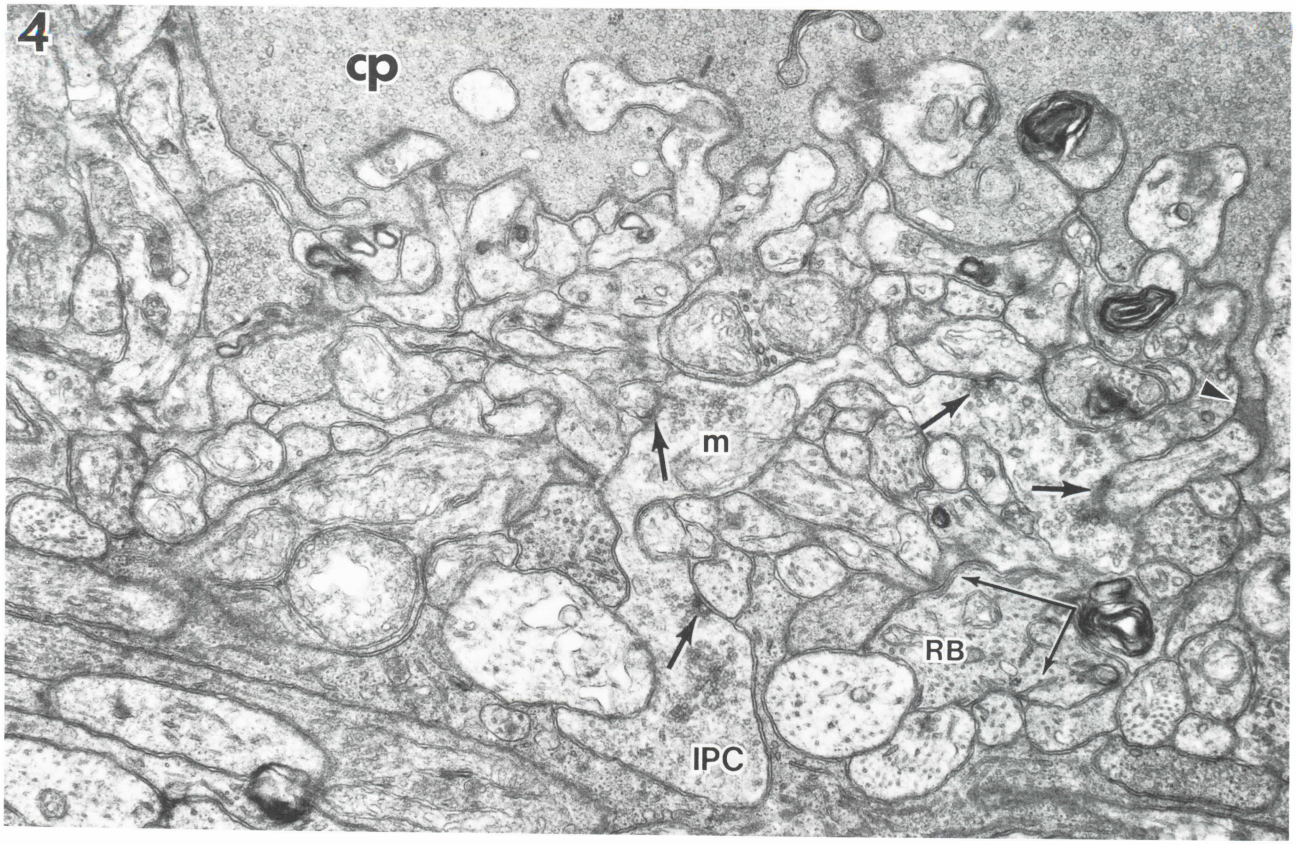


Fig. 4. A dilated IPC process rises in the OPL beneath a cone pedicle. It makes four synapses (arrows) onto an equal number of unidentified cone bipolar dendrites, one of which reaches the cone pedicle (arrowhead). A rod bipolar cell dendrite contains SER in the configuration known as a "helical organelle" (thin arrows). x21,000.

The process must arise from within the IPL since it did not ascend directly from a cell body in the INL. Unfortunately, the process was traced completely back to the beginning of the series of sections (to the left in Fig. 9) and then lost in the IPL after tracing it for 836 sections (to the right in Fig. 9). Selected micrographs from the series are shown in Figure 10. Their locations on the diagram in Figure 9 are indicated by the thin dashed lines and identified by the appropriate figure number. The process began in our series (Fig. 9, thick arrow #1) as a thin connective just entering the INL at the IPL border. It maintains this narrow diameter through the INL. Just after entering the OPL the process gives rise to an appendage (at thick arrow #2 in Fig. 9) that ascends toward the receptor terminals. This appendage has two major lobes, both of which are presynaptic and one of which gives rise to a long thin extension paralleling the main process and forming a synapse onto an invaginating cone bipolar dendrite near its end. Overall, the synapses formed by the process in the OPL are clustered into three major groups: one occurring at the site of the first major appendage (thick arrow #2, Fig. 9); another at the second ascending appendage (the synapse at the end of the long thin branch is part of this cluster); and a third just before the process reenters the INL (to the far right of Fig. 9, between "10C" and "10E"). All three of these clusters occur beneath cone pedicles. In all, the process is presynaptic at 19 sites in the OPL; three of these have rod bipolar dendrites and the rest have cone bipolars as their postsynaptic element. Six of the cone bipolar processes were identified positively as invaginating cone bipolars. At "10B" in Figure 9 the process has changed orientation so that it appears as an elongated profile in Figure 10B. At the right end of Figure 9 there is a swelling containing several of the characteristic vesicular clusters. Figure 10C–F shows sections through this extended dilation; all five synapses occurring here are onto cone bipolars, and three of these are onto the same identified invaginating bipolar dendrite. Figure 10F shows the process as it emerges from the synaptic dilation and just before it leaves the OPL. The process then reenters the INL (Fig. 9, thick arrow #3), and eventually enters the IPL (Fig. 9, thick arrow #4). The narrow diameter and tortuous contour of the process, coupled with the large number of similarly appearing processes in the IPL, made it impossible to follow it further in this series with any confidence.

Development

We have found OPL synapses in the posterior pole of developing human retinas at various ages. Whereas these synapses were well developed in the 17-week specimen, they first appear at 10.5 weeks. This is soon after the OPL first forms and concurrent with the first evidence of photoreceptor synapses, but about 10 weeks before the initial appearance of outer segments (Johnson et al., '85). We have not found evidence for these synapses in the retina of a 10-week specimen. In general, these developing synapses do

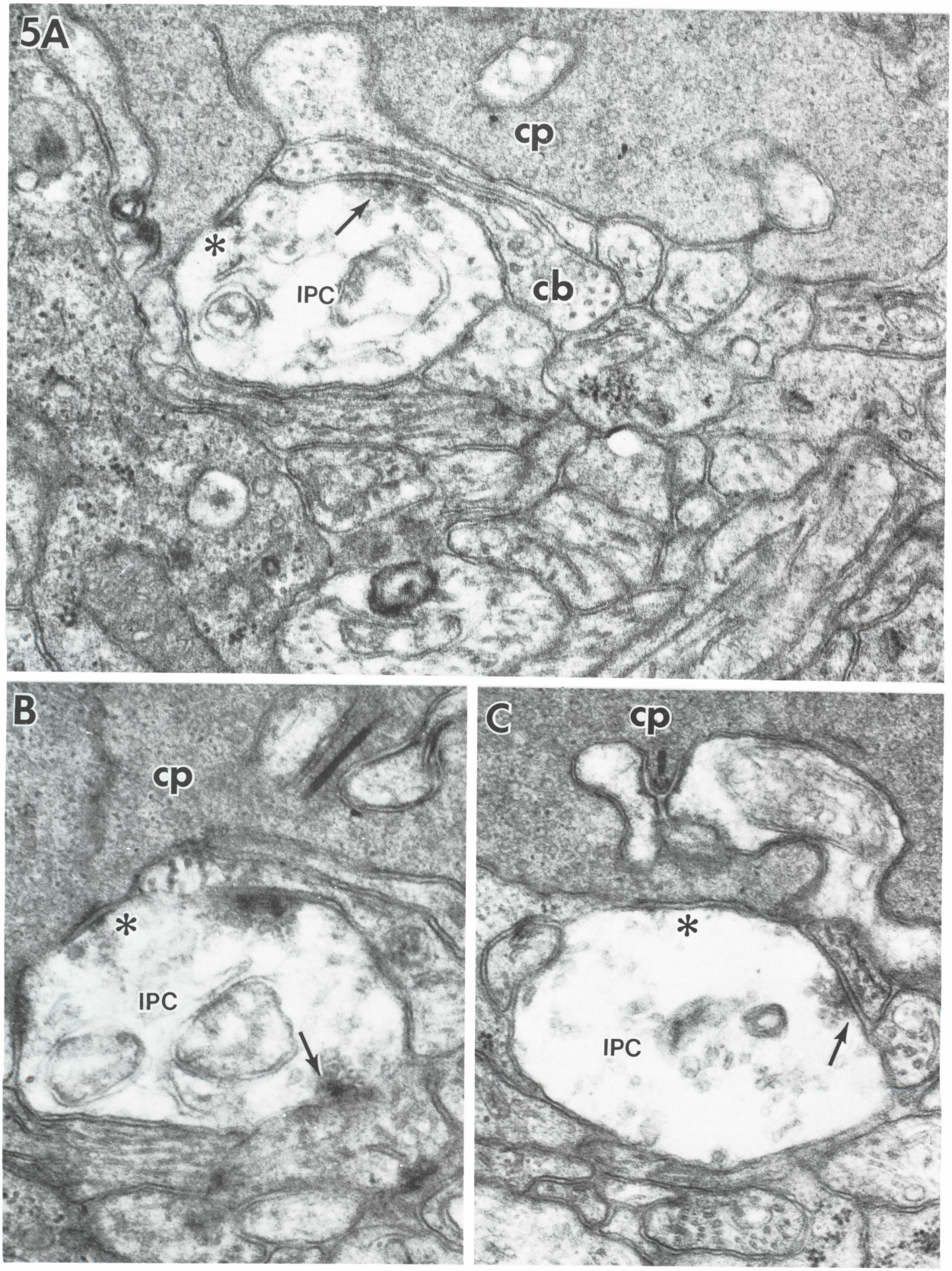


Fig. 5. A. A dilated IPC process not only is presynaptic (arrow) to a cone bipolar cell dendrite, but also makes a specialized contact (*) onto the cone pedicle that it directly apposes. B. Four sections serial to A, the IPC process is presynaptic to a different bipolar dendrite (arrow)—both the conventional synapse and the specialized contact (*) shown in A are still apparent. C. Another example of an IPC dilation that both apposes a cone pedicle and is presynaptic (arrow) to a bipolar cell dendrite. In comparison to A and B, the region of specialized contact (*) with the cone is more extensive. A = x42,000, B = x59,000, C = ×52,000.

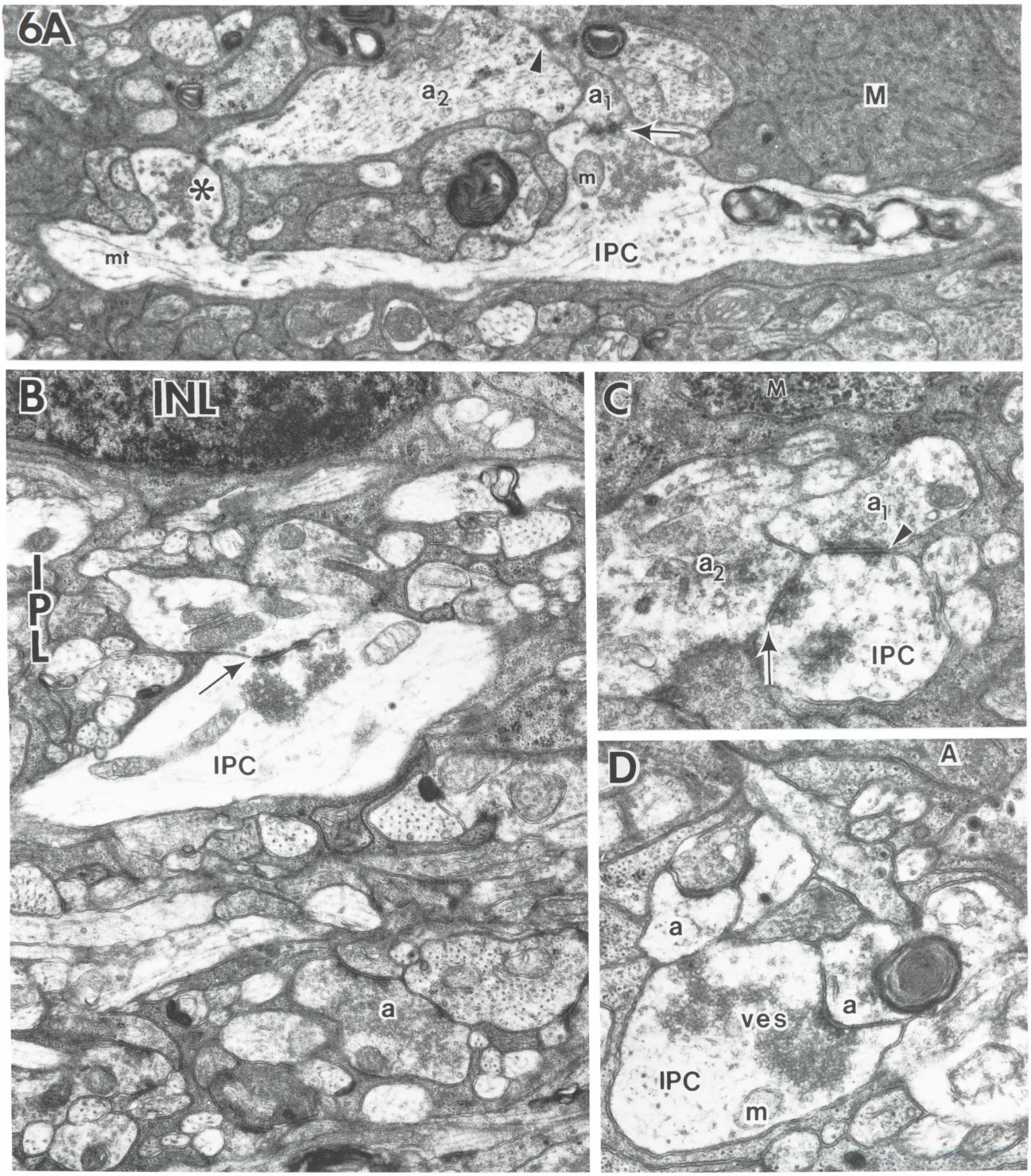


Fig. 6. A. An IPC process at the scleral edge of the IPL runs in the plane of section and is presynaptic to two amacrine cell processes. One of them (a_1), is itself presynaptic (arrowhead) to the other amacrine process (a_2), which is also contacted by (*) the same electronlucent IPC process. B. A dilated IPC process in the outer IPL is presynaptic (arrow) to a presumed amacrine cell process. Compare the localized cluster of vesicles in the IPC process with the more uniformly distributed vesicles typically seen at an amacrine cell synapse. C. An IPC process in the outer IPL is postsynaptic (arrowhead) to one amacrine cell process (a_1) while being presynaptic (arrow) to a second (a_2). D. In the outermost layer of the IPL, directly beneath the basal cytoplasm of an amacrine cell perikaryon, an IPC process is presynaptic to two amacrine cell processes. Presynaptic cytoplasm contains the characteristic cluster of vesicles, spanning the two synaptic sites, and a mitochondrion. A = x13,500, B = x16,000, C = x31,000, D = x29,000.

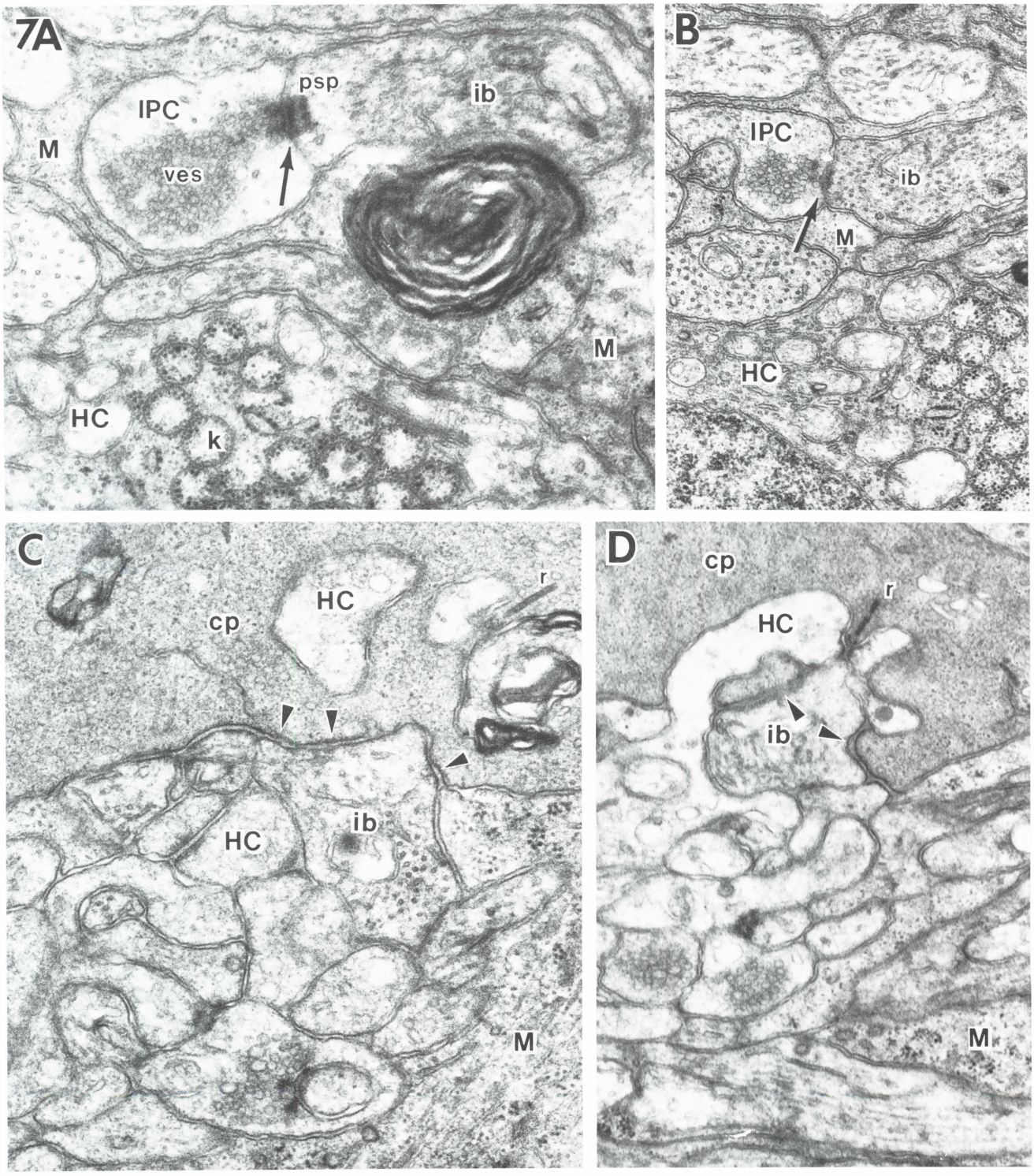


Fig. 7. Micrographs showing a serially traced process of an invaginating cone bipolar cell. This ascending dendrite is postsynaptic to an IPC process at three closely adjacent sites. The bipolar process branches only once within the OPL and ultimately forms the central element of several triads in at least two neighboring cone pedicles. This sequence spans 234 sections. A. In the OPL, a dilated IPC process is presynaptic (arrow) to an invaginating cone bipolar cell dendrite containing a postsynapic plate parallel to the synapse. Membrane densifications are symmetrical on the apposed membranes. Presynaptic cytoplasm contains a central, dense cluster of synaptic vesicles continuous with those at the synapse. Both the IPC process and its postsynaptic target are isolated from other neurons by Müller cell cytoplasm. This synapse is located at the inner border of the OPL, directly above a horizontal cell containing a characteristic form of rough endoplasmic reticulum, Kolmer's organelle. B. Seventeen sections from A. At the third synaptic site (arrow) between the IPC process and the invaginating cone bipolar, the postsynaptic membrane is not underlain by the postsynaptic plate. C. Sections (197) from B. As the invaginating cone bipolar process contacts the cone pedicle, it dilates and makes a specialized contact (arrowheads) with it. The process is postsynaptic to the synaptic ribbon and participates with lateral horizontal cell processes in the triad. D. Twenty sections from C. The invaginating bipolar process forms the central element of a cone triad. It retains the specialized contacts with the cone pedicle laterally (arrowheads). Other features labeled as in C. A = x44,000, B = x28,000, C = x32,000, D = x26,000.

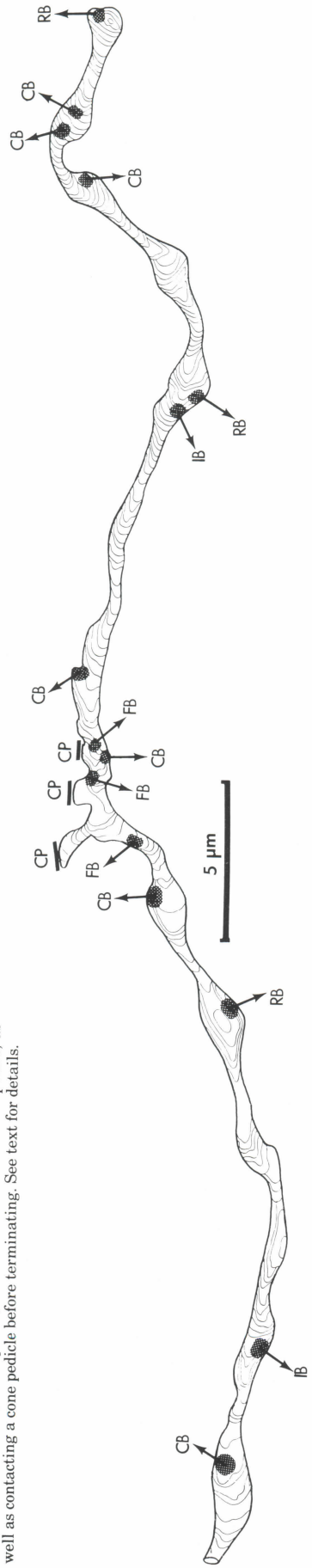


Fig. 8. An IPC process, reconstructed from 415 serial sections, rises from the inner OPL to the outer OPL where it synapse with invaginating cone bipolars, rod bipolars, flat bipolars, or other unidentified cone bipolars, as well as contacting a cone pedicle before terminating. See text for details.

not appear as clearly defined as synapses in the adult retina, nor do they occur in particularly pale-appearing processes. Most commonly they occur as a cluster of vesicles adjacent to dense pre- and postsynaptic membranes separated by a widened extracellular cleft (Fig. 11A,B). Occasional synapses show either a small cluster of vesicles remote from the synaptic site (Fig. 11C) or well-defined presynaptic projections (Fig. 11D)—both characteristics of the synapses described above in the adult retina. Somatodendritic synapses also occur at the 10.5-week stage (Fig. 11E–H), although we cannot assign a certain identity to the pre- or postsynaptic cells at this stage. Such synapses do, however, appear at the margin of the INL and OPL and seem to be presynaptic to bipolar dendrites.

DISCUSSION

In this study we have been able to demonstrate that one type of synapse in the OPL of the human retina is made by processes that ascend from the IPL and run through the INL and OPL. These synapses have a unique morphology making them easily recognized in the OPL, INL, and IPL. Furthermore, the presynaptic processes have the same ultrastructure as that found in processes assigned to interplexiform cells in other species (Kolb and West, '77; Marc and Lam, '81; Pourcho, '81; Kleinschmidt and Yazulla, '84). Figure 12 shows a summary diagram illustrating the distribution of this synaptic type in the human retina. Based on their locations and the fact that they are made by processes that cross the INL, we are assuming that they are made by IPCs. None of the seven processes we followed across the INL ascended directly from a cell body and thus must be arising from within the IPL, a common feature of IPC processes in many species (Boycott et al., '75; Oyster and Takahashi, '76; Marc and Lam, '77; Pourcho and Goebel, '83).

The morphology of these synapses is distinctive, but they also have characteristics of common chemical synapses: the symmetrical densification of the pre- and postsynaptic membranes, conical presynaptic projections, and a synaptic cleft containing slightly electron-dense material. The processes bearing the presynaptic structures are distinctive in both of our specimens by their electron-lucent cytoplasm and the presence of very thin connections between swellings that are invariably presynaptic. Pale cytoplasm is characteristic for IPCs in the cat (Kolb and West, '77) and toad (Kleinschmidt and Yazulla, '84), although apparently not for those described by Frederick et al. ('82) in humans. The synaptic connections we have described here for the pale processes closely resemble those described for cat IPCs by Kolb and West ('77), inasmuch as they have their synaptic output in the OPL onto both rod and cone bipolar cells, with invaginating cone bipolars forming the majority of the postsynaptic elements (Fig. 12). IPCs of the cat are apparently postsynaptic to other IPCs in the OPL; we have not yet found this to be the case in the human. Our data suggest that in the IPL the IPC processes are mainly pre- and postsynaptic to amacrine cells, with only some evidence that they may be infrequently presynaptic to cone bipolar cells. In the cat, the IPCs described by Kolb and West ('77) and Nakamura et al. ('80) were presynaptic to all bipolar cell types as well as to amacrine cells. The IPCs described by Frederick et al. ('82) in the human retina were pre- and postsynaptic only to amacrine cells in the IPL.

The major difference between the circuitry of the IPCs described here and those described by Frederick et al. ('82)

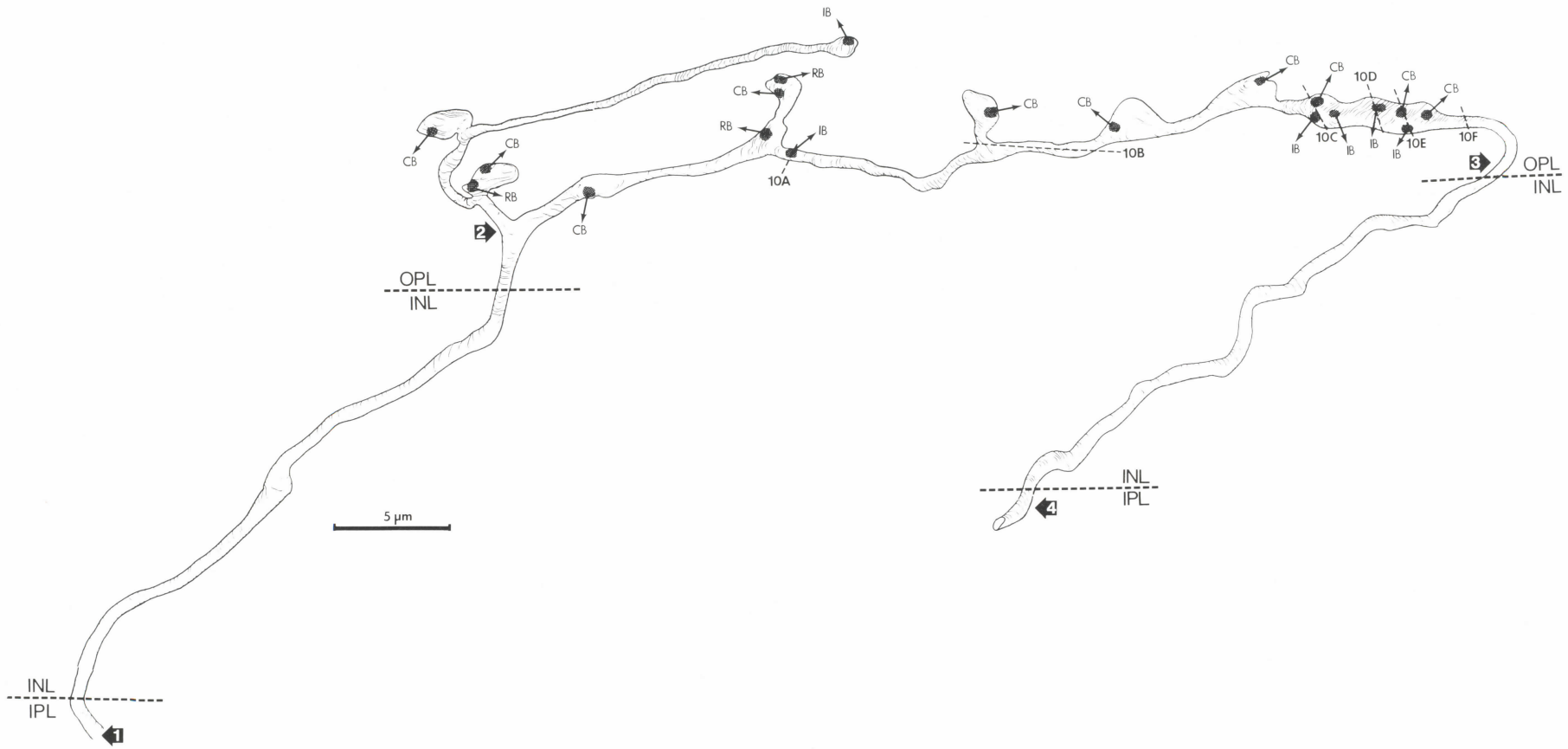


Fig. 9. Another serially reconstructed (836 sections) IPC process rises from the IPL to the OPL and returns to the IPL. In the OPL it runs almost exclusively along the innermost border of that layer. It is presynaptic to the same classes of bipolar cells listed in Figure 8. See the text for further information. The relative positions of the micrographs shown in Figure 10 are indicated by 10A,F on the diagram. The numbered thick arrows are referred to in the text.

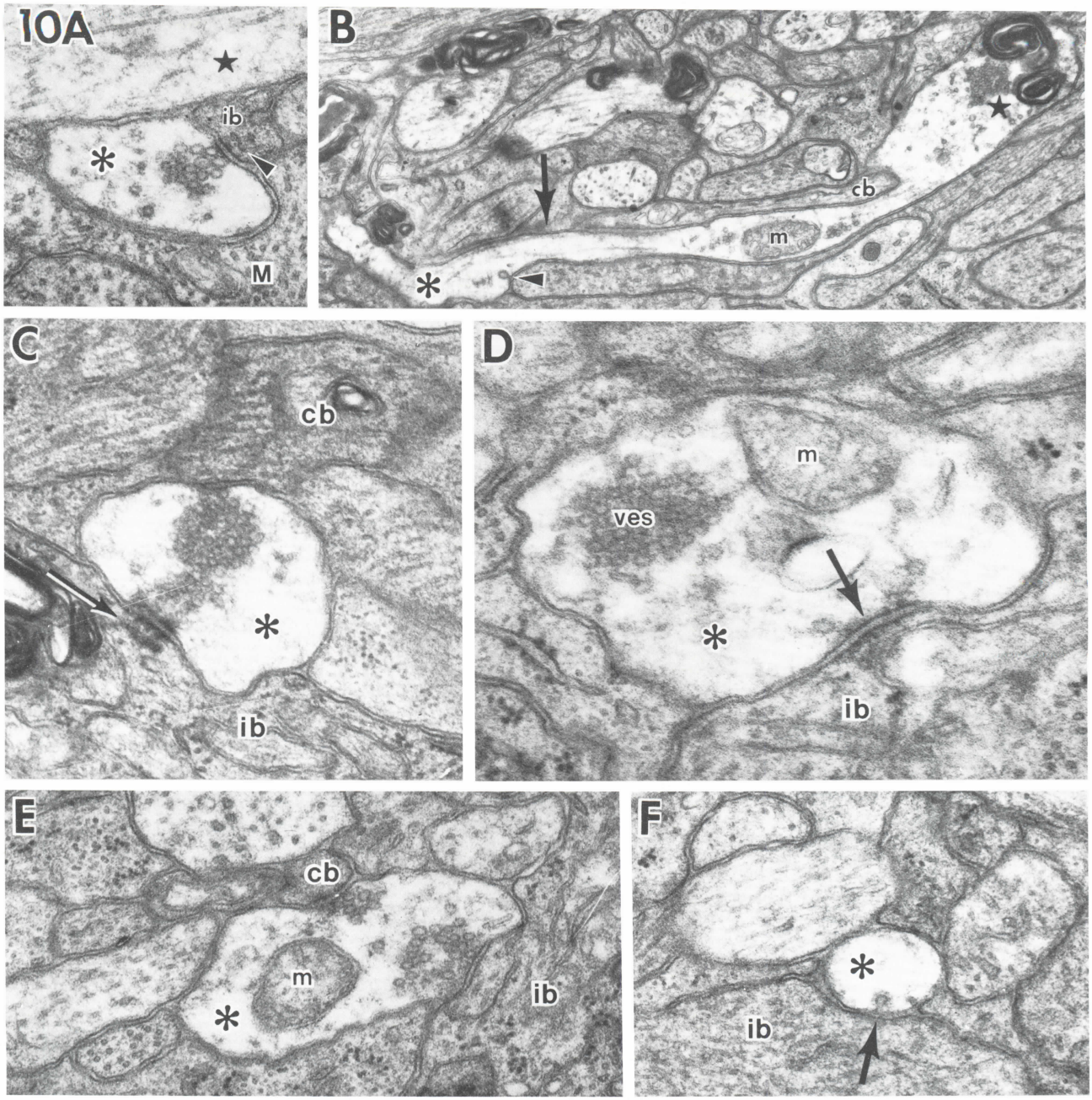


Fig. 10. Micrographs of the serially traced IPC process (*) diagrammed in Figure 9. The process makes 19 synapses within the OPL. The relative positions of these micrographs are indicated on Figure 9. A. Sections (331) from the beginning of the series. A slightly dilated IPC process (*) is presynaptic at this site only to an invaginating bipolar dendrite. A small postsynaptic plate is shown (arrowhead). The IPC process also directly apposes, but never forms a synapse with, an unidentified process (star). B. Ninety-six sections from A. The IPC process (*) travels in the plane of section for a short distance. The process originates at the asterisk. It connects to its left with a short branch leading to a synaptic knob (see "10B", Fig. 9) out of this plane of section. The IPC process proceeds to the right of the asterisk. The thin connective (arrow) opens onto one of two synaptic dilations. The first contains a mitochondrion flanked on either side by small vesicular clusters. A short constriction of this first dilation opens onto the second, larger dilation. It contains a vesicular cluster (star) which is part of a synapse onto a bipolar cell process (cb) in an adjacent plane of section. Note coated pit (arrowhead) in IPC process. C. Fifty-two sections from B. The dilated IPC process (*) forms characteristic synapses with two bipolar cell processes. A vesicular cluster spans the two synaptic sites. The IPC makes only this single synapse with the cone bipolar cell dendrite indicated as "cb". The lower synapse is the first of a series of three onto the branching dendrite of an invaginating bipolar cell. Note its postsynaptic plate (arrow). D. Twenty-five sections from C. The extended IPC dilation (*) is presynaptic, at a second locus, to the invaginating bipolar cell dendrite shown in C. The vesicular cluster connects out of the plane of section with the synapse (arrow). E. Thirteen sections from D. The IPC dilation (*), pictured above in C and D, is presynaptic at a third locus to the same invaginating bipolar, as well as to another cone bipolar onto which it makes this sole synapse. F. Forty-three sections from E. The IPC process (*) again narrows just before leaving the OPL and descending to the INL. The bipolar process indicated in C, D, and E is shown. Arrow indicates a coated pit. A = x45,000, B = x18,000, C = x40,000, D = x60,000, E = x55,000, F = x40,000.

occurs in the OPL. The processes described by them are presynaptic to horizontal cells while those described by us are presynaptic to bipolar cells. Thus, in the OPL the IPC processes identified by us make connections similar to the IPCs in the cat retina (Kolb and West ('77), whereas those described by Frederick et al. ('82) make connections similar to dopamine-containing IPCs in teleosts and cebus monkey (Dowling and Ehinger, '75). This difference may be explained by recent evidence in a number of vertebrate retinas for two types of IPCs containing different neurotransmitters (Dowling and Ehinger, '78; Oyster and Takahashi, '77; Marc and Lam, '81; Marc, '82). In fact, the cells described by Frederick et al. ('82) are thought to be dopaminergic while Hendrickson et al. ('85) have recently found in the human retina a population of GABA-containing neurons in the amacrine cell layer with ascending processes. Furthermore, we have seen a rare type of OPL synapse (not shown) with a morphology more similar to the synapses described by those authors. Also, the cells described by Frederick et al. ('82) all seem to have their ascending processes arising directly from a cell body whereas ours all ascend from processes in the IPL and their cells apparently do not form specialized contacts with the cone pedicles. Thus, it seems likely that there are two classes of IPCs in the human retina with differing circuitries and different neurotransmitters.

A new finding of this study is that IPCs appear to make specialized junctions onto the base of cone pedicles. In other studies, IPCs were seen terminating close to or abutting cone terminals but not actually making contacts with them (Laties, '72; Boycott et al., '75; Kolb and West, '77; Dowling and Ehinger, '75; Kleinschmidt and Yazulla, '84). We have found well over a dozen of these light-staining processes forming specialized junctions with cones (in half of these the same lobe of the IPC process is also presynaptic to a bipolar dendrite). These processes do not enter the synaptic invaginations of the cone pedicle, but rather make contacts resembling those made between flat bipolar cells and photoreceptors (Kolb, '70; Boycott and Kolb, '73). On morphological evidence alone it is not possible to determine if this is a site of transmission between the photoreceptor and the IPC. However, because the morphology of this contact is the same as the contact between photoreceptors and flat bipolars, we suggest that both the IPC and the cell type postsynaptic to it (cone bipolars) are receiving input from the same photoreceptor cell. There is a similar synaptic arrangement in the IPL of the vertebrate retina where a bipolar cell is presynaptic to both an amacrine cell and a ganglion cell dendrite and the amacrine cell is often, in turn, presynaptic to the ganglion cell as well (Dowling and Boycott, '66).

In the carp retina the IPC is thought to play a role in regulating the receptive field properties of horizontal cells, and its synapses are uniquely situated to do so (Dowling and Ehinger, '75; Hedden and Dowling, '78; Cohen and Dowling, '83). In the cat, it has been suggested that IPCs synapses are strategically located to feed back information to bipolar cells from the IPL (Kolb and West, '77). The processes that we have described in the human retina would appear to be similarly arranged to feed back information to bipolar cell dendrites in the OPL, but in addition they may be in a position to feed forward information to the bipolars directly from the cones. Naturally, the validity of such a circuit requires demonstration that the site of contact between the light-staining processes studied here and the cones is a physiologically active one.

Development

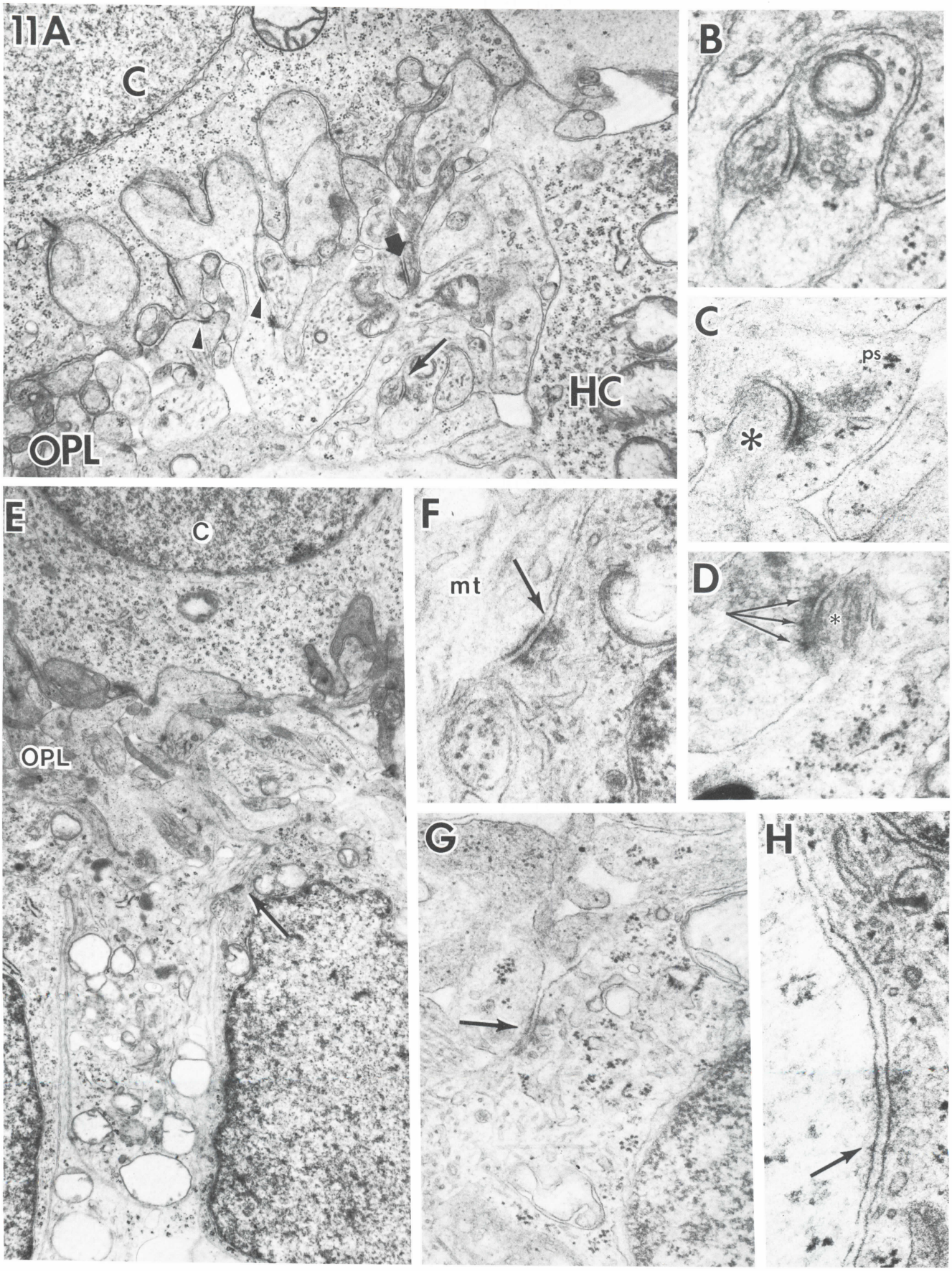
The most complete studies of synaptogenesis in human retina are those of Hollenberg and Spira ('73) and Spira and Hollenberg ('73). In neither of these reports are chemical synapses, other than those made by photoreceptors, described in the OPL. This is not surprising considering their relative infrequency and the fact that they had not been described in adult human retina at that time. Our data indicate that these synapses develop very early in the sequence of events leading to formation of the outer retina. In our 10-week specimen, the OPL was barely discernable and no OPL synapses were found at the posterior pole of the eye. At 10.5 weeks, however, there are photoreceptor ribbon synapses, a narrow, loosely packed OPL, and chemical synapses within the OPL. Our data agree with those of Hollenberg and Spira ('73) as well as with those reported for other species (Olney, '68; Blanks et al., '74; Smelser et al., '74; Spira, '75; Cragg, '75; McArdle et al., '77), that synaptogenesis in the retina precedes outer segment formation. Moreover, our data suggest that synaptogenesis may occur in the OPL even earlier than previously believed. Hollenberg and Spira ('73) reported that the first synapses occur in cone terminals at 83 mm (12 weeks) while we find them to be present in significant numbers at 61 mm (10.5 weeks). This confirms the idea that the human retina develops precociously in comparison to nonprimate mammals (Spira and Hollenberg, '73).

Conclusions

In this paper we provide evidence that synapses within the outer plexiform layer of the human retina are fairly common. At least some of them occur in processes that are synaptic in both plexiform layers and, thus, most likely belong to the class of retinal cells known as interplexiform cells. There is only one previous description of synapses in the human OPL, that of Frederick et al. ('82).

If the processes we have described here are in fact receiving synaptic input directly from the cones, then this represents the second pathway in the primate retina for transmission from photoreceptors to the inner retina by cells other than bipolars. Mariani ('82) described a "biplexiform cell" in the macaque retina that has a cell body residing in the ganglion cell layer and dendrites extending into the OPL where they enter the rod synaptic invagination. These cells also receive synaptic input from rod bipolars and amacrine cells in the IPL and have been proposed to play a role in on-off circuitry in the primate retina (Zrenner et al., '83). We do not believe that the processes we have described arise from biplexiform cells (Mariani, '82) for two reasons. First, we have never found them to enter rod invaginations nor to even approach the layer of rod terminals. Secondly, these processes are *presynaptic*; "biplexiform" cells are supposedly a class of ganglion cells inasmuch as they are only *postsynaptic* within the retina and have an axon that enters the optic nerve (Mariani, '82). It seems, therefore, that primates have unique retinal circuitry by comparison to other vertebrates, with cells other than bipolar and horizontal cells, namely, IPCs and biplexiform cells, receiving direct input from photoreceptors.

Because the detailed study of IPCs is relatively new and because these cells, in some species, seem to be particularly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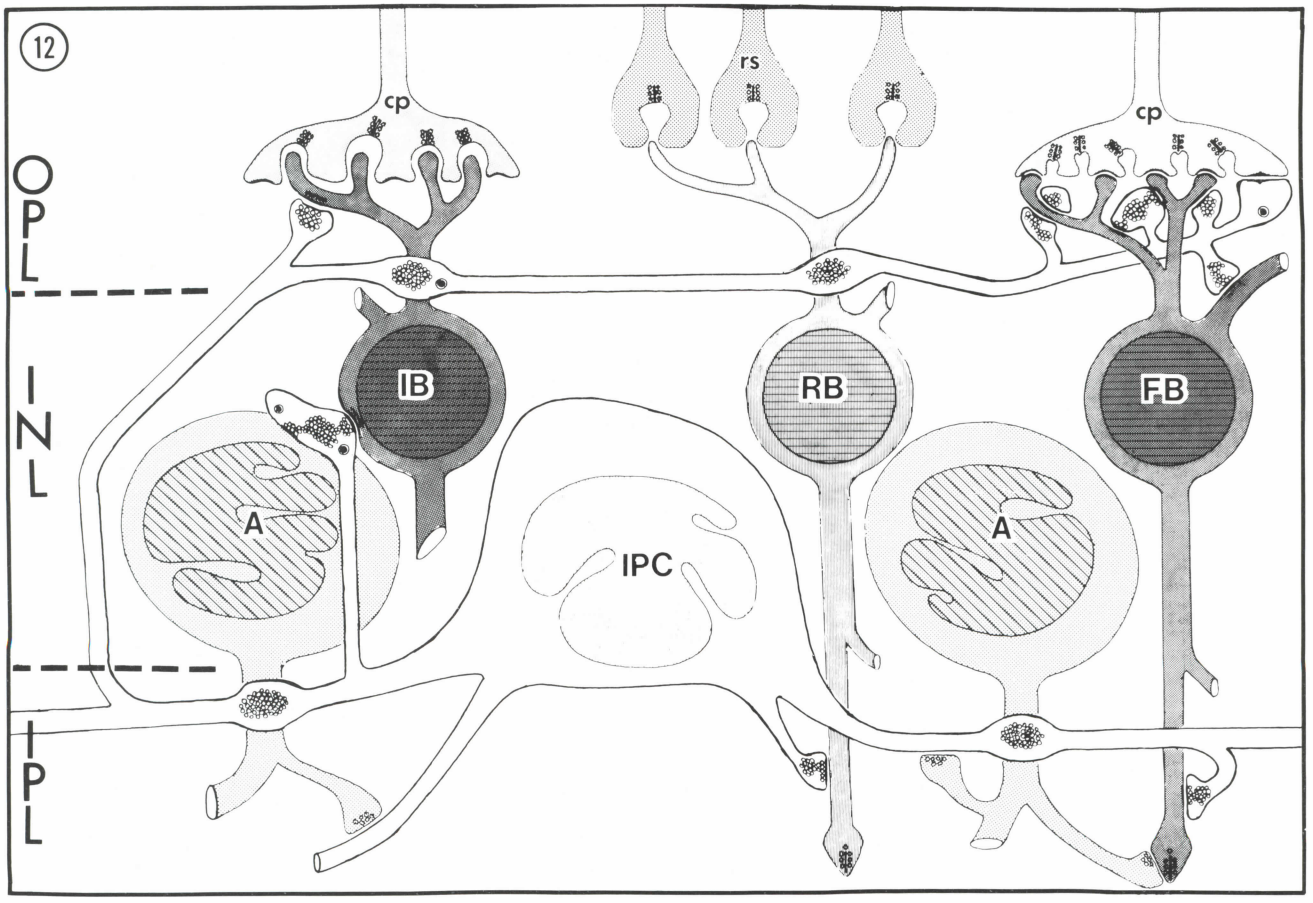

Fig. 12. Diagram of the likely synaptic connections of the IPCs described here. These cells have processes that ascend from the IPC to the OPL where they make synapses with bipolar cells as well as form junctions with cone pedicles. We have identified invaginating, flat, and rod bipolar cells as postsynaptic to these processes in the OPL. IPCs are also presynaptic within the INL to both bipolar and amacrine cells. In the IPL they appear to be both pre- and postsynaptic to amacrine cells and less frequently presynaptic to bipolar cells.

Fig. 11. OPL synapses from a 10.5-week fetal human retina. A. A thin OPL separates a developing cone terminal from a developing horizontal cell. A synapse (arrow), having similar morphology to those described for IPC synapses in the adult, lies near the inner margin of the OPL. Other junctions in the OPL include those with electron-dense membranes but a normal extracellular cleft (arowheads) and those with a widened cleft, electron-dense membranes, but no vesicles (thick arrow). Note the loose packing of the OPL at this stage. B. Higher magnification of the synapse in A. The postsynaptic process contains microtubules and has the appearance of bipolar dendrites in the adult. C. Another synapse containing similar components to those described in B. The postsynaptic process is indicated by the asterisk. D. Another synapse similar to those in Figure 11A and C, showing, in addition, three presynaptic dense projections (arrows) surrounded by vesicles. The postsynaptic process (*) contains microtubules. E. A cell at the outer edge of the INL forms a synapse (arrow) with a dendrite of a cell located deeper in that layer. A portion of a developing cone is shown at the top of the figure. F. The synapse in E at higher magnification (arrow). G. A somatodendritic synapse (arrow), possibly at an earlier stage of development than that in F. Pre- and postsynaptic membrane densification is less pronounced. There are presynaptic vesicles but the cleft is not widened. H. A somatodendritic synapse (arrow) at the margin of the OPL at a very early stage of differentiation. There is only faint densification of pre- and postsynaptic membranes, very few presynaptic vesicles, and no widening of the cleft. A = x20,000, B = x55,000, C = x45,000, D = x50,000, E = x11,000, F = x50,000, G = x36,000, H = x82,000.

resistant to Golgi impregnation techniques (Boycott et al, '75; Oyster and Takahashi, '77), it seems likely that we have only begun to describe this cell type and understand its role in the retina.

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