

## Interplexiform cells of the mammalian retina and their comparison with catecholamine-containing retinal cells

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[Plates 26 and 27]

Retinal interplexiform cells have processes that branch within both the inner and outer plexiform layers. Their morphology is described from Golgi-preparations of cat, rhesus macaque and squirrel monkey retinae. Comparisons are made with similar cells, known to be catecholamine-containing, which have been observed histofluorometrically in the teleost fish and New World monkeys. It is concluded that there may be more than one pharmacological type of interplexiform cell.

In addition an inner nuclear layer plexus of fibres is described for the first time from Golgi-material of the squirrel monkey's retina. Electron microscopy reveals that this plexus synapses within the inner nuclear layer on to bipolar and amacrine cells. It is compared with the catecholamine-containing inner nuclear layer plexus of New World monkeys.

### INTRODUCTION

After fixation in formaldehyde vapour, nerve cells containing certain catechol- and indoleamines fluoresce when excited with short wavelengths (420 nm). This technique has shown, in representative species of teleosts (Ehinger, Falck & Laties 1969) amphibia and reptiles (Scheie & Laties 1971), birds (Ehinger 1967; Hauschild & Laties 1973), and mammals (Ehinger & Falck 1969*a, b*), that catecholamine-containing nerve cells are a general feature of vertebrate retinae. The cells most commonly observed to fluoresce have their perikarya in the amacrine cell layer. Photoreceptor, bipolar and horizontal cells do not show catecholamine-fluorescence, nor do the great majority of ganglion cells.

The identity of retinal nerve cells described by fluorescence methods with those observed by other methods, especially the Golgi-method, has not been established

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(Ehinger & Falck 1969*a, b*; Laties 1972). At least some of the cells which fluoresce are certainly not amacrine cells as usually defined by light microscopy because they have processes which go to the outer plexiform layer, although the distribution of their processes within the inner plexiform layer resembles that of amacrine cells (Cajal 1892; Boycott 1974). Such cells are prominent in teleostean (Ehinger, Falck & Laties 1969), and New World monkeys' retinae (Laties & Jacobowitz 1966; Ehinger & Falck 1969*b*).

Amacrine-like cells with processes in both the inner and the outer plexiform layers represent a new class of retinal nerve cell that had, until recently, only tentatively been described by Golgi-methods. Cajal (1892) illustrated two cells from the dog's retina (his figure 2*g*, plate 5) that might fit this general description. In a teleost he described 'special stellate' cells (his figure 4, plate 1), with processes in both plexiform layers. And in a frog (his figure 3*h*, plate 2) he showed part of a cell which may belong to this class. The cell in the frog's retina could, however, be a different type of cell, one resembling it has been called an oligopolar cell in teleosts (Parthe 1972). Recently Gallego (1971*a*) in the cat retina, and Dawson & Perez (1973) in the dolphin retina, have identified more clearly in Golgi-material cells similar to those described by Cajal in the dog. In the retina of primates Polyak (1941, 1957) described centrifugal bipolar cells, some of which may be comparable to these cells (Gallego 1971*a, b*). Gallego (1971*b*) has introduced the term *interplexiform cell* and we propose its acceptance rather than the terms duplex amacrine which was used in our preliminary communication (Laties 1972), or centrifugal bipolar (see discussion).

The previous reports from Golgi studies of what may have been interplexiform cells, together with the data from fluorescence experiments, suggest that a cell type connecting the two plexiform layers is present in many, perhaps all, vertebrate retinae. The sporadic nature of the Golgi reports, the incompleteness of the descriptions and the appearance of the cells in the material to be described here, show that this type of cell is uncommonly refractory to Golgi techniques and that, when it does react, it usually is poorly impregnated. Thus the data to be presented are inevitably limited. Their presentation now can be justified in several ways: (1) we have been unable to improve impregnation using a wide variety of Golgi-procedures, (2) the interplexiform cells described from fluorescence microscopy may well differ pharmacologically from those seen in Golgi-preparations, (3) it is becoming clear that interplexiform cells are a distinctive class of retinal nerve cell. In this respect, Dowling & Ehinger (1975) have shown that the processes of catecholamine-containing interplexiform cells of the retinae of goldfish and *Cebus* monkey receive synapses in the inner plexiform layer and are presynaptic to horizontal and bipolar cells within the outer plexiform layer. These interplexiform cells thus provide a centrifugal pathway from the inner to the outer plexiform layers.

We have also observed a system of fibres that synapse within the inner nuclear layer of the squirrel monkey's retina. This will be referred to as the *inner nuclear*

*layer plexus*. It has not previously been described as a distinctive entity in Golgi-material of any vertebrate retina, although fluorescing fibres have been observed to form a plexus within the inner nuclear layer of several New World monkeys (Latties & Jacobowitz 1966; Ehinger & Falck 1969*b*).

#### METHODS

Most of the Golgi-material used in this study has been accumulated over the past decade. Much of it has been used in previous papers (Boycott & Dowling 1969; Kolb 1970, 1974; Boycott & Kolb 1973*a, b*; Boycott & Wässle 1974). There, Golgi-Cox, Golgi-rapid and Golgi-Kopsch procedures and their modifications are described. But none of the retinæ processed in these ways show recognizable interplexiform cells; although retrospectively what may be pieces of them have sometimes been identified in Golgi-rapid material (page 360). Together with more recent material, a total of about 250 cat and 80 rhesus monkey retinæ have been examined.

Although it was with the Golgi-rapid method that Gallego (1971*a, b*) observed his interplexiform cell, it is perhaps of some significance, in explaining why these cells have for so long remained undescribed, that all our interplexiform cells come from retinæ processed by the Colonnier (1964) modification of the Golgi-Kopsch method. This method uses glutaraldehyde instead of formaldehyde in the initial fixative. However we found no interplexiform cells when the retinæ were fixed directly in the glutaraldehyde-dichromate mixture. By chance it was discovered that fixation in phosphate-buffered-glutaraldehyde for periods of 2-6 weeks, followed by the usual glutaraldehyde-dichromate and silver procedures, was more likely to stain interplexiform cells. Our procedure has been described in Boycott & Kolb (1973*a*) where it was thought more likely to stain cone bipolar cells in the cat's retina. Interplexiform cells in a dolphin's retina were also stained in this way but after periods of only two days in the buffered glutaraldehyde (Dawson & Perez 1973). The buffered glutaraldehyde procedure does not guarantee staining of interplexiform cells; of 15 cat retinæ used only 9 had interplexiform cells. For rhesus monkey the results were similar, except that only 2 retinæ (of different individuals) out of 10 showed interplexiform cells. Of the 9 cat eyes containing interplexiform cells one had only one cell stained, each of the other retinæ had many; for the cat our description is based on about 100 interplexiform cells. Of the rhesus monkey retinæ one had 5 interplexiform cells stained and another only one. About 200 rabbit retinæ have been processed by all the procedures described for the cat and the monkey. There were no recognizable interplexiform cells in the rabbits' retinæ including the 10 processed by initial fixation in buffered glutaraldehyde. These data serve to illustrate the poor yield of cells from even the 'best' material and show that a failure to stain interplexiform cells in the rabbit's retina has to be interpreted cautiously. As the results section makes clear for *all retinæ*, the quality of those interplexiform cells that were stained was poor.

Because Ehinger & Falck's (1969*b*) results had shown there were likely to be numerous interplexiform cells in New World monkeys, we used 10 retinæ of the squirrel monkey (*Saimiri sciureus*) initially fixed in buffered glutaraldehyde for 14–18 day periods. These were more readily available than *Cebus* monkeys whose retinæ, we now know, might have been preferable (see discussion). Six of the 10 retinæ had portions of the inner nuclear layer plexus stained but only two cells in two different retinæ were identifiable as possibly interplexiform.

The retinæ with interplexiform cells came from adult cats 9 months and older and 9 month old rhesus macaques. The retinæ of squirrel monkeys and rabbits were from young adults. All retinæ were immersion-fixed on the sclera after removal of the front half of the eye and vitreous. Except for the squirrel monkeys and rabbits, which were killed with an overdose of barbiturate, the majority of the animals were obtained as described in Boycott & Kolb (1973*a, b*) at the termination of the experiments of other workers. The drawings were made, and perikarya are given as the mean of the long and short-axes, as described in Boycott & Wässle (1974). The dimensions of the retinal fields are the maximum observed for individual cells.

The sections for the Golgi-stained material were cut in celloidin at about 100  $\mu\text{m}$  thick. Those for fluorescence microscopy were cut in paraffin wax at between 10 and 20  $\mu\text{m}$ . In both instances the sizes of the cells are such that they are inevitably cut many times. Fragmentation of the cells examined by fluorescence microscopy is, of course, greater and comparisons of the morphology of cells observed by the two methods is inevitably limited by this factor.

For transmission electron microscopy the retinæ were fixed in osmium tetroxide and processed as described in Dowling & Boycott (1966). Golgi-impregnated cells used for electron microscopy were treated as described by Kolb (1970, 1974) and the same precautions taken to obtain isolated cells as are described there.

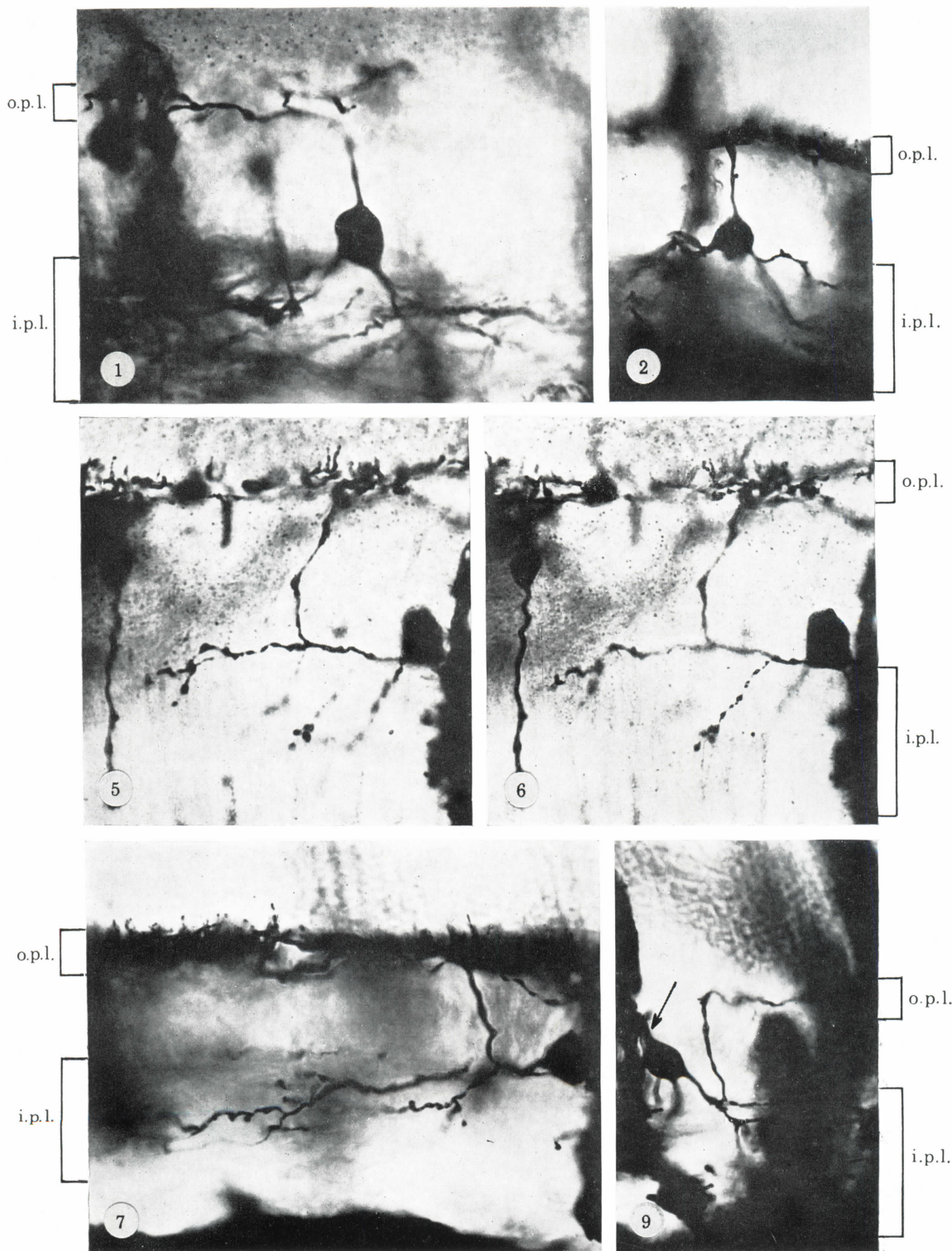
Our observations on the catecholamine-containing cells were carried out by the procedures described in the quoted papers of Ehinger, Falck and Laties.

## RESULTS

### *General description of interplexiform cells in the cat's retina*

Figures 1, 5–7 and 9, plate 26 and figures 3, 4, 8, 10 and 11 show cells representative of the main morphological variations we have observed in over 100 interplexiform cells from the cat's retina. The perikarya of the interplexiform cells are in that layer of the inner nuclear layer where the amacrine cell perikarya are situated. Their diameters vary between 9 and 12  $\mu\text{m}$ . They are thus, except in the Golgi preparations, not yet discriminable from amacrine cell perikarya. The cell in figure 2 and those in figures 3 and 4 illustrate a type of interplexiform cell with a single process to the outer plexiform layer arising on the scleral side of the perikaryon. The perikarya of figures 5–7, plate 26, and figure 8 have no scleral process but processes that arise as branches from within the inner plexiform layer. The





FIGURES 1, 2, 5-7, 9. For description see opposite.

(Facing p. 357)

morphology of the cells illustrated in figures 5–8 rather closely resembles that of the fluorescing interplexiform cells described in teleosts by Ehinger *et al.* (1969). Sometimes there appeared to be only one process from a single cell ascending through the inner nuclear layer (figures 5, 6 and 7, plate 26), at other times up to 3 or 4 ascending processes were seen (figure 10). Figure 9, plate 26 and figures 10 and 11 shows cells which have processes to the outer plexiform layer both from the perikaryon and from the inner plexiform layer processes. These differences in origin of the scleral processes i.e. whether they come from the perikarya, from processes within the inner plexiform layer or from both according to the individual cell being considered, seem likely to be genuine. But we could not decide whether the differences between individual interplexiform cells in the cat's retina represented different morphological classes, or differences in their positions in the retina relative to the central area (Boycott & Kolb 1973*b*; Boycott & Wässle 1974). Nor, because of section thickness and variations in staining, could we decide whether there are consistent differences in the numbers of processes from individual cells.

Measurement of the extent of branching of the interplexiform cells within the plexiform layers was limited by their poor staining. Within the inner plexiform layer quite thick processes (2–4  $\mu\text{m}$  in diameter) may have an irregularly beaded appearance; this is often characteristic of poor staining (figures 5 and 6, plate 26). The thicker processes may branch irregularly into fine fibres (about 0.5  $\mu\text{m}$  or less in diameter) as shown in figures 8 and 10, but only traces of such fine fibres could be seen on cells like those in figures 3, 4 and 11. Sometimes the branching covers a greater retinal field within the inner plexiform layer than the outer (figures 3 and 8), sometimes the reverse (figure 10). The cell described by Gallego (1971*a, b*) may be an extreme example of erratic staining, thus giving a much larger extent

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#### DESCRIPTION OF PLATE 26

Interplexiform cells seen in vertical sections of retinae, processed by the buffered glutaraldehyde Golgi-procedure all magn.  $\times 600$ . Intense impregnation of masses of glia and clumps of poorly stained cells and fibres are characteristic of this procedure when used on retina. o.p.l., outer plexiform layer; i.p.l., inner plexiform layer.

FIGURE 1. Rhesus monkey, another focus of this cell is in Laties (1972), all other figures from the cat.

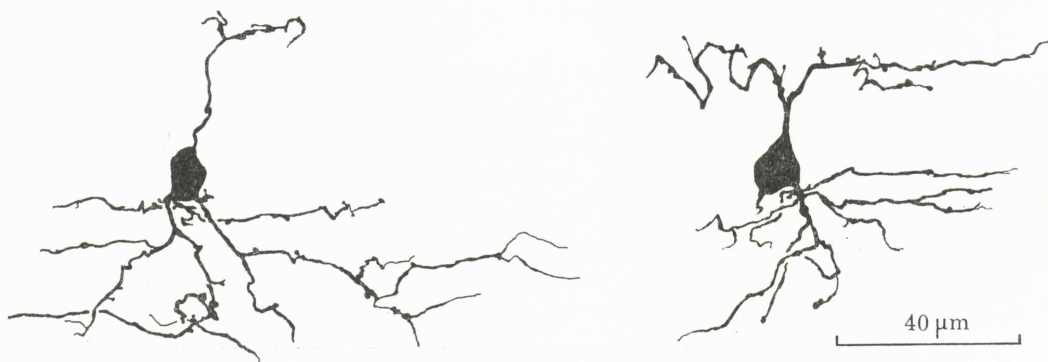
FIGURE 2. A cell whose morphology resembles those of the monkey.

FIGURES 5 AND 6, two focuses of a branch from an inner plexiform layer process ascending to the outer plexiform layer. The two focuses also illustrate well the appearance of understaining.

FIGURE 7. The cell shows processes extending through the thickness of the inner plexiform layer, as well as a fibre ascending to the outer plexiform layer. It resembled the cell shown in figure 8.

FIGURE 9. shows an interplexiform cell with a process (arrow) to the outer plexiform layer from the perikaryon and from the inner plexiform layer. It resembled the cell shown in figure 10.

of branching in the outer than the inner plexiform layer than we have observed. Quite often we found cells where the field occupied by the processes was about equal in both layers but even then we did not feel confident we were seeing the full extent of the processes. However we are sure that within the inner plexiform layer the processes of the cells are not usually confined to a single stratum. The cells in figures 2, 5, 7 and 9, plate 26, are not unistratified. The cells in figures 8 and 10 clearly show processes running through most of the strata of the inner plexiform layer. This was also true of the cells in figures 3 and 4, although they were in oblique sections where it was harder to determine their exact level of branching. Of all the cells observed only one, that illustrated in figure 11, showed evidence for branching confined to a single stratum. With this one exception, which seems therefore likely to be understained, the distribution of the processes of interplexiform cells within the inner plexiform layer most closely resembles that of diffuse amacrine cells (Cajal 1892; Boycott & Dowling 1969). This was also Gallego's (1971*a, b*) conclusion.



FIGURES 3 and 4. Two interplexiform cells from a cat's retina each showing a single process to the outer plexiform layer from the scleral side of the perikaryon. These two cells and that of figure 11 were sectioned for electron microscopy because the processes in the other plexiform layer showed small terminals we judged might be likely to make connections with the photoreceptors. All text figures are at the same magnifications and details are in the text.

In the same way estimation of the extent of branching of the interplexiform cells within the outer plexiform layer is made difficult by apparent variations in the stainability of the processes. For example, the cells of figures 4 and 11 show extensive branching when compared with what appears to be the more restricted branching shown by the cells in figures 3 and 10. The scleral processes of the cell in figure 9 are clearly incompletely stained. However, from an assessment of all the cells we have observed, we suspect that the processes of individual interplexiform cells occupy equal retinal fields within the two plexiform layers. Where several processes ascend to the outer plexiform layer from a single cell, it is likely, as is suggested by figure 11, that their branches overlap. The more restricted field



of individual processes, indicated by a cell such as that of figure 10, is likely to be due to poor impregnation. Measurements of the diameter of spread of the fields in either layer, using cells selected as having the 'best' stained processes, gave fields between 100 and 250  $\mu\text{m}$  in diameter. These dimensions differ considerably from what was reported by Gallego (1971 *a, b*). From one cell he measured a maximum field of 60  $\mu\text{m}$  in the inner plexiform layer and estimated a field of 400–500  $\mu\text{m}$  in the outer plexiform layer. Dawson & Perez (1973) followed the processes of several cells in the outer plexiform layer for up to 100  $\mu\text{m}$ . However it may be there are larger and smaller field sizes (see page 361).

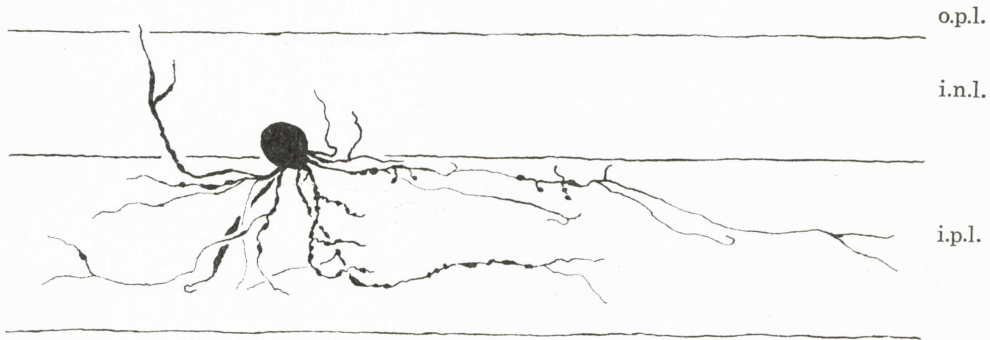


FIGURE 8. Interplexiform cell in a well-oriented section of a cat's retina showing the variation in thickness of the processes in the inner plexiform layer. o.p.l., outer plexiform layer; i.n.l., inner nuclear layer; i.p.l., inner plexiform layer.

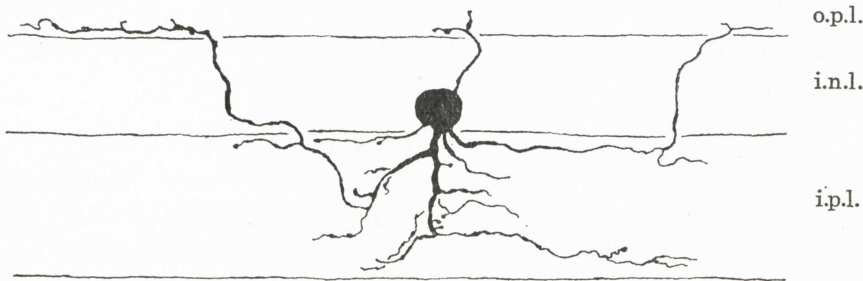


FIGURE 10. An interplexiform cell from a cat's retina.

#### *Frequency of interplexiform cells in the cat's retina*

The frequency with which the interplexiform cells stain in most Golgi-preparations can give the impression that they are rare cells. Some of our sections from individual retinæ strongly suggest that this is not so. On two occasions as many as five interplexiform cells have been found in a single 100  $\mu\text{m}$  thick section which transected most of the hemisphere of the retina. In addition, on three occasions stained interplexiform cells were observed sufficiently close together for it to be clear that their processes were overlapping. These observations are good evidence that interplexiform cells must be quite numerous in the cat's retina, and are

probably distributed throughout it. Dawson & Perez (1973) reached the same conclusion for the interplexiform cells of the dolphin's retina.

*Cajal's 'ascending nerve fibrils'*

Beginning with his earliest paper on the mammalian retina Cajal (1891) illustrated 'ascending nerve fibrils' (Cajal 1892, 1911) which, from an unknown origin had the appearance of terminating in the outer plexiform layer. Figures 12 and 13, plate 27, illustrate a nerve fibre which resembles the branching of an outer plexiform layer process of some of the interplexiform cells. Like Cajal we have observed



FIGURE 11. A cat's interplexiform cell used for electron microscopy (see figures 3 and 4). It also showed, as does a process in figure 8, that sometimes the outer plexiform layer processes branch during their passage through the inner nuclear layer.

these fibres fairly frequently in Golgi-rapid material and also in Golgi-Colonnier material. Their origin has never been ascertained. With the discovery of interplexiform cells it is perhaps reasonable to suggest that they may be ascending processes of interplexiform cells the rest of which were not stained. However, the spread of these processes in the outer plexiform layer was always small in both Cajal's and our material.

*Interplexiform cells in rhesus and squirrel monkey's retinæ*

We found only 6 interplexiform cells in the retina of rhesus monkey and two in squirrel monkey. The cells had the morphology of those illustrated in figure 1, plate 26 and figure 14. There were no indications of fibres ascending from the inner plexiform layer processes of any of the six cells, nor have we observed fibres of the kind illustrated in figures 12 and 13, plate 27. All the monkey's interplexiform cells resembled those of the cat which have a single scleral process from the perikaryon to the outer plexiform layer.

In the rhesus monkey all six cells had their processes obscured by glia but it was clear that they extended through several strata of the inner plexiform layer. Both cells from the squirrel monkey's retina had processes reaching near to the ganglion cell perikarya (figure 14); the diameter of spread of the processes of both cells within the outer and inner plexiform layers was about equal. The same problems of inequality of staining of processes in the inner and outer plexiform layers as have been discussed for the cat (page 358) were encountered with all the six cells of the rhesus macaque. For the rhesus monkey the maximum retinal fields

occupied in either layer were between 200 and 300  $\mu\text{m}$ , for squirrel monkey between 50 and 80  $\mu\text{m}$ . The perikaryal diameters were about 12  $\mu\text{m}$  for the rhesus monkey and 8  $\mu\text{m}$  for the squirrel monkey. As with the cat our data could not be specified with respect to the central area. It is known for horizontal cells in the rhesus monkey's retina (Boycott & Kolb 1973*b*) and for two types of ganglion cell in the cat's retina (Boycott & Wässle 1974) that the dimensions of their dendritic fields and perikarya can increase by factors of between 5 and 10 with increasing retinal eccentricity. It is possible that the fields of the interplexiform cells and their perikaryal dimensions may vary in a similar manner. Thus larger and smaller interplexiform cell field sizes than we have given here may be found in all these animals. No cells which could be interpreted as interplexiform cells were described in *Aotes* retina by Ogden (1974).

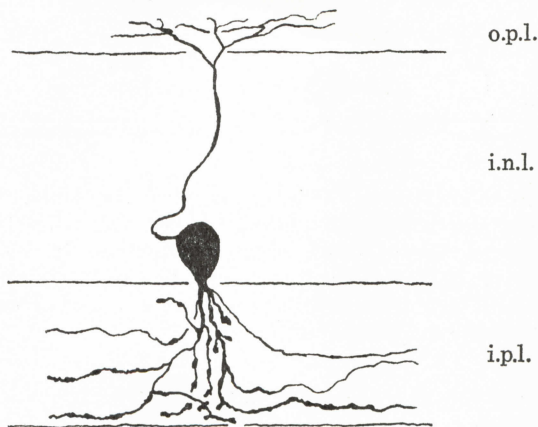


FIGURE 14. A possible interplexiform cell from a squirrel monkey's retina. The abrupt manner in which the horizontally running processes are represented as ending is because the cell was framed by impenetrably stained glia. The morphology of this cell resembles some of those described as centrifugal bipolars by Polyak (1941); see, for example, those of figures 61B and 66B and E from the retina of chimpanzee.

#### *Inner nuclear layer plexus of the squirrel monkey*

Within the inner nuclear layer of the squirrel monkey the Golgi-staining showed an extensive system of fibres passing in between the perikarya. There appears to have been no previous description of such a structure from Golgi-material in any vertebrate retina. Pending proof of the origin of the fibres they will be referred to as the inner nuclear layer plexus.

Figure 15 is an example of how these fibres may appear in vertical sections. Lengths up to 100  $\mu\text{m}$  were observed in a single section but more often only shorter pieces were found. On the course of the fibres occasional swellings about 1.0  $\mu\text{m}$  in diameter were seen. The fibres as observed in Golgi-preparations appeared to have no beginning and no end, and there was no regular branching pattern. A single fibre varied between so fine as to be barely resolvable in a thick section and a maximum of about 2.0  $\mu\text{m}$  in diameter. The fine branches could sometimes

be traced to the edge of the outer and inner plexiform layers, where they were then lost.

By electron microscopy of the inner nuclear layer we found bundles of fine fibres, individual members of which synapsed on to perikarya of that layer. Occasional loops of amacrine processes have been observed to be presynaptic within the inner nuclear layer and somato-somatic synapses have been described in the cat (Fisher 1972). But an extensive system of bundles of synapsing fibres appears not to have been observed previously. We suppose that the single fibres observed in the

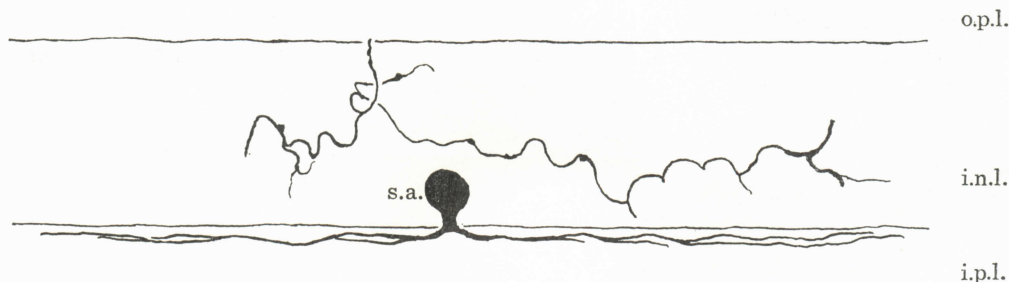


FIGURE 15. Diagram of an example of the inner nuclear layer plexus when Golgi-stained. As electron microscopy shows (figure 16 plate 27) such a single fibre is probably a bundle of fibres. A stratified amacrine cell (s.a.), whose processes are in the outer stratum of the inner plexiform layer, is illustrated. The distribution of its processes at the outer edge of the inner plexiform layer resembles the distribution of the processes of the adrenergic junctional cells in many mammalian retinæ.

Golgi-material and the bundles observed in the electron microscope are equivalent.

Figure 16, plate 27, shows within the inner nuclear layer of the squirrel monkey a bundle of nerve fibres containing individual fibres of  $0.1\text{--}0.3\ \mu\text{m}$  in diameter. Although small in diameter serial sections showed that an individual fibre could increase to a diameter of about  $1.0\ \mu\text{m}$  and, when it did so, it usually made synaptic contact with a perikaryon or dendrite. Figure 17, plate 27, shows synapses on to a perikaryon in the amacrine cell layer, others were observed on to the perikarya and the part of the dendrite near the perikaryon of bipolar cells. The synapses in the amacrine cell layer could have been onto amacrine or

#### DESCRIPTION OF PLATE 27

FIGURES 12 AND 13. Two focuses of an 'ascending fibril of Cajal'. For details see page 360. Golgi-rapid (magn.  $\times 600$ ). Cat.

FIGURE 16. Transverse section through a bundle of fibres of the inner nuclear layer plexus running horizontally between the perikarya of the inner nuclear layer. This shows 16 fibres ranging in size from about  $0.1\ \mu\text{m}$  in diameter to  $0.3\ \mu\text{m}$ . The overall diameter of the bundle was about  $0.75\ \mu\text{m}$ . Squirrel monkey.

FIGURE 17. Fibres from a bundle of the inner nuclear layer plexus (thick arrow) enlarging to form synapses (thin arrows) on the perikaryon of an amacrine or interplexiform cell. Squirrel monkey.

interplexiform cells. There is, as yet, no way to distinguish between the perikarya of the two cell types. The problem of the possible origin of the fibres of the inner nuclear layer plexus is discussed on page 366.

*Combined light and electron microscopy of interplexiform cells*

Our attempts to identify the synaptic relationships of Golgi-impregnated interplexiform cells were limited by the fact that those fixation procedures which produced interplexiform cells adequate for light microscopy, did not give fixation of the surrounding tissues suitable for identification of synapses presynaptic to the stained cell. The fixation was adequate, however, for an attempt to see if there were invaginating connexions with the photoreceptors. Two rhesus monkey interplexiform cells and five from the cat were examined. Figures 3, 4 and 11 illustrate three of the cells used. The results showed that neither rods nor cones receive invaginating processes from interplexiform cells. Occasionally, as illustrated in figure 19 of Laties (1972), an interplexiform cell process came very close to a cone pedicle but we could not obtain evidence that such processes were forming flat contacts with the cones in the manner of flat bipolar cells in the rhesus monkey and the cat (Kolb 1970; Boycott & Kolb 1973*a*). Thus it is likely that the synapses of the Golgi-stained interplexiform cells, like the synapses of the amine-containing interplexiform cells of goldfish and *Cebus* monkey, are not with the photoreceptors but within the outer and inner plexiform layers.

#### DISCUSSION

Whatever doubts may exist as to the adequacy with which they have been stained, there is no doubt from our observations, together with those of Gallego (1971*a, b*) and Dawson & Perez (1973), that there is a previously inadequately recognized class of nerve cell in the mammalian retina with perikarya at the inner edge of the inner nuclear layer and processes within both the outer and inner plexiform layers. The morphology of the processes within the inner plexiform layer resemble those of diffuse amacrine cells (see also Gallego 1971*a, b*). For this reason, and because the retinal field occupied is between about 100 and 250  $\mu\text{m}$  in the four species (cat, rhesus monkey, squirrel monkey and dolphin) in which they have now been described in some detail, they are to be considered distinct from bipolar cells (Gallego 1971*a, b*; Dawson & Perez 1973). It is likely that some of the cells which Polyak (1941, 1957) described as centrifugal bipolars included interplexiform cells (see figure 14); but Polyak thought that centrifugal bipolars contacted photoreceptors and he included in their morphological characterization many cells which are certainly amacrine. For these and other reasons (see Boycott & Dowling 1969) we think that Gallego's (1971*a, b*) term is a more suitable one. As described in the introduction, the evidence from previous and from the present Golgi-studies, together with that of the fluorescence observations, suggests that

there may well be homologous interplexiform cells in retinae from all classes of vertebrates.

Our initial expectation was that the interplexiform cells from the Golgi-stained material would be homologous with the catecholamine-containing interplexiform cells. A cell such as that in figures 5 and 6, plate 26 from the cat's retina has obvious morphological resemblances to the fluorescing interplexiform cells described by Ehinger *et al.* (1969) in the teleostean retina. However, although the cells may be morphologically homologous in the sense that they have processes in both plexiform layers and generally a perikaryon at the inner edge of the inner nuclear layer, they almost certainly differ pharmacologically in different species. The reason for this conclusion is that no significant numbers of fluorescent fibres have been found, either in the inner nuclear or the outer plexiform layers of the cat's retina (Ehinger 1966; Laties & Jacobowitz 1966; Ehinger & Falck 1969*a*; Laties 1972). Yet we have stained many interplexiform cells by the Golgi-method in the cat's retina and they are clearly a significant proportion of the population of retinal nerve cells (see page 359). Thus their processes would have been unlikely to have been missed had they been catecholamine-containing, especially since Laties & Ehinger in unpublished work have independently tried many times in recent years to demonstrate catecholamine-containing inner nuclear layer and interplexiform connexions in the cat's retina. Treatment of retinae with 5,6 dihydroxytryptamine, methyl dopamine and monoamine oxidase inhibitors, all of which increase the probability of revealing catecholamine-containing cells by fluorescence microscopy (Dahlström, Haggendal & Atack 1973), have not found the cells. The only type of catecholamine-containing cell with a perikaryon in the inner nuclear layer in the cat's retina is the adrenergic junctional cell. The processes of this cell are confined to the outer stratum of the inner plexiform layer. Since there are no fluorescing fibres external to this layer in the cat's retina and the processes of the cat's interplexiform cells are not confined to a single stratum of the inner plexiform layer (page 358), it can be concluded that, within the limits of sensitivity of the histofluorometric method (Norberg 1967; Björklund, Falck & Owman 1972), this retina has no catecholamine-containing interplexiform cells. Ehinger (unpublished) has made similar observations on the rabbit's retina and in addition, by electron microscopy, has been unable to observe degeneration in the outer plexiform layer of retinae loaded with 5,6 dihydroxytryptamine.

The situation in many other mammals is similar. No catecholamine-containing fibres have been observed in the outer plexiform layer of retinae of the dog, guinea-pig, mouse and flying-fox; although these retinae, like those of all mammals, have adrenergic junctional cells whose processes are within the inner plexiform layer. (Laties & Jacobowitz 1966; Ehinger 1966; Ehinger & Falck 1969*a*; Laties 1972; Ehinger 1973.) But there is as yet no consistent general picture because the white rat has some catecholamine-containing fibres that reach the outer plexiform layer (Ehinger & Falck 1969*a*; Laties unpublished) and occasional processes of this kind are observed in rhesus monkey (Laties 1972). Furthermore fluorescent

fibres are regularly observed in the outer and inner nuclear layers of several genera of New World monkeys, where they may be present in a considerable density, particularly in *Cebus* monkey (Laties & Jacobowitz 1966; Ehinger & Falck 1969b).

Clearly from our comparison of the Golgi-stained retinæ of the cat with those examined histofluorometrically, absence of catecholamine-containing interplexiform connexions does not mean a retina has no interplexiform cells. For example, we have not stained interplexiform cells in the rabbit's retina; yet this does not prove that they are absent. The data given in the methods section, together with the fact that interplexiform cells have been discovered only comparatively recently in Golgi-material, show that negative evidence from Golgi-material may well be unreliable. Indeed there is circumstantial evidence for the presence of interplexiform cells in the rabbit from the data of Fisher & Boycott (1974). They were unable to relate about 30% of the presynaptic structures observed in the rabbit's outer plexiform layer to a cell type, and therefore suggested that some of these could be from interplexiform cells.

That interplexiform cells are presynaptic in the outer plexiform layer has been shown by Dowling & Ehinger (1975). Degeneration of these fibres was induced by loading the retina with 5,6-dihydroxytryptamine in *Cebus* monkey and goldfish. The interplexiform cell processes were found to be pre- and post-synaptic in the inner plexiform layer and presynaptic to horizontal and bipolar cells in the outer plexiform layer. Perhaps, although they are pharmacologically different, similar synaptic relationships are likely for the Golgi-stained interplexiform cells we have described here.

Not only do New World monkeys among mammals have the greatest frequency of catecholamine-containing fibres in the outer plexiform layer, they also have a catecholamine-containing plexus of fibres in the inner nuclear layer. In *Cebus* monkey, Ehinger & Falck (1969b) described cells whose perikarya are at various levels within the inner nuclear layer and which, therefore, appeared to be distinct from adrenergic junctional cells. They called these cells adrenergic pleomorph cells. Because in *Cebus* monkey there is a great density of catecholamine-containing fibres in both the inner nuclear and outer plexiform layers, Ehinger & Falck (1969b) suggested that the adrenergic pleomorph cells were providing the majority of fluorescing processes in these layers. In spider monkey, however, we have only rarely seen adrenergic pleomorph cells, yet there is an inner nuclear layer plexus and numerous fluorescing processes in the outer plexiform layer. In squirrel monkey, in which pleomorph cells have not been observed, there is also an inner nuclear layer plexus, although there are fewer fluorescing processes in the outer plexiform layer. It is, therefore, difficult to assign either the inner nuclear layer plexus, or fluorescing outer plexiform layer processes, exclusively to adrenergic pleomorph cells. These observations suggest that the retinæ of the different genera of New World monkeys may have different proportions of catecholamine-containing cells and indicate a possible interpretation of the adrenergic pleomorph cells. In squirrel

monkey, which has the least outer plexiform layer fluorescence, virtually all fluorescing perikarya are found along the inner edge of the inner nuclear layer (Ehinger & Falck 1969*b*, figure 3, p. 367), indicating that the catecholamine-containing interplexiform cells have their perikarya in that layer. The spider monkey would seem to be intermediate in that it has more fluorescing outer plexiform layer processes together with a few pleomorph cells. In the *Cebus* monkey the catecholamine-containing outer plexiform layer processes are at a higher density correlated with more numerous adrenergic pleomorph cells. On the evidence of such a series adrenergic pleomorph cells may tentatively be regarded, not as a unique feature of the *Cebus* monkey retina, but as an example of interplexiform cell perikarya displaced from the inner edge of the inner nuclear layer.

The inner nuclear layer plexus fibres have been identified in Golgi-prepared retinæ and by electron and fluorescence microscopy. Regardless of the methodology used the composition and origin of this plexus is obscure. Clearly, from the description given above, some of the fibres must be processes of catecholamine-containing interplexiform cells ascending to the outer plexiform layer; but there may also be fibres in the plexus which are not catecholamine-containing. At present all we can attempt to interpret are those fibres which fluoresce.

Ehinger & Falck (1969*b*) did not think that the fluorescent plexus was exclusively associated with the adrenergic pleomorph cells. We have seen in monkey and spider monkey, as Ehinger & Falck (figure 4, p. 368), have observed in their platyrrhine material, that fluorescent fibres leave the inner plexiform layer and loop around perikarya in the inner nuclear layer, often forming pericellular baskets. Such fibres may also ascend through several cell body diameters of that layer. They probably give rise to at least some of the synapses we have described in that layer and may well, having passed through it, turn back into the inner plexiform layer.

Because they are so fine these fibres are difficult to resolve and trace by light microscopy. They could have any of three possible origins. They could be: (1) processes of alloganglion and eremite cells (Ehinger 1966; Laties & Jacobowitz 1966; Ehinger & Falck 1969*a, b*). But these authors have not been able to trace the processes of such cells as going anywhere other than within the inner plexiform layer. So it is unlikely that they contribute to the inner nuclear layer plexus; (2) centrifugal fibres, since these have been observed in other vertebrates to push in between and synapse on the perikarya of amacrine cells (Cajal 1892, 1911; Dowling & Cowan 1966). But Ehinger & Falck (1969*a*) have shown that there is no fluorescence in the optic nerves even when they have been crushed for 24 h prior to fixation and examination. So the catecholamine-containing fibres of the retina have their origin within and are confined to the retina and are not centrifugal from the brain; (3) processes from adrenergic junctional cells. These are the cells in the retina that are most likely to be forming the fluorescent component of the inner nuclear layer plexus. It is from these amacrine cells that we suggest most of the fluorescing fibres of the inner nuclear layer plexus arise.



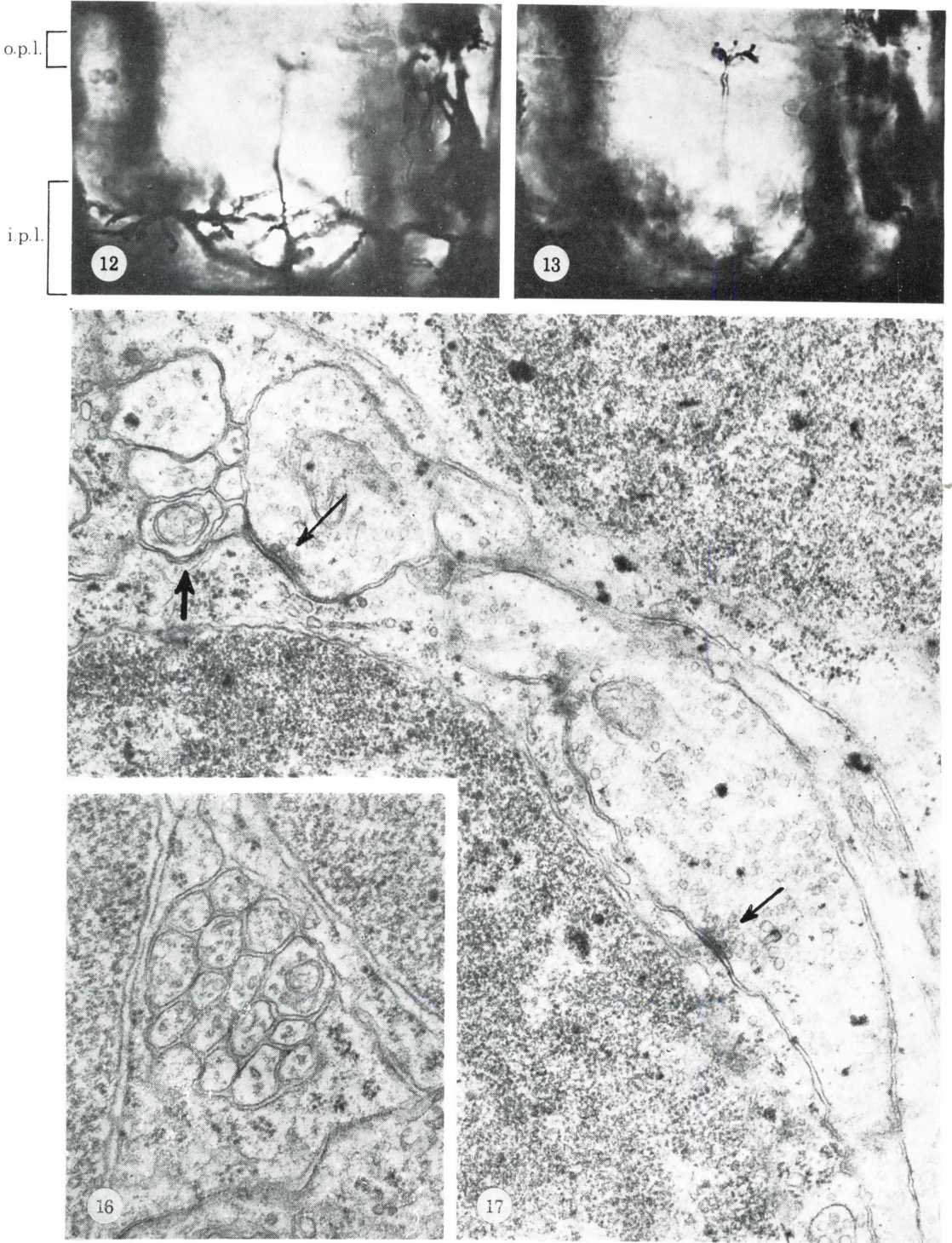
In many species of mammals there is no inner nuclear layer plexus and on first inspection the fluorescing processes of the adrenergic junctional cells are confined to the inner plexiform layer (Ehinger 1966; Laties & Jacobowitz 1966; Ehinger & Falck 1969*a, b*; Laties 1972). However both in Golgi-preparations and by electron microscopy, loops of amacrine processes into the inner nuclear layer have been seen to make synapses there. There has been no published systematic description of such processes, but Ehinger (1966) illustrated in several mammals fluorescent processes from adrenergic junctional cells that pushed between the perikarya just at the inner edge of the inner nuclear layer. It is tempting to suggest that in all mammals there are some synapses within the inner nuclear layer onto the perikarya of bipolar, amacrine, and/or interplexiform cells; and that the difference between most mammals and New World monkeys is one of the degree to which this occurs. This suggestion has received further support from an ape's retina. Laties (unpublished) has obtained preliminary evidence for the human retina of fluorescent fibres in the inner nuclear layer similar to those of squirrel and spider monkeys. Why New World monkeys and an ape should apparently differ significantly from many other mammals in the depth of the distribution of the synapses within the inner nuclear layer of the retina remains to be seen.

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FIGURES 12, 13, 16, 17. For description see opposite.

(Facing p. 362)