Proc. R. Soc. Lond. B. **201**, 57–72 (1978)
Printed in Great Britain

Synaptic organization of the inner plexiform layer of the retina of *Xenopus laevis*

By Karen A. Goldman† and S. K. Fisher
Department of Biological Sciences, University of California, Santa Barbara, California 93106, *U.S.A.*
(*Communicated by B. B. Boycott, F.R.S. – Received* 26 *September* 1977)

[Plates 1–6]

The inner plexiform layer (i.p.l.) of the retina of the South African clawed frog, *Xenopus laevis*, was studied by electron microscopy. Photomicrographs of single sections revealed synaptic morphologies comparable to those in other vertebrate retinae. In a partial serial reconstruction of a bipolar terminal, however, some unusual arrangements were found. The bipolar terminal made some synapses that at first examination appeared much like conventional synapses, but subsequent sections always revealed an extremely small ribbon. Many of the ribbon synapses were found to contact more than two postsynaptic processes; up to six processes postsynaptic to one ribbon contact were seen. A reciprocal synapse was not evident at each ribbon synapse.

Montages of the entire width of the inner plexiform layer were constructed from sections cut from four different locations across the retina. The numbers of conventional and ribbon synapses per unit volume of tissue were determined. The synaptic densities found in *Xenopus* were much lower than those reported for other frogs. Differences in synaptic densities from the four locations were found to be statistically insignificant. The overall amacrine/bipolar synapse ratio was 6.8/1. The synaptic densities in the inner plexiform layer did not change when the tissue was stained with lead citrate alone rather than with uranyl acetate and lead citrate.

The functional significance of the morphological and quantitative synaptic arrangements in *Xenopus* i.p.l. is discussed, and the synaptic organization is compared to that of other amphibia and vertebrates.

Introduction

Quantitative electron microscopic studies of the relative synaptic densities in the inner plexiform layer (i.p.l.) have furthered the understanding of the intricate synaptic connections found there (Dowling 1968; Dubin 1970). Two morphological types of synapses are found in the i.p.l., those made by amacrine cells (termed 'conventional') and those made by bipolar cells (termed 'ribbon') (Dowling & Boycott 1965, 1966; Raviola & Raviola 1967; Dubin 1970). The relative frequencies of these synapses have been correlated to the relative input of information received

† Present address: Department of Physiology, University of California, San Francisco, California 94143, U.S.A.

by ganglion cells from amacrine and bipolar cells. A high amacrine to bipolar synapse (A/B) ratio has been found in various species, among them the frog, where physiologically 'complex' ganglion cell receptive fields, often sensitive to the presence or direction of movement, predominate (Dowling 1968; Dubin 1970). The rôle of amacrine cells in the mediation of these complex receptive field properties has been supported as well by studies of physiological responses from retinal neurons (Werblin & Dowling 1969; Norton, Spekreijse, Wagner & Wolbarsht 1970). In other species, such as the monkey or the cat, where ganglion cells with concentric and antagonistic centre-surround receptive fields are most common, lower A/B ratios have been found (Dowling 1968; Dubin 1970). This correlation of A/B ratio with receptive field type has been demonstrated in a variety of species (Dubin 1970; West & Dowling 1972).

Recently, however, surprisingly different results have been reported from i.p.l. synaptic density studies by different investigators with the same species. Chernenko & West (1976) discussed this problem in their report of anatomical plasticity of the rat i.p.l. They reasoned that these different values for absolute densities of synapses should not be surprising in as much as such differences, in part, could be due to differences in methodology; including fixation, staining, photomicrography, sampling, the criteria used for identifying synapses, or bias in scoring synapses. Moreover, differences in the animals themselves, reflecting their age or pigmentation could also yield differences in the data. Despite these possible sources of variation, Chernenko & West argued that different investigators using the same species and the same manipulation of the visual environment should produce relative changes in synaptic density which are in the same direction and of similar magnitude. Nevertheless, this was not the case in studies which they reviewed on the i.p.l. of the rat. There is similar lack of consistency in both absolute and relative values of i.p.l. synaptic densities in recent developmental studies of *Xenopus laevis* (L. J. Fisher 1976; Tucker & Hollyfield 1977). Calculation of the A/B ratios of the adult frogs used in these studies yields values ranging from 8.8/1 (L. J. Fisher 1976) to 2.8/1 (Tucker & Hollyfield 1977),† values lying at the high and low ends of A/D ratios found in a rank of nine different species by Dubin (1970). Although it may be very difficult, it is evident that reproducible values for absolute synaptic densities are essential for a physiological or phylogenetic interpretation. Moreover, some dissimilarity in the pattern of synaptic development in these studies (L. J. Fisher, 1976; Tucker & Hollyfield 1977) suggests the difficulties in even obtaining consistent relative values of synaptic density.

We have investigated the variation of i.p.l. synaptic densities between four retinal regions in *Xenopus laevis* (the aquatic African clawed frog), using the methods and criteria established by Dubin (1970). We felt that such regional variation might be a source of the inconsistencies in studies of synaptic densities,

† The amacrine synaptic densities used in these calculations are the combined single and serial conventional synaptic densities. The A/B ratio from Tucker & Hollyfield's (1977) study is that of the adult frogs reared on a normal light/dark schedule.

especially developmental studies, when the eye is small and the area sampled might extend across a large portion of the eye. Although the regional synaptic densities do vary considerably, we found that the variation is statistically insignificant. As a prerequisite to this study we examined i.p.l. synapses in serial sections to determine the synaptic arrangements made by amacrine and bipolar cells. This allowed us to compute an A/B ratio from synapses identified in single sections. Finally, in order to compare our counts with those of other studies, we considered the effect of staining method on synaptic density as done by Yazulla (1974) on the pigeon.

Methods

The eyes were dissected from an adult male *Xenopus* and the lenses were removed to facilitate penetration of the fixative. A notch was made in the ciliary margin at the superior pole so that the orientation of the eye could be determined after embedding. The eyes were fixed on ice in veronal acetate buffered 1.3 % OsO_4 with 15 mg/ml sucrose and 0.133 mg/ml $CaCl_2$. They then were dehydrated in a series of graded ethanol–water solutions, transferred to propylene oxide, and embedded in Araldite 6005. The right eye was sectioned until a plane containing the superior and inferior poles plus the optic nerve was reached and 1 μm sections, stained in methylene blue and azure II, were prepared for light microscopy. At numerous points between the optic nerve and the ora serrata, the width of the i.p.l. was measured by two independent observers. Four small blocks, their locations shown in figure 1, were carved from the retina and remounted on blank blocks with structural adhesive. Silver–grey sections (approximately 700 Å† thick) were cut from each of the four blocks, collected either on 200-mesh copper grids or 0.2 % formvar coated slot grids, and stained for 20 min in 1 % aqueous uranyl acetate (Watson 1958) and 10 min in lead citrate (Reynolds 1963). Sections used for comparison of staining procedure were stained with lead citrate only. Sections were examined either on a Siemens 1A or 101 electron microscope.

Large photomontages of the i.p.l. extending from the edge of the inner nuclear to the edge of the ganglion cell layer were constructed. At each location, three subsamples, comprising a total area of 2500–3000 μm^2, were examined. The montages were printed at a final magnification $\times 30000$ and scored for the presence of conventional and ribbon synapses according to the criteria established by Dubin (1970). True synaptic densities (i.e., not biased by synaptic size) were calculated with the modified Abercrombie correction (Abercrombie 1946; Dubin 1970). Synaptic lengths were measured to the nearest 0.5 mm with a Bausch and Lomb 7X ocular micrometer. For measuring purposes the length of a conventional synapse was considered to be that distance along the membrane where both membrane specialization and synaptic vesicles coincided. Curved synapses were measured from end to end along a straight line, in accordance with the simplifying assumptions of the modified Abercrombie correction. A Fortran computer program (Goldman 1975) was used to aid in the compilation and analysis of the data.

† 1 Å = 0.1 nm = 10^{-10} m.

Results

(*a*) *General morphology*

In all sections examined by light microscopy, the *Xenopus* retina appeared to be completely uniform with no evidence of a specialized central area. Although there were local variations, the width of the i.p.l. appeared to be generally uniform except for the marked thinning at the ora serrata (figure 1, plate 1).

When examined in single thin sections, the *Xenopus* i.p.l. is similar to that in other vertebrate species. Examples of typical synaptic arrangements found in *single sections* are shown in figures 2–5, plate 1. Bipolar processes, characterized by the presence of synaptic ribbons, formed dyad and sometimes triad arrangements. Small bipolar processes were often completely filled with vesicles (figure 4); larger ones usually had vesicles primarily near the ribbons (figure 3), with mitochondria and small profiles of endoplasmic reticulum in the centre. Amacrine processes made conventional synapses onto bipolar terminals and other unidentified processes (figures 2, 4, 5). In most, but not all amacrine processes, vesicles were generally found only in the area immediately surrounding the synapse. Large amacrine processes also contained mitochondria, but profiles of endoplasmic reticulum were not common. Glycogen granules were more numerous in bipolar processes. Reciprocal synapses (figure 4) and serial conventional synapses (figure 5) were seen occasionally.

A partial reconstruction of a bipolar terminal and its many adjacent processes was made from the block closest to the optic nerve on the inferior side (figure 1). Since the shape of the terminal changes considerably throughout the series, and the surrounding processes move and completely interweave, the exact proportions and three-dimensional interrelation of all the processes could not be reduced to two dimensions. It was possible, however, to portray all of the synaptic connections of the bipolar terminal and its surrounding processes within this series (figure 6). This single bipolar terminal made 12 ribbon synapses in this series but no conventional synapses. A synapse which at first had seemed to be conventional took on the dyad configuration with the appearance of a short ribbon when followed serially (figures 7–12, plate 2). It was usually possible, however, to recognize a ribbon synapse in individual sections, even when the ribbon was not in that section, because the synapse throughout would appear to contact more than one postsynaptic process (figures 13–29, plates 3–6). Other bipolar terminals examined serially also made only ribbon synapses, and in micrographs of single sections we never saw conventional synapses in processes which also made ribbon synapses. Thus, for quantitative purposes all synapses identified as conventional in single sections were counted as amacrine synapses, and all ribbon synapses as bipolar synapses.

In single sections it was unusual to find a ribbon synapse with more than three postsynaptic processes. When examined serially, however, two to six possible postsynaptic processes were found (figure 6) on the basis of the presence of mem-

brane densification. Figures 13–15 and 16–29, plates 3, 4, 5 and 6 show serial micrographs through three ribbon synapses contacting four, five, and six postsynaptic processes. Despite the large number of processes postsynaptic to this bipolar terminal, only four reciprocal conventional synapses could be identified (figure 6). Some of the postsynaptic processes which made reciprocal contacts were quite long and were postsynaptic to more than one ribbon synapse. Serial synapses were observed between the processes surrounding the bipolar terminal. In general, the processes postsynaptic to each ribbon synapse formed complex, interlocking connections with each other and with processes postsynaptic to adjacent ribbons.

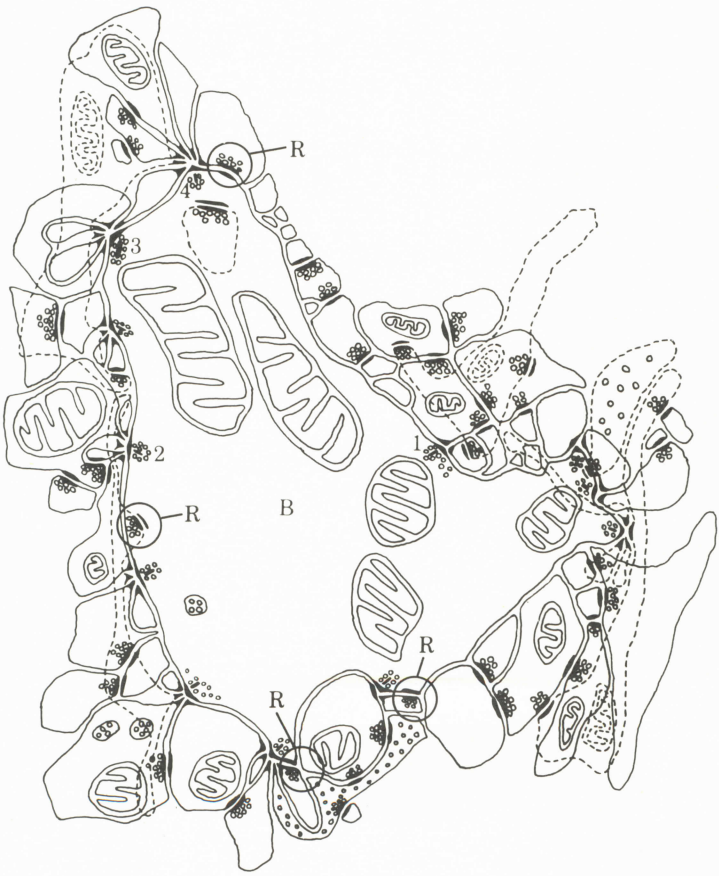


FIGURE 6. A semi-diagrammatic serial reconstruction of a bipolar terminal (B) and its surrounding processes. The serial reconstruction was made from 36 sections extending through about 2.5 μm. The exact proportions and three-dimensional interrelations of all the processes could not be reduced to two dimensions, but the synaptic connections are accurately portrayed. The dotted lines indicate processes which occurred either above or below the processes drawn with solid lines. The four reciprocal conventional synapses made onto this terminal are labelled 'R'. Electron micrographs of the numbered ribbon synapses are shown in the following figures. (Magnification equivalent to ×15000.)

(*b*) *Quantitative electron microscopy*

The results of the quantitative study of synaptic densities are shown in condensed form in tables 1 and 2. Table 1 shows the synaptic lengths and densities before, and table 2 after, modified Abercrombie correction. The data shown for each retinal location are actually the combined results of three subsamples each about 900 μm^2 in area, which were used in order to examine the variability of synaptic lengths and densities both within and between locations. For both conventional and ribbon synapses, neither the differences in measured lengths between subsamples nor between locations were statistically significant (tested by the unbalanced nested analysis of variance, a test used when samples are of unequal size – see Gates & Shiue 1962; Gower 1962; Tietjen & Moore 1968). Thus, for the entire retina the best estimates of uncorrected conventional and ribbon synaptic lengths are 2670 and 1280 Å, respectively.

Unbiased comparisons of the uncorrected synaptic densities were possible since the synaptic lengths did not vary across the retina. There appeared to be con-

---

Description of plate 1

Figure 1. A vertical section through the eye of an adult male *Xenopus laevis*. The four lettered regions were sampled for electron microscopy. (*a*) Superior peripheral retina. (*b*) Superior retina near optic nerve. (*c*) Inferior retina near optic nerve. (*d*) Inferior peripheral retina. S, superior; I, inferior; ★, optic nerve. (Magn. × 40.)

Figures 2–5. Examples of i.p.l. synapses in the *Xenopus* retina.

Figure 2. A conventional (amacrine) synapse (arrow). (Magn. × 45000.)

Figure 3. A ribbon (bipolar) synapse (arrow) contacting two postsynaptic processes in a dyad arrangement. (Magn. × 30000.)

Figure 4. Reciprocal synaptic arrangement. A ribbon synapse (arrow) contacts two postsynaptic processes, one of which makes a conventional synapse (small arrow) back onto the original bipolar terminal. (Magn. × 45000.)

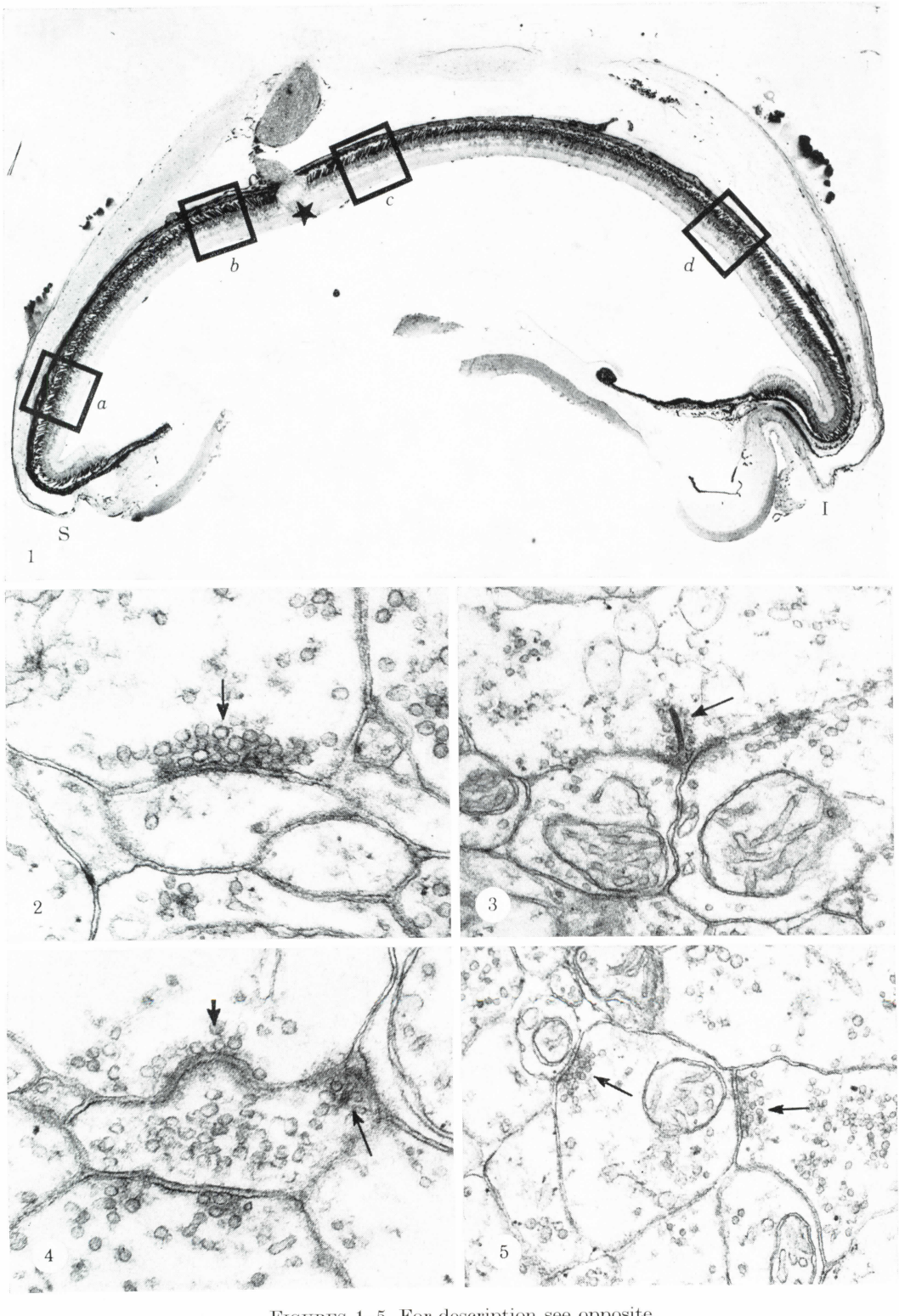
Figure 5. Serial synaptic arrangement. Two amacrine processes form a chain of conventional synapses (arrows). (Magn. × 30000.)

Description of plate 2

Figures 7–12. Serial sections of a bipolar ribbon synapse. In the first four serial sections (figures 7–10) this synapse appears to be a conventional synapse (arrow). In the fifth section (figure 11) a very small ribbon (short arrow) appears. In this section the synapse appears to be a dyad. The ribbon no longer appears in the sixth (figure 12) and last section showing evidence of synaptic specialization (arrow). This synapse is labelled 1 in figure 6. (Magn. × 45000.)

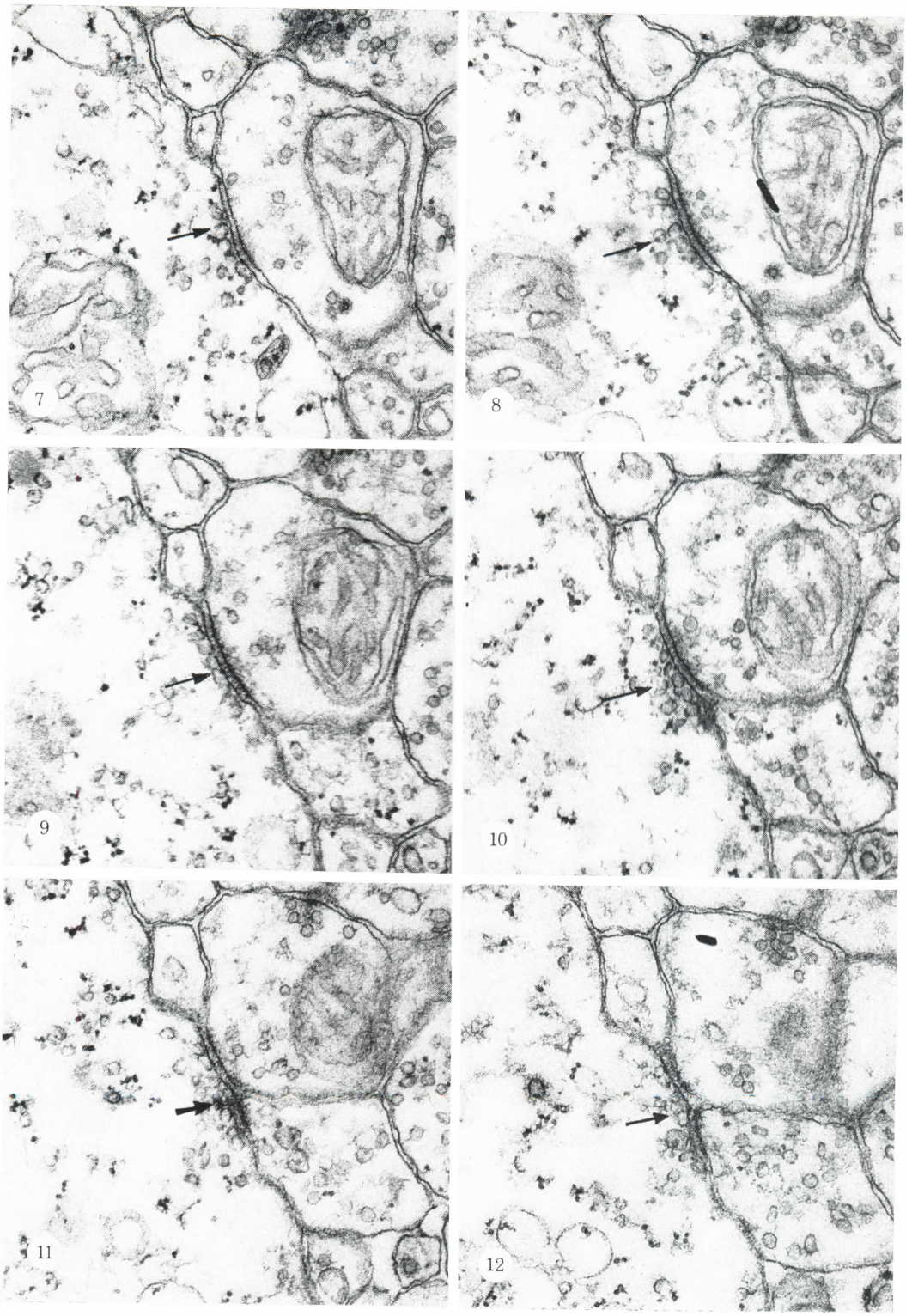
Description of plate 3

Figures 13–15. Serial sections through a ribbon synapse with four postsynaptic processes. The ribbon is not visible in figure 15 but the membrane densification remains. A fifth process is contacted by this ribbon synapse but is not shown in the series. This synapse is labelled 2 in figure 6. (Magn. × 40000.)

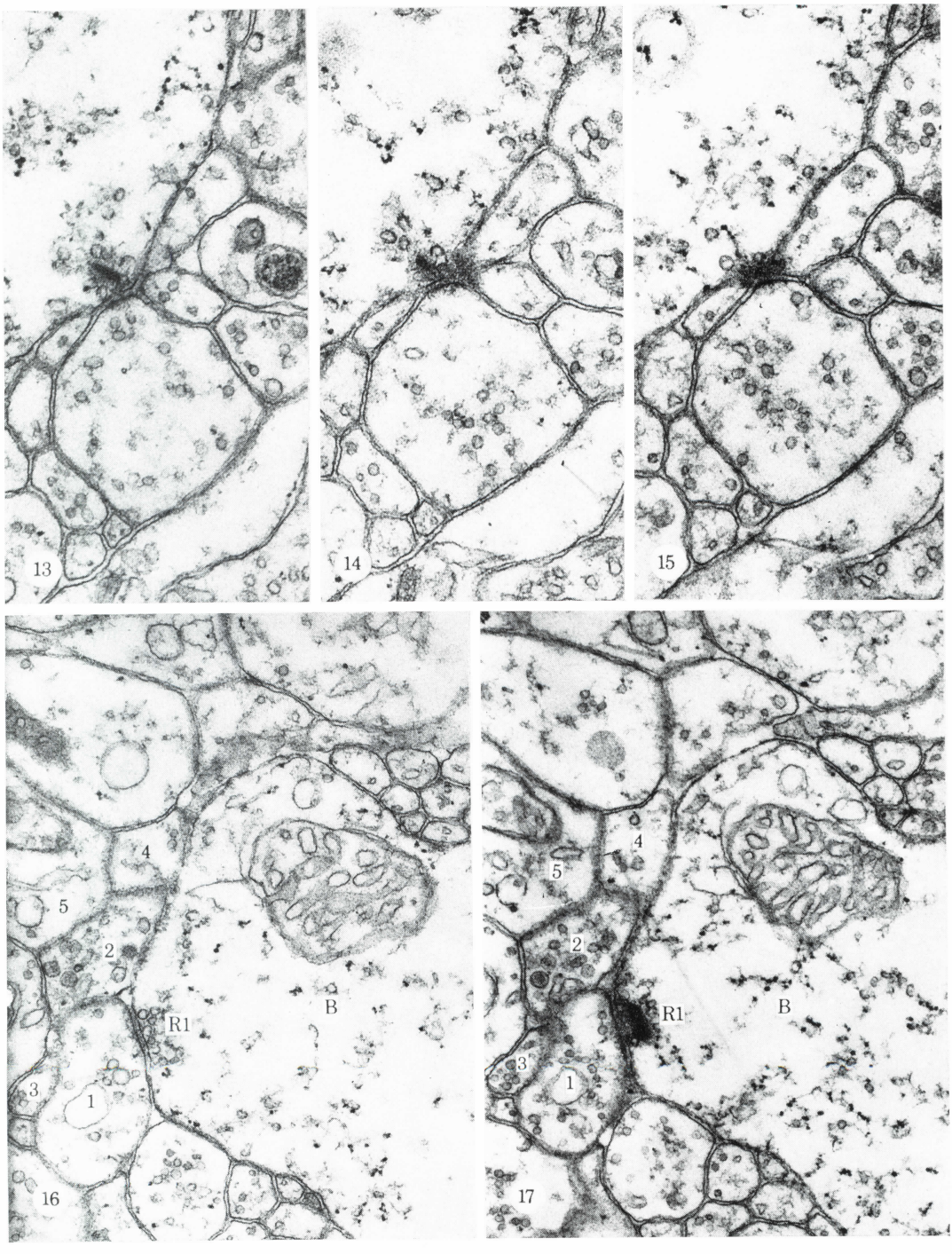


FIGURES 1–5. For description see opposite.

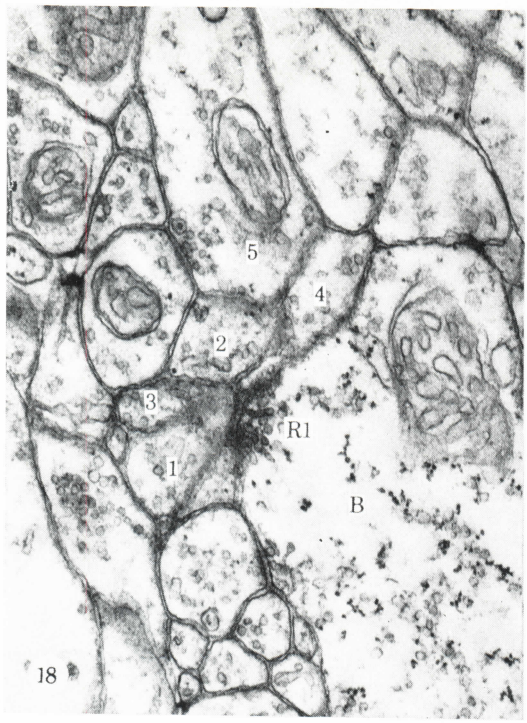
(*Facing p.* 62)

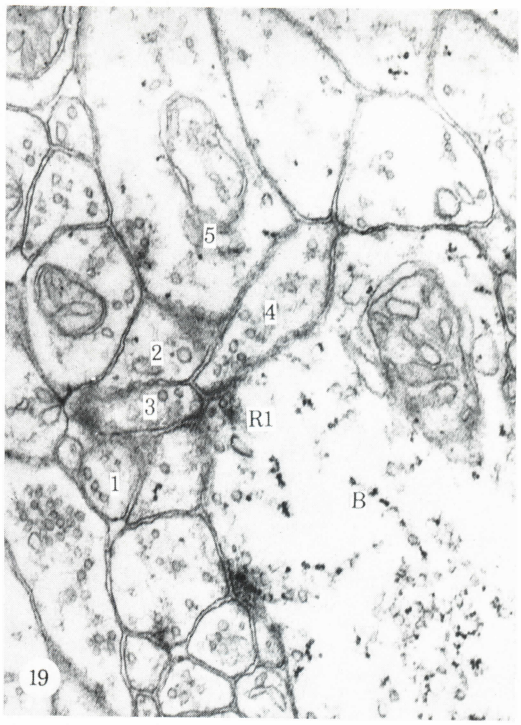


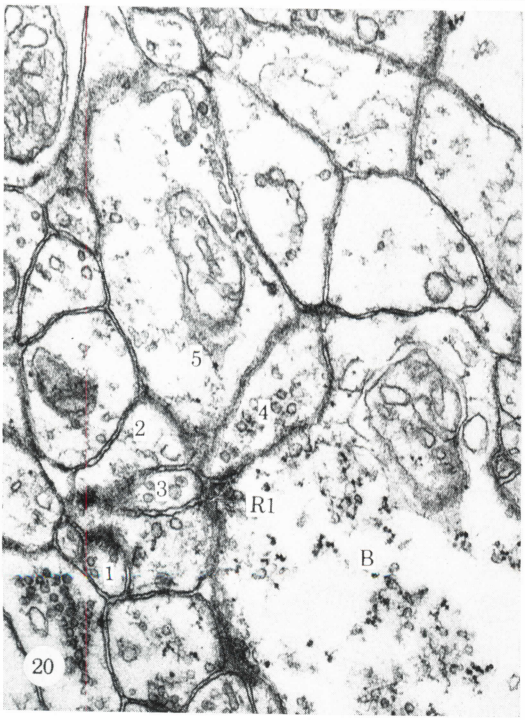
FIGURES 7–12. For description see p. 62.

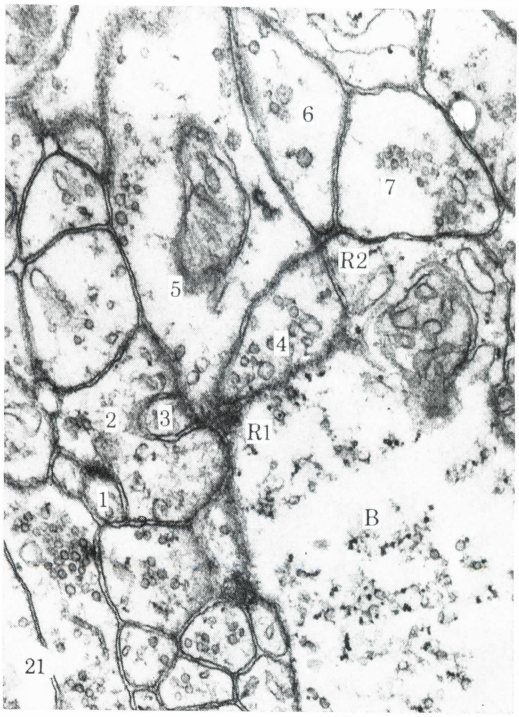


FIGURES 13–17. For description see pp. 62–63.

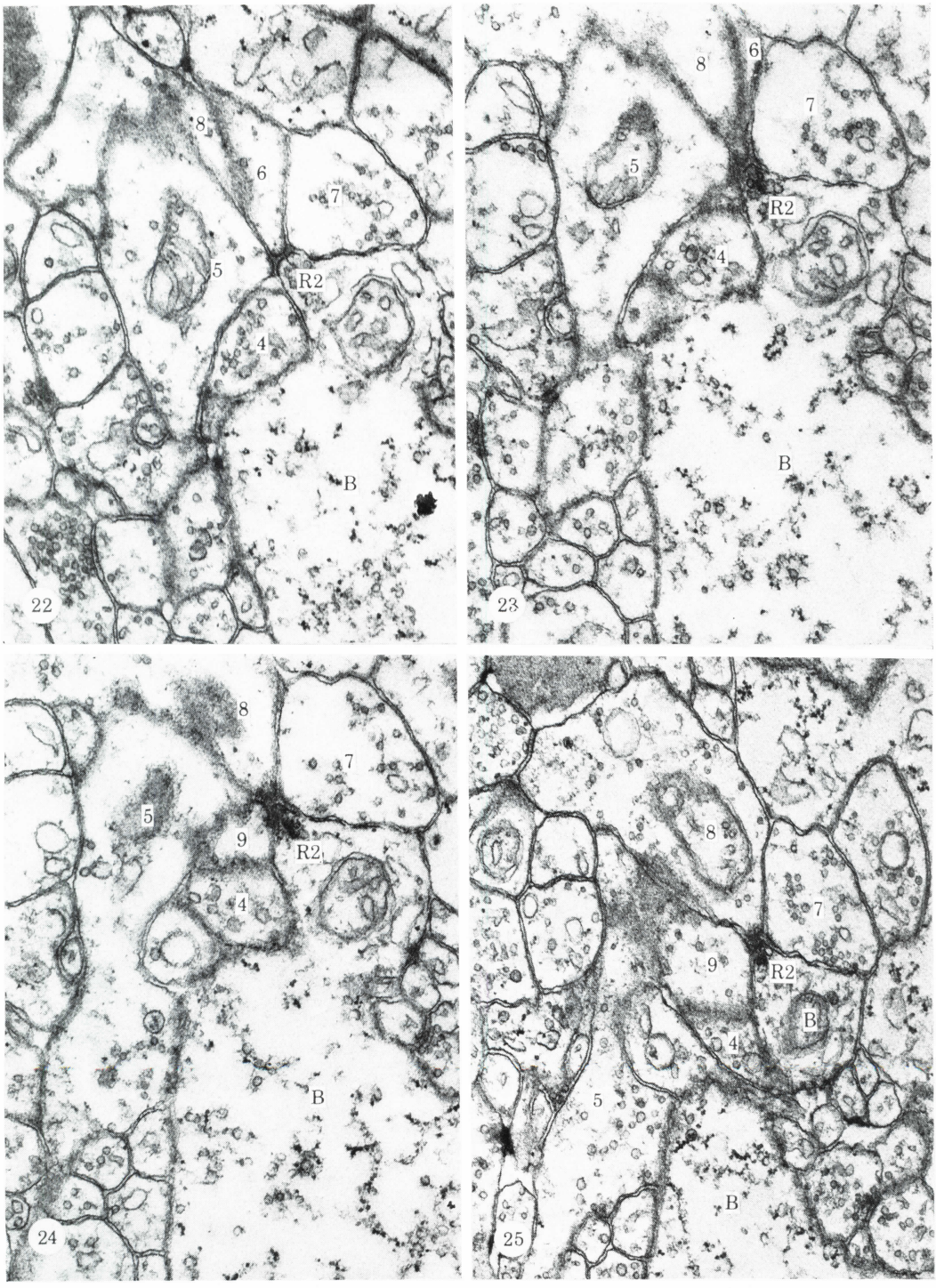




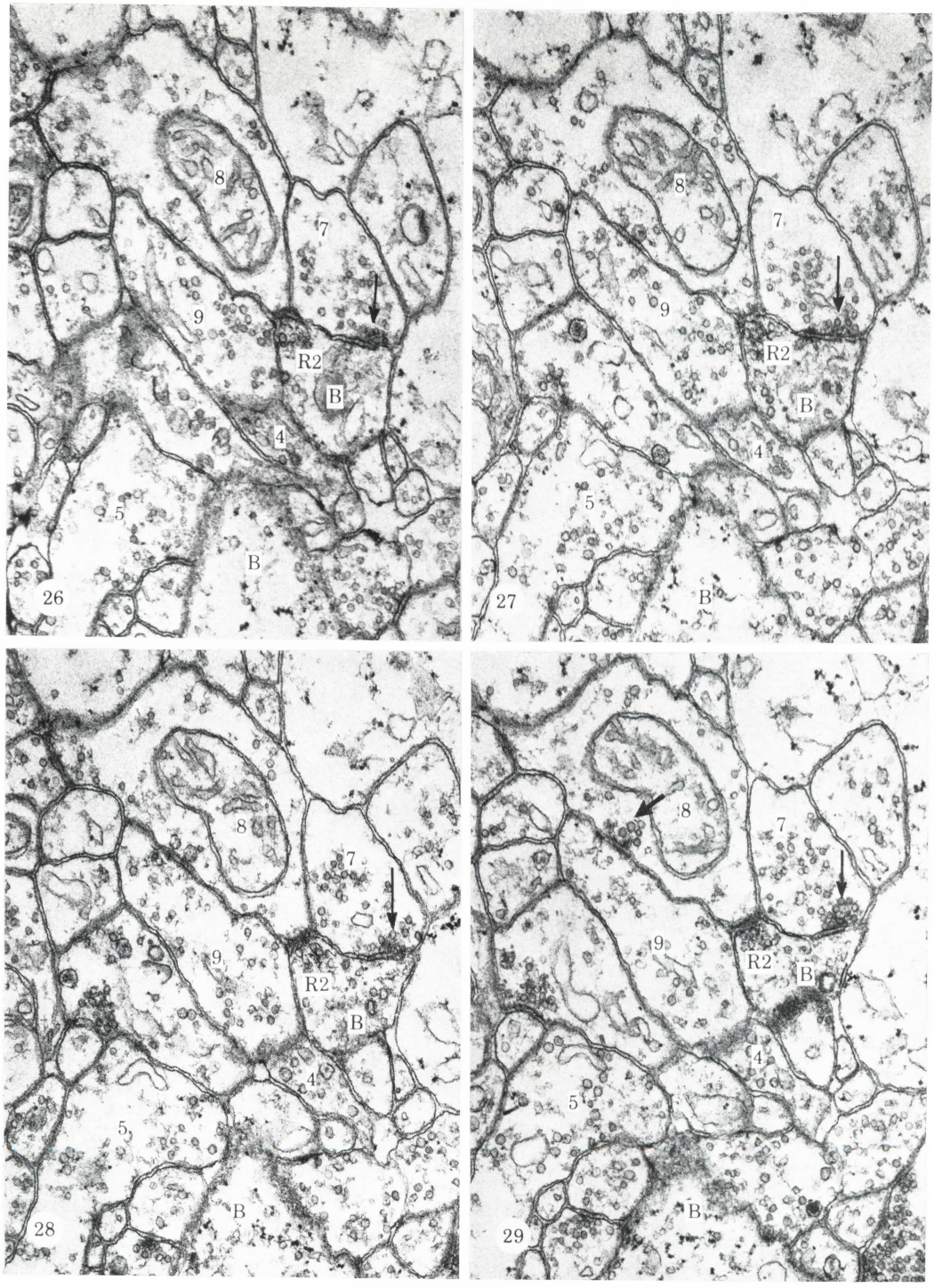




FIGURES 18–21. For description see p. 63.



FIGURES 22–25. For description see p. 63.



FIGURES 26–29. For description see opposite.

siderable variation between synaptic densities at different locations (table 1), but since the subsample densities also varied greatly, differences due to location proved statistically insignificant ($p > 0.05$) when tested by an analysis of variance. Thus, while locally variable, the overall densities of both conventional and ribbon synapses appear to be uniform across the retina. The result of the local variability is that large samples are necessary to get reliable estimates of the synaptic density.

We noticed that the densities of conventional synapses in both central samples were higher than in the two peripheral samples. The higher incidence of serial conventional synapses in the central regions (table 1) also hinted at increased amacrine participation in the circuitry of the central retina. To make sure that there were no statistically significant differences between particular pairs of locations, we tested the differences in density of conventional synapses between central and peripheral pyramids with the 'least significant difference' method and the Q-method (Snedecor & Cochran 1969). These tests showed that there were no statistically significant differences between any central and peripheral locations ($p > 0.05$); in fact even when the combined data from the two central samples were compared to the combined data of the peripheral samples, the difference in conventional synaptic density was not significant ($p > 0.05$) when tested by the S-method of all contrasts (Scheffe 1953, 1959; Guenther 1973).

True synaptic lengths, densities and A/B ratios at each location were calculated with the modified Abercrombie correction (table 2). Corrected A/B ratios ranged from 4.9/1 at the superior peripheral location to 8.4/1 at the inferior peripheral location. The average corrected A/B ratio for the whole retina was 6.8/1, compared to 10.8/1 before correction.

Yazulla (1974) found that staining procedure had no effect on conventional synaptic density in pigeon i.p.l., but ribbon density in material stained with lead citrate only was 60 % of that in tissue stained with both uranyl acetate and lead citrate. Thus, since staining procedure may have a large effect on the A/B ratio, and many of the species examined by Dubin (1970) were stained with lead citrate only, we investigated the effect of staining procedure on synaptic densities in

---

DESCRIPTION OF PLATES 3–6

FIGURES 16–29. Serial sections of ribbon synapses with five and six postsynaptic processes. In sections shown in figures 16–21, ribbon synapse R1 contacts five postsynaptic processes numbered 1–5. The ribbon itself only appears in figure 17 but membrane densities and aggregations of vesicles are visible in the other sections. Note that process 2 appears to be postsynaptic to R1 twice, both in figure 16 and figure 21. R1 is labelled 3 in figure 6. In serial sections shown in figures 21–29 a ribbon synapse (R2) contacts six postsynaptic processes numbered 4–9. The ribbon is visible in figures 23–25, but on the basis of the membrane densities and aggregations of vesicles it appears that synaptic contact is maintained throughout figures 21–29. R2 is labelled 4 in figure 6. In figures 25–29 the original bipolar terminal (B) is bisected by other processes, so that it appears as two separate processes. In figures 26–29 process 7 makes a reciprocal conventional synapse (arrow) back onto the bipolar terminal. Process 8 makes a conventional synapse onto process 9 (short arrow) in figure 29. (Magn. × 30 000.)

TABLE 1. QUANTITATIVE SYNAPTIC DATA, BEFORE MODIFIED ABERCROMBIE CORRECTION

		amacrine (conventional) synapses				bipolar (ribbon) synapses			
location	area μm^2	number of synapses	mean length Å	density synapses per μm^2	percentage serial configuration	number of synapses	mean length Å	density synapses per μm^2	ratio amacrine/ bipolar synapses
superior peripheral	2465	236	2620	0.096	2.1 %	28	1170	0.011	8.4/1
superior near optic nerve	2705	337	2590	0.125	3.3 %	30	1280	0.011	11.2/1
inferior near optic nerve	2791	322	2830	0.115	5.6 %	29	1300	0.010	11.1/1
inferior peripheral	2897	308	2630	0.106	2.6 %	24	1390	0.008	12.8/1
combined data	10858	1203	2670	0.111	3.5 %	111	1280	0.010	10.8/1
inferior near optic nerve (stained with lead citrate only)	2460	340	2410	0.138	3.8 %	23	1270	0.009	14.8/1

Xenopus. Some sections taken from the sampling regions next to the optic nerve on the inferior side (see figure 1) were stained only with lead citrate. Micrographs of i.p.l. from these sections had somewhat less contrast than those of material stained with uranyl acetate and lead citrate, making both types of synapses slightly more difficult to see. Synapses were scored as they had been on the doubly stained material, and the mean synaptic lengths determined. Data from i.p.l. stained only with lead citrate are shown in tables 1 and 2. Both conventional and ribbon lengths were slightly shorter than the double stained synaptic lengths, but testing by the unbalanced nestled analysis of variance showed that the difference

TABLE 2. QUANTITATIVE SYNAPTIC DATA, AFTER MODIFIED ABERCROMBIE CORRECTION

		amacrine (conventional) synapses		bipolar (ribbon)		
location	volume μm^3	corrected length Å	density synapses per μm^3	corrected length Å	density synapses per μm^3	ratio amacrine/ bipolar synapses
superior peripheral	173	3110	0.367	1320	0.074	4.9/1
superior near optic nerve	189	3080	0.488	1460	0.062	7.8/1
inferior near optic nerve	195	3380	0.431	1480	0.065	6.6/1
inferior peripheral	203	3130	0.402	1590	0.048	8.4/1
combined data	760	3180	0.414	1460	0.061	6.8/1
inferior near optic nerve (stained with lead citrate only)	173	2850	0.559	1450	0.056	10.0/1

was not significant even at the $p = 0.05$ level. Similarly, the differences in synaptic densities between single and doubly stained material were not significant. Thus, the staining procedure appears to have no statistically significant effect on synaptic counts in *Xenopus*, although if the effects were slight, they would have been masked by the high local variability in the i.p.l. The results of our staining study did not justify any modifications of our interpretation of synaptic densities in *Xenopus* i.p.l.

DISCUSSION

(*a*) *General morphology*

We have found that, in *Xenopus*, amacrine cells make conventional synapses and bipolar cells make ribbon synapses. Our quantitative results show that the average corrected length of the ribbons (1460 Å) is longer than that reported by Dubin (1970) for the frogs *Rana pipiens* and *R. catesbeiana*, but at the short end of the range for all other species examined. Wong-Riley (1974) suggested that i.p.l. ribbons may either participate in synaptic transmission, have a metabolic function, or be vestigial structures. It is difficult to imagine how the unusually

short ribbons found in *Xenopus* i.p.l. could function in synaptic transmission as a guide for vesicle release (Gray & Pease 1971); many of the vesicles surrounding small ribbons in *Xenopus* i.p.l. appear to interact directly with the synaptic membrane as do vesicles in conventional synapses (see figures 7–12). Moreover, bipolar cells have been found to make conventional synapses frequently in the tiger salamander (Wong-Riley 1974). Perhaps this is part of an evolutionary trend in amphibians towards disappearance of the ribbons; in some species the ribbons are short, and in others a portion have disappeared completely, leaving synapses which appear to be conventional in the bipolar terminals. Conversely, these findings could be evidence of the evolution of ribbon synapses in amphibian bipolar terminals.

Ribbon synapses in the i.p.l. of most species have been found to contact either two or three postsynaptic processes, in configurations termed dyads or triads, respectively (Dowling & Boycott 1966; Dowling 1968, 1970; Boycott 1974). In *Xenopus*, we have found that ribbon synapses may contact two to six postsynaptic processes. Ribbons contacting more than three postsynaptic processes were previously reported by Allen (1969), who occasionally saw four postsynaptic processes per ribbon in his serial section study of the human i.p.l.; and Wagner (1973), who published one micrograph of a ribbon contacting five postsynaptic processes in the cichlid fish, *Nannacara*. More than three postsynaptic processes per ribbon have not been reported in other serial section studies of the i.p.l. (human: Foos & Miyamasu 1973; dogfish: Witkovsky & Stell 1973; salamander: Wong-Riley 1974). On the other hand, the dogfish (Witkovsky & Stell 1973) and the tiger salamander (Wong-Riley 1974) both have a proportion of ribbons synapses that contact only *one* postsynaptic process. Thus, the number of processes postsynaptic to a ribbon synapse appears to exhibit some species variability.

It should be noted that identification of processes postsynaptic to a ribbons hinges on the membrane densification, rather than the ribbon itself, since it may not appear in all sections of a series through a synaptic complex (see figures 7–30). If this were not considered, postsynaptic processes might be missed should a section not include the ribbon. This is particularly important in *Xenopus* and other species which have relatively short i.p.l. ribbons.

A number of important physiological functions have been hypothesized for the reciprocal synapse, but there exists some confusion in the literature as to the frequency of reciprocal relationships between ribbon and amacrine-to-bipolar conventional synapses. Dowling & Boycott (1966) noted that in single sections of primate i.p.l. the number of ribbon synapses is very close to the number of amacrine-to-bipolar synapses; they suggested that this might be because all ribbon and amacrine-to-bipolar synapses were in a one-to-one reciprocal relation, which would be evident in serial sections. In the frog, Dowling (1968) suggested that for every ribbon synapse there was a reciprocal conventional synapse. More recently, however, studies with serial sections have revealed that both ribbon and amacrine-to-bipolar synapses may exist outside the reciprocal relation. Allen

(1969) found in his serial section study of bipolar terminals in the human i.p.l. that although most of the ribbon synapses had reciprocal contacts, many other amacrine-to-bipolar synapses were not associated with a ribbon. Such amacrine-to-bipolar synapses, as well as those in a reciprocal relation, could form part of the pathway for lateral interaction between bipolars in the i.p.l. (Boycott 1974). Other serial section studies of the human (Foos & Miyamasu 1973) and dogfish (Witkovsky & Stell 1973) i.p.l. suggest that many ribbon and amacrine-to-bipolar synapses are not reciprocally related. Similarly, in *Xenopus* two of the amacrine processes synapsing on the bipolar terminal shown in figure 6 were not contacted by ribbons; at least half of the ribbons were not contacted by a reciprocal synapse. The number of reciprocal relationships detected depends partly on the length of the series; the longer the series the more reciprocal contacts will be revealed. In addition there is the question of the physiological effectiveness of these reciprocal synapses made at some distance from the ribbons; if effective over certain (unknown) distances, some of the amacrine-to-bipolar synapses in *Xenopus* could act reciprocally on more than one ribbon (see figure 6). If effective over longer distances, some of the ribbon and amacrine-to-bipolar synapses within this series might have reciprocal relations with synapses outside the series. Functions proposed for reciprocal synapses in the i.p.l. include acting as gain control devices (Dowling 1967) or acting to translate the sustained signals of bipolar cells into their transient counterparts in amacrine cells (Werblin & Dowling 1969).

(*b*) *Synaptic densities*

(i) *Variation of synaptic densities across the retina*

We observed that the *Xenopus* retina is uniform, with no specialized central area, and constant i.p.l. thickness throughout. Denton & Pirenne (1951, 1954) and Saxén (1954) also reported that the retina appears uniform, with little variation in receptor density, and an even distribution of receptor types. Our finding that in *Xenopus* i.p.l. the densities of both ribbon and conventional synapses are statistically uniform across the retina is consistent with these light microscopic observations of no specialized retinal regions. Although the variations in synaptic densities are statistically insignificant, the densities do vary considerably despite the large areas examined at each location. The variations appear to be a function of changes in density occurring over small distances, requiring the examination of very large areas to obtain a reliable estimate of average synaptic density. Densities at different locations ranged from 0.367/μm^3 to 0.488/μm^3 for conventional, and from 0.048/μm^3 to 0.074/μm^3 for ribbon synapses. This range of variation is insufficient to account entirely for the differences in synaptic densities between the studies by Fisher (1976) and Tucker & Hollyfield (1977) of adult and developing *Xenopus*. L. J. Fisher (1976) reported that the combined single + serial conventional synaptic density of his juvenile adult frogs was *ca.* 662/1000 μm^3; the ribbon synaptic density was 75/1000 μm^3. Tucker & Hollyfield (1977) reported that the combined single + serial conventional synaptic density of their adult frogs on a

12/12 light/dark schedule was 292/1000 μm^3; the ribbon synaptic density was 106/1000 μm^3. Other factors which may contribute to disparities between these studies are methodological differences, such as the fixative used (Tucker & Hollyfield used glutaraldehyde with OsO_4 for postfixation, whereas we and L. J. Fisher used OsO_4 only); or differences in the visual environment, the ages of the animals, their hormonal levels, or the season when they were sacrificed. Differences in the visual environment during development are thought to affect synaptic densities in *Xenopus* (Tucker & Hollyfield 1977), and Wagner (1973) has shown that even short term light/dark changes may affect ribbon synaptic density in the i.p.l. of *Nannacara*. Thyroxine is thought to affect serial conventional synaptic density in *Rana* (L. J. Fisher 1972). Whether the other factors listed above actually affect synaptic densities is unknown.

Synaptic counts done on a number of species by Dubin (1970) showed that a high A/B ratio is found in animals, such as the frog, where physiologically complex ganglion cell receptive fields predominate, whereas a low A/B ratio is associated with a high proportion of receptive fields with centre-surround organization. The regional variations in synaptic density which we observed in the *Xenopus* i.p.l. resulted in a range of A/B ratios from 4.9/1 to 8.4/1, suggesting a considerable range of receptive field physiology when compared to Dubin's (1970) scale. In previous studies of variation of synaptic densities across the retina, A/B ratios were constant across the retina (Dubin & Turner 1977), or were constant within physiologically and anatomically specialized regions but varied between the regions (Yazulla 1974; Okabe 1976). In contrast, *Xenopus* has no systematic variation in A/B ratios with respect to locations examined here, but instead a high degree of variability at each location. This may reflect an overall uniform distribution of receptive field types across the retina, as was the case in *Rana* (Maturana, Lettvin, McCulloch & Pitts 1960; Pomeranz & Chung 1970; Pomeranz 1972), but local variations in the density of different ganglion cell receptive field types within each region.

(ii) *Correlation of synaptic densities with receptive field types*

The corrected A/B ratio of 6.8/1 which we found in *Xenopus* i.p.l. agrees well with the A/B ratios which Dubin (1970) found in *Rana catesbeiana* (6.3/1) and *R. pipiens* (6.4/1 and 9.5/1); implying that the physiological characteristics of the ganglion cell receptive fields in *Xenopus* may be similar to those in *Rana*. The receptive field properties of *Xenopus* ganglion cells have not been examined in detail, but a number of reports suggest that they are similar to those of *Rana* (Chung, Gaze & Stirling 1972, 1973; Chung, Bliss & Keating 1974; Chung, Keating & Bliss 1974). The quantitative synaptic organization of *Xenopus* i.p.l. differs significantly from other anurans in one respect, however; the densities of both ribbon and conventional synapses are low. The low ribbon density in *Xenopus* (0.061/μm^3) is particularly significant since Dubin (1970) found that ribbon densities of terrestrial species, from primate to pigeon, generally fell within the

range of 0.1–0.2/μm^3. Like *Xenopus*, however, all aquatic species that have been examined, have low synaptic densities (mudpuppy: Dowling & Werblin 1969; carp: Witkovsky & Dowling 1969). It is not known whether the lower synaptic densities affect the nature of processing of visual information in the i.p.l., or whether they are simply a reflexion of a low density of visual cells in these species. In any case, these low densities have the consequence that it is necessary to examine very large areas to produce reliable quantitative data.

The percentage of conventional synapses in serial configuration has also been suggested as an indicator of the type of ganglion cell receptive fields in a species (Dowling 1968; Dubin 1970). Dubin (1970) has shown that there is a correlation between the percentage of synapses in serial configuration and the A/B ratio in the terrestrial species he studied. It is interesting that in *Xenopus*, which has a high A/B ratio but low synaptic density, the percentage of conventional synapses in serial configuration is small (3.5 %) compared to that found in *Rana* (12.9–14.5 %) (Dubin 1970). It seems likely, on the basis of other morphological and physiological data, that this smaller percentage does not indicate a real difference in synaptic arrangement for visual processing, but most likely reflects the low amacrine synaptic density in *Xenopus*, causing the serial arrangement to be encountered less frequently in a single section.

Discussion of the A/B ratio and its correlation with ganglion cell receptive field type cannot be closed without mention of recent studies which suggest caution in the physiological interpretation of the A/B ratio. We have already discussed variation in synaptic density, possibly due to environmental or procedural factors, or just local variation, making it difficult to obtain a reliable A/B ratio. In addition, there are other problems in correlating this ratio to physiological properties. Foremost is that the ranking of species by complexity of retinal processing of visual information is no longer clear cut, and may even be different from the order Dubin (1970) assigned them (Stone & Fukuda 1974). Studies of the cat have shown that some (8 %, Cleland & Levick 1974) of its ganglion cell receptive fields are complex, like the rabbit's (in the rabbit, 40 % are non-concentric; see Oyster 1968). Another problem is the possibility that in some species there may be a high proportion of amacrine cells which produce transient signals, while in others a high proportion may produce sustained signals. Early recordings from amacrine cells suggested that they always produced transient responses (Werblin & Dowling 1969; Kameko 1970). Recent evidence suggests that in some species many or most amacrine cells produce sustained responses, and may even have centre-surround organization (Toyoda, Hashimoto & Ohtsu 1973; Kaneko 1973; Naka & Carraway 1975; Naka & Ohtsuka 1975; Naka, Marmarelis & Chan 1975; Kolb & Famiglietti 1974). Input from such amacrine cells might not be expected to produce complex ganglion cell receptive fields, hence a high A/B ratio in these species might not indicate a predominance of complex receptive fields.

The authors wish to thank Dr Katherine Esau for use of her electron microscope, Mr R. Gill for technical assistance, and Dr M. Dubin and Professor B. B. Boycott for their discussion and suggestions throughout the course of the project. The work was supported by a U.S. Public Health Service N.I.H. grant no. EY00888 to SKF.

References

Abercrombie, M. 1946 Estimation of nuclear population from microtome sections. *Anat. Rec.* **94**, 239–247.

Allen, R. A. 1969 The retinal bipolar cells and their synapses in the inner plexiform layer. In *The retina* (ed. B. R. Straatsma, M. O. Hall, R. A. Allen & F. Crescitelli), pp. 101–143. Los Angeles: University of California Press.

Boycott, B. B. 1974 Aspects of the comparative anatomy and physiology of the vertebrate retina. In *Essays on the nervous system* (ed. R. Bellairs & E. G. Gray), pp. 223–257. Oxford: Clarendon Press.

Chernenko, G. A. & West, R. W. 1976 A re-examination of anatomical plasticity in the rat retina. *J. Comp. Neurol.* **167**, 49–62.

Chung, S. H., Bliss, T. V. P. & Keating, M. J. 1974 The synaptic organization of optic afferents in the amphibian tectum. *Proc. R. Soc. Lond.* B **187**, 421–447.

Chung, S. H., Gaze, R. M. & Stirling, R. V. 1972 The maturation of toad visual units. *J. Physiol., Lond* **230**, 57–58*P*.

Chung, S. H., Gaze, R. M. & Stirling, R. V. 1973 Abnormal visual function in *Xenopus* following stroboscopic illumination. *Nature New Biol.* **246**, 186–189.

Chung, S. H., Keating, M. J. & Bliss, T. V. P. 1974 Functional synaptic relations during the development of the retino-tectal projection in amphibians. *Proc. R. Soc. Lond.* B **187**, 449–459.

Cleland, B. G. & Levick, W. R. 1974 Properties of rarely encountered types of ganglion cells in the cat's retina and an overall classification. *J. Physiol., Lond.* **240**, 457–492.

Denton, E. J. & Pirenne, M. H. 1951 The spectral sensitivity of the toad *Xenopus laevis*. *J. Physiol., Lond.* **115**, 66*P*.

Denton, E. J. & Pirenne, M. H. 1954 The visual sensitivity of the toad *Xenopus laevis*. *J. Physiol., Lond.* **125**, 181–207.

Dowling, J. E. 1967 The site of visual adaptation. *Science, N.Y.* **155**, 273–279.

Dowling, J. E. 1968 Synaptic organization of the frog retina: An electron microscopic analysis comparing the retinas of frogs and primates. *Proc. R. Soc. Lond.* B **170**, 205–228.

Dowling, J. E. 1970 Organization of vertebrate retinas. *Invest. Ophthal.* **9**, 655–680.

Dowling, J. E. & Boycott, B. B. 1965 Neural connections of the retina: Fine structure of the inner plexiform layer. *Cold Spring Harb. Symp. quant. Biol.* **30**, 393–402.

Dowling, J. E. & Boycott, B. B. 1966 Organization of the primate retina: Electron microscopy. *Proc. R. Soc. Lond.* B **166**, 80–111.

Dowling, J. E. & Werblin, E. S. 1969 Organization of retina of the mudpuppy, *Necturus maculosus*. I. Synaptic structure. *J. Neurophysiol.* **32**, 315–338.

Dubin, M. W. 1970 The inner plexiform layer of the vertebrate retina: A quantitative and comparative electron microscopic analysis. *J. Comp. Neurol.* **140**, 479–506.

Dubin, M. W. & Turner, L. 1977 Anatomy of the retina of the mink (*Mustela vison*). *J. Comp. Neurol.* **173**, 275–288.

Fisher, L. J. 1972 Changes during maturation and metamorphosis in the synaptic organization of the tadpole retina inner plexiform layer. *Nature, Lond.* **235**, 391–393.

Fisher, L. J. 1976 Synaptic arrays of the inner plexifrom layer in the developing retina of *Xenopus*. *Develop. Biol.* **50**, 402–412.

Foos, R. Y. & Miyamasu, W. 1973 Synaptic analysis of inner plexiform layer in human retina. *J. Comp. Neurol.* **147**, 447–454.

Gates, C. E. & Shiue, C. 1962 The analysis of variance of the s-stage hierarchal classification. *Biometrics* **18**, 529–536.

Goldman, K. A. 1975 The synaptic organization of the inner plexiform layer of the retina of *Xenopus laevis*. M.A. thesis, University of California, Santa Barbara.

Gower, J. C. 1962 Variance component estimation for unbalanced hierarchal classifications. *Biometrics* **18**, 537–542.

Gray, E. G. & Pease, H. L. 1971 On understanding the organization of the retinal receptor synapses. *Brain Res.* **35**, 1–15.

Guenther, W. C. 1973 *Concepts of statistical inference*, 2nd ed., pp. 360–366. New York: McGraw-Hill.

Kaneko, A. 1970 Physiological and morphological identification of horizontal, bipolar and amacrine cells in goldfish retina. *J. Physiol., Lond.* **207**, 623–633.

Kaneko, A. 1973 Receptive field organization of bipolar and amacrine cells in the goldfish retina. *J. Physiol., Lond.* **235**, 133–153.

Kolb, H. & Famiglietti, E. V. 1974 Rod and cone pathways in the inner plexiform layer of cat retina. *Science, N.Y.* **186**, 47–49.

Maturana, H. R., Lettvin, J. Y., McCulloch, W. S. & Pitts, W. H. 1960 Anatomy and physiology of vision in the frog (*Rana pipiens*). *J. gen. Physiol.* **43** (suppl.), 129–175.

Naka, K.-I. & Carraway, N. R. G. 1975 Morphological and functional identifications of catfish retinal neurons. I. Classical morphology. *J. Neurophysiol.* **38**, 53–71.

Naka, K.-I., Marmarelis, P. Z. & Chan, R. Y. 1975 Morphological and functional identifications of catfish retinal neurons. III. Functional identification. *J. Neurophysiol.* **38**, 92–131.

Naka, K.-I. & Ohtsuka, T. 1975 Morphological and functional identifications of catfish retina neurons. II. Morphological identification. *J. Neurophysiol.* **38**, 72–91.

Norton, A. L., Spekreijse, H., Wagner, H. G. & Wolbarsht, M. L. 1970 Responses to directional stimuli in retinal preganglionic units. *J. Physiol., Lond.* **206**, 93–107.

Okabe, S. 1976 Electron microscopic studies on the inner plexiform layer of the retina. Report 2. Calculative studies on synapses of different parts of the human retina. *Soc. Ophthal. Japonica* **80**, 126–131.

Oyster, C. W. 1968 The analysis of image motion by the rabbit retina. *J. Physiol., Lond.* **199**, 613–635.

Pomeranz, B. 1972 Metamorphosis of frog vision: Changes in ganglion cell physiology and anatomy. *Expl Neurol.* **34**, 187–199.

Pomeranz, B. & Chung, S. H. 1970 Dendritic-tree anatomy codes form-vision physiology in tadpole retina. *Science, N.Y.* **170**, 983–984.

Raviola, G. & Raviola, E. 1967 Light and electron microscopic observations on the inner plexiform layer of the rabbit retina. *Am. J. Anat.* **120**, 403–426.

Reynolds, E. S. 1963 The use of lead citrate at high pH as an electron opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.

Saxén, L. 1954 The development of the visual cells. Embryological and physiological investigations on amphibia. *Suomal. Tiedeakat. Toim.* A, IV, **23**, 1–95.

Scheffe, H. 1953 A method for judging all contrasts in the analysis of variance. *Biometrika* **40**, 381–400.

Scheffe, H. 1959 *The analysis of variance*, pp. 66–72. New York: John Wiley and Sons.

Snedecor, G. W. & Cochran, W. G. 1969 *Statistical methods* (sixth edition), pp. 271–275, 291–294. Ames: Iowa State University Press.

Stone, J. & Fukuda, Y. 1974 Properties of cat retinal ganglion cells: A comparison of W-Cells with X- and Y-cells. *J. Neurophysiol.* **37**, 722–748.

Tietjen, G. L. & Moore, R. H. 1968 On testing significance of components of variance in the unbalanced nested analysis of variance. *Biometrics* **24**, 423–429.

Toyoda, J.-I., Hashimoto, H. & Ohtsu, H. 1973 Bipolar-amacrine transmission in the carp retina. *Vision Res.* **13**, 295–307.

Tucker, G. S. & Hollyfield, J. G. 1977 Modifications by light of synaptic density in the inner plexiform layer of the toad, *Xenopus laevis*. *Expl. Neurol.* **55**, 133–151.

Wagner, H.-J. 1973 Darkness-induced reduction of the number of synaptic ribbons in fish retina. *Nature New Biol.* **246**, 53–55.

Watson, M. L. 1958 Staining of tissue sections for electron microscopy with heavy metals. *J. Biophysic. Biochem. Cytol.* **4**, 475–479.

Werblin, F. S. & Dowling, J. E. 1969 Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* **32**, 339–355.

West, R. W. & Dowling, J. E. 1972 Synapses onto different morphological types of retinal ganglion cells. *Science, N.Y.* **178**, 510–512.

Witkovsky, P. & Dowling, J. E. 1969 Synaptic relations in the plexiform layers of carp retina. *Z. Zellforsch. mikrosk. Anat.* **100**, 60–82.

Witkovsky, P. & Stell, W. K. 1973 Retinal structure in the smooth dogfish *Mustelus canis*: Electron microscopy of serially sectioned bipolar cell synaptic terminals. *J. Comp. Neurol.* **150**, 147–168.

Wong-Riley, M. T. T. 1974 Synaptic organization of the inner plexiform layer in the retina of the tiger salamander. *J. Neurocytol.* **3**, 1–33.

Yazulla, S. 1974 Intraretinal differentiation in the synaptic organization of the inner plexiform layer of the pigeon retina. *J. Comp. Neurol.* **153**, 309–324.