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Synaptic connexions made by horizontal cells within the outer plexiform layer of the retina of the cat and the rabbit

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[Plates 56–61]

Two ultrastructurally distinctive types of horizontal cells are described in the retinae of the cat and the rabbit. Evidence is presented that they have different synaptic connexions in the outer plexiform layer. The majority of the presynaptic structures identified in the outer plexiform layer of the rabbit (as defined on page 320) belong to a neurofilamentous type of horizontal cell. It is suggested that the cat may be the same. No synapses have been identified on to, or from, the second, predominantly neurotubular, type of horizontal cell. No chemical synapses on to, or between, horizontal cells have been found. Thus input of this kind to both types of horizontal cells is as yet only known to be from the photoreceptors. All positively identified postsynaptic processes were the dendrites or perikarya of bipolar cells. Other cell types that are possibly pre- or postsynaptic in the outer plexiform layer are discussed.

Introduction

Two distinctive morphological types of horizontal cell have been described from Golgi preparations of the domestic cat's retina (Dowling, Brown & Major 1966; Boycott 1975). They are illustrated here in figure 1. The A-type horizontal cell has relatively coarse dendrites, from which arise finer processes that end in aggregates of terminals. This type of cell almost certainly has no axon. The B-type horizontal cell has thinner main dendrites, which also branch to end in similar aggregates of terminals, and an axon that always arises from one of the dendrites. For most of its length this axon has a diameter of 0.5–1.0 μm. After an irregular course in the outer plexiform layer it abruptly enlarges to a diameter of 3–4 μm; then with branches of decreasing diameter forms an extensive axon terminal system. The morphological types of horizontal cells of the rabbit's retina are substantially similar to those of the cat (Dowling *et al.* 1966; Boycott, unpublished). In the cat the dendritic terminals of both A- and B-type cells synapse with cones as the lateral elements of the cone triads, and the processes of the B-type cell's

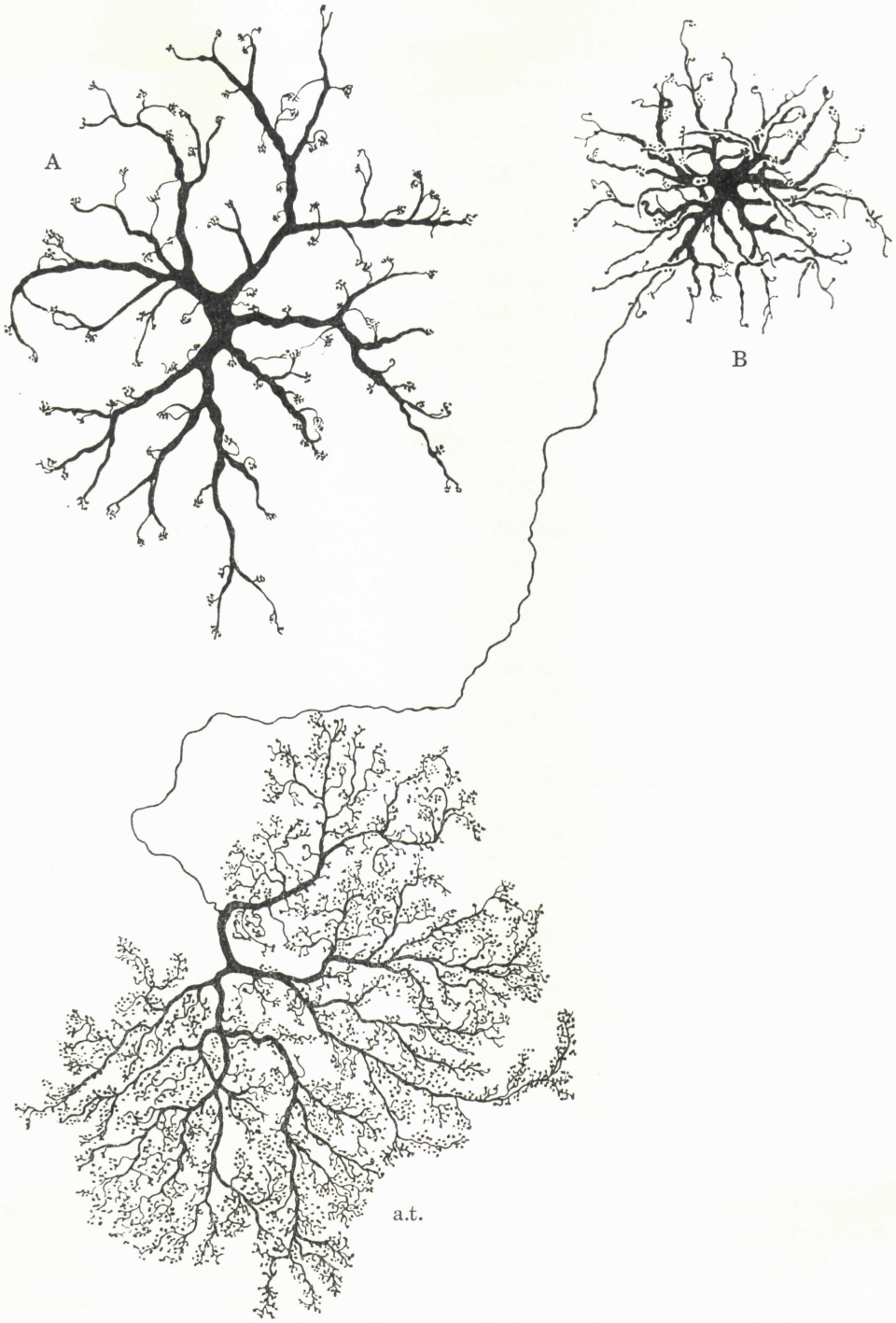


FIGURE 1. Golgi-stained A- and B-type horizontal cells from whole mounts of the retina of the domestic cat. The A-type cell (Golgi–Cox) is viewed from the ganglion cell side, and the B-type cell (Golgi–Colonnier) from the photoreceptor side. The former had a dendritic spread of about 250 μm × 250 μm and the latter about 150 μm × 150 μm. a.t., axon terminal system. For details see page 317. (From Boycott 1974, modified from Boycott 1975.)

axon terminal system occupy analogous positions in the rods (Kolb 1974). Dowling *et al.* (1966) showed that the processes of the horizontal cells in the cat and the rabbit form synapses with bipolar cells and suggested that there might be synapses between horizontal cells. They had no evidence to show whether both types or only one type, of horizontal cell formed any of the synapses in the outer plexiform layer.

From what is known of horizontal cell physiology in the cat there is no particular reason to expect two morphologically distinctive cells of the kind described above (Steinberg 1969; Niemeyer & Gouras 1973). General discussions of the role of the horizontal cells in the organization of ganglion cell receptive fields have often been predicated on the assumption of a morphologically homogeneous population (see, for example, Dowling 1970; Werblin 1972; Naka & Nye 1971; Boycott 1974). Clearly, if the role of horizontal cells in visual processing is to be understood, it is important to anatomize their synaptic relations. The purpose of the present paper is to relate the observable synapses of the outer plexiform layer to the appropriate horizontal cell types. Because of the dimensions of the cells this has had to be done indirectly. The smallest A-type cell in the cat is in the central area and has dendritic field dimensions of about 100 μm × 100 μm, while the comparable parameter of a B-type cell is about 80 μm × 80 μm. For cells towards the periphery these dimensions increase to a maximum of about 250 μm × 250 μm for the A-type cell, and 175 μm × 150 μm for the B-type. In addition each B-type cell has an axon and an axon terminal system. The latter has dimensions that are about twice those of the dendritic field of the parent cell (Boycott 1975). Thus a complete ultrastructural reconstruction of even one of these cells would be very difficult. Equivalent measurements for the rabbit's horizontal cells show them to be larger (Honrubia & Elliott 1969; Boycott 1975). It seemed to us, therefore, that a feasible approach for an attempt to relate synapses to horizontal cell types would be to take advantage of light microscopical observations which suggested possible ultrastructural differences between A- and B-type cells.

Since Cajal (1904), many authors, by using neurofibrillar† methods (see, for example, Gallego 1965, 1971; Honrubia & Elliott 1969; Boycott 1975), have stained a neurofibrillar class of horizontal cells in the retina of both the cat and the rabbit. Dowling *et al.* (1966), Gallego (1971) and Boycott (1975) have identified cells stained in this way with the A-type horizontal cells observed by Golgi methods. Boycott has also shown (unpublished) that in the cat and the rabbit no other nerve cell types contributing processes to the outer plexiform layer react with neurofibrillar methods. Cajal (1904, 1911, 1928) was explicit that nerve cells which stain with certain reduced silver methods were reacting with the stain because they contained bundles of neurofibrillae. Boycott, Gray & Guillery (1961),

† The term is used here in the sense discussed by Guillery (1970) to distinguish reduced silver methods primarily staining neurofibrillae from other reduced silver methods. It recognizes that such methods also stain other components of nerve cells to a greater or lesser degree.

Gray & Guillery (1966), Guillery (1970) and Potter (1971) have discussed the evidence which shows that, ultrastructurally, such cells can be expected to possess neurofilaments.

Horizontal cells can be identified in light microscopical sections of retinae fixed for electron microscopy (Dowling *et al.* 1966). For the reasons given above we thought that by sampling them it might be possible to obtain criteria for two ultrastructurally distinct populations: one containing neurofilaments (therefore A-type), and one with few or no filaments (therefore presumably the B-type). Given such differences, an attempt could then be made by examination of the pre- and postsynaptic cytoplasm of the processes forming the outer plexiform layer synapses, to determine which types, and possibly which parts, of the horizontal cells were involved. It was also hoped that some ultrastructural features of the large part of the axon of the B-type cell's axon terminal system would be recognized (figure 1). In this latter respect we have not yet been successful.

Materials and methods

Eyes were dissected from adult domestic cats and rabbits killed with an overdose of Nembutal. The anterior portion of the eye was removed at the *ora serrata* together with the vitreous humour and the posterior part immersed in fixative. The retina was left attached to the sclera.

Fixation was at room temperature for 1.5 h in 2.5% glutaraldehyde buffered to pH 7.4 in 0.067 M sodium cacodylate with 0.05% calcium chloride added. After fixation the tissue was washed for 2 h in 0.067 M sodium cacodylate buffer with 45 mg/ml of sucrose added, then postfixed for 1 h in 2.0% osmium tetroxide at room temperature, buffered to pH 7.4 with veronal acetate containing 0.05% calcium chloride and 45 mg/ml of sucrose. The tissue was dehydrated in a graded ethanol series and embedded in Araldite. While in absolute ethanol the retina, together with the attached sclera, was cut into pieces about 5 mm square for embedding. Sections approximately 1 μm thick were stained for light microscopy with either toluidine blue, or a mixture of azure II and methylene blue. Sections for electron microscopy were placed either on copper mesh grids, or formvar-coated slot grids and stained with uranyl acetate and lead citrate.

Terminology

In current usage the term outer plexiform layer usually includes the neuropil between the inner nuclear layer and the terminals of the photoreceptors. Thus when reference is made to synapses in the outer plexiform layer it is often taken to include synapses with the photoreceptors as well as synapses within the neuropil. For this paper it is necessary to make a distinction and the phrase 'synapses in the outer plexiform layer' excludes synapses with the photoreceptors, about which there is here no information. Except where stated the term synapse refers

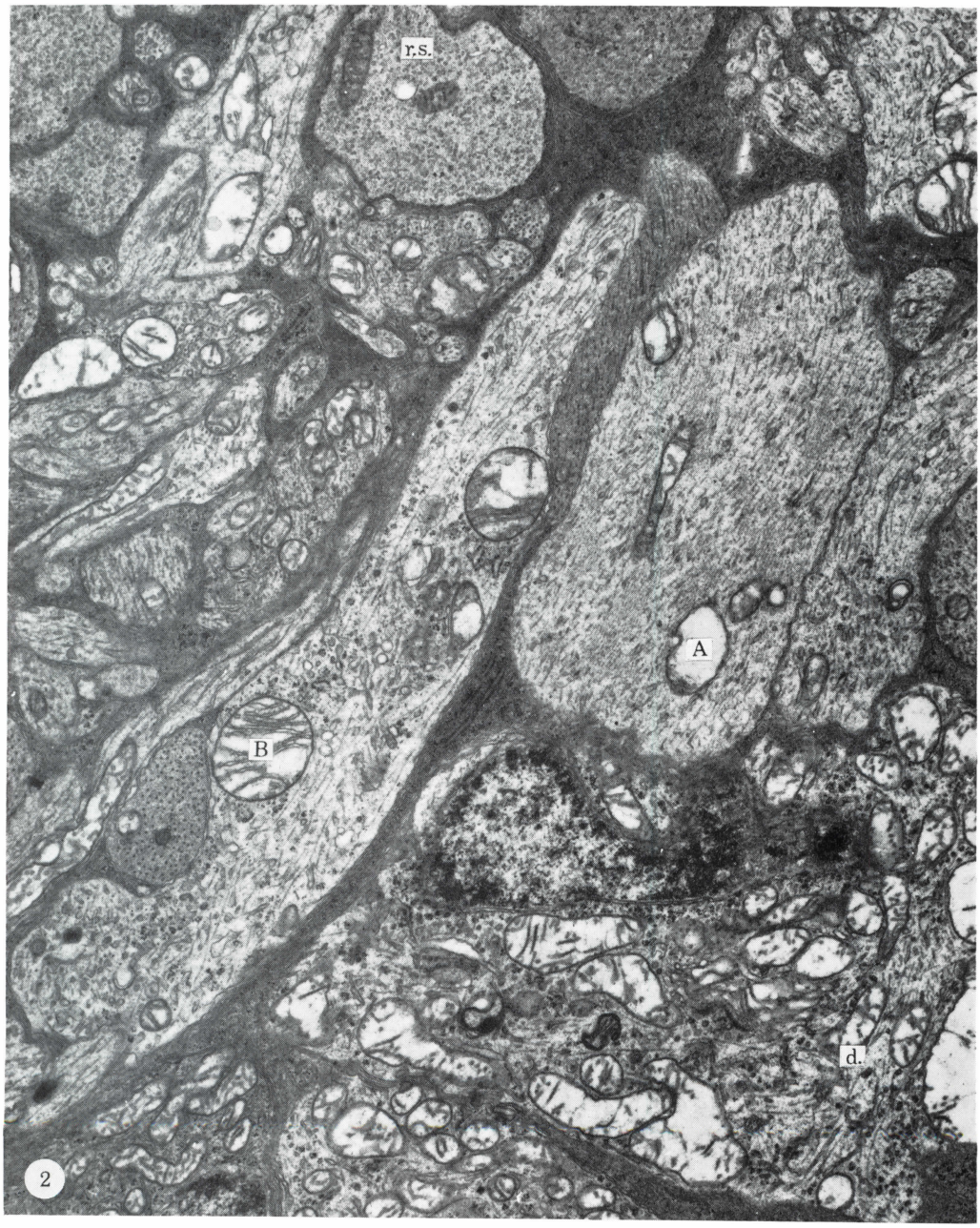
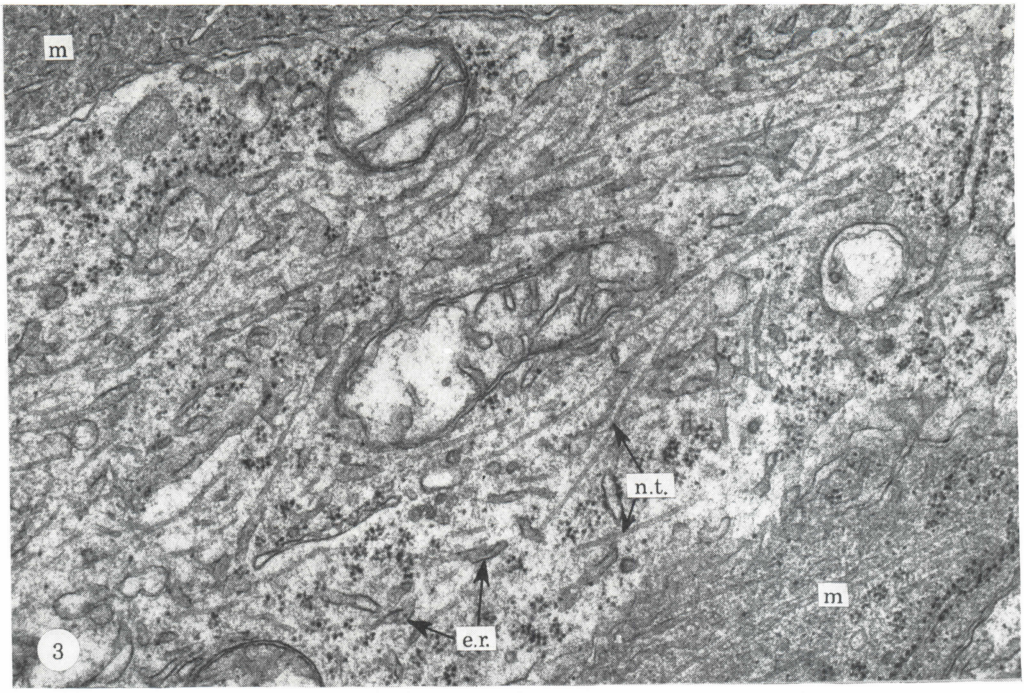
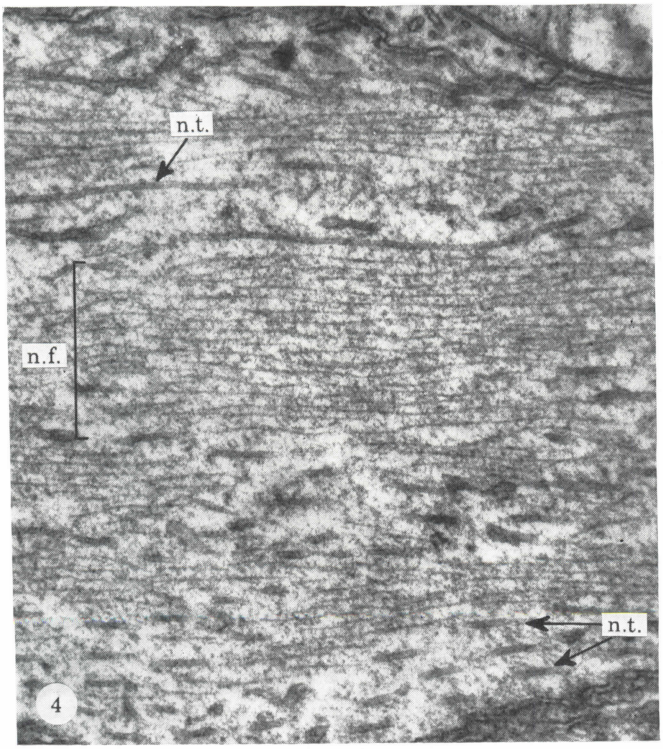


FIGURE 2. Low-power electron micrograph of the outer plexiform layer of the cat's retina to show dendrites of an A-type and a B-type horizontal cell. The streaks in B going from bottom left to top right are neurotubules; the grey patches in A are obliquely cut bundles of neurofilaments (for details see plate 57). r.s., rod spherule; d., bipolar cell dendrite. (Magn. × 12 500.)

(*Facing p.* 320)





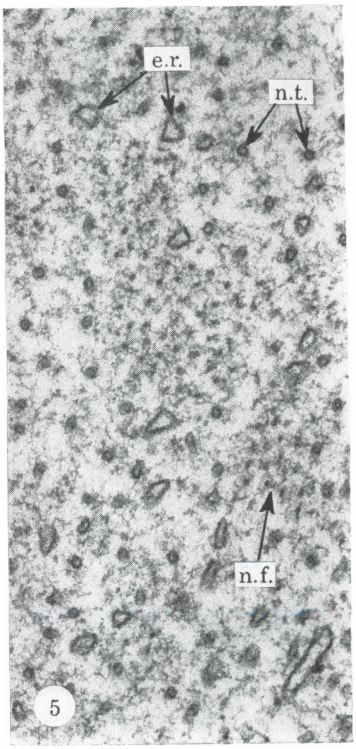


FIGURE 3. The cytoplasmic structure of a dendrite of a cat's B-type horizontal cell near its origin from the perikaryon, n.t., neurotubules; e.r., smooth endoplasmic reticulum. The electron-dense cytoplasm at either corner is that of Müller's cell (m). (Magn. $\times 35\,000$.)

FIGURE 4. Longitudinal section through a main dendrite of a cat's A-type horizontal cell, n.f., bundle of neurofilaments. (Magn. $\times 41\,500$.)

FIGURE 5. Transverse section across a process comparable to that of figure 4. The endoplasmic reticulum often appears triangular in cross-section. (Magn. $\times 57\,000$.)

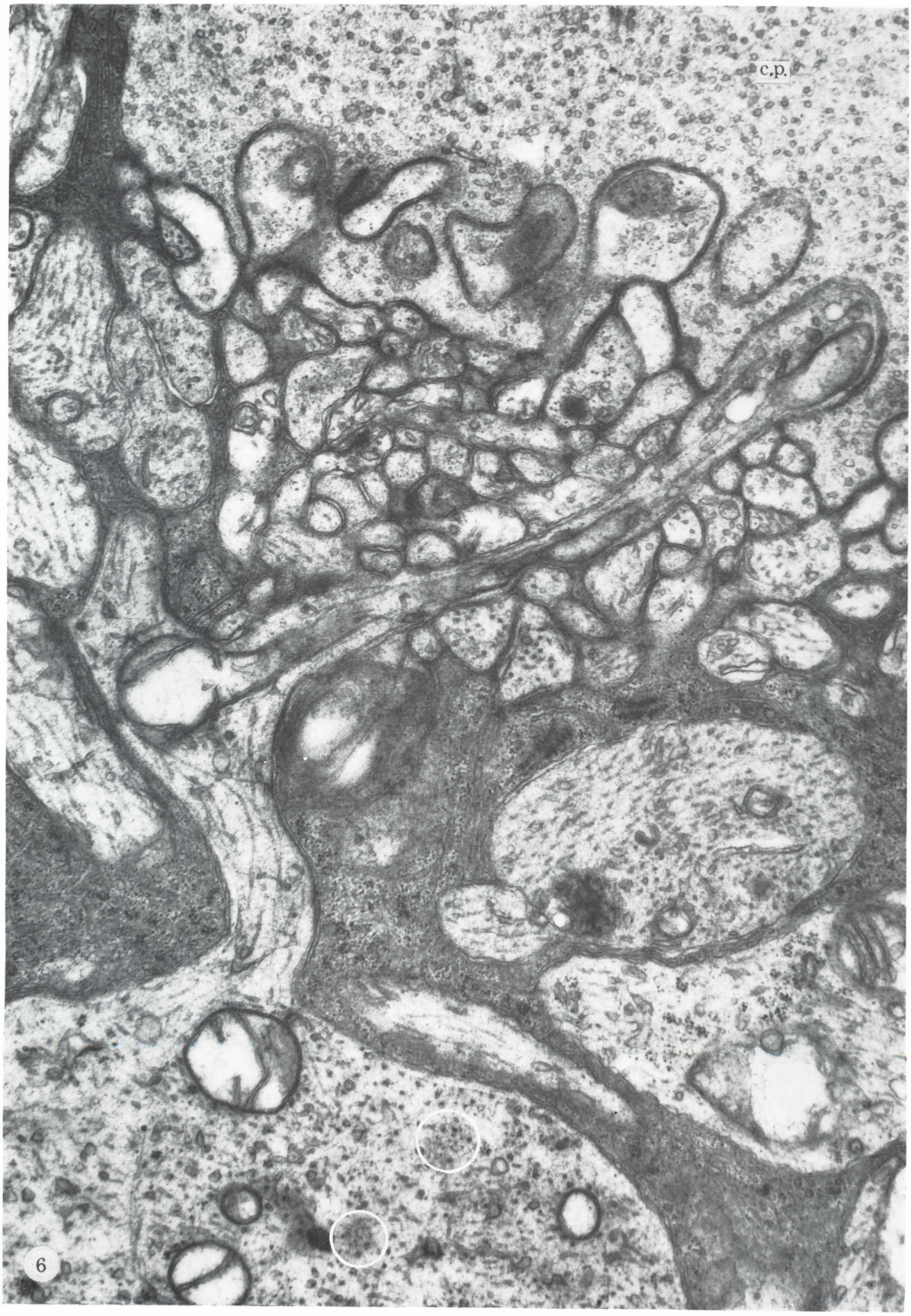
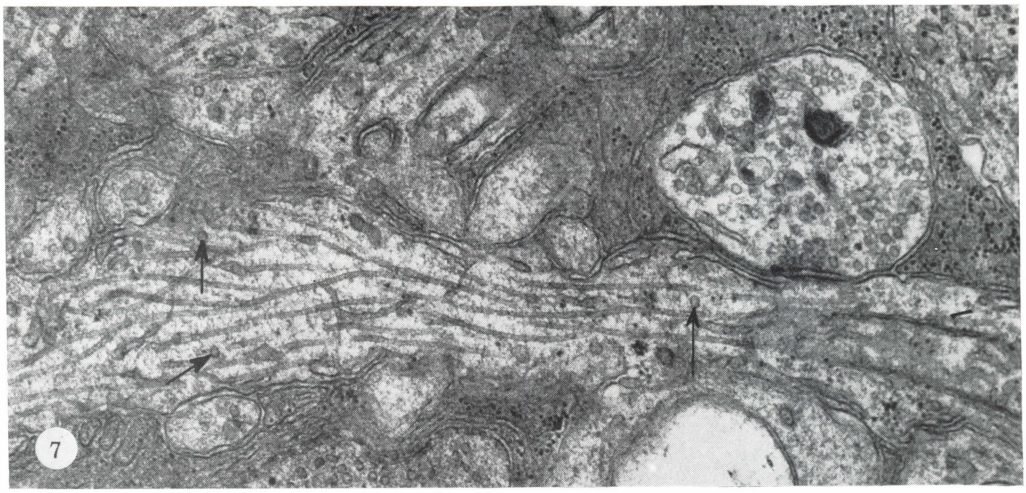
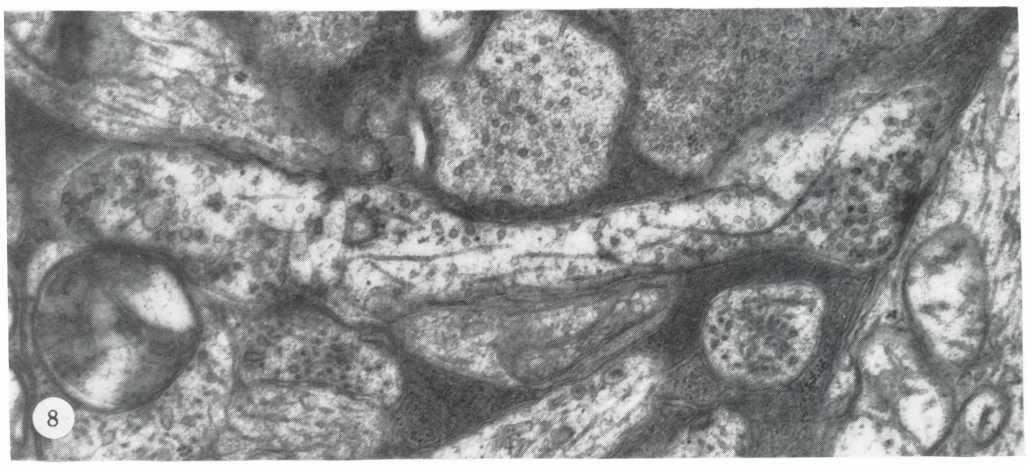
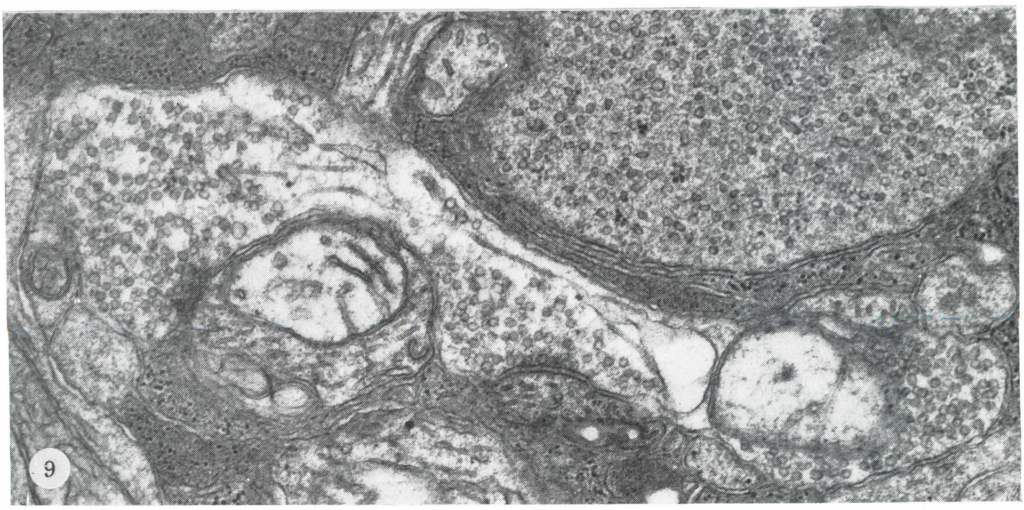


FIGURE 6. Section through a main dendrite (bottom left) of a cat's A-type horizontal cell; a branch without neurofilaments is shown passing through the outer plexiform layer to a cone pedicle (c.p.). Two bundles of neurofilaments are ringed. (Magn. × 24000.)







FIGURES 7–9, Cat's outer plexiform layer. Figure 7, a synapse on to a bipolar cell dendrite so identified in serial section. Arrows point to vesicles sometimes seen in the cytoplasm of bipolar cell dendrites. (Magn. × 37 000.) Figures 8 and 9, two processes each presynaptic at two places for comparison with the process contacting the cone in figure 6, plate 58 (for details see page 322). (Figure 8, magn. × 31 000; figure 9, magn. × 33 000.)

only to structures with aggregations of vesicles associated with membrane specializations (see, for example, Gray & Guillery 1966; Pappas & Purpura 1972). These are usually regarded as chemical synapses distinct from other specializations between processes, such as gap junctions, that are commonly presumed to be electrical (Pappas & Purpura 1972). Concerning the latter this paper has no observations to report.

Results

(*a*) *Outer plexiform layer of the cat's retina*

In 1 μm thick sections stained for light microscopy horizontal cells are distinguished from other nerve cells by their relatively large size at the outer edge of the inner nuclear layer and the pallor of the perikarya and larger processes. Their nuclei are relatively large and in fortunate sections show a characteristically big, densely stained, nucleolus. At first it seemed likely that A- and B-type cells could separately be recognized by relative differences in the size and position of the perikarya in the inner nuclear layer. Because the sections were thin relative to the sizes of the cells, this was not a reliable means of identification; nonetheless when sampling the cells two ultrastructurally distinct populations were found by electron microscopy.

Figure 2, plate 56, shows an electron micrograph of the two kinds of horizontal cell processes at low magnification. That labelled A contains neurofilaments, while B does not; a better resolution of these structures is given in figures 3–5, plate 57. The processes in figures 4 and 5 clearly have a neurofilamentous cytoplasm, while that in figure 3 contains no neurofilaments but has a greater density of neurotubules. Thus, for the reasons given in the introduction, the neurofilamentous processes are referred to the A-type of horizontal cell of light microscopy and the non-neurofilamentous processes to the B-type horizontal cell. A more detailed discussion of this concordance will be given in Boycott (1975). Having observed these differences between the processes it was then appreciated that the nucleus of an A-type cell is likely to show deep indentations (an example is illustrated in figure 3 of Dowling *et al.* 1966), while the nucleus of the B-type cell is without such indentations, and appears more rounded.

Ultrastructurally both the A- and B-type cell perikarya show mitochondria, many free ribosomes, and both smooth and rough endoplasmic reticulum. The Golgi apparatus of the A-type cell tends to remain near the nucleus and not to extend beyond the limits of the perikaryon; this organelle in the B-type cell may extend into the primary dendrites. Likewise granular endoplasmic reticulum is more often found in the primary dendrites of the B-type than the A-type of cell. Farther away from the cell body there appears to be virtually no granular endoplasmic reticulum in either cell type but smooth endoplasmic reticulum is found in the dendrites of both types.

The clearest contrast between the two cells types is in the presence or absence of neurofilaments (figure 2, plate 56; figures 3–5, plate 57). In the main dendrites of

the A-type cell a majority of the neurofilaments are grouped into bundles (figures 2, 4, 5). No further organelles are observed within a bundle, but neurotubules and tubular endoplasmic reticulum occur between the neurofilamentous bundles (figures 4, 5). Such tubular endoplasmic reticulum could be confused with the neurotubules except that it is always much less regular in outline and of a greater diameter than the neurotubules. In the perikaryon of the A-type cell the neurofilaments may be found as bundles or, sometimes, as single filaments. No bundles of neurofilaments and certainly no unequivocal suggestion of isolated neurofilaments, has been found in any part of a B-type cell's cytoplasm. That cytoplasm is characterized by a higher density of neurotubules and smooth endoplasmic reticulum. The latter, although tending to be tubular, has a less regular profile than the smooth endoplasmic reticulum of an A-type cell (figures 3–5).

The juxtaposition of the neurofilaments in the A-type cell as discrete groups forms them into bundles of a size that would be sufficient, when stained with silver, to be observable by light microscopy (Boycott *et al.* 1961; Guillery 1970). Such horizontal cells when stained with neurofibrillar methods do not, as Golgi-preparations do, show the fine processes from the main dendrites which connect the cell to the cones (see figure 1 and Boycott 1975). Figure 6, plate 58, suggests an ultrastructural reason for this. There the main dendrite of the A-type cell is identifiable by the presence of bundles of neurofilaments in the cytoplasm; a process from the dendrite is shown to go through the outer plexiform layer to a cone terminal. This process has no neurofilaments and therefore would not be expected to stain with neurofibrillar stains. Processes without neurofilaments were frequently observed leaving neurofilamentous A-type cell dendrites, but that of figure 6 was the only one we were able to observe continuous with a cone. The ultrastructure of the processes leaving the neurofilamentous dendrites show the presence of neurotubules, mitochondria, uncoated and, occasionally, coated vesicles of about 35 nm diameter. Other organelles, whose nature is difficult to interpret are characteristically present. They could represent expanded smooth endoplasmic reticulum and/or portions of a poorly fixed mitochondrion. The general background to the cytoplasm is electron-lucent.

B-type horizontal cells were identified as described on page 321 but we did not have the fortune to encounter a plane of section showing the connexion of an identified dendrite with a cone. Thus, although all the branches of known B-type cell processes closely resembled the neurotubular structure of the parent dendrite, we do not know the ultrastructure of the finer processes. It is certainly not true that all outer plexiform layer processes containing a high density of neurotubules are B-type cell processes. Bipolar cell dendrites also are characterized by the presence of neurotubules and a general ultrastructure resembling the cytoplasm of B-type horizontal cell processes (figure 7, plate 59). Another class of profiles without neurofilaments, with a sparse population of neurotubules and a varied number of synaptic vesicles were found in the outer plexiform layer. Such processes sometimes formed synapses; of which figures 7–9, plate 59, and figures 13–15,

plate 61, are representative examples. They show both pre- and postsynaptic membrane densities, an increase in the width of the inter-cellular space to about 25 nm between the pre- and postsynaptic surfaces, and electron-opaque material in that gap. On the presumed presynaptic side, the synaptic vesicles show a definite aggregation close to the site of membrane specialization. In general, such vesicles are about 35 nm in diameter. Significant numbers of oval profiles among the synaptic vesicles were never observed, although all the material was fixed in glutaraldehyde. Thus in the cat, and it was the same for the rabbit, there is as yet no evidence for more than one morphological type of chemical synapse. It can be seen from a comparison of the presynaptic processes in figures 8 and 9 with the dendritic branch in figure 6, plate 58, that, apart from the more numerous synaptic vesicles in the presynaptic processes, the ultrastructure of the three profiles is very similar. All three processes are without neurofilaments but contain a few neurotubules as well as organelles that could be smooth endoplasmic reticulum, or portions of mitochondria (see discussion of figure 6 above). The presynaptic profile in figure 7 can be interpreted as a transverse section through this type of process. The presynaptic profiles in figures 13–15, plate 61 less easily fit such an interpretation. Because of the ultrastructural similarities of many of the presynaptic processes in the cat's outer plexiform layer to the process in figure 6, plate 58, we wish to suggest that the A-type cell in the cat may be a major presynaptic structure in that layer (page 326). This suggestion would be less plausible were it not that in the rabbit's outer plexiform layer the majority of synapses we have observed are clearly from neurofilamentous horizontal cell processes.

(*b*) *Outer plexiform layer of the rabbit's retina*

The ultrastructural features differentiating the two horizontal cell types in the rabbit are essentially the same as those in the cat. Also the cytoplasm of the bipolar cell dendrites in the rabbit, like that in the cat, contains many neurotubules; so that they too are difficult to discriminate from dendrites of the B-type horizontal cells unless traced to identifiable perikarya. The rabbit's retina was the first mammalian retina used by Cajal (1904) to demonstrate neurofibrillar-staining horizontal cells and, as with the homologous cell in the cat, it too can be identified as the A-type cell observable in Golgi impregnations (Dowling *et al.* 1966; Boycott 1975). There are distinct neurofibrillar bundles within the cytoplasm of these cells (Cajal 1904, 1911; Honrubia & Elliott 1969). But unlike the cat the rabbit's A-type cells at the ultrastructural level only occasionally show bundles of filaments that would be observable by light microscopy (figures 10–12, plate 60). This may have been due to the methods of fixation used, although these were the same for both animals. Because it has been sufficient for present purposes to have established neurofilamentous and non-neurofilamentous types of horizontal cells, we have not undertaken a systematic investigation of the effects of varied fixation procedures on the filamentous components of these cells.

Figures 10–12, plate 60, show that the synapses in the rabbit's outer plexiform

layer are essentially similar to those of the cat. A detailed difference was that in the rabbit the vesicles appeared always to be aggregated nearer the area of membrane specialization (compare figures 8 and 9, plate 59, with figures 10–12, plate 60). The notable difference between the cat's and the rabbit's outer plexiform layer synapses was that the majority of the presynaptic processes we observed in the rabbit contained neurofilaments.

A count was made of the total number of synapses observed in the rabbit's outer plexiform layer during the course of this work. Of 75 synapses from different parts of the retina, 56 of the presynaptic processes were neurofilamentous, 14 were not. Thus there was direct evidence that 80 % of the presynaptic processes were from one type of horizontal cell (the A-type). In nine different instances scored as from A-type cells, the profiles bearing the synaptic specializations were followed in serial section. In three of the serial sets some individual sections showed the synaptic site but the presynaptic cytoplasm did not show filaments. It was only when the presynaptic processes were followed in serial section that filaments were found and their identity with A-type cell processes demonstrated. This is because neurofilaments are not always distributed in the cytoplasm in such a manner that they can be observed in all the planes of section through a synapse. None of those synapses scored as not on neurofibrillar processes were followed in serial section. It is possible, therefore, that the percentage of synapses associated with the A-type cell is higher than the figures given suggest. In the cat 96 synapses were observed, eight of them in serial section; not one had any indication of filaments in the presynaptic processes, even in serial section. Thus, although the majority of the synapses in the rabbit could readily be classified as from one of the two ultrastructural classes of cell, we obtained no evidence further than that given in Dowling *et al.* (1966) that horizontal cells in the cat's outer plexiform layer are presynaptic.†

Discussion

The results show that there are two ultrastructural types of horizontal cell in the retinae of cats and rabbits. For the reasons given in the introduction they are referred to the A and the B morphological types of light microscopy.

† The methods of fixation used by (Dowling *et al.* 1966) left the processes of the horizontal cells very 'empty'. Through the courtesy of John Dowling, we were able to examine their original plates. A very few presynaptic filaments were found in the presynaptic cytoplasm of the majority of the synapses in the rabbit. In their cat material no presynaptic filaments were found with one exception, a portion of which was published as their figure 4*a*. The presynapatic process was very large and some filaments were found well away from the synaptic site. This one example is the only direct evidence so far that A-type cells carry synapses in the cat's outer plexiform layer. Assuming the interpretation given on page 326 that presynaptic processes of the cat's A-type cells are on the non-neurofilamentous processes going to the cones, then this example from the material of Dowling *et al.* could be interpreted as an exception in which a synapse is from the main dendrite, or as an oblique section through one of those processes that included also a portion of the main dendrite.

(a) Cell types presynaptic in the rabbit's outer plexiform layer

The ultrastructural evidence in the rabbit is that the greater majority of the presynaptic processes (80 %) are from the A-type cells; the rest remain unclassified. When the presynaptic processes are neurofilamentous it follows that they are from those parts of an A-type cell which stain with neurofibrillar methods (i.e. the main dendrites of the cell). No presynaptic processes were found involving any positively identified dendrites of the rabbit's B-type cells.

None of the 20 % of presynaptic processes that remained unclassified had an ultrastructure resembling known B-type horizontal cell dendritic processes any more closely than that of figure 16, plate 61. Others were like the adjacent figure 17, which has no B-type cell features although it has (see legend) a feature sometimes found near A-type cell synaptic sites. Aside from B-type cell dendrites there are at least three other types of processes in the outer plexiform layer which could be the presynaptic elements of the synapses we were unable to classify.

(1) The axon terminal systems of the B-type horizontal cells, together with the axons, form a considerable proportion of the processes in the outer plexiform layer. These have not been identified ultrastructurally, but recently Boycott & Hopkins (unpublished) have found processes in the neuropil near the rod spherules which contain synaptic vesicles. These are likely to be the finer branches of the axon terminal systems near where they enter the rods. They run close to other processes that are probably rod bipolar cell dendrites but no synapses have been observed between the two. These presumptive axonal processes have not yet been followed to a structure that could represent the enlarged part of the axon terminal system (figure 1). At present this remains ultrastructurally unidentified and clearly might be a type of process involved in synapses within the outer plexiform layer.

(2) The cytoplasm of the dendrites of identified bipolar cells in both the rabbit and the cat does show occasional, scattered, synaptic vesicles (figure 7, plate 59), sometimes a coated vesicle, less often an aggregation of vesicles and even, in the soma, a ribbon surrounded by vesicles. None of these synaptic structures have ever been seen aggregating at the surface of the cell to suggest a synaptic site. No processes with the density of neurotubules usually seen in bipolar cell processes have been observed to be presynaptic. Thus it is unlikely that bipolar cells are presynaptic in the outer plexiform layer. Polyak's (1941) conception of a centrifugal bipolar cell, which was criticized on other grounds by Boycott & Dowling (1969), receives no support from the present study.

(3) The interplexiform cells (Gallego 1971) have processes in the inner plexiform layer whose general morphology resembles that of some kinds of amacrine cells but these cells also have processes which ascend to the outer plexiform layer. Cells of this type are found in a variety of vertebrates (Ehinger, Falck & Laties 1969; Laties 1972). Among mammals they were first mentioned by Cajal (1892) in the dog; they have now been observed in the cat, the rhesus monkey (Gallego 1971;

Boycott *et al.* 1975) and the dolphin (Dawson & Perez 1973), but not in the rabbit. Presumably interplexiform cells have some synaptic relationships in the outer plexiform layer but they are as yet known only from light microscopy and therefore their synaptic contacts remain unknown. (See, however, p. 331.)

Allen (1970, p. 118) briefly mentioned that he observed an occasional synaptic ribbon in the human outer plexiform layer. We have observed two such ribbons in the cat and three in the rabbit but are uncertain as to whether they are borne by basal processes of the cones, or some other structure perhaps processes of the interplexiform cells. Basal processes are not usually supposed to enter deeply into the outer plexiform layer in mammals, although they do so in other vertebrates (Cajal 1892). Nor have these receptor processes been observed to contain ribbons (Missotten 1965; Missotten & Van Den Dooren 1966; Cohen 1972). In our material there were ultrastructural indications on the membranes that the ribbons might represent a presynaptic site but they were observed so infrequently that we could not decide. Accordingly we have not included them in our synaptic data.

(*b*) *Cell types presynaptic in the cat's outer plexiform layer*

The cat and the rabbit have homologous horizontal cell types (Dowling *et al.* 1966; Honrubia & Elliott 1969; Boycott 1975), yet none of the synaptic profiles we have seen in the cat's outer plexiform layer contain neurofilaments. Thus we have no direct evidence for the cat that A-type cells are presynaptic (see footnote page 324). Differences between species in the ratios of the synapses from different cell types are to be expected (Dubin 1970; Boycott 1974). In the present comparison the direct evidence is that 80% of the observed synapses in the rabbit and less than 1% in the cat, are from the A-type cell. The density of synapses in the outer plexiform layers of the two animals has not been measured but when working at the sections we were equally likely to find synapses in the outer plexiform layer of either animal. Thus if most of the synapses in the cat's outer plexiform layer are not from A-type horizontal cells they must come from the processes of other cell types. We have found no evidence that they come from B-type cell dendrites, since the presynaptic processes have no resemblance to known B-type cell ultrastructure. Their identification with the processes of other cell types that

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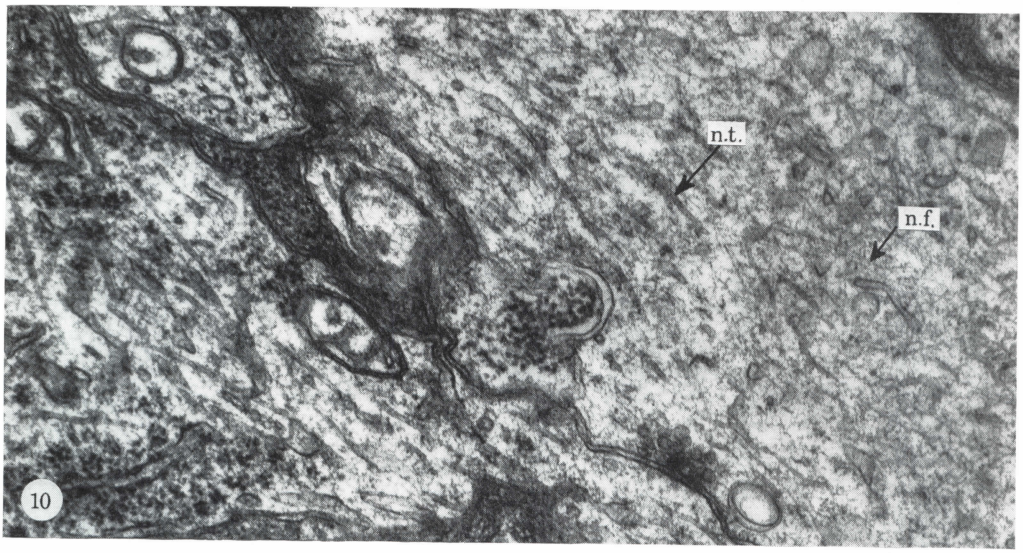
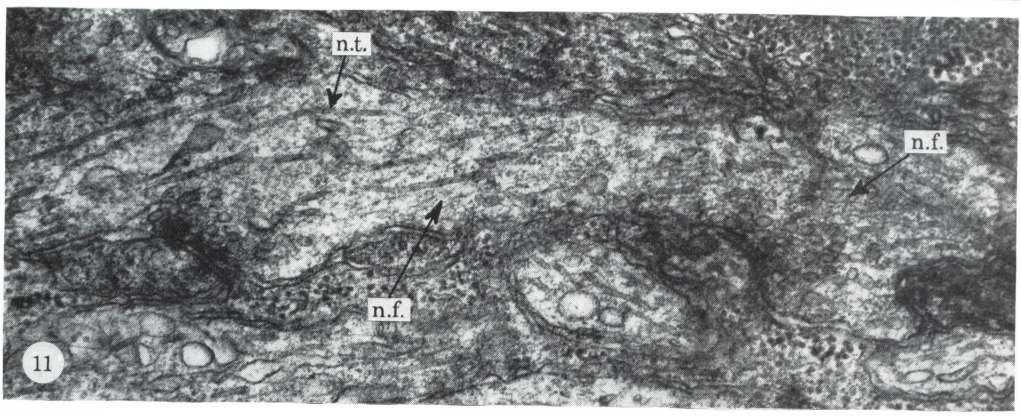
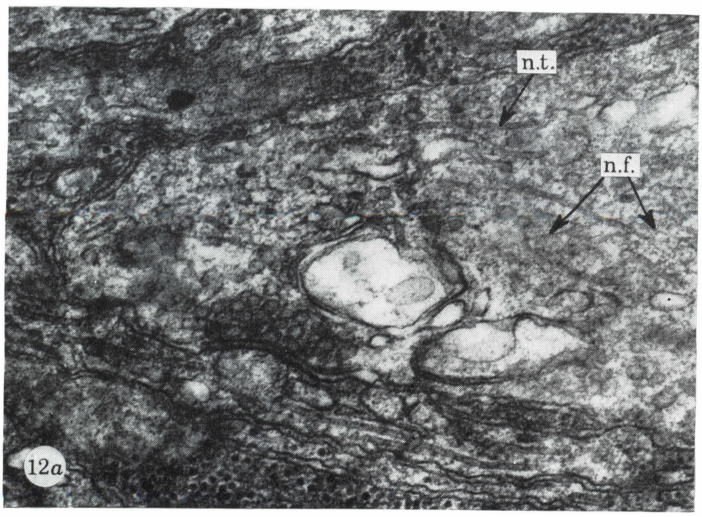
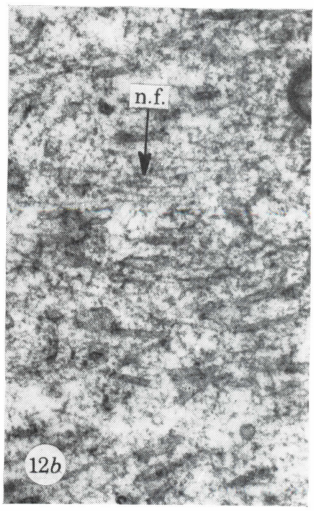
DESCRIPTION OF PLATE 60

FIGURES 10–12. Main dendrites of three different A-type cells of the rabbit to show that these processes contain neurofilaments (n.f.) and neurotubules (n.t.) and are presynaptic. The postsynaptic structure of figure 10 was identified in serial section as a bipolar cell. In figure 11, five sections further along, another presynaptic aggregation of vesicles was found in the same process. Figure 12*b*, higher magnification of the A-type cell cytoplasm of figure 10 to show the neurofilaments (n.f.). The arrows point to examples of the filaments which in the rabbit, always had low contrast and seemed more dispersed when compared with those of the cat treated in the same way. (Magn. in order of figures: ×36000; ×44500; ×44000; ×42000.)



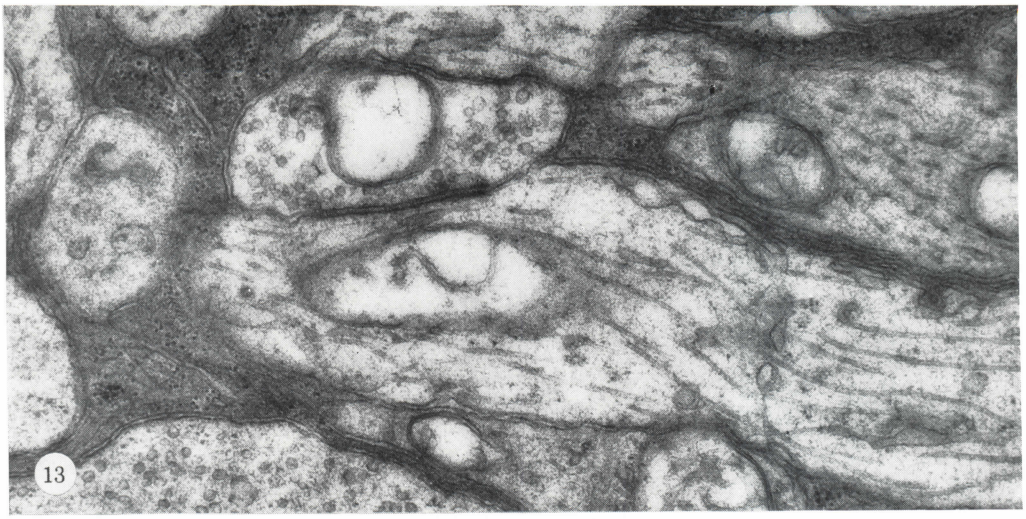


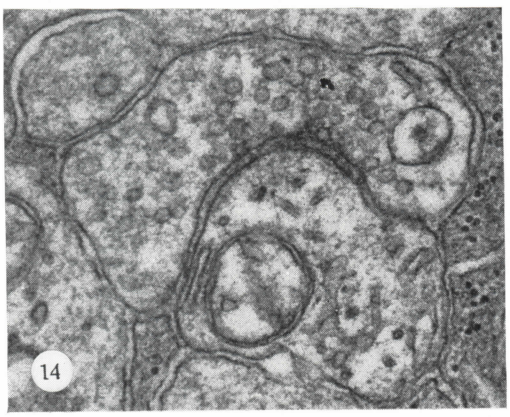


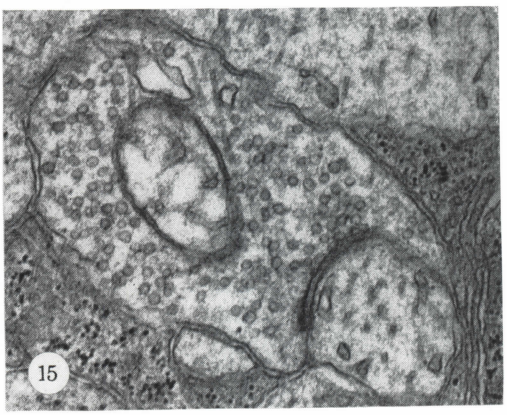


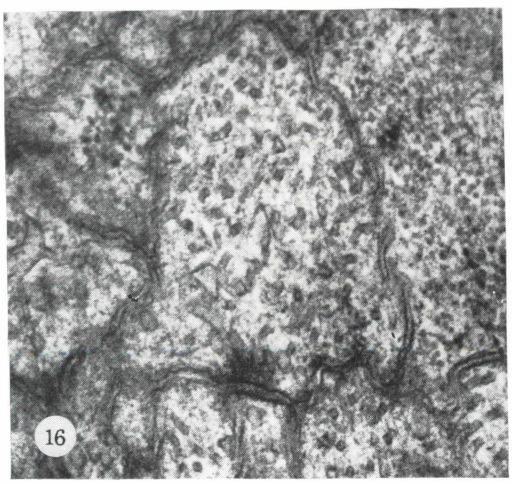
FIGURES 10–12. For description see opposite.

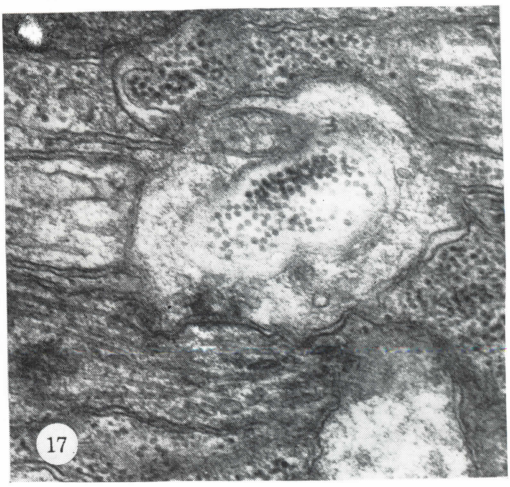
(*Facing p.* 326)











FIGURES 13–15. From the cat outer plexiform layers.

FIGURES 16 and 17. From the rabbit outer plexiform layers. All these illustrations are of presynaptic processes, which have not been identified with a particular cell type. In figure 17, the granular structure near the point of synaptic contact was quite often found associated with synaptic regions of the rabbit's A-type cell cytoplasm, see for example, figure 10. (Magn. in order of figures $\times 41\,500$; $\times 55\,500$; $\times 32\,000$; $\times 50\,500$; $\times 42\,000$.)

may be in the outer plexiform layer is open to the same comments as those given on page 325 for the unclassified synapses of the rabbit. For the reasons given below we feel that it is unlikely that the outer plexiform layers of the cat and the rabbit would differ as radically as the observations we have presented suggest.

In the outer plexiform layer of simian retinae no synapses of the kind described here have been observed (Dowling & Boycott 1966; Allen 1970; E. Raviola, personal communication; Boycott & Hopkins, unpublished). At present this seems to be a valid difference between these animals and the cat and the rabbit. There is another difference apparent at the level of light microscopy. In simian retinae (Polyak (1941) described only one morphological type of horizontal cell and this has since been confirmed by Boycott & Kolb (1973*a*) for the rhesus monkey. There is no evidence in those papers for a cell of the morphology and ultrastructure of the A-type cell of cats and rabbits. Because of sampling errors in electron microscopy it is difficult to evaluate a claim for the absence of synapses particularly in an area like the outer plexiform layer; because of the uncontrollable nature of the Golgi-staining reaction it is difficult definitively to decide that a particular kind of cell is absent (see, for example, Boycott & Kolb 1973*b* and page 331). However, assuming that these species differences are genuine, then the simplest interpretation of the present data in comparative anatomical terms is to suppose that the absence of an A-type cell and the apparent absence of synapses in the outer plexiform layer of rhesus monkey are correlated. This interpretation is only meaningful if the cat's A-type cells have synapses in the outer plexiform layer.

Figure 6, plate 58, shows a process from a cat's A-type cell to the cone pedicles. The similarity between this process and those bearing more synaptic vesicles and showing sites of synaptic contact, figures 7–9, plate 59, has been pointed out on page 322. We think that these similarities suggest that the A-type cell in the cat is indeed presynaptic in the outer plexiform layer; and that many of the outer plexiform layer synapses in the cat involve the processes between the cones and the main dendrites of the A-type cells. Thus on this interpretation the rabbit would differ from the cat primarily in having the majority of its A-type cell synapses borne on the main dendrites of the cell. Such an interpretation also emphasises, as has been pointed out by Boycott & Kolb (1973*a*), the seeming difference between simian and other mammalian retinae.

Figures 13–15, plate 61, show examples of synapses in the cat's outer plexiform layer, the profiles of whose processes do not show organelles that enable them to be classified in the manner suggested above. The arguments for the elements to which they could be attached are essentially those used when discussing the unclassified synapses of the rabbit.

(*c*) *Cell types postsynaptic in the outer plexiform layer of the rabbit and the cat*

The postsynaptic processes found in the outer plexiform layer have neurotubules but never neurofilaments in their cytoplasm. Figures 7 and 8, plate 59; figure 10,

plate 60; figure 13, plate 61 show postsynaptic processes that were traced to the perikaryon of a bipolar cell. In the cat a total of 10, and in the rabbit 5, bipolar processes were identified in this way. In addition four synapses were made directly in the cat on to a bipolar cell perikaryon; none were observed in the rabbit. These observations confirm the conclusion of Dowling *et al.* (1966) that bipolar cells are postsynaptic in the outer plexiform layer of the cat and the rabbit. Together with the evidence of the preceding discussion it is now certain that some bipolar cells are postsynaptic to A-type horizontal cells in the rabbit and it is suggested that this is so in the cat. Boycott & Kolb (1973*b*) described the relative positions of the perikarya of the different bipolar cell types in the inner nuclear layer. However none of our observations have permitted a certain judgement of whether the synapses described here were on to rod, or to cone, or to both kinds of bipolars.

Many synapses have now been observed in the outer plexiform layer where the postsynaptic processes look like the positively identified bipolar cell processes. However, because of the resemblance of these processes to B-type horizontal cell cytoplasm, the possibility of the B-type cell being postsynaptic to the A-type cell is not excluded. For the reason that their ultrastructure is as yet uncharacterized, the axon terminal system of the B-type cell and the processes of the interplexiform cells are also possible postsynaptic structures. No neurofilamentous processes have been observed to be postsynaptic.

(*d*) *Synapses between horizontal cells*

Dowling *et al.* (1966) did not find conclusive evidence for chemical synapses between horizontal cells, although they thought this likely because of the presence of scattered vesicles in processes postsynaptic to known horizontal cell processes. It is possible that some of the postsynaptic processes they saw were bipolar cell dendrites because these are sometimes observed containing scattered vesicles (see above). We found no clear evidence of the presence of chemical synapses between horizontal cells of the same type. The possibility of synapses between A and B-type cells is discussed above. However, synapses between different morphological types of horizontal cell have not been described in other vertebrate classes. Nor have chemical synapses between horizontal cells of the same morphological type been observed (Stell 1972; Lasansky 1972), except in the carp retina (Witkovsky & Dowling 1969).

Electrical coupling between horizontal cells of the same morphological type has been demonstrated in goldfish (Kaneko 1971) and correlated with the presence of gap junctions in dogfish (Stell 1972; Witkovsky & Stell 1973). In mammals electrical coupling of horizontal cells has not been reported. Gap junctions between unspecified horizontal cells in the cat's retina were first claimed by Sobrino & Gallego (1970). More recently, E. Raviola (personal communication) has found gap junctions between horizontal cells in both the rabbit and simian retinae, and J. A. Sobrino & J. E. Dowling (personal communication) have observed them in the cat. Our material was not prepared in such a way that convincing identification

of gap junctions would have been possible. However, a common observation was the presence between two A-type cell processes of extensive areas of closely apposed membranes flanked by desmosomal-like contacts. These junctions were substantially as described by Raviola & Raviola (1967) in the rabbit.

Among the mammals the types of nerve cells contacting the photoreceptors are reasonably well known for feline and simian retinae (Boycott & Dowling 1969; Kolb 1970; Boycott & Kolb 1973*a*, *b*; Kolb 1974), although there are many questions yet to be answered (Boycott 1974); similar data are becoming available for other retinae (Stell 1972; Lasansky 1971, 1973). Especially in the turtle retina physiological data suggest that synaptic interactions occur between the neural elements at their sites of contact with the photoreceptors and between these and the photoreceptors (Baylor, Fuortes & O'Bryan 1971; O'Bryan 1973). The present paper has not been concerned with the possible ultrastructural correlates of such interactions (see, for example, Lasansky 1971, 1973). Its purpose has been to examine the likely synaptic relations of the types of cells with processes in the outer plexiform layer and most particularly the horizontal cells. It has shown that the synaptic relationships within the outer plexiform layer of different morphological types of horizontal cells are not necessarily the same.

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Note added in proof 21 *May* 1974

Dowling, J. E. & Ehinger, B. (Association for Research in Vision and Ophthalomoagy abstracts, Spring Meeting 1974) have recently injected the eye of goldfish and *Cebus* monkey with 5,6 dihydroxytryptamine. This alters the fine structural appearance of the adrenergic interplexiform cells. In this way the authors have shown that the processes of interplexiform cells are postsynaptic to bipolar cells in the inner plexiform layer and presynaptic to horizontal and bipolar cells in the outer plexiform layer. In goldfish these synapses are common but even though the synaptic terminals in the *Cebus* monkey are marked out by their de-generating appearance owing to the procedures used, they are hard to find (J. E. Dowling, personal communication) and therefore may be inferred to be relatively uncommon. For this and other reasons to be discussed in Boycott *et al.* (1975), these new observations do not invalidate the arguments presented here. Presumably these new synapses may be represented among our unclassified synapses in the cat and the rabbit.