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Synaptic organization of the frog retina: an electron microscopic analysis comparing the retinas of frogs and primates

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[Plates 20 to 27]

The synaptic contacts in the inner and outer plexiform layers of the frog retina have been identified and studied by electron microscopy. In the inner plexiform layer, two types of synaptic contact were recognized. One type, believed to be the synaptic contact of the bipolar terminals, is characterized by a synaptic ribbon in the presynaptic cytoplasm. At such ribbon contacts, there are ordinarily two postsynaptic elements, both of which usually contain numerous synaptic vesicles and appear morphologically identical. The second type of synaptic contact in the inner plexiform layer has a more conventional morphology and is observed very much more frequently than are the ribbon contacts. It is characterized by a dense aggregation of synaptic vesicles clustered close to the presynaptic membrane and is thought to be the synaptic contact of the amacrine processes. The conventional synapses are presynaptic to ribbon-containing processes, ganglion cell dendrites, and other amacrine cell processes. Reciprocal contacts between processes making ribbon synapses, and processes making conventional synapses are often observed. Serial synapses between morphologically identical processes, presumably amacrine processes, are frequently seen; and up to four synapses in series between five adjacent processes have been observed. These findings suggest that in the inner plexiform layer of the frog: (1) bipolar terminals synapse primarily with amacrine processes; (2) amacrine processes synapse extensively with the processes of other amacrine cells; and (3) ganglion cells are driven primarily by the amacrine cells.

In the outer plexiform layer, processes penetrate into invaginations in the bases of the receptor terminals and lie in close proximity to the synaptic ribbons of the terminals, where the processes presumably receive synaptic input from the receptors. Elsewhere in the outer plexiform layer, knob-like processes, probably from horizontal cells, make conventional synaptic contacts with other horizontal cell processes and probably with bipolar dendrites.

Introduction

Recent physiological studies have revealed much about the analysis and coding of information in the visual system. In the cat and the monkey, for example, it has been shown that along the visual pathway the neurons at each succeeding level respond preferentially to increasingly more complex stimuli. That is, cells in the retina and lateral geniculate body respond vigorously to small spots of light projected onto the retinal visual fields (Kuffler 1953; Hubel & Wiesel 1960, 1961, 1966); but in the visual cortex, cells respond best to more specific stimuli such as lines or edges (Hubel & Wiesel 1959, 1962). Other cells in the visual cortex respond vigorously to highly oriented and moving figures or shadows and hardly at all to spots of light. Thus, a primary function of the visual system is to enable individual neurons to code certain critical features of the visual image.

Studies on the visual system of the frog have aroused considerable interest because many ganglion cells in this amphibian retina already demonstrate a

complexity of response behaviour that is comparable to neurons in the visual cortex of the cat (Maturana *et al.* 1960; Lettvin *et al.* 1961; Grüsser–Cornehls, Grüsser & Bullock 1963). For example, many ganglion cells in the frog retina respond preferentially to oriented edges and to movement. In addition, there are complex features of the responses of ganglion cell neurons of the frog, such as erasability, that have not as yet been observed anywhere in the cat visual system.

One might expect to observe a considerable elaboration of neuronal relationships in the frog retina to account for the complexity of ganglion-cell behaviour. However, examination of the frog retina with the light microscope has revealed few obvious differences of structure or cell types that can be correlated with functional complexity. It is true that the inner plexiform layer in the frog shows a suggestion of layering, which has been recently emphasized by Lettvin and his colleagues (Lettvin *et al.* 1961); but the significance of such layering in the inner plexiform layer is unclear at the present time (Boycott & Dowling, in preparation).

Figure 1 is a slightly modified drawing from Cajal (1911) showing the cell types in the frog retina as revealed by Golgi-stained material. The receptor, horizontal, bipolar, amacrine, and ganglion cells are typical of such cells in vertebrate retinas.

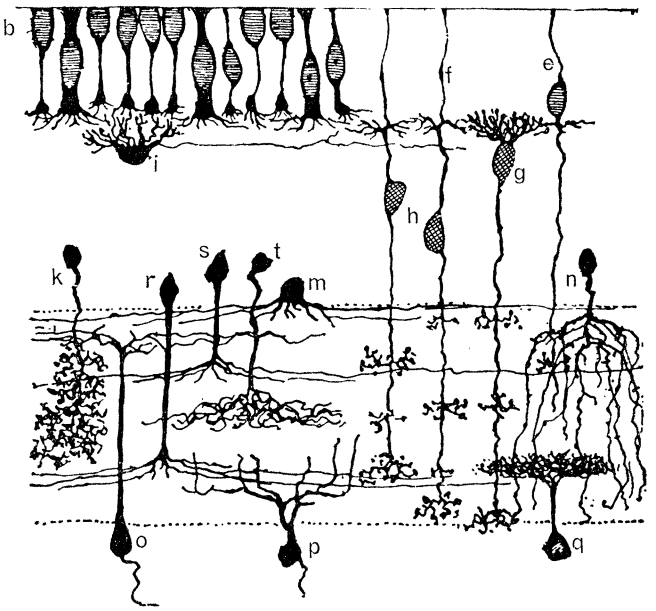


FIGURE 1. Drawing showing typical cells in a frog retina stained by Golgi method. Slightly modified from Cajal (Cajal 1911). Receptors (b); horizontal cell (i); bipolar cells (h, g); displaced bipolar cell (e); Landolt club (f) [distal process found on many of the bipolar cells]; amacrine cells (k, r, s, t, m, n); displaced amacrine (q); ganglion cells (o, p).

The processes of many of the cells in the inner plexiform layer are somewhat finer and more numerous than are observed in the primate retina; but beyond these observations, light microscopy provides few clues for understanding the anatomical basis for the complex physiology of the frog retina.

Rencetly, the synaptic organization of the central area of the primate retina has

been studied by electron microscopy (Dowling & Boycott 1966). In the inner plexiform layer of primates the bipolar terminals are large and easily identified in electron micrographs. The synapses of the bipolar terminals are characterized by densely staining synaptic ribbons or bars that are found close to the plasma membrane of the terminal and at right angles to it. The ribbons typically point between two postsynaptic processes. A cluster of synaptic vesicles surrounds each ribbon, and membrane densities can be seen (with suitable fixation) on the presynaptic membrane adjacent to the ribbon and also on both postsynaptic elements. This dual synaptic contact of the bipolar terminal is called a 'dyad'. In primates, it is usually possible to identify one of the postsynaptic elements as a ganglion cell dendrite, and the other postsynaptic element as an amacrine cell process.

The amacrine processes in the primate retina were found to contain synaptic vesicles and to make synaptic contacts with the bipolar terminals, ganglion cell dendrites and perikarya, and other amacrine cell processes. As compared with the ribbon synapses of the bipolar terminals, the amacrine synaptic contacts have a more conventional morphology. The synapses of the amacrine processes are characterized by a dense aggregation of synaptic vesicles on the amacrine-cell side of the contact and by densities on both the presynaptic and postsynaptic membranes. More than half of the amacrine synapses in the primate retina are on the bipolar terminals; and often an amacrine synapse on a bipolar terminal is observed adjacent to a ribbon synapse of a bipolar terminal. This arrangement suggests a reciprocal junction or feedback between the amacrine process and the bipolar terminal. Serial sections show that probably all bipolar terminals have reciprocal synaptic contacts with amacrine processes.

In the outer plexiform layer of primates, synaptic ribbons are found above invaginations in the receptor terminals; and these ribbons are believed to indicate the site of synaptic contact of the receptors. In cone terminals, three processes penetrate into each invagination. The central element is a dendrite of a midget bipolar cell, whereas the two lateral elements appear to come from horizontal cells (Missotten 1965; Stell 1965). In rod terminals, four or more processes penetrate into a single invagination; the more central processes are again bipolar dendrites, and the lateral processes are derived from the horizontal cells (Missotten 1965). Besides these relatively specialized synaptic contacts, there is also a presumed synaptic contact (without clear morphological specializations) between the flat bipolar dendrites and the bases of the cone pedicles (Missotten 1965). No other synaptic contacts in the outer plexiform layer of primates have been identified. In some mammals, such as the rabbit or the cat, synapses made by horizontal cells have been observed (Dowling, Brown & Major 1966). These synapses are between horizontal cell processes and bipolar dendrites and between horizontal cell processes.

The present investigation was undertaken to study the synaptic contacts of the frog retina and to see in what ways they might be similar to, and different from, the synaptic contacts in the primate retina. In particular, some understanding of the synaptic contacts and cells involved in establishing the complex physiological properties of the frog ganglion cells was sought.

Methods and materials

Medium-sized frogs (*Rana pipiens*) were decapitated, the eyes excised, and the anterior portion of the eye dissected away. The central portion of the back of the eye was immersed in cold 1·5 % OsO_4 buffered with 0·1 M veronal acetate containing 0·7 ml. of 1 % $CaCl_2$ and 30 mg/ml. of sucrose. After 20 min in the cold, fixation was continued for 1 h at room temperature. The tissues were dehydrated in a graded series of ethanol–water mixtures. When the tissues reached absolute alcohol, they were further dissected into pieces about 5 mm on each side and were flat embedded in Araldite or Epon epoxy resin. Sections were cut with glass knives on an MT-2 Porter–Blum microtome, stained for 10 to 30 min with lead citrate, and observed in an RCA EMU-3G electron microscope.

Observations

Inner plexiform layer

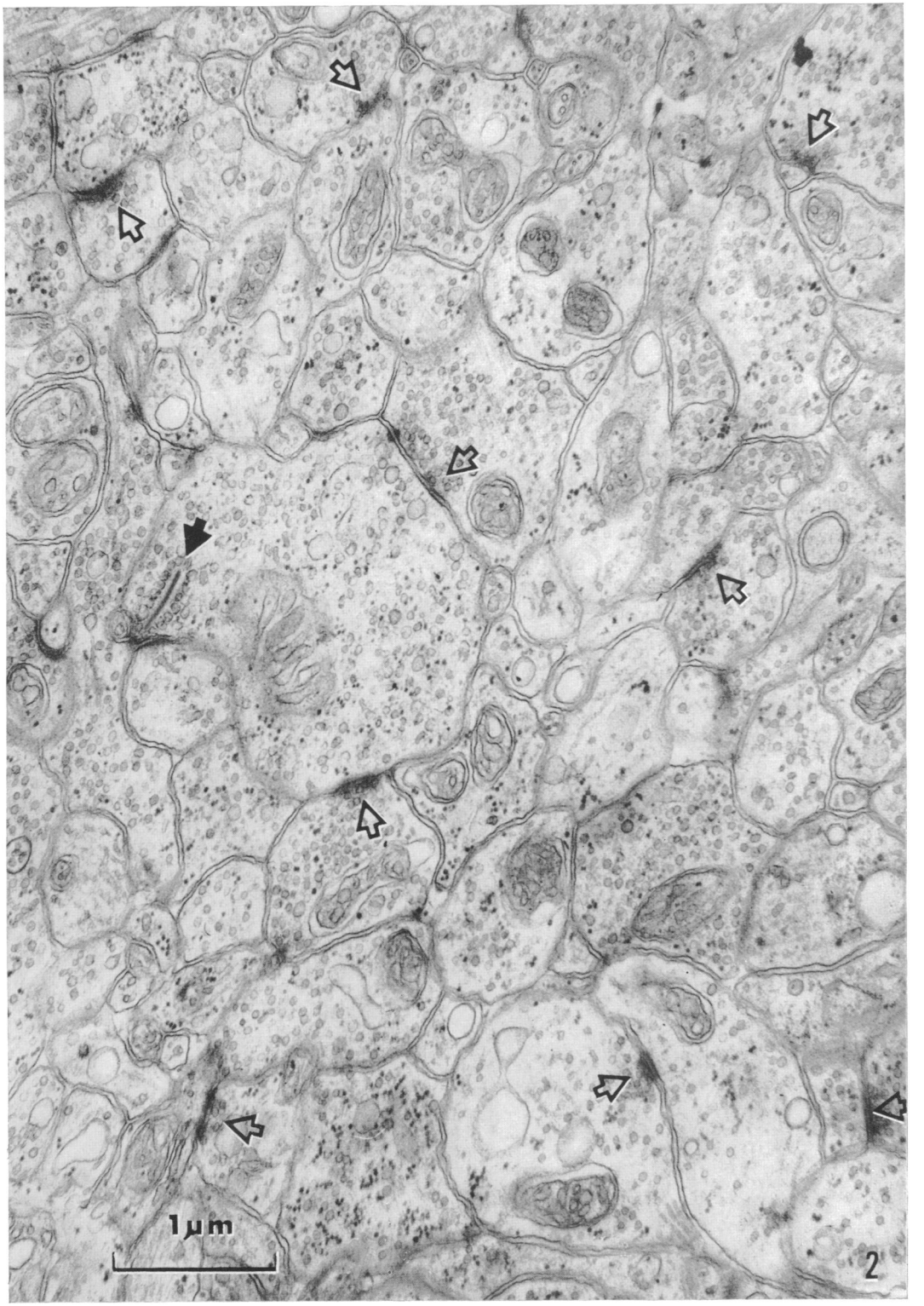
Three striking features of the inner plexiform layer in the frog are immediately obvious in electron micrographs (figure 2, plate 20). (1) The majority of the processes are small and fairly uniform in size, especially in comparison with the processes in the inner plexiform layer of primates. In the frog, processes are seldom larger than 1·5 to 2 μm in diameter, and the average profile is usually only about 0·5 μm in diameter. In the central region of the primate retina numerous processes may be as large as 6 to 8 μm in diameter, and the processes are most often about 1 to 2 μm in diameter. (2) In the frog the great majority of the processes in the inner plexiform layer contain numerous synaptic vesicles. Relatively few processes are without such vesicles and can be considered as possible dendrites. (3) Numerous synapses are observed scattered throughout the inner plexiform layer, many more than are seen in equivalent areas in the primate retina. For example, table 2 shows that there are approximately 3 times as many synaptic junctions per unit area in the frog as there are in the primate retina.

Synaptic contacts

Two types of presumed synaptic contacts are readily distinguished in the inner plexiform layer of the frog. These are morphologically similar to the two types of axodendritic synaptic contacts found in the inner plexiform layer of primates. The first type is characterized by a synaptic ribbon surrounded by a cluster of synaptic vesicles in the presynaptic cytoplasm close to the plasma membrane of the process (figure 3*a*, plate 21). The ribbon is usually directed between two

Description of plate 20

Figure 2. Low-power electron micrograph of portion of inner plexiform layer in frog. The processes are quite uniform in size and usually contain synaptic vesicles. Probable synaptic contacts are marked by open arrows; the lone ribbon contact, by a filled arrow. Glycogen particles are scattered throughout all the processes in frog retina. (× 25 000.)



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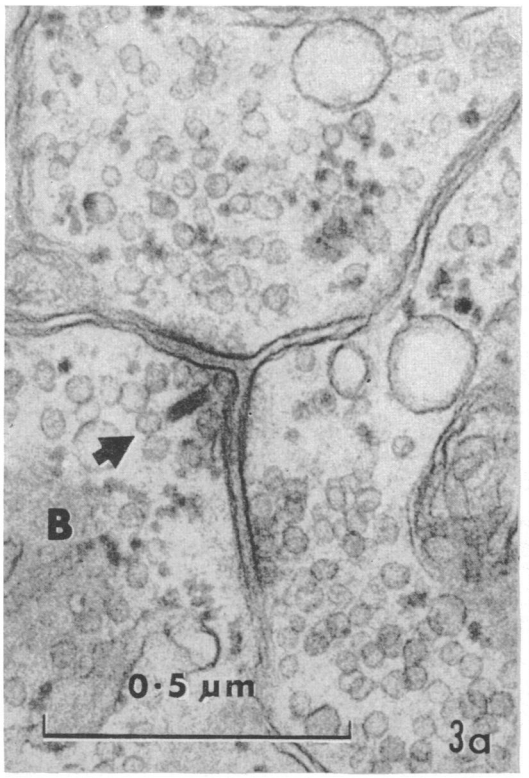

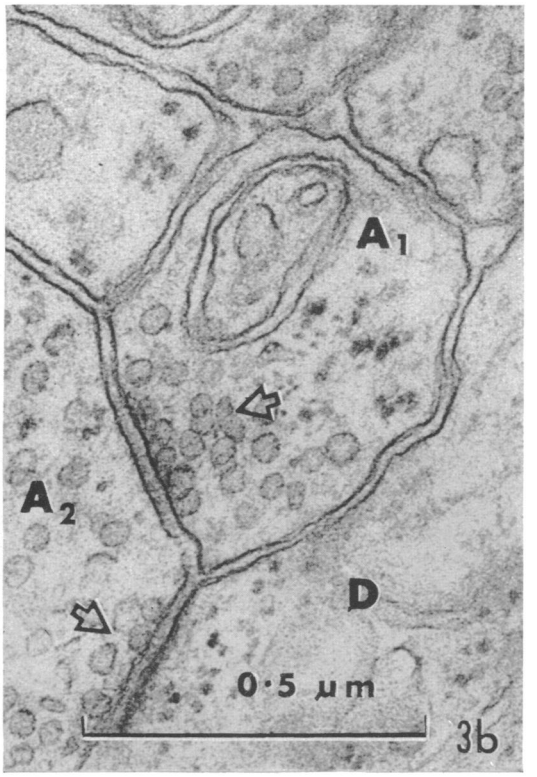

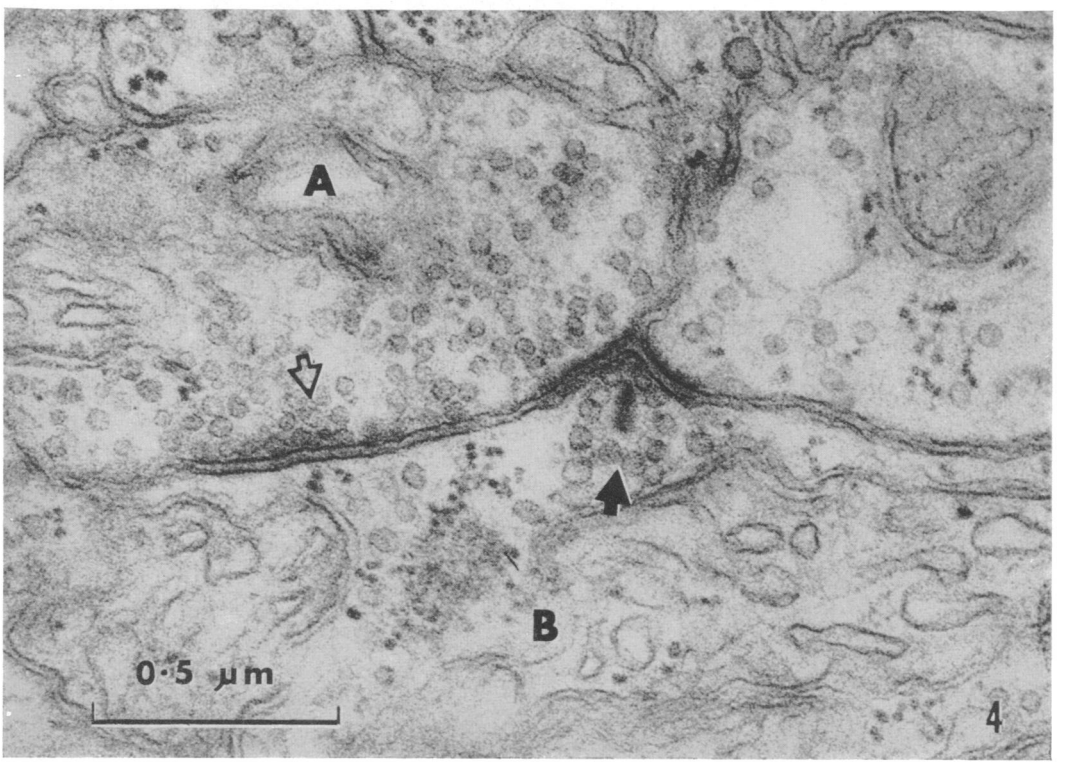

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postsynaptic elements, and membrane densities* are observed on the presynaptic membrane and on both postsynaptic elements. Thickening* of the postsynaptic membrane is also usually observed, and the cleft between pre- and postsynaptic elements is wider than the space ordinarily observed between processes at non-synaptic sites.

Similar ribbon contacts in the primate retina were identified positively as belonging to the bipolar terminals (Dowling & Boycott 1966). In guinea pigs (Allen, in preparation), rabbits (Raviola & Raviola 1967), teleost fishes (Goodland 1966), and rats (Dowling, unpublished) it also has been shown that bipolar terminals possess synaptic ribbons and make similar ribbon contacts. In the frog, the bipolar terminals are small; and as yet it has not been possible to identify positively the ribbon contacts as belonging to bipolar terminals. Since in all known cases the bipolar terminals have been shown to contain ribbons, it seems likely that in the frog also the ribbon contacts belong to the bipolar terminals; and there is some evidence supporting this assumption. For example, some of the larger processes in the inner plexiform layer of the frog contain ribbons, and these processes correspond in size to the larger bipolar terminals observed in Golgi-stained material.

In no process possessing a ribbon synapse has a synaptic contact of a second type been observed. Since the ribbons in the inner plexiform are small, however, an oblique or peripheral section near a ribbon does sometimes give the appearance of a dense cluster of vesicles near the plasma membrane in a process containing a synaptic ribbon, thus suggesting a second type of contact in the process. All such observations have been sufficiently equivocal so that I believe processes possessing

* At most synapses, the membranes on both the pre- and postsynaptic elements stain more densely; this will be referred to here as 'membrane densities'. In addition, cytoplasmic material is frequently seen associated with the postsynaptic membrane, giving the membrane a somewhat thickened appearance; this will be referred to as 'membrane thickening'.

Description of plate 21

Figure 3. Typical synaptic contacts in frog inner plexiform layer. Figure 3*a* shows a ribbon junction, thought to be a synaptic contact of a bipolar terminal (filled arrow in B). The ribbons usually point between two postsynaptic processes, and membrane densities are seen on the presynaptic membrane and the membranes of both postsynaptic elements. Note that both postsynaptic processes contain numerous synaptic vesicles and appear morphologically similar. Figure 3*b* shows a typical conventional contact, thought to be a synaptic contact of an amacrine process (open arrow in A_1). These contacts are characterized by an aggregation of synaptic vesicles clustered close to the presynaptic membrane, and densities on both the pre- and postsynaptic membranes. In this micrograph, fine filaments can be distinguished crossing the synaptic cleft. This conventional contact is on a process (A_2) that also contains synaptic vesicles; and a second synaptic contact is suggested between this process (arrow in A_2) and a third process (D), which contains no vesicles (a dendrite?). The thickening of the postsynaptic membrane on D appears greater than that of A_2. (× 81 000).

Figure 4. A typical reciprocal contact in the inner plexiform layer. At the ribbon (filled arrow), process B appears to make synaptic contact with two vesicle-containing processes. One of the processes (A) makes a conventional contact (open arrow) back onto B about 0·5 μm from the ribbon contact. (× 65 000.)

ribbons make only ribbon contacts. Thus, it will be assumed here that the ribbon contacts are those of the bipolar terminals and that bipolar terminals make only ribbon contacts.

The second type of synaptic contact in the inner plexiform layer of the frog has a more conventional morphology as compared with the ribbon synapse (Gray & Guillery 1966), and this type of junction will be referred to as a 'conventional synapse' (figure 3*b*, plate 21). A conventional synapse is characterized by a dense aggregation of synaptic vesicles clustered close to the presynaptic membrane, and densities on both the pre- and postsynaptic membranes. Occasionally the postsynaptic membrane exhibits some thickening, but this is not usually the case. It is my impression that the thickening on the postsynaptic element is seen when the contact is an axodendritic contact. 'Axoaxonic contacts'* usually show densities on both the pre- and postsynaptic membranes, but no significant thickening at the postsynaptic membrane.

Similar conventional synapses in the primate retina were shown to belong to amacrine cell processes. In the frog, also, the amacrine cells make such synaptic contacts. Figure 7, plate 23 shows an amacrine cell with one process extending deep into the inner plexiform layer. The cell is identified as an amacrine primarily on the basis of its position along the inner margin of the inner nuclear layer, its rounded nucleus and abundant cytoplasm. (Bipolar perikarya, on the other hand, are found deeper in the inner nuclear layer, usually have oval nuclei, and have sparse cytoplasm.) Scattered clusters of synaptic vesicles are observed in the amacrine cell process, and three probable conventional contacts are marked by arrows. In processes positively identified as amacrine processes (e.g., figure 7) no synaptic ribbons have ever been observed. Thus, it is likely that amacrine processes make synapses only of the conventional type. Since the bipolar terminals appear to make ribbon contacts, it also seems clear that most, if not all, the conventional synapses observed in the inner plexiform layer of the frog are amacrine cell synapses; and here this is assumed to be the case.

In the synaptic cleft of both the ribbon and conventional junctions, structural details can be observed (figure 3, plate 21). Occasionally these can be resolved as fine filaments running between pre- and postsynaptic membranes. In other instances, when the resolution seems poorer, filaments cannot be distinguished in the synaptic cleft; and only an intermediate, dense line may be seen. Observations of such structured material in synaptic clefts have been made before at synapses in several different parts of the nervous system, but as yet its interpretation is unclear (De Robertis 1964; Van der Loos 1963).

Ribbon synapses. The ribbon synapses in the inner plexiform layer of the frog are observed about as frequently per unit area as in the primate retina (see below and table 2). As in primates, there are usually two postsynaptic elements associated with one ribbon. On rare occasions there may be three in the frog; and when this occurs, membrane densities and thickening are observed on all three postsynaptic processes.

* The term 'axoaxonic contacts' is used here to describe synaptic contacts between two processes both of which contain synaptic vesicles (see Dowling & Boycott 1966).

In primates it was shown that at most ribbon synapses one postsynaptic process contains synaptic vesicles and is an amacrine process; whereas the second postsynaptic process usually contains ribosomes, no synaptic vesicles, and is a ganglion cell dendrite. In the frog, on the other hand, both postsynaptic elements usually contain numerous synaptic vesicles; and most often the two postsynaptic elements are morphologically indistinguishable (figure 3*a*, plate 21).

In other instances in the frog, however, one postsynaptic element may contain fewer synaptic vesicles than the other (figure 4, plate 21); and occasionally, one or both postsynaptic processes may contain no synaptic vesicles. In order to make a

TABLE 1. SYNAPTIC VESICLES IN POSTSYNAPTIC PROCESSES AT RIBBON SYNAPTIC CONTACTS

	primate			frog		
	no.		%	no.		%
1. numerous vesicles in both processes	8	14	21	15	22	73
2. numerous vesicles in one process; a few vesicles in the other	6			7		
3. one process with no vesicles; other process with vesicles	47	53	79	6	8	27
4. both processes with no vesicles	6			2		

more objective statement, the postsynaptic elements at 67 ribbon contacts in the primate inner plexiform layer were compared with the postsynaptic elements at 30 ribbon contacts in the frog. The data were obtained from random micrographs of the frog and primate inner plexiform layers, and only those micrographs were used that showed well-oriented ribbon contacts with readily distinguishable postsynaptic elements. The postsynaptic elements were scored on the basis of whether both elements contained numerous vesicles, whether one process contained few vesicles or none, or whether both contained none. The results are presented in table 1.

In primates the usual situation is that one of the postsynaptic elements contains synaptic vesicles and the other process contains none. In the frog, however, in 73 % of the cases both processes contained vesicles that appeared to be synaptic vesicles. In primates in those processes not containing vesicles, ribosomes can almost always be seen, confirming that in primates one postsynaptic element is ordinarily a ganglion cell dendrite. In the few instances in primates that both postsynaptic elements contained some vesicles, most of these micrographs were from the peripheral retina rather than from the central or foveal regions.

In the frog, small ganglion-cell dendrites do not usually contain ribosomes. They therefore cannot be identified by this criterion. They do often contain vesicles, which are usually larger than synaptic vesicles, but which occasionally might be confused with synaptic vesicles (see below, and Cohen 1967). Thus, it is possible that in some of the instances in which one postsynaptic process contains only a few vesicles, this process could be a ganglion cell dendrite (table 1). Even assuming that this is so in all cases in which a postsynaptic process contains only a few

vesicles, this still leaves half of the cases, in which both postsynaptic elements at the dyads in the frog have abundant synaptic vesicles and appear morphologically identical (table 1 and figure 3*a*, plate 21). In addition, at many dyads in the frog, both postsynaptic elements are observed making synaptic contacts of the conventional type (figure 5, plate 22). It is clear, therefore, that at numerous dyad synaptic contacts in the frog, both postsynaptic elements contain synaptic vesicles; and it is likely that both are amacrine cell processes. It is possible that, in some instances, one of the postsynaptic elements containing numerous synaptic vesicles might be another bipolar terminal, but no ribbons have yet been observed in a postsynaptic element at a dyad contact in the frog.

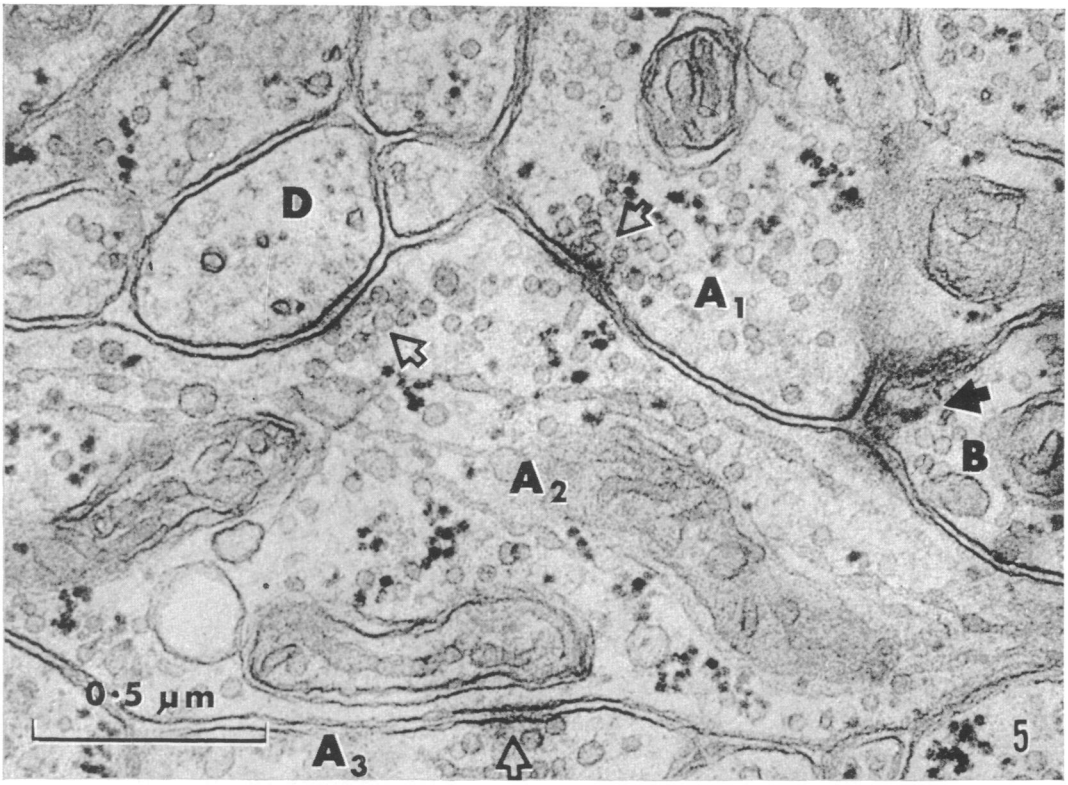
Numerous instances of reciprocal contacts between processes making ribbon synapses and processes making conventional contacts are observed in the frog. Figure 4 (plate 21) shows a typical example. In this micrograph, one postsynaptic process of the dyad complex contains numerous synaptic vesicles, the other only a few. The process containing numerous synaptic vesicles makes a typical conventional contact back onto the ribbon-containing process about 0·5 μm from the ribbon. In some instances, in which both postsynaptic processes contain synaptic vesicles, a double reciprocal contact has been seen feeding back onto the ribbon-containing process. Such observations suggest that in the frog, as in primates, there is a reciprocal synaptic arrangement at every ribbon synapse.

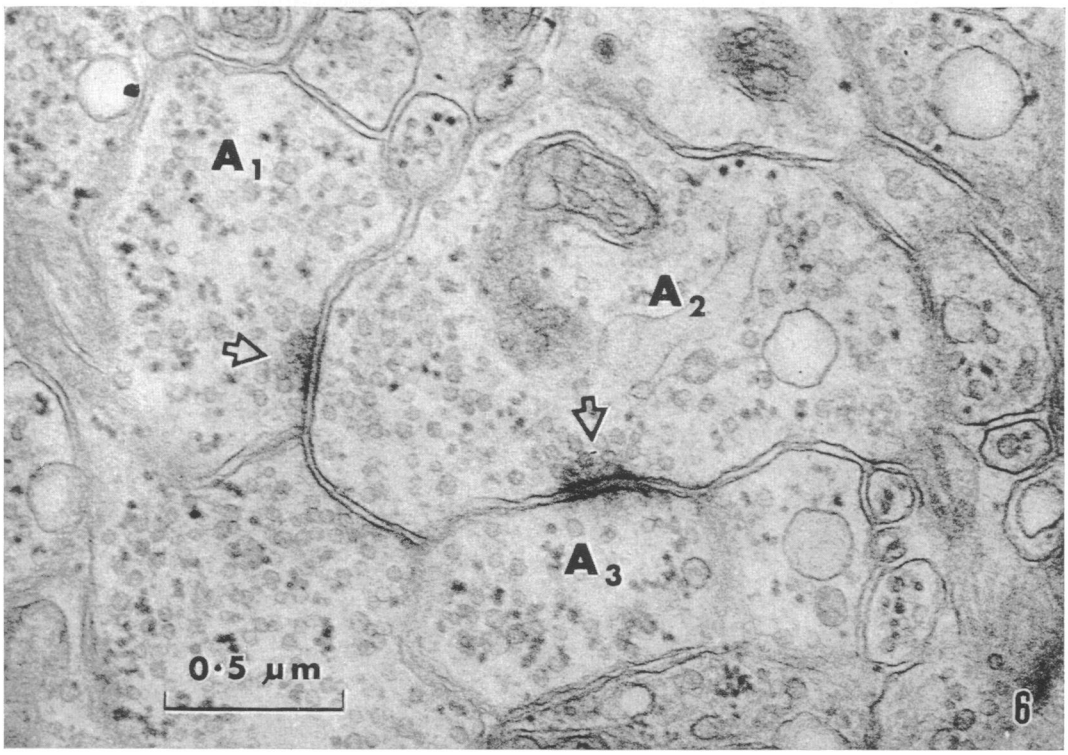
Figure 5 (plate 23) shows how most of the inner plexiform layer of the frog may be organized. In this micrograph, a ribbon-containing process (*B*) makes synaptic contact with two morphologically identical processes, both of which contain synaptic vesicles and both of which make conventional contacts. One of the postsynaptic processes (A_1) makes a conventional synaptic contact onto the second process (A_2) which in turn makes a conventional synaptic contact onto a third process, which does not contain synaptic vesicles and, therefore, might be a ganglion cell dendrite (*D*). In addition, another process (A_3) makes a conventional contact on the second postsynaptic process. This may be interpreted as follows. At the dyad junctions of the bipolar terminals in the frog, both postsynaptic elements are morphologically identical amacrine processes. The amacrine processes make conventional synapses among themselves and onto ganglion cell dendrites. If this is true, then in the frog many bipolar terminals may not make direct contacts with ganglion cell dendrites as is the usual case in the primate retina, but

Description of plate 22

Figure 5. A micrograph illustrating typical synaptic relations between processes in the inner plexiform layer. The ribbon-containing process (B) appears to contact two morphologically identical processes (filled arrow), both of which contain synaptic vesicles. One of these processes (A_1) makes a conventional contact (open arrow) on the second (A_2) which in turn makes a conventional contact (open arrow) on a third process (D) which contains no synaptic vesicles (a dendrite?). In addition, a fourth process (A_2) appears to make a conventional contact (open arrow) on process A_2. (× 62 000.)

Figure 6. Serial synapses between three morphologically identical processes. Process A_1 makes a conventional contact on process A_2 (arrow), which makes a conventional contact on process A_3. Note that process A_3 also contains synaptic vesicles. (× 47 000.)





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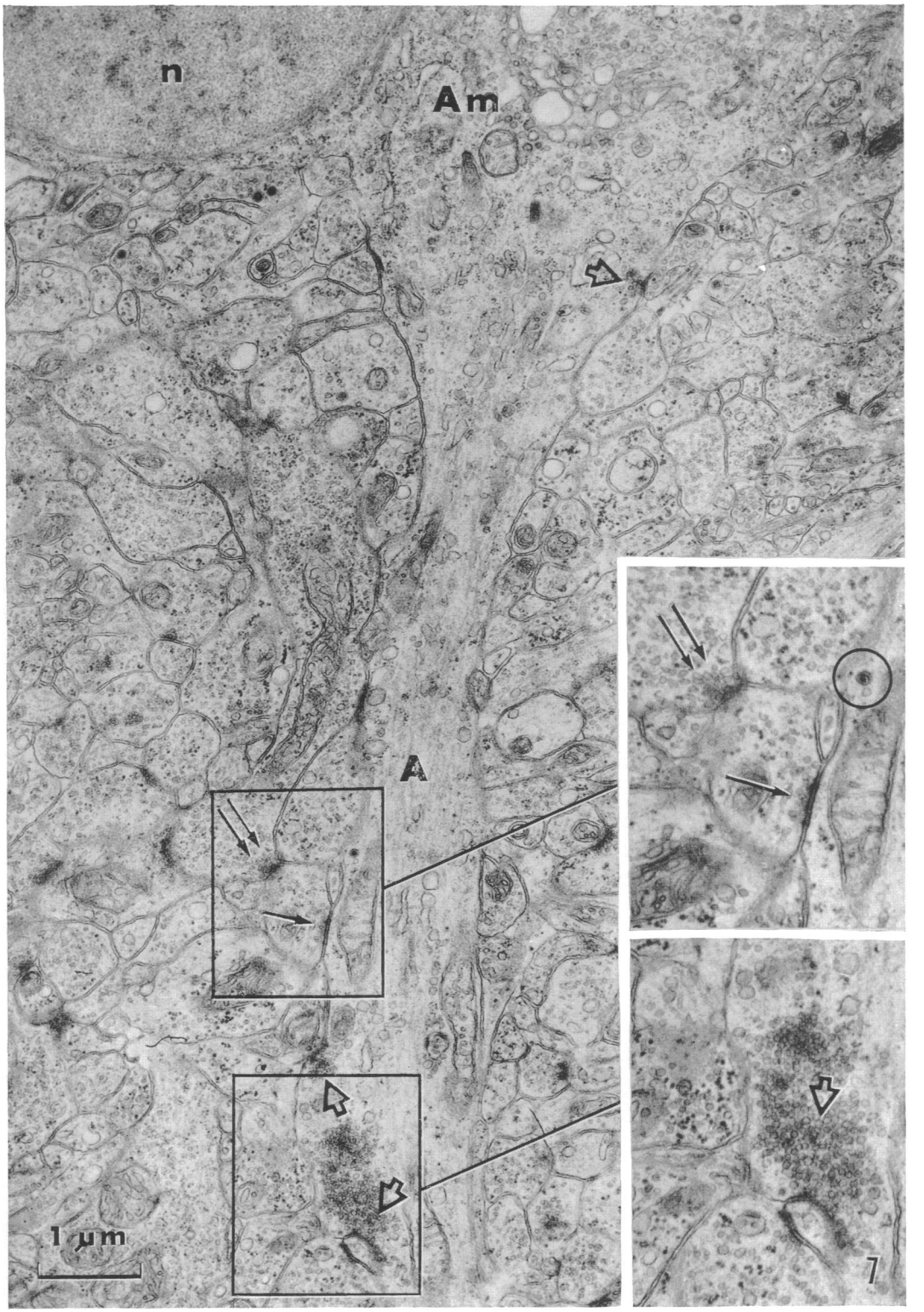

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rather make contacts with amacrine processes which are interposed between the bipolar terminals and ganglion cell dendrites (see discussion).

Conventional synapses. There are very many more conventional contacts in the frog inner plexiform layer than there are ribbon contacts. Indeed, about 90 % of the synaptic contacts in the frog inner plexiform layer are of the conventional type (see below, and table 2). The majority of conventional contacts in the frog are on processes that also contain numerous synaptic vesicles. Some of these postsynaptic structures contain ribbons and are presumably bipolar terminals, but many postsynaptic processes do not contain ribbons and do themselves make conventional synapses. Such 'serial synapses' (Kidd 1962) between morphologically identical, presumably amacrine, processes are observed frequently in the frog and represent one of the most striking and probably significant differences between the frog and the primate inner plexiform layers (figures 6, 7 and 8, plates 22, 23 and 24). In primates, serial contacts between amacrine processes have been seen only once or twice; but in the frog, about 15 % of the observed conventional synapses are organized in series (table 2). This estimate is made from single sections; and it is likely that if consecutive sections were studied, even more processes making conventional junctions would be observed to participate in serial synapses.

Figure 6 (plate 22) shows a typical serial synapse between two morphologically identical processes. The second process makes its contact on a third process that also contains synaptic vesicles and appears similar to the other presynaptic processes. This suggests that there may be serial synapses between three or more processes, and this has been observed (figures 7 and 8, plates 23 and 24). Figure 8 shows an instance in which there are three serial synapses between four adjacent processes. In addition, the last process in the chain makes a conventional contact back onto the third process. Such observations of reciprocal contacts between two processes that both make conventional synapses have been rare; only three such examples have been found so far. This contrasts with the large number of reciprocal contacts that are observed at the ribbon synapses in both frogs and primates.

In two instances, four synapses have been seen in series between five adjacent processes. Again the last processes in the series contained vesicles, suggesting that there may be even more synapses in such sequences. There is no evidence as to whether the processes in a chain of serial synapses are all from different cells. It is possible that several different processes in such a sequence might be from the same cell, and that such a chain of contacts represents reciprocal synapses between two

Description of plate 23

Figure 7. An amacrine cell process (A) extending from the cell soma (Am) well into the inner plexiform layer. One conventional contact is observed on the amacrine process (thin arrow and top insert), and three conventional contacts made by the amacrine process are marked by open arrows. One of the conventional contacts made by the amacrine process is enlarged in the second insert. The amacrine process shows synaptic vesicles only adjacent to the synaptic contacts. Note that a third process is apparently making a contact on the process that contacts the amacrine process (double thin arrows). One granulated vesicle is present in the amacrine cell process (circle). Amacrine cell nucleus (n). (× 16000.)

cells. If that were the case, however, it might be supposed that more instances of reciprocal contacts between processes that make conventional contacts would be seen. Since that is not so, it seems likely that in most instances of serial synapses the adjacent processes are from different cells.

Besides finding conventional contacts on vesicle-filled processes, numerous instances of conventional contacts on ganglion cell dendrites (figure 10, plate 25) and occasionally on the perikarya of ganglion cells are found (see below). Also, occasionally a ganglion cell dendrite can be followed for several microns, and numerous conventional synaptic contacts are observed on it (figure 11, plate 25).

Granular synaptic vesicles

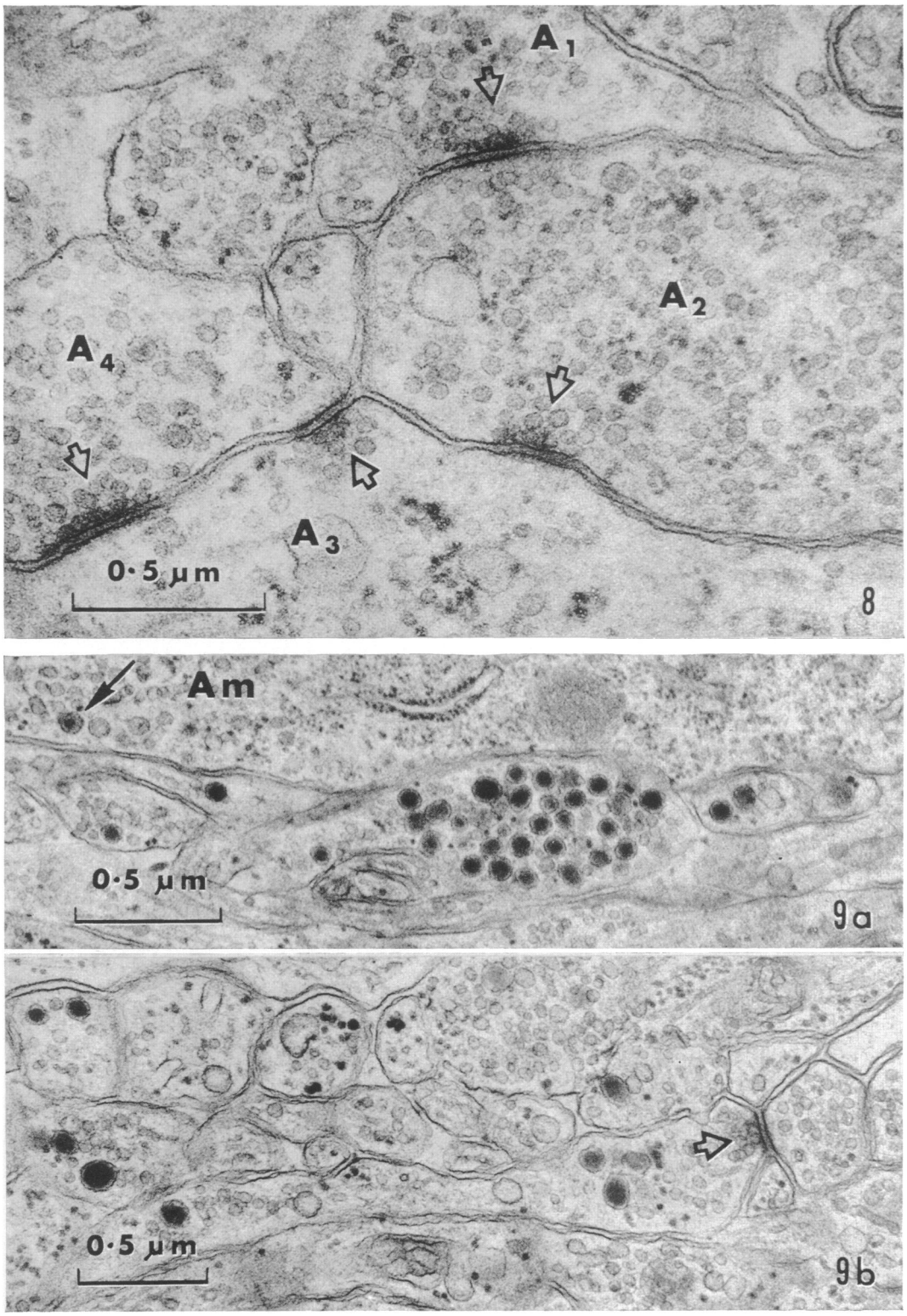
In occasional processes containing typical, 300 to 500 Å agranular synaptic vesicles, large granulated vesicles are also observed (figure 9, plate 24). These vesicles closely resemble the type I granulated vesicles described by Grillo & Palay (1963) in autonomic nerve processes. Processes containing the large granulated vesicles are seen most frequently near the outer margin of the inner nuclear layer, although occasionally they are seen elsewhere in the inner plexiform layer. Such large granulated vesicles are also occasionally seen in the somata of cells that lie along the inner margin of the inner nuclear layer (figure 9*a*, plate 24). They have never been observed in processes containing synaptic ribbons. In a few instances, processes containing the large granulated vesicles have been observed making conventional synaptic contacts (figure 9*b*, plate 24), but the granulated vesicles are never observed immediately adjacent to the site of synaptic contact.

Recently several workers (Malmfors 1963; Häggendal & Malmfors 1963, 1965; Ehinger 1966; Laties & Jacobowitz 1966) have demonstrated that a few amacrine cells in vertebrate retinas show strong fluorescence when the retinas are prepared by the method of Falck (1962). These cells are large amacrines whose processes usually run in the outer part of the inner plexiform layer just under the inner nuclear layer. The fluorescence is ascribed to an amine within these cells, probably dopamine. It seems likely that the large granulated vesicles observed here are amine-containing vesicles, and that these vesicles are present in some of the large amacrine cells and their processes (Grillo & Palay 1963; Richardson 1964). The significance of these observations is at present unclear.

DECRIPTION OF PLATE 24

FIGURE 8. Complex serial synaptic arrangement between four adjacent processes. Process A_1 makes a conventional contact with A_2, which makes a conventional contact with A_3, which makes a conventional contact on A_4. Process A_4 makes a conventional contact back on A_3. Reciprocal contacts between processes making conventional contacts are observed only very rarely. (× 60000.)

FIGURE 9. Processes in inner plexiform layer containing large, granulated synaptic vesicles. (*a*) A large process containing numerous granulated vesicles plus many agranular vesicles is observed adjacent to an amacrine cell soma (Am). Occasional granulated vesicles are also observed in nearby processes and in the cytoplasm of the amacrine cell (thin arrow). (× 45000.) (*b*) One process containing granulated vesicles is observed making a synaptic contact of the conventional type (arrow). The synaptic vesicles clustered close to this contact are all of the agranular type. (× 40000.)



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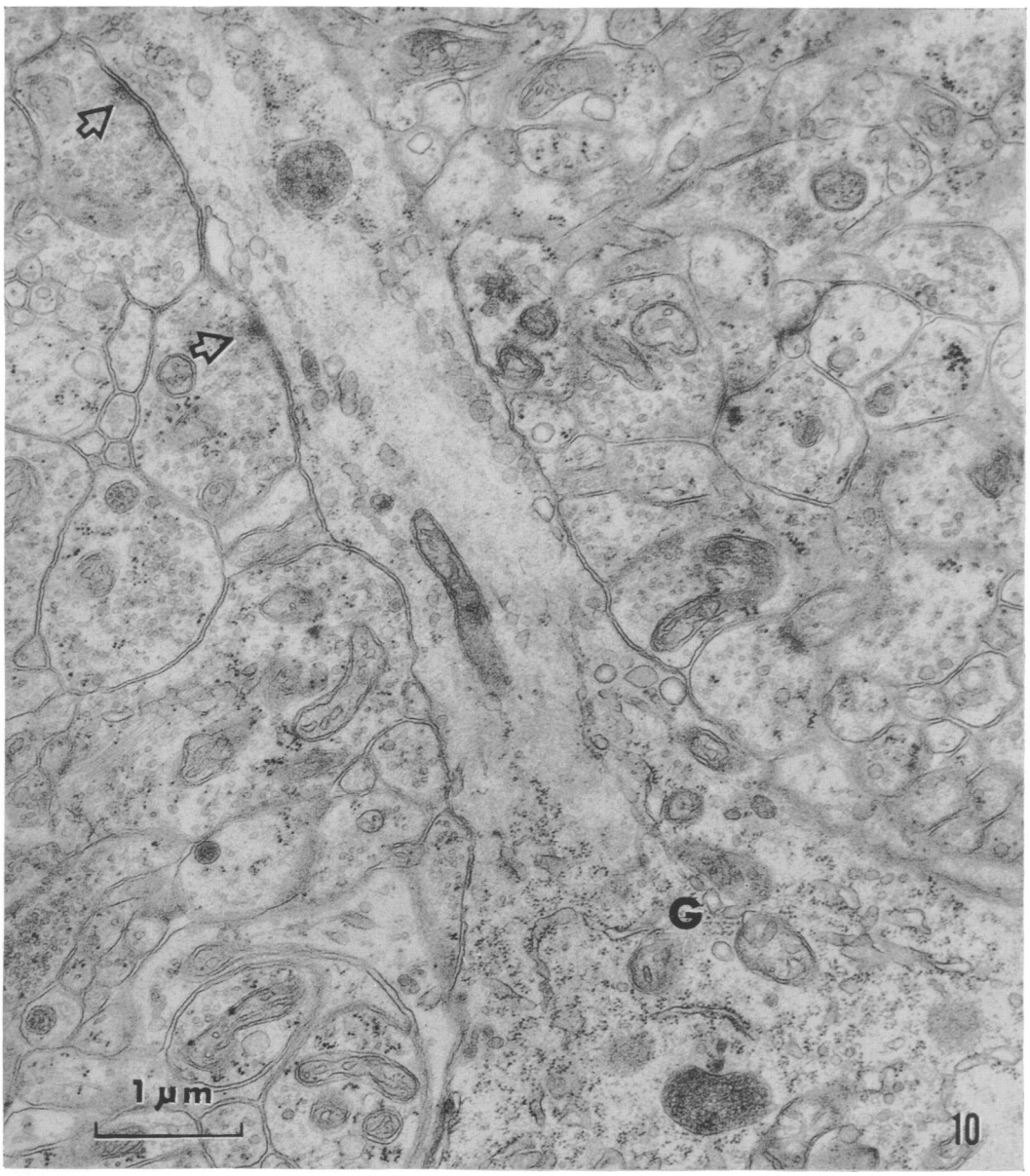

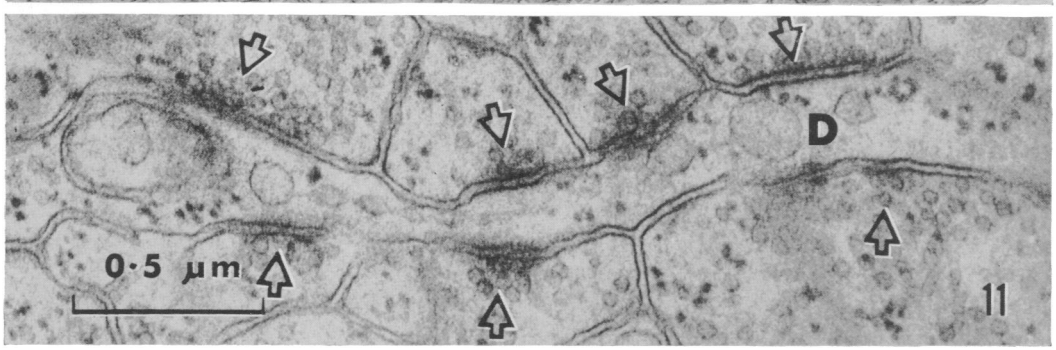

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Ganglion cells and dendrites

As explained above, most dendrites of ganglion cells in the frog contain few, if any, ribosome-like particles. Figure 10 (plate 25) shows a portion of a frog ganglion cell with a large dendrite extending into the inner plexiform layer. Numerous ribosomes are seen in the perikaryon, but the dendrite contains relatively few such particles over most of its length. Deeper in the inner plexiform layer, ganglion cell dendrites seldom show any ribosomes; and these dendrites usually appear quite 'empty', with only occasional profiles of smooth endoplasmic reticulum, mitochondria and glycogen. Unfortunately, the other processes observed in the inner plexiform layer also show these cytoplasmic organelles.

Thus, identification of isolated ganglion cell dendrites remains difficult in the frog. A process is tentatively identified as a ganglion cell dendrite in the inner plexiform layer of the frog only when the process can be followed for several microns (μm) and no synaptic vesicles are observed over this length. Figure 11 (plate 25) shows one process that is probably a ganglion cell dendrite. For several microns, this process contains no vesicles that by current criteria would be classified as synaptic vesicles. What is interesting about such presumed dendrites is that they are often surrounded by numerous vesicle-containing processes that make synaptic contact of the conventional (amacrine) type with the dendrites (figure 11, plate 25). In the primate retina an amacrine process is occasionally observed making a conventional contact on a ganglion cell dendrite, but in primates such dendrites are never seen covered with numerous conventional synapses. Another difference between primates and frogs is that, in primates, few contacts of any kind are observed on the large ganglion-cell dendrites close to the perikaryon. In frogs, conventional contacts are frequently seen on large ganglion-cell dendrites, often quite close to the cell perikaryon (figure 10, plate 25).

In the ganglion cell perikarya of the frog, in addition to numerous ribosomes, one observes mitochondria and various sizes and shapes of profiles of endoplasmic reticulum with or without ribosomes. Small clusters of vesicles, which are probably small profiles of endoplasmic reticulum, are noted quite often in the cytoplasm. Some of these vesicles may be as small as about 600 Å in diameter, and they resemble synaptic vesicles (figure 14, plate 27). On one occasion a cluster of such vesicles was observed near the plasma membrane of the cell, and this arrangement appeared suggestive of a synapse (insert, figure 14). However, measurement of these vesicles showed most of them to be consistently larger than most synaptic vesicles in the

Description of plate 25

Figure 10. A portion of a ganglion cell (G) with a dendrite extending into the inner plexiform layer. Few ribosomes are observed in the dendrite although there are numerous ribosomes in the cell soma. Two processes are seen making conventional contacts on the dendrite (arrows). (× 20000.)

Figure 11. A presumed ganglion-cell dendrite (D) in the inner plexiform layer. The dendrite is surrounded by processes, most of which are making conventional contacts with it (arrows). (× 50000.)

inner plexiform layer, and thus this single observation is considered fortuitous and not an indication that ganglion cell perikarya or their dendrites make presynaptic contacts.

Occasionally a process is seen making a conventional synapse on the perikaryon of a ganglion cell, but no ribbon-containing processes have been seen contacting a ganglion cell perikaryon. Thus there is no evidence that axosomatic contacts between bipolar dendrites and ganglion cells exist in the frog.

Comparative numbers of synapses in inner plexiform layers of frogs and primates

In order to quantify the frequency of various types of synaptic contacts in frogs and primates, two large montages through the inner plexiform layer of frog retinas were prepared. The montage areas in both cases were from central retina, and the retinas were from different frogs. The synaptic contacts in these montages were identified and counted, along with the synaptic contacts in a single montage of parafoveal human retina. Parafoveal retina was chosen for comparison because it is the most central area of retina in primates that contains both rods and cones. The montage of the human retina covered an area of 1890 μm^2; and the two frog montages had a combined area (of inner plexiform layer) of 1527 μm^2. The two frog montages gave identical results and were combined. The results are given in table 2.

TABLE 2. INCIDENCE OF TYPES OF SYNAPTIC CONTACTS IN INNER PLEXIFORM LAYER

type of contact	human (parafoveal retina)			frog (central retina)		
	no.	%	incidence	no.	%	incidence
ribbon	52	36	0·028/μm^2	36	10	0·024/μm^2
conventional	88	62	0·046/μm^2	281	78	0·184/μm^2
serial conventional	2	2	0·001/μm^2	44	12	0·028/μm^2
	142	100	0·075/μm^2	361	100	0·236/μm^2
	montage area = 1890 μm^2			montage areas = 1527 μm^2		

Table 2 shows that in central retina of the frog there are approximately 3 times as many synapses per unit area as there are in the parafoveal region of the human retina. However, the incidence of ribbon contacts is about the same in the frog and human retinas, so that the significant difference between the two is in the numbers of conventional (amacrine) synapses. The frog retina has about 4 times more conventional synapses per unit area than does the human retina. In addition, the frog has numerous conventional synapses in series; and, as noted above, such arrangements are only rarely observed in primates.

From these montages, it is possible to calculate the numbers of contacts per square millimetre of tissue encompassing the inner plexiform layer. In man, whose inner plexiform layer is about 30 μm thick, this number is 2900000 synaptic contacts/mm^2 (Dowling & Boycott 1966). In the frog, the inner plexiform layer is about 35 μm thick, which means there are about 10400000 contacts/mm^2 of inner plexiform layer.

Kidd (1962) made counts of the incidence of various types of synaptic contacts in the inner plexiform layers of cats and pigeons. The incidence of ribbon contacts found by Kidd in both cat and pigeon retinas was 0·022 ribbon contacts/μm^2, which matches our results closely. However, Kidd reported that there are approximately twice as many conventional contacts per unit area in cats as we have found for primates, and about twice as many conventional contacts in pigeons as are reported here for frogs. Kidd did not prepare montages through the entire thickness of the inner plexiform layer in order to make his counts, but apparently used single pictures from scattered and selected areas. To estimate more accurately the frequency of contacts in cats, a montage through the thickness of the inner plexiform layer was prepared and scored. The area of the montage was 735 μm^2 and the results obtained are as follows: ribbon contacts, 0·0026/μm^2; conventional contacts, 0·057/μm^2; and serial contacts 0·006/μm^2. These frequencies of types of synaptic contacts in the inner plexiform layer of the cat match very closely our results obtained from the montage of human retina.

In pigeons, we have not yet succeeded in preparing montages of sufficient quality to make accurate counts of contacts. Examinations of numerous individual sections indicate that there are very many more conventional contacts than ribbon contacts, in about the same proportion as is observed in the frog. There are also a significant number of serial contacts in pigeons. The total number of contacts per unit area in pigeons appears at least as great as in frogs, and it seems likely (from the individual sections so far examined) that there may be even more contacts per unit area in pigeons than in frogs. Thus, the cat retina resembles closely the primate retina in terms of numbers and arrangements of contacts, while the pigeon retina is strikingly similar to that of the frog.

Lettvin and his colleagues have recently suggested that in the inner plexiform layer of the frog the layering which is observed with the light microscope may indicate where certain critical operations occur in processing of visual information in the frog (Lettvin *et al.* 1961). Attempts to see whether certain synaptic arrangements occur more frequently at one level or another in the inner plexiform layer have not as yet revealed any patterns of layering in our montages.

Outer plexiform layer

The outer plexiform layer in the frog is relatively thin and does not appear more elaborate in electron micrographs than the outer plexiform layer of primates or of most other mammalian retinas (figure 2, plate 20). Indeed, there is a striking lack of complexity of the outer plexiform layer in the frog when compared with the inner plexiform layer.

Receptor terminals of both rods and cones may be readily identified by following them back to the receptor cell nucleus and the inner or outer segments. The terminals of the rods and cones are not obviously different in their fine structural morphology (Evans 1966). Along the bases of the terminals, both show invaginations into which processes from the outer plexiform layer penetrate. Above each invagination is a synaptic ribbon, surrounded by a cluster of synaptic vesicles (figure 13*a*, plate 27). The ribbons in the receptor terminals of the frog are quite

long, often having a length of 0·5 μm or more. These contrast with the short synaptic ribbons (0·1 to 0·2 μm) found in the inner plexiform layer (compare figures 3*a*, plate 21 and 13*a*, plate 27). In primates the ribbons in the receptor terminals are often slightly longer (0·3 to 0·4 μm) than those in the bipolar terminals (0·2 to 0·3 μm) but the difference is not great.

The arrangement of the processes in the invaginations of the receptor terminals is similar to that seen in other vertebrate retinas. Two of the penetrating processes come to lie laterally in the invagination, on either side of the ribbon which sits in a ridge of terminal cytoplasm extending downward into the invagination. One or more of the elements are placed centrally in the invagination and lie directly underneath the ribbon. In primates the central elements usually do not penetrate into the invagination as deeply as the lateral elements, and the central ones never come closer than 800 to 1000 Å to the presynaptic membrane underlying the ribbon in the receptor terminal. In the frog, however, the central elements do lie close to the presynaptic membrane, within about 200 to 300 Å.

In retinas of both fishes and primates it has been shown that the lateral elements in the invaginations of the receptor terminals are derived from the horizontal cells, and that the central elements are bipolar cell dendrites (Stell 1965; Missotten 1965). Such identification has not as yet been made in the frog, but there is no reason to suggest that in the frog a different arrangement exists. It is generally assumed that the ribbon in the receptor terminal indicates the site of synaptic contact of the receptors. In the frog, one usually notes some densification on the presynaptic (terminal) membrane, and densification and thickening on the membranes of all presumed postsynaptic processes. This membrane specialization seems limited to the region surrounding the ridge of cytoplasm that extends into the invagination in which the synaptic ribbon lies. At the tip of this ridge is a slightly rounded, nipple-like protrusion; and the membrane specialization is particularly noticeable surrounding this region (figure 13*a*, plate 27).

Occasionally, processes are observed contacting the bases of the receptor terminals and making superficial contacts on the terminals, similar to the superficial contacts of the flat bipolars on the cone pedicles in the primate retina (figure 13*b*, plate 27). These contacts in frogs, like those in primates, show no clear morphologic specializations on either side of the contact; that is, neither ribbons nor aggregations of vesicles are seen. The processes making such superficial contacts dent the surface of the pedicle, and at that point the membranes on both sides of the contact appear somewhat denser and more distinct. In the frog, the membrane on the receptor side often appears denser. Another feature of these contacts is the presence

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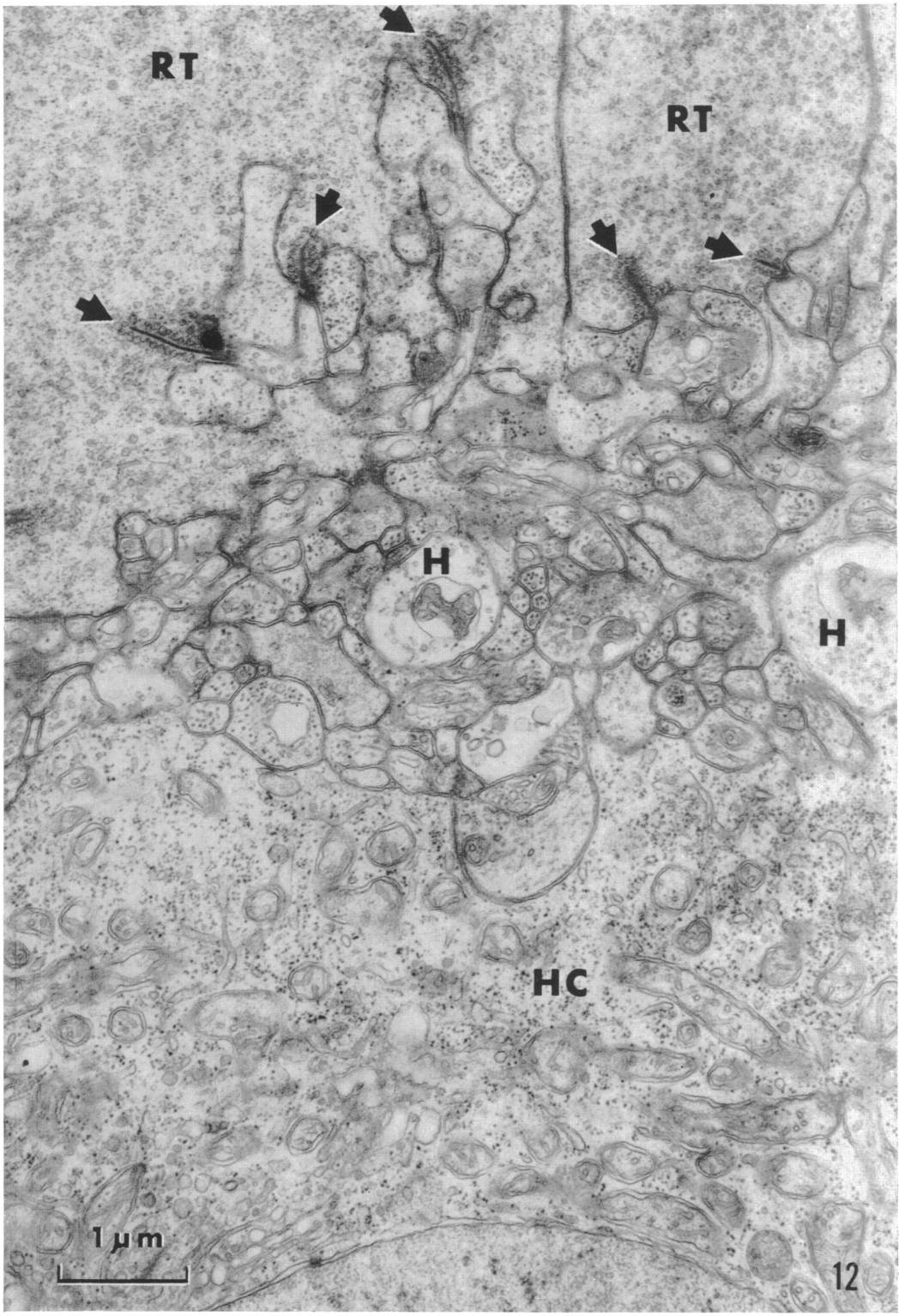
Description of plate 26

Figure 12. A low-power electron micrograph of the outer plexiform layer in a frog. Synaptic ribbons in the receptor terminals (RT) are marked by filled arrows. Two large synaptic-vesicle-filled processes (H), believed to be horizontal cell processes, are observed in the plexiform layer. A large cell, probably a horizontal cell (HC), fills the lower portion of the micrograph. (× 20000.)



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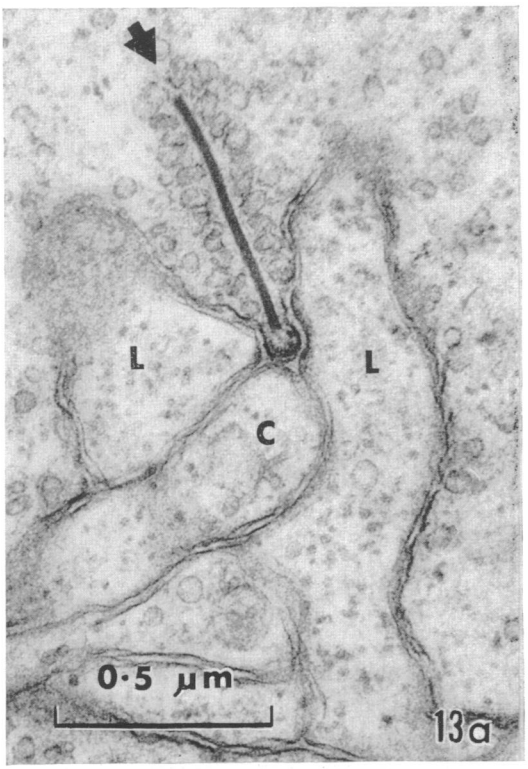

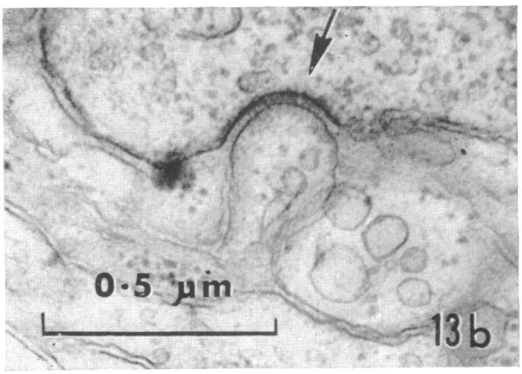

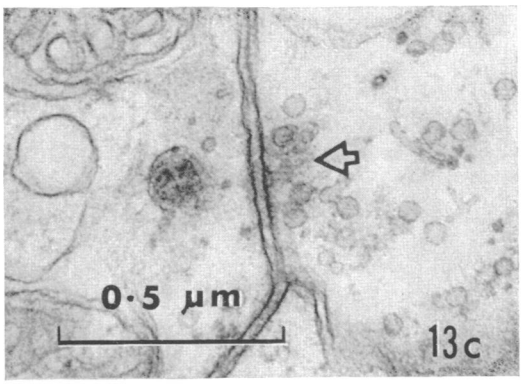

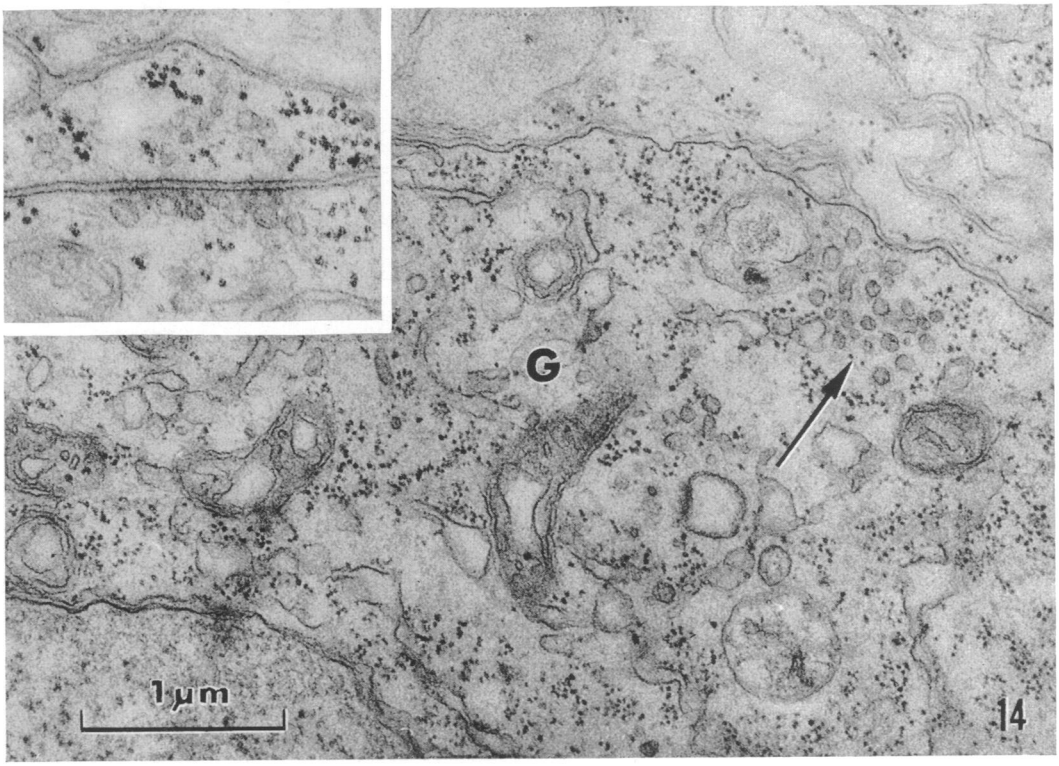

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of fine filaments extending between pre- and postsynaptic membranes, similar to the filaments seen at most synaptic contacts in the inner plexiform layer. The origin of the processes making superficial contacts on the receptor terminals is unclear; they may be bipolar dendrites, horizontal-cell processes, or processes from adjacent receptors. In the frog, fine processes from the receptors extend laterally in the outer plexiform layer, perhaps to make interreceptor contacts. These fine processes are seen readily in Golgi-stained preparations (figure 1), but they have not yet been identified in the electron microscope.

Elsewhere in the outer plexiform layer, rather large processes that appear quite 'empty' except for a few synaptic vesicles and mitochondria are observed making conventional synaptic contacts (figures 12 and 13*c*, plates 26 and 27). These contacts are characterized by a group of synaptic vesicles clustered close to the presumed presynaptic membrane. Some densities of the membrane on both sides of the contact are usually noted, as well as some material in the synaptic cleft. These synaptic contacts in the outer plexiform layer of the frog appear quite similar to synaptic contacts of the horizontal cells observed in the outer plexiform layer of the rabbit and the cat. In preliminary studies of Golgi-stained frog retinas, Boycott and I have observed that on the dendrites of the horizontal cells there are swellings that usually attach to the dendrite by a thinner stalk. These swellings appear to correlate in size with the processes observed with the electron microscope to make synaptic contacts. Since neither the bipolar dendrites nor receptor terminal processes exhibit such knobs in Golgi-stained material, it is likely that the synaptic contacts in the outer plexiform layer are those of horizontal cell processes.

These synaptic contacts are often observed between processes that are morphologically identical, suggesting that many of the contacts are between horizontal cell processes. Contacts are also occasionally seen on smaller processes, of different morphology, that are probably bipolar cell dendrites. No contacts of these

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Description of plate 27

Figure 13. Typical synaptic contacts in the outer plexiform layer. (*a*) A ribbon contact of the receptor terminals (filled arrow). The ribbon, surrounded by a cluster of synaptic vesicles, lies in a ridge of presynaptic cytoplasm. Processes from the outer plexiform layer penetrate into the invagination and end laterally (L) or centrally (C) in close proximity to the ribbon. Some membrane densification is observed on both the pre- and postsynaptic membranes adjacent to the nipple-like protrusion at the tip of the presynaptic ridge of cytoplasm. (× 57 000.) (*b*) A typical superficial contact on the base of a receptor terminal (thin arrow). No ribbon or aggregation of vesicles is seen on either side of the contact. Some membrane densities are observed on both sides of the contact, and that on the receptor side appears somewhat greater. Filaments are observed in the cleft between the two membranes. (× 62 000.) (*c*) A conventional contact between two presumed horizontal cell processes (open arrow). This contact is characterized by a cluster of vesicles on the presynaptic side of the contact and some membrane densities on both pre- and postsynaptic sides. Filamentous material is often observed in the synaptic cleft of both ribbon and conventional contacts in the outer plexiform layer. (× 60 000.)

Figure 14. A portion of a ganglion cell soma (G). Numerous vesicles, interpreted as profiles of endoplasmic reticulum, are scattered throughout the cytoplasm. Some of these vesicles may be as small as 600 to 800 Å in diameter (thin arrow). The insert shows a cluster of such vesicles along the cell membrane. (× 27 000.)

processes have ever been seen on the receptor terminals, although in one or two instances such processes have been observed adjacent to receptor terminals, separated by only the usual 200 Å gap.

In summary, processes from both bipolar and horizontal cells penetrate into invaginations in the bases of the receptor terminals and come to lie in close proximity to the synaptic ribbons of the terminal. In addition, other (unidentified) processes make superficial contacts on the bases of the receptor terminals. Elsewhere in the outer plexiform layer, knobs apparently originating from the horizontal cell processes make conventional contacts with other horizontal cell processes and with bipolar cell dendrites. All the types and arrangements of contacts seen in the outer plexiform layer of the frog have been seen in the outer plexiform layer of many other vertebrates, including the cat and rabbit; and it does not appear that the outer plexiform layer in frogs is greatly different from the outer plexiform layer of these other vertebrates.

Discussion

The novel and striking findings encountered in the frog retina involve mainly the inner plexiform layer and the amacrine cells.

(1) There are abundant conventional synapses, presumably amacrine cell junctions, in the frog inner plexiform layer; about 4 times as many of this type of contact per unit area are found in frogs as in primates. Many of these contacts are with processes that also may be identified as amacrine cell processes. Serial synapses between two, three or more such amacrine cell processes are frequently observed. Thus, a first conclusion is that there is a considerable amount of synaptic interaction between amacrine processes in the inner plexiform layer of the frog.

(2) At the ribbon synapses of the bipolar terminals the two postsynaptic elements in the majority of cases in the frog contain numerous synaptic vesicles, appear morphologically identical, and are probably both amacrine cell processes. This contrasts with the usual situation in the primate retina, in which the two postsynaptic elements at the dyads are morphologically distinct and appear to have a different origin. In primates, one process is usually an amacrine process and the other a ganglion cell dendrite. Thus, a second conclusion is that in most instances in frogs the bipolars do not contact ganglion cell dendrites directly, but instead feed onto the amacrine cells.

(3) Numerous conventional (amacrine) junctions are also observed on ganglion cell dendrites in the frog retina. This also differs from the primate retina, in which there are relatively few amacrine-ganglion contacts—many fewer of these contacts, for example, than direct bipolar-ganglion cell contacts. A third conclusion, therefore, is that the ganglion cells in the frog are primarily influenced by the amacrine cells and not by direct bipolar contacts.

An interpretation of the above observations and conclusions is that in the frog the amacrine cells are true interneurons, interposed between the bipolar terminals and ganglion cells. Thus, the bipolars at many of the ribbon contacts may feed only onto amacrine processes; the processes synaptically interact with each other in the inner plexiform layer and contact the ganglion cell dendrites. Thus, the complexity

of ganglion-cell response behaviour in the frog could be accounted for by the extensive synaptic interactions occurring between the amacrine cells that are interposed between the bipolar terminals and ganglion cell dendrites.

It has been suggested previously that the amacrine cells are involved in the receptive field organization of the ganglion cells in retinas of vertebrates such as primates or cats (Dowling & Boycott 1966; Gallego 1965). This suggestion was based on observations that the dendritic spread of the ganglion cells is not of sufficient width to account for the entire diameter of the receptive field. This is especially clear in the foveal region of the primate retina, where many of the ganglion cells (the midget ganglion cells) have very narrow dendritic fields. Where comparisons have been made in cat and monkey retinas, only the centres of the receptive field correspond to the ganglion cell dendritic spread (Dowling & Boycott 1966; Gallego 1965; Brown & Major 1966). This implies that the antagonistic surround of the receptive field is mediated via an interneuron, presumably the amacrine cells. Thus, our view of receptive field organization in primates or cats is that the centres of the fields are mediated by direct bipolar-ganglion contacts, whereas the periphery is mediated by indirect contacts from bipolars to ganglion cells through the amacrines.

In the frog, simple centre-surround receptive fields are not found. Instead, a variety of receptive field organizations of considerable complexity have been described. In correlation with this, there are few direct bipolar-ganglion cell contacts, but numerous amacrine-amacrine and amacrine-ganglion cell contacts. Thus, it is likely that the amacrine cells play a larger role in receptive field organization in the frog as compared with the primate; and perhaps most of the responses throughout the receptive field of the ganglion cells in the frog are mediate through the amacrine cells.

A diagram incorporating the findings and interpretations of the frog retinal structure is figure 15. In the outer plexiform layer, processes from bipolars and horizontal cells penetrate into invaginations in the receptor terminals and come to lie under the synaptic ribbons in the terminals. Other processes make superficial contacts on the bases of the cone pedicles; in figure 15 these are drawn as bipolar dendrites, although this has not been established as yet. Deeper in the outer plexiform layer, swellings attached to horizontal cell processes make contacts with ascending bipolar dendrites and other horizontal cell processes (not shown in diagram).

In the inner plexiform layer, bipolar terminals make contacts at the synaptic ribbons. At many of the contacts both of the postsynaptic elements are amacrine cell processes, many of which feed back onto the bipolar terminal in a reciprocal synaptic arrangement. There is extensive synaptic interaction among the amacrine processes, indicated by numerous serial synapses in the inner plexiform layer; and there are many contacts of the amacrine processes on the ganglion cell dendrites. A few large granulated vesicles are present in some of the amacrine cell processes.

It is probable that there are at least some direct bipolar-ganglion cell contacts in the frog (see table 1), and such synaptic arrangements are included in the drawing. Assuming there are some direct bipolar-ganglion contacts, another interpretation of

our findings could be that every bipolar terminal makes at least a few direct contacts with ganglion cells, although the majority of its contacts are with amacrine processes. There is no way at present to decide between these alternatives; in the diagram the former interpretation is presented, that some bipolars make only direct contacts and others make only indirect contacts. In either case, it is

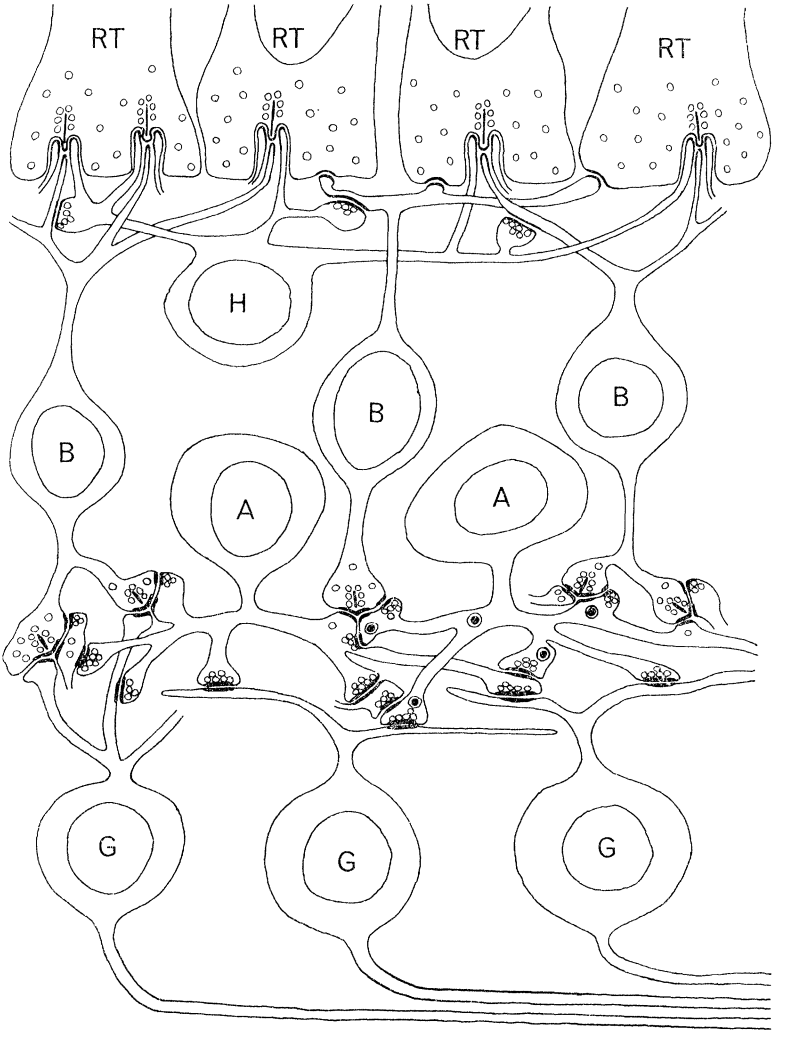

FIGURE 15. A summary diagram of the contacts in the frog retina. See text for details. Receptor terminals (RT), horizontal cell (H), bipolar cells (B), amacrine cells (A), ganglion cells (G).

abundantly clear that in the frog the majority of bipolar synapses are with amacrine cells, and thus the ganglion cells are mainly reached indirectly through the amacrine cell processes.

It is interesting in this regard to recall that Cajal (1937) pointed out long ago that the great complexity and diversity of the human cerebral cortex appeared to

correlate with a great abundance and variety of 'short axon' neurons, cell types believed to play roles as interneurons in the cortex. Here, in the retina of the frog, the complexity of response behaviour of the ganglion cells is best explained by the wealth and variety of interactions among the processes of the amacrine cells, the interneurons of the inner plexiform layer of the retina.

Vertebrate retinal structure and function

In our laboratory the synaptic organization of the retinas of frogs and primates has now been examined in some detail, whereas the retinal synapses in cats, pigeons, ground squirrels, and rabbits have been looked at briefly. It is appropriate to

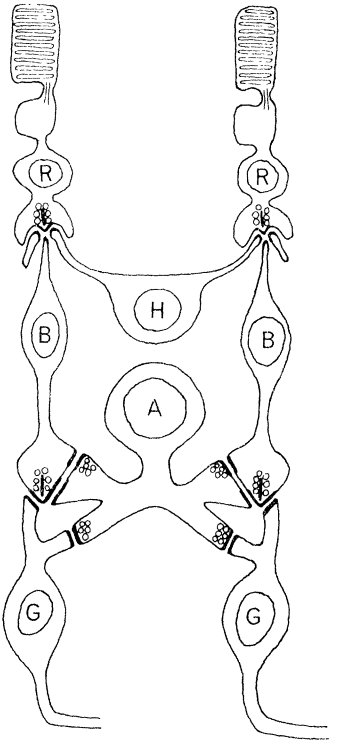

FIGURE 16. A summary diagram of the synaptic contacts found in all vertebrate retinas so far examined. See text for details. Receptors (R), horizontal cell (H), bipolar cells (B), amacrine cell (A), ganglion cells (G).

consider the synaptic features common to all the vertebrate retinas so far examined, to compare the differences in synaptic organization of retinas of various species, and to see how the synaptic organization correlates with the known physiology.

In figure 16, those synaptic features that are common to all the above retinas are drawn. There is no attempt to indicate any spatial array of processes or of synaptic contacts, but only to show the probable synapses and synaptic pathways between the various cell types in the vertebrate retina. For simplicity, the amacrine-amacrine synapses, which are present in all retinas so far examined, have been omitted. In the outer plexiform layer, processes from the bipolar and horizontal

cells penetrate into invaginations in the receptor terminals, presumably to receive input from the receptors. A variable number of bipolar processes enter centrally into the invaginations, while two horizontal cell processes terminate laterally in the invaginations. In most of these retinas, occasional synapses between horizontal cell processes and between horizontal cell processes and bipolar cell dendrites are observed in the outer plexiform layer. In primates, however, no such contacts have been found. Thus the fundamental synaptic pathways and functions of the horizontal cells remain obscure. It is known that in all these retinas horizontal cell processes extend between receptor terminals, and that these processes strategically terminate in the invaginations of the receptor terminals. Therefore they are conveniently situated to regulate transmission between receptor and bipolars. At the present time, however, this possibility is only speculation.

In the inner plexiform layer, the bipolar terminals of all these retinas contain synaptic ribbons and made dyad synaptic contacts. At least one of the postsynaptic processes at the dyad contact is an amacrine process; and in all the retinas the amacrine processes synaptically feed back onto the bipolar terminal just adjacent to the ribbon contact of the bipolar. The amacrine cell processes extend laterally in the inner plexiform layer, connecting adjacent bipolar terminals in a reciprocal synaptic relationship. They also make feed-forward contacts onto the ganglion cells directly. Thus the amacrine cells provide both a feedback function onto bipolar terminals and a pathway for lateral and reciprocal interactions between bipolar terminals and ganglion cell dendrites.

It is interesting to note that the same sort of feedback and lateral interconnexions postulated here as existing in the inner plexiform layer of all vertebrate retinas are already known to be present in the primitive eye of *Limulus*, which is perhaps the most fully analysed visual system of any at present (Hartline, Ratliff & Miller 1961; Ratliff, Hartline & Miller 1963; Ratliff, Hartline & Lange 1966; Purple & Dodge 1965, 1966). In *Limulus*, fine processes from the eccentric-cell axons extend laterally to end on adjacent eccentric-cell axons. Also, some of these processes appear to feed back onto the axon from which they originated. All of these connexions in *Limulus* appear to be inhibitory; and Hartline, Ratliff, and their colleagues, have demonstrated how this system of lateral inhibition can account for enhanced border or edge contrast and also perhaps explain the Mach Band phenomenon (Ratliff & Hartline 1959). Much less is known about feedback or self-inhibition in *Limulus*, and little can be said concerning its function. It does seem suited to play some sort of gain-control or adaptation role in the visual system, and suggestions that feedback plays such a function in the vertebrate eye have recently been made (Rushton 1965; Dowling 1967).

The synaptic features common to all the vertebrate retinas that have been studied, as drawn in figure 16, represent probably the most elementary synaptic organization of the vertebrate retina. This drawing comes closest to describing the central region of the primate retina, which appears to have the simplest and most fundamental synaptic organization of all the retinas so far studied. In other retinas, additional synaptic features and complexities are observed. For example, in cats and rabbits, synapses of the horizontal cells are found. In rabbits and

ground squirrels numerous instances of the bipolars contacting only vesicle-filled (presumably amacrine) processes are observed. Also, in rabbits and ground squirrels there are proportionally more conventional (amacrine) contacts as compared with primates, and serial synapses are frequent.

The most complex retinas that have been examined are those of frogs and pigeons, which have relatively few direct contacts between bipolars and ganglion cells in the inner plexiform layer, but abundant amacrine-amacrine and amacrine-ganglion cell synapses. If a scale is made evaluating the synaptic organization of these retinas from 'simple' to 'complex', the sequence reads as follows: primates and cats, simple; rabbits and squirrels, intermediate; frogs and pigeons, complex. It is clear that the trend toward complexity of retinal structure correlates with less encephalization of neural function; so that in man and monkeys, in which the cerebral cortex is best developed, the retina is simplest. In frogs and pigeons, in which the cortex is least developed, the retina is most complex.

Finally we might compare the physiology of these retinas with their structure; and thus far the correlation of anatomical complexity appears to agree very well with physiological complexity. For example, the simplest receptive field organization is probably that of many of the ganglion cells found near the foveal region of primates. The evidence suggests that the receptive fields are made up of two concentric and antagonistic zones. The central zone of these foveal receptive fields is usually very small, perhaps driven by one or only a few cones; and these tiny central zones seems always to be excitatory (Hubel & Wiesel 1966). The surround is much larger and is inhibitory. (This situation appears not too different qualitatively from that in *Limulus*, in which stimulation of the unit being recorded from always results in excitation of that unit, whereas stimulation of the surrounding units always induces inhibition.)

The receptive fields of more-peripheral primate ganglion cells and cat ganglion cells are also relatively simple. Again, most of the ganglion cells behave quite like one another and might thus be described as 'homogeneous' or 'uniform'. The receptive fields are also made up of two concentric and antagonistic zones, and the cells can be maximally driven by spots of light (Kuffler 1953; Hubel & Wiesel 1960). However, the centres of these receptive fields are usually larger than those found in the foveal region, and the ganglion cells may be either excited or inhibited when light is shone on the field centre. The peripheral response, always antagonistic to the centre, may also be either inhibitory or excitatory.

In an intermediate group with regard to complexity of ganglion cell receptive fields are the retinas of rabbits and ground squirrels (Barlow, Hill & Levick 1964; Barlow & Levick 1965; Michael 1965). About half of the ganglion cells in these retinas have simple receptive fields as in cats or monkeys, but many of the other ganglion cells have receptive fields with more complex properties. Directionally sensitive receptive fields are found in both rabbits and ground squirrels, for example; and the rabbit possesses a few ganglion cells with receptive fields of complex (frog) type (Levick 1967).

In a final group with regard to complexity of ganglion cell responses are the retinas of frogs and pigeons. Few, if any, of the receptive fields in these latter

retinas appear to be of the simple concentric variety. To stimulate maximally these cells requires a variety of stimuli; simple spots of light work poorly (Maturana *et al.* 1960; Lettvin *et al.* 1961; Grüsser–Cornehls, Grüsser & Bullock 1963; Maturana & Frenk 1963). The cells in these retinas may be described as 'inhomogeneous' or 'nonuniform', in the sense that individual cells are responsive to quite specific features of the visual image.

In summary, the complexity of the physiological responses of the retinal ganglion cells correlates with the complexity of the synaptic organization of the retina, particularly that in the inner plexiform layer. Further anatomical analysis will perhaps increase understanding of how the responses of the ganglion cells and other cells in the visual system are evoked by the interplay of excitatory and inhibitory synapses in the plexiform layers. It is difficult to see how this information can be obtained solely by the physiological methods now available to us. For example, it seems doubtful that one could ever understand the complex synaptic interactions occurring among the amacrine cell processes in the inner plexiform layer of the frog by single-unit recordings from any one of the cells contributing processes to the inner plexiform layer. It is questionable whether even multiple-cell recordings could yield this information. To understand the functioning of the plexiform layers in the retina (or plexiform layers elsewhere in the nervous system), additional techniques must be employed; and the analysis of synaptic organization by electron microscopy is clearly a promising approach.

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