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Cellular and synaptic organization in the lamina of the dragon-fly *Sympetrum rubicundulum*

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[Plates 1–6]

The cellular and synaptic organization within the cartridges of the lamina of the dragon-fly have been analysed by light- and electron-microscopy. The ommatidium contains eight retinular (photoreceptor) cells all of which project to a single cartridge in the lamina. Six of the retinular cells terminate within the cartridges of the lamina (retinular terminals R 1–5 and R 8) and two pass through to the medulla (long visual fibres R 6 and R 7). The retinular terminals are arranged in pairs, which are matched in size, position and length. One pair terminates in the distal portion of the cartridge, while the remaining two pairs terminate at its proximal border. The cartridge also includes two large and two small axons of monopolar cells (M I, M II and M III, M IV respectively) and several unidentified minor profiles. The spatial arrangement of the main elements within a cartridge is constant through most of the depth of the lamina.

All synapses within the cartridges contain similar elongate presynaptic specializations and have either a dyadic or triadic arrangement of postsynaptic elements. Analyses of serial sections reveal three principal types of synaptic configurations: *A* is a triadic synapse in which retinular cells (R 1–5 and R 8) are presynaptic to monopolar elements, usually with M II as the central postsynaptic process and M I as the lateral processes; *B*, found only in the proximal lamina, is a dyadic feedback synapse from M I upon one pair of retinular terminals (R 5 and R 8); and *C* is a triadic synapse in which the long visual fibre (R 7) is presynaptic to M II, the central postsynaptic element. Thus each cell type in the lamina is involved in specific synaptic connections and this results in different synaptic relationships for the two large output axons of the lamina, M I and M II.

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Introduction

Few regions of neuropile appear to be organized with quite the level of anatomical precision that is observed in the optic lobes of insects. Much of the precision results from the grouping of photoreceptor (retinular) cells in structurally identical units, ommatidia, which are disposed across the retina in a regular array. Retinular cell axons leave the retina and project in an ordered way upon second-order neurones within the structurally identical cartridges of the lamina, the first optic neuropile. Axons from the lamina project to the second optic neuropile, the medulla.

Two aspects of the projection pattern between retina and lamina have been distinguished. The first of these is the pattern by which axon bundles connect between the retina and the distal face of the lamina, and a recent comparative account stresses a uniformity of projection amongst a broad spectrum of insects. That is, axon bundles between retina and lamina invariably maintain their retinotopic order without inversion or intermingling (Meinertzhagen 1976). The second of these patterns involves the distribution of individual retinular axons within the lamina neuropile. Here there are species differences, although in all cases the distribution of retinular elements within cartridges of the lamina relates by some simple spatial transposition to the pattern of their receptor cell bodies in the retina. The most frequently encountered example, that of the fused-rhabdome ommatidium where the photoreceptive organelles of each receptor group are united, is the homotopic projection of retinular axons from a single ommatidium to a single cartridge (Meinertzhagen 1976) (figure 1). In other groups where the cells of the retinula have divergent fields of view, for example in the open-rhabdomere ommatidium of the fly (Kirschfeld 1967), retinular terminals are distributed among a group of cartridges such that each cartridge receives axons from cells with the same field of view (Trujillo-Cenóz 1966; Braitenberg 1967).

Clearly the organization of the lamina cartridge needs to be considered in relation to the structural composition of the overlying ommatidia. So far studies of cartridge organization have centred largely upon species of higher Diptera, the flies, for which there now exists detailed documentation of cell types (Strausfeld 1970, 1971, 1976*a*), their ultrastructure and synaptic interconnections (Trujillo-Cenóz 1965; Boscheck 1971; Campos-Ortega & Strausfeld 1973; Strausfeld & Campos-Ortega 1973). These studies reveal not only the rather specialized dipteran open-rhabdomere organization with the divergent distribution of retinular axons (Trujillo-Cenóz 1966; Braitenberg 1967) but also a relative simplicity in the lamina both of the morphology of cell types and the cartridge organization (Strausfeld 1976*a*). The primacy of the fly in these studies prompted us to examine lamina organization in another insect group with different characteristics. With the exception of some recent studies on the bee (Ribi 1974, 1975*a*, *b*; Sommer & Wehner 1975) descriptions of the lamina in

insects other than the fly have in general been limited and fail to standardize upon a single species.

The dragon-fly was selected from among insects with both acute vision and visually interesting behaviour for a number of reasons. First, the availability of electrophysiological recordings of receptor (Autrum & Kolb 1968; Horridge 1969;

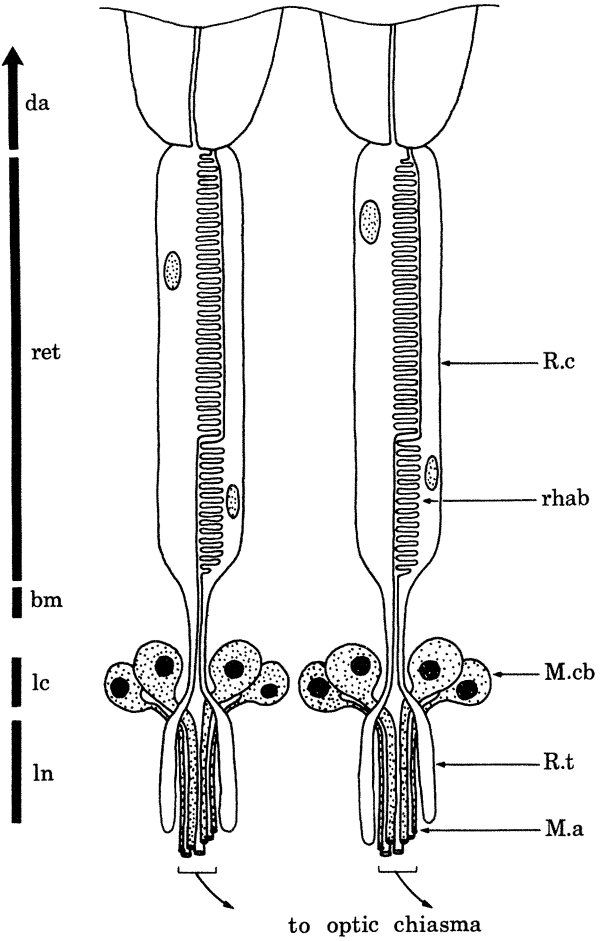

FIGURE 1. Schematic diagram to show the general principles of cell arrangement in the retina and lamina of an insect eye with a fused rhabdome. The rhabdomeres of individual retinular cells are tiered within the fused rhabdom. da, dioptric apparatus; ret, retina; bm, basement membrane; lc, lamina cortex; ln, lamina neuropile; R.c., retinular cell body; R.t., retinular terminal; M.cb, monopolar cell body; M.a, monopolar axon.

Laughlin 1973) and monopolar cell responses (Laughlin 1973, 1975) suggested that structural and functional correlations might be made. Secondly, the observations of Dowling & Chappell (1972) on the clarity of synaptic ultrastructure in the dragon-fly ocellus suggested that synapses in the dragon-fly lamina might be especially easily recognized. An additional interesting feature is the cellular

organization of odonate ommatidia (Horridge 1969; Ninomiya, Tominaga & Kuwabara 1969; Eguchi 1971). Within the fused-rhabdome of each ommatidium the rhabdomeres of individual receptors are united, although at any one level not all are represented, i.e. the rhabdome is tiered (figure 1) (Grenacher 1897), an arrangement which may have important consequences for receptor sensitivity (Snyder, Menzel & Laughlin 1973; Gribakin 1975).

We were especially concerned with three separate issues that underlie connectivity: (1) the cellular organization of identified neural elements, (2) the spatial pattern of synaptic interconnections between these identified neuronal elements, and (3) the configuration of their juxtaposed processes at points of synaptic intimacy. On the continued assumption that synapses identified morphologically are indeed the sites of the physiological synaptic interactions (e.g. Dowling & Boycott 1966), knowledge of the patterns of structural connectivity in the lamina is obviously a crucial step in understanding the transfer of visual information centrally from the retina. Although invertebrate synapses tend to be inconspicuous and their recognition has often been problematic, several studies have provided a number of criteria for presumptive synapses (Lamparter, Steiger, Sandri & Akert 1969; Trujillo-Cenóz 1969; Boschek 1971; Dowling & Chappell 1972). These include (1) presynaptic electron-opaque specializations with associated synaptic vesicle clusters, (2) filamentous material between pre- and postsynaptic membranes and (3) subsynaptic webs on postsynaptic membranes. An additional interesting feature of many invertebrate synapses is the presence of multiple postsynaptic elements (Lamparter *et al.* 1969). In the fly lamina Boschek (1971) has further observed that some of the postsynaptic elements are processes from glial cells. This latter finding is surprising and prompted us to examine especially carefully the postsynaptic elements at synapses in the dragon-fly lamina.

Materials and methods

Adult male and female dragon-flies (*Sympetrum rubicundulum*) were taken by net from a lakeside habitat in Lincoln, Massachusetts, in late autumn. The eye and adherent optic lobe were dissected from the head capsule under a drop of chilled fixative. The mouthparts and frons were excised with a razor blade and the posterior face of the head capsule dissected off to reveal the brain. The head was bisected into left and right halves; each half was kept separate and sliced with a new razor blade into horizontal longitudinal sections 0.5–1 mm thick. Only slices from the ventral eye half, left or right, were used in this study. The ventral retina has smaller facets than the dorsal retina and its ommatidia have more screening pigment.

Most of the material was fixed over ice in 2% osmium tetroxide buffered with 0.14 M veronal acetate, pH 7.3, containing 0.02% calcium chloride and 2.25% sucrose for a period of $1\frac{1}{2}$ h. Some material was prefixed cold (4 °C) for 2 h with 2.5% glutaraldehyde in 0.067 M cacodylate buffer, pH 7.3, containing 0.05%

calcium chloride, washed for several hours in the same buffer containing 4.5% sucrose, and postfixed with buffered osmium tetroxide as described above. Material was dehydrated in a graded ethanol series (10 min per step), infiltrated with propylene oxide (1 h), and embedded in Epon (Luft 1961) or in an Epon+Araldite mixture (Mollenhauer 1964).

Polymerized blocks were orientated with the head axes identified and in all cases sections were cut in a plane transverse to the lamina. For purposes of cell reconstruction, embedded specimens were cut serially on glass knives in ribbons of 1 μm thickness, and stained with toluidine blue for light microscopy. A single complete series of several hundred 1 μm sections was photographed with bright field optics using a Zeiss planopochromat 63/1.4 oil-immersion objective. To provide details of synaptic and cytoplasmic structure at high resolution, ultra-thin (grey) sections were cut on glass or diamond knives and examined singly on uncoated grids in the electron microscope. For analysis of synaptic connectivity patterns, grey to silver sections were cut serially with diamond or glass knives and the ribbons mounted in known orientation on single slot, formvar-coated grids. In both the distal and proximal lamina, two long series (approximately 75 sections) and two short series (approximately 25 sections) were studied. All series from the proximal lamina were cut from the same block and all series from the distal lamina from a second block, so ensuring consistent orientation of section series, crucial for cell numbering. Comparison of orientation between blocks was deduced by comparison with the light micrograph series. For each block the body axes, anterior and ventral, were recorded.

RESULTS

Cellular organization of the retinula and its axon bundle

In the dragon-fly the retinula, those photoreceptors grouped within one ommatidium, has a fused rhabdome. The receptor cells are arranged in a characteristic spatial pattern within an ommatidium, always of such constancy as to allow recognition of individual cells by criteria of size and shape. In addition, at a basal level, there is a consistent orientation of retinulae from ommatidium to ommatidium. There are eight receptor (retinular) cells, and their arrangement in the basal portion of the ommatidium is shown diagrammatically in figure 2*b*. Detailed structural analyses of the dragon-fly ommatidium are given by Eguchi (1971) and Horridge (1969); the cells are numbered here according to the system used by Horridge on the same species (from figures 21B and 18F of his 1969 paper). We have interpreted his observations in the following way and would emphasize some additional features. The retinular cell pattern appears generally symmetrical: three pairs of cells (R 5 and R 8; R 1 and R 4; R 2 and R 3) are arranged in a bilateral configuration, each member of a pair being situated opposite its partner. The retinula is, however, asymmetrical: cells R 6 and R 7 are an unmatched pair, situated at the posterior pole of the retinula cross-section

with R 6 ventral to R 7. Cell R 6 is slender and has been seen only in the basal position of the ommatidium, while cell R 7 is larger and is visible at all levels. Axons of R 7 and probably R 6 pass through the lamina and are thus the so-called long visual fibres (Strausfeld 1976*a*). The axons of the other three retinular cell pairs all terminate in the lamina.

The retinula of each ommatidium gives rise to a single axon bundle (figure 1). A series of light micrographs (figure 2) shows that every receptor of the retinula contributes an axon to the bundle although that of cell R 6 is extremely slender and occasionally difficult to resolve in individual sections. Within the bundle, the retinular cell axons maintain a constant spatial pattern. Each axon bundle projects exclusively to a single cartridge and the array of cartridges is in precise retinotopic order. Evidence for this pattern is only compelling after examining the trajectories of axon bundles through a complete micrograph series from retina to lamina, since individual axon bundles cannot often be distinguished in the fibre tracts between the two regions. Axon bundles separate from each other in the cortical zone of the lamina where they pass between the cell bodies of the monopolar neurones (figure 4, plate 1).

The orientation of retinular cell axon bundles and of the cross-sectioned axis of the cartridge with which each connects varies, i.e. both axon bundles and cartridge elements twist about their longitudinal axes. Clockwise and anti-clockwise twists have both been observed but the magnitude and direction of torsion has not been studied extensively to reveal a pattern. Two points of consistent orientation are located at (1) the basement membrane, where retinula orientation has R 7 in the most posterior position with reference to the body axis, and (2) in the proximal third of the lamina, where R 7 of each cartridge now occupies an anterior position, a net rotation of 180°.

Cellular organization of the cartridge

The cartridge, the unit structure of the lamina, comprises retinular elements from a single ommatidium, a number of morphologically distinguishable mono-

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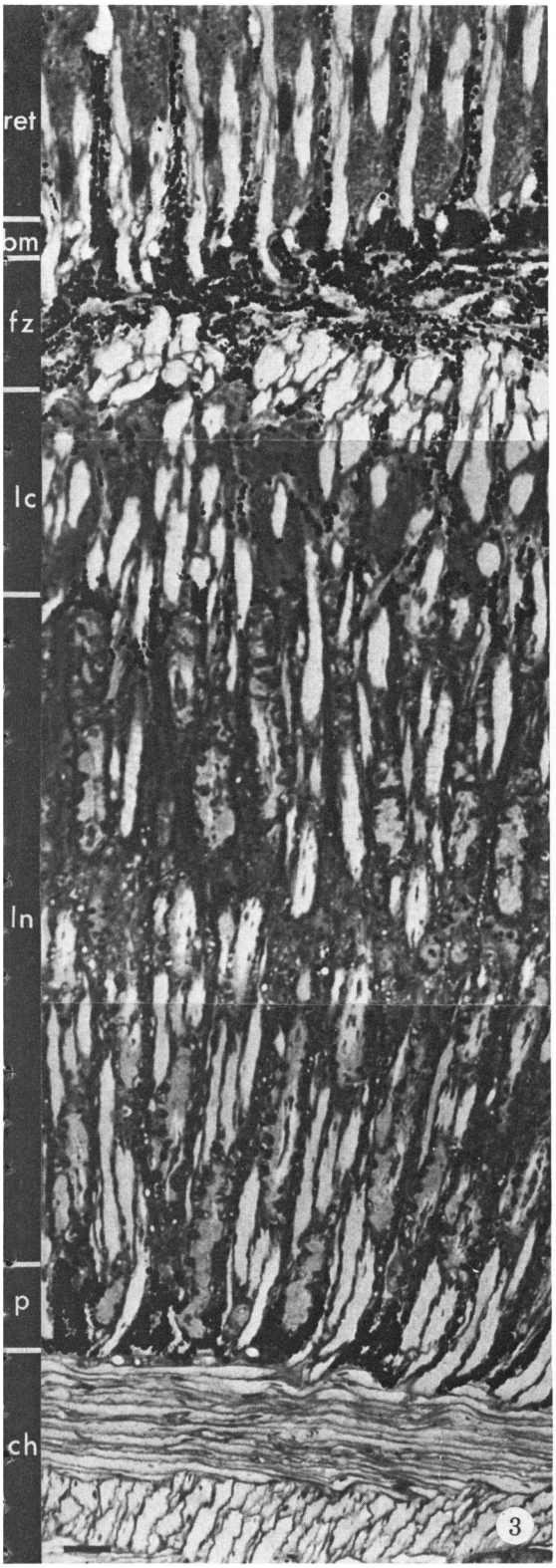
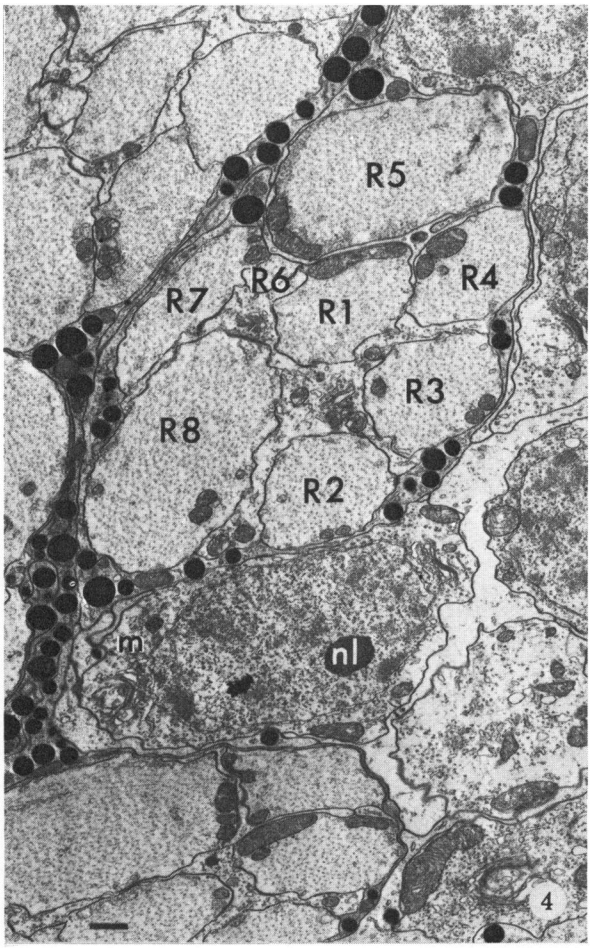
Description of plate 1

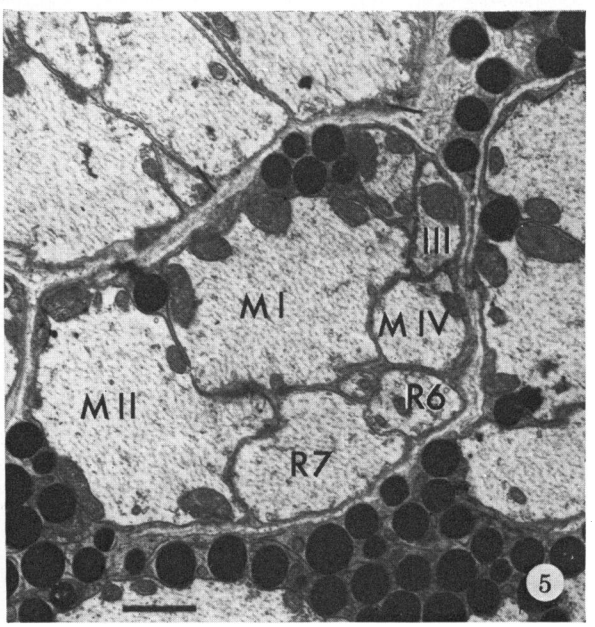
Figure 3. Longitudinal section through the lamina of the compound eye of *Sympetrum rubicundulum*. ret, retina; bm, basement membrane; fz, fenestration zone; lc, lamina cortex; ln, lamina neuropile; ch, chiasma; p, pigment band; T, tracheole. Light micrograph, calibration bar 10 μm.

Figure 4. Transverse section through the cell body layer of the lamina of the dragon-fly to show a single retinular axon bundle. Compare with the diagrammatic representation of an axon bundle in section 20 and 50 in figure 2*b*. Adjacent is a monopolar cell body (m) with its conspicuous nucleolus (nl). Electronmicrograph; calibration bar 1 μm.

Figure 5. Transverse section through the most proximal region of the dragon-fly lamina showing the axon bundle connecting a single cartridge with the chiasma. At this level the cartridge contains the monopolar axons M I–IV and the two long visual fibres. The remaining elements are unidentified. Electronmicrograph, calibration bar 1 μm.







Figures 3–5. For description see opposite.

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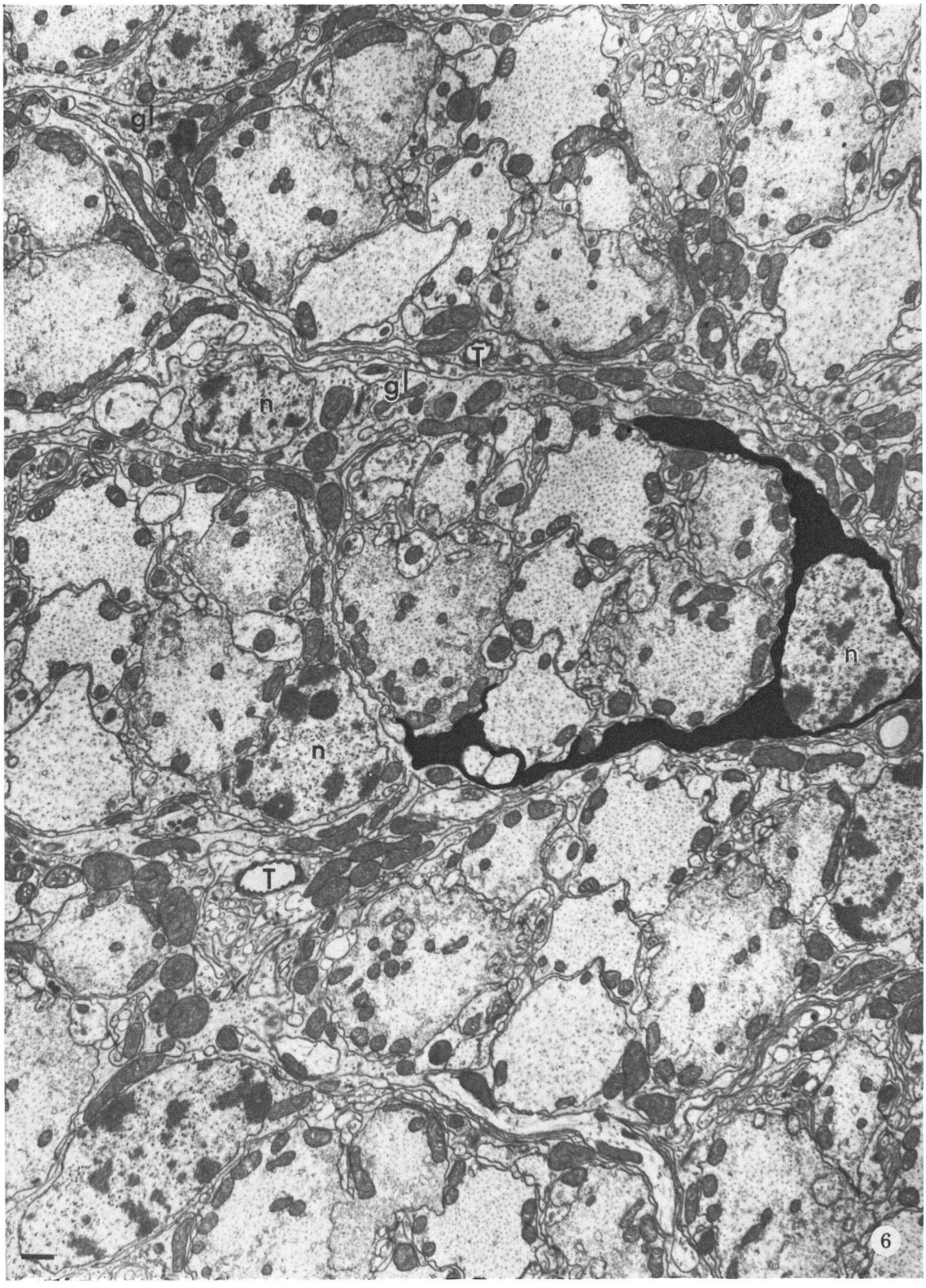

FIGURE 6. For description see opposite.

polar elements which arise from cell bodies in the cortex of the lamina and components of centrifugal elements derived from the medulla (Cajal & Sánchez 1915; Strausfeld 1976*a*). In addition processes from amacrine and tangential cells may interconnect cartridges (Strausfeld 1976*a*). Separation of the cartridges in the lamina is provided by glial processes which surround the entire structure. The investment by one glial cell which partially surrounds a cartridge has been shaded in figure 6, plate 2.

Within individual cartridges the organization of elements has been analysed from a series of light micrographs. The results of these analyses are summarized in figures 2*a*, *b*. They reveal characteristic differences in the diameters of the elements and a stratification of the depths of termination of the retinular axons. In both these respects members of retinular cell pairs are matched and will be treated accordingly in the following description.

Retinular terminals R 2 and R 3 are short, end in the distal third of the lamina, and have short bifid tips (figures 2*a*, *c*). They are the first axon pair to enlarge within the lamina and acquire the cytoplasmic characteristics of terminals, visible in light micrographs as modulations of staining intensity and changes in the distribution of mitochondria. Retinular cells R 1 and R 4 terminate in the proximal lamina; their terminals occupy the position in the cartridge periphery which has been occupied by the terminals of R 2 and R 3 in the distal lamina. The tips of the terminals of R 2 and R 3 coincide with the initial enlargement of R 1 and R 4 in the way depicted in figure 2*c*. Retinular terminals R 5 and R 8 also terminate in the proximal lamina and are the stoutest pair in the cartridge. In general, the diameter of a terminal varies in proportion with the diameter of its axon in the ommatidial bundle.

Axons R 6 and R 7 occupy a polar position in the cartridge cross-section; in the proximal lamina their position is in the anterior sector of the cartridge. Retinular axon R 7 is easily traced from ommatidium to chiasma because of its large diameter. By contrast, R 6 is extremely slender and is traced as a continuous element only with uncertainty. It occupies a position alongside R 7 and a slender profile is always seen in this same position through to the chiasma. The evidence is compatible with R 6 being a second long visual fibre.

There are two stout central axons found in all cartridges. These profiles are labelled monopolar I (M I) and II (M II) throughout. In several instances we have traced these fibres from their central position in the cartridge to cell bodies in the lamina cortex. Nuclei of the monopolar somata (figure 3, plate 1) have

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DESCRIPTION OF PLATE 2

FIGURE 6. Transverse section through the proximal lamina of the ventral eye of the dragon-fly. Nuclei (n) are of three glial cells which together invest a single cartridge; one of these glial cells has been shaded. The section is at the level of a layer of glial cell nuclei. Tracheoles (T) are present through the depth of the lamina. Electronmicrograph, calibration bar 2 μm.

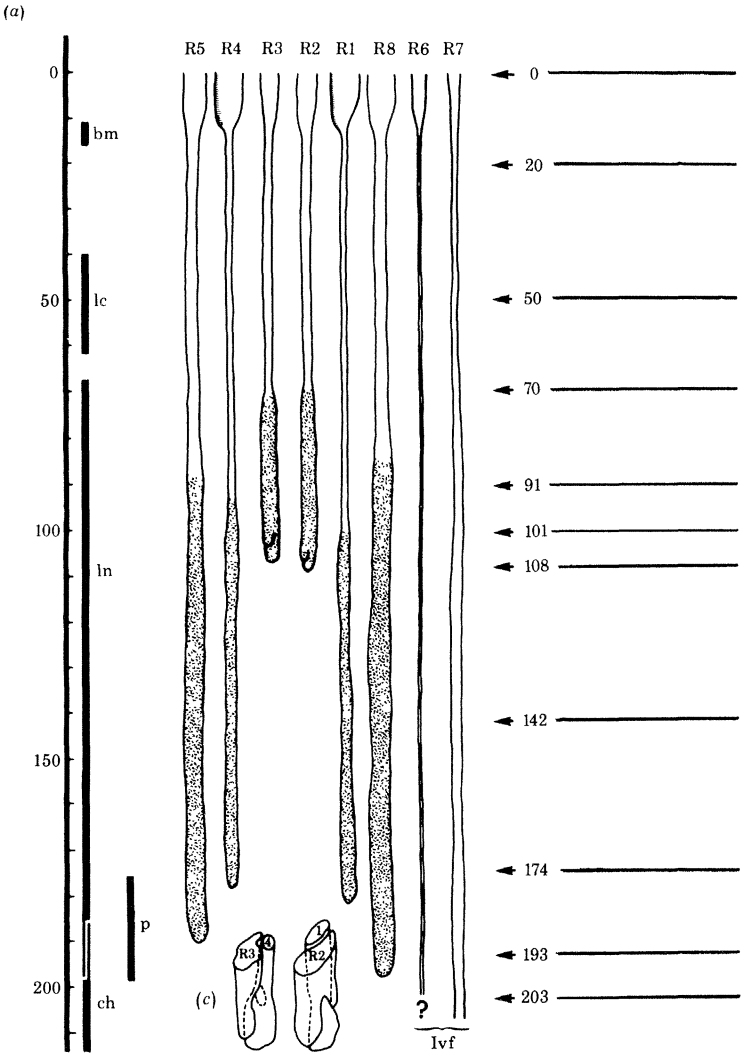
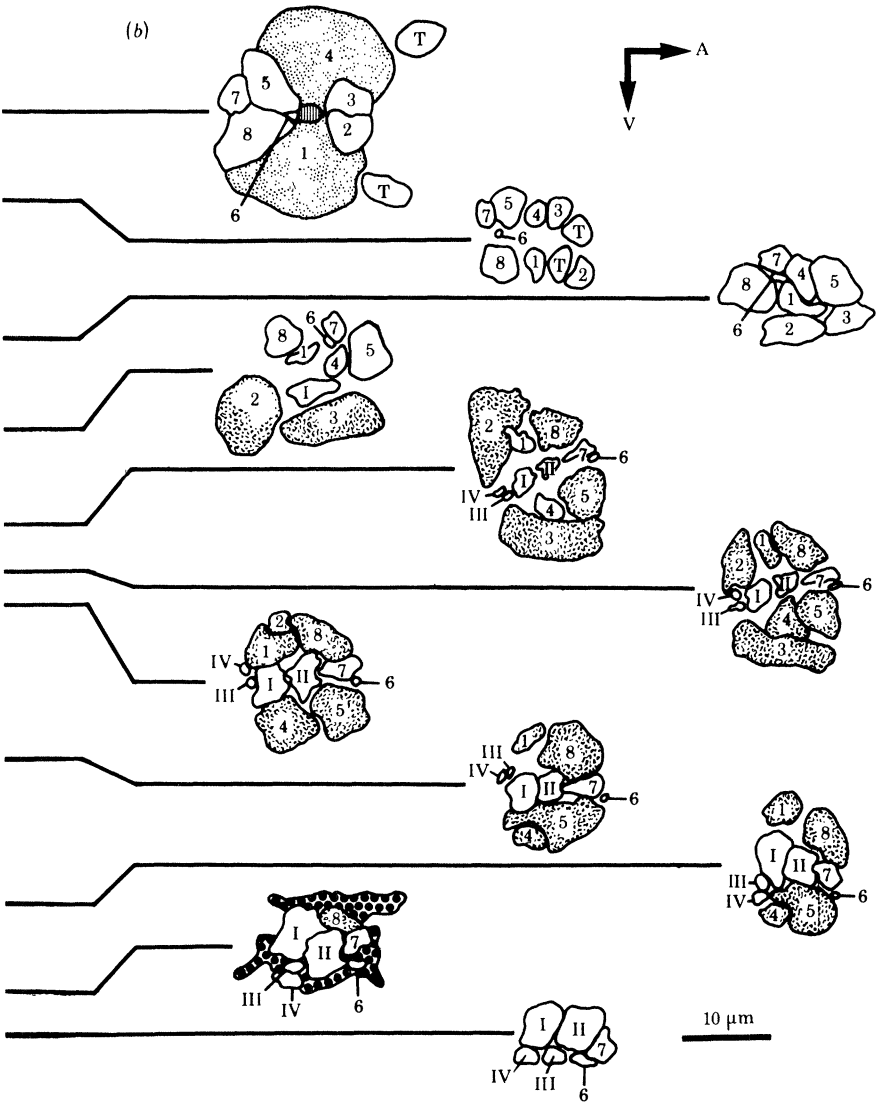


FIGURE 2. A diagrammatic summary of the main findings from serial photomicrography of the retinular terminal morphologies and their arrangement at different depths in a typical cartridge from the ventral region of the right eye.

(*a*) The retinular axons drawn from their identified cells of origin, R 1 to R 8, as reconstructed from consecutive photomicrographs of 1 μm sections numbered 0 to 200 along the ordinate. Also indicated on the ordinate are the positions of the basement membrane (bm), lamina cortex (lc), lamina neuropile (ln), chiasma (ch) and a dense pigment band (p.). The region of enlargement and cytological appearance characteristic of the retinular axon terminals are indicated for R 1–5 and R 8 by stippled areas. The sequence of retinular cells from left to right represents the anticlockwise sequence of their positions in the retinula. There is correspondence between the depth of termination of the shallow bifid endings of the retinular terminal pair R 2 and R 3 and the shoulders of the terminals of their respective neighbours R 1 and R 4 (see also *c*). The axons of R 7 and probably of its slender partner R 6 are the long visual fibres (lvf). Morphologies of axon and terminal are schematic and so comparable with a Golgi impregnate only in general characteristics. [*Legend continued on facing page*



(*b*) Tracings from serial light micrographs of the retinula (section 0), its axons bundle (sections 20 and 50), its underlying cartridge (sections 70, 91, 101, 142, 174 and 193) and cartridge axon bundle (section 203) from which the outlines in (*a*) were constructed. Arabic numerals identify retinular profiles and roman numerals monopolar profiles. Stippling indicates the high staining intensity of profiles that is consonant with terminal ultrastructure. Associated with R 2 and R 3 in the basal portion of the retinula are two tracheoles (T) which communicate more centrally with the tracheae of the fenestration zone. R 1 and R 4 are enlarged in the basal portion of the retinula and have densely staining peripheral cytoplasm with a clear central area. The cytoplasm of the six remaining retinular cells is clear at this level (section 0). In R 2 and R 3 of section 70 the transformation from axon to terminal is incomplete. The magnification in (*b*) is approximately twice that in (*a*). (*c*) The mutual relationships of the terminals R 2 and R 3 and their respective neighbours R 1 and R 4. As the latter pair expand to form their terminal they push between the parted ends of R 2 and R 3.

a single large conspicuous nucleolus which is visible not only in electron micrographs but also in serial light micrographs. This has facilitated identification of monopolar cell bodies particularly in the dorsal lamina where they are especially large. Nucleolar counts in the cortex of the dorsal lamina reveal a minimum of four somata per cartridge; this (assuming uniformity in the dorsal and ventral lamina) suggests two monopolar elements within each cartridge in addition to M I and M II. Two slender axons which travel the length of the lamina and enter the chiasma have tentatively been identified as monopolar elements M III and M IV, even though they are too fine to be traced with certainty to somata in the lamina cortex.

R 7, M II and M I are aligned in cartridge cross-sections with an orientation which is initially variable, but in the proximal two thirds of the lamina becomes anteroposterior with respect to the body axis (figure 2). Associated with them are R 6 (next to R 7) and M III and M IV (next to M I) (figures 2, 7 and 8, plate 3, and figure 9). Around the periphery of these central elements, retinular terminals R 1–5 and R 8 always maintain their relative positions without interweaving.

Axon bundles leaving the cartridge at the chiasmal interface comprise at least R 7, M I, M II, M III and M IV and probably R 6 (figures 2 and 5, plate 1). Remaining fibres (at least two in figure 5) are unaccounted for but may include centrifugal elements which have been observed in a wide variety of insect groups.

Ultrastructure of the cartridge

The main elements in electronmicrographs of cartridge cross-sections from the distal (figure 7) and proximal (figure 8) lamina have been identified according to their relative positions in the cartridge as determined by serial light microscopy. The presence of the terminals R 2 and 3 throughout the distal third of the cartridge is the criterion by which the distal lamina is distinguished from the proximal. The cytoplasmic appearance of the retinular terminals (R 1–5 and R 8) is quite distinct from that of the large axons that pass through the lamina (R 7, M I and M II). These distinctions are particularly clear in figures 8 and 15, plate 5, and also in figure 7 for R 2 and R 3 (which assume their terminal ultrastructure more distally in the cartridge). The retinular terminals show an even distribution of mitochondria and their cytoplasm contains pleomorphic vesicular material

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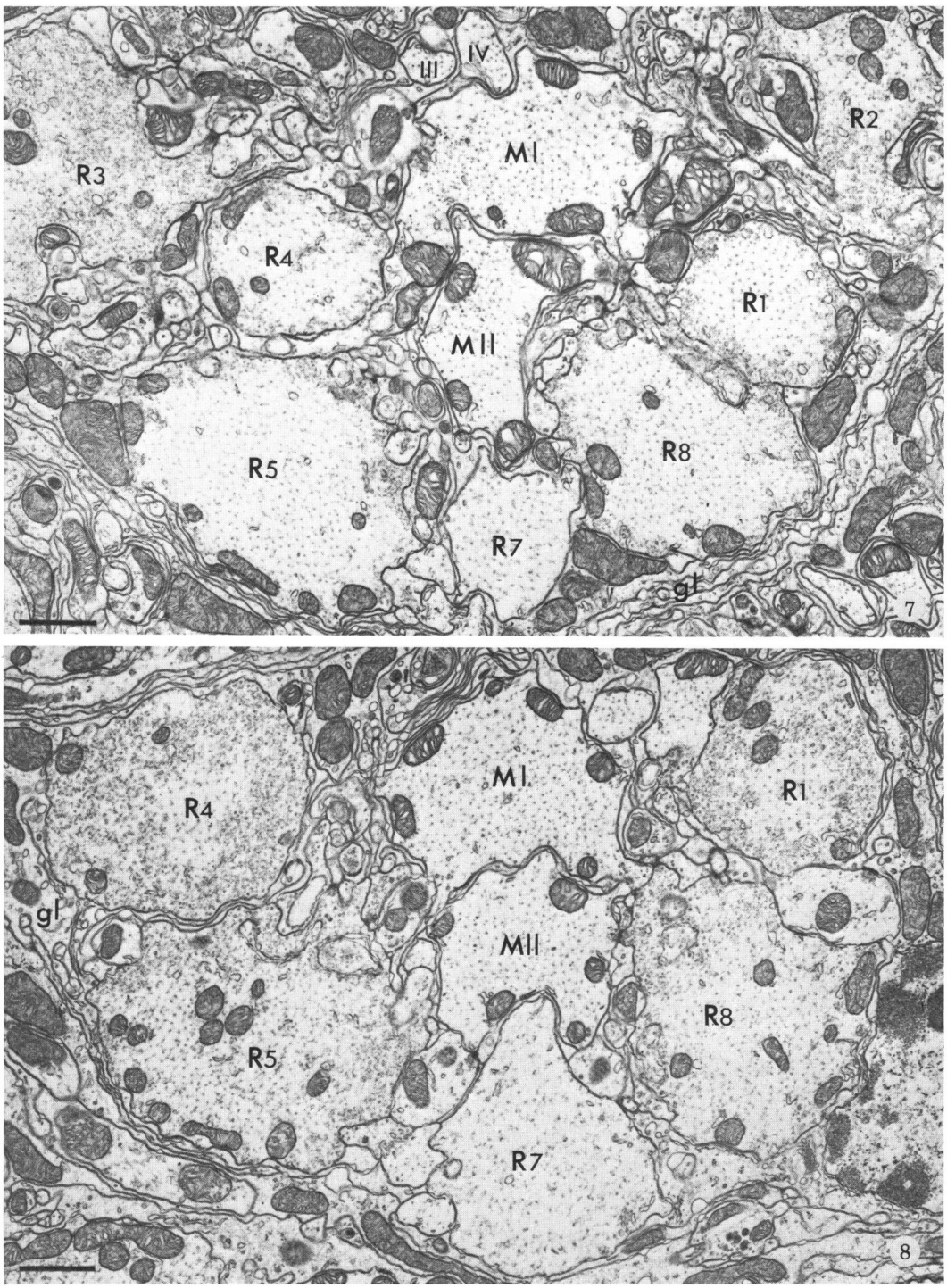
Description of plate 3

Figure 7. Transverse section through a single cartridge in the distal lamina of the ventral eye of the dragon-fly. At this level three pairs of retinular terminals are present, although only R 2 and R 3 show terminal ultrastructure (see text). A process of M I is clearly visible. Electronmicrograph, calibration bar 1 μm.

Figure 8. Transverse section through a single cartridge in the proximal region of the same lamina shown in figure 7. R 2 and R 3 terminated distal to this section. Electronmicrograph, calibration bar 1 μm.



FIGURES 7 AND 8. For description see opposite.

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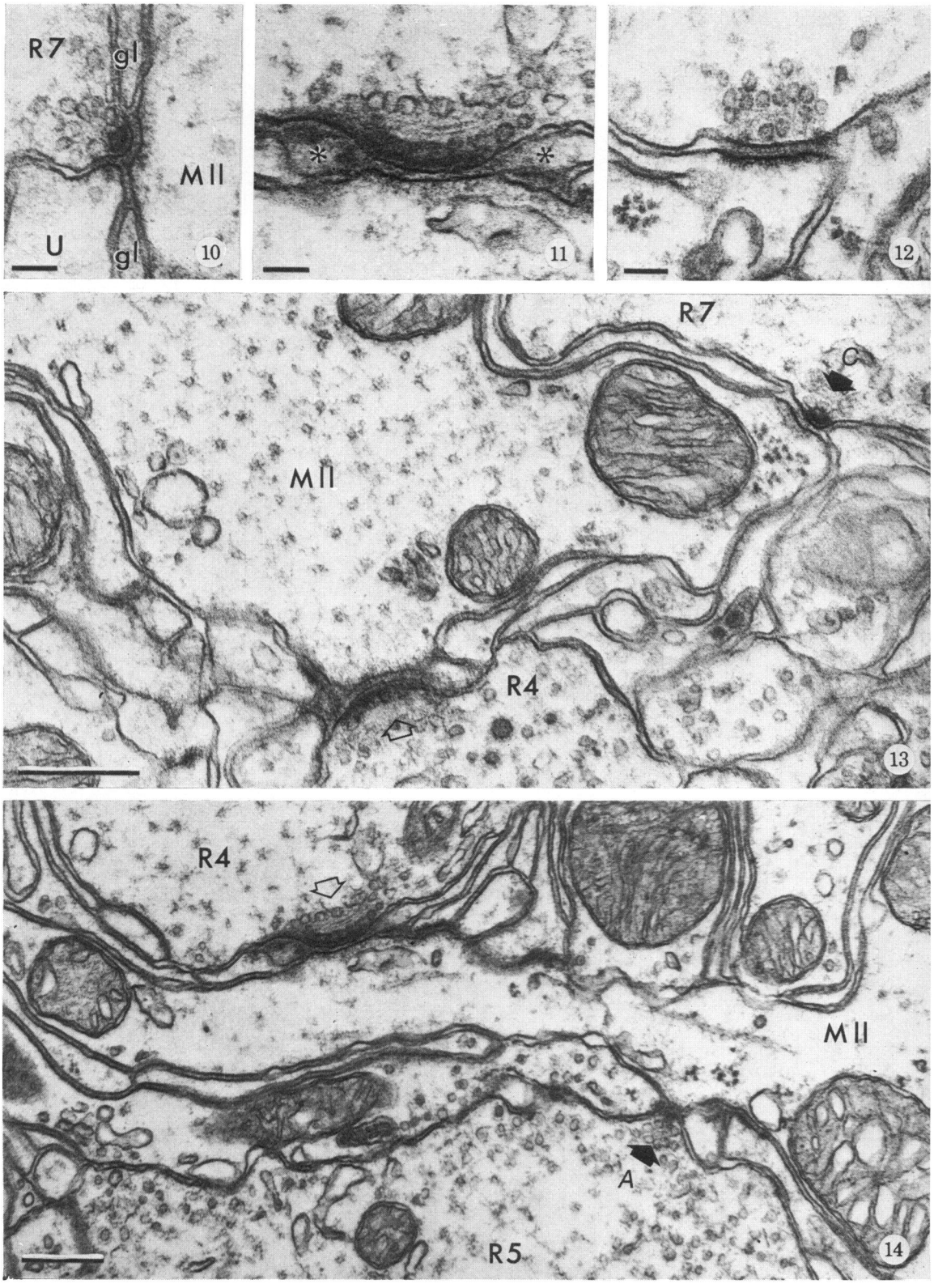


FIGURES 10–14. For description see opposite.

throughout. The mitochondria of the monopolar and long visual fibres, by contrast, are situated around the periphery, vesicles are few (those present are always round and grouped in clusters near the cell membrane, e.g. M I in figure 15), and numerous microtubules are present in the cytoplasm which has a much clearer appearance than that of the retinular terminals.

Serial section analysis shows that numerous small processes extend from the monopolar elements and wind between the retinular cell terminals. Few processes come from retinular terminals and those that do are short and relatively coarse. In single electronmicrographs the processes from both monopolar axons and retinular terminals are seen as small profiles (figures 7 and 8). Their identity can only be established by tracing back to their parent elements through serial sections because not only is their position variable but also their ultrastructure is no indication of cell of origin. For example, the processes of the monopolar axons may contain numerous vesicles despite the absence of vesicles in the parent axon. The intertwining of these processes among the main elements in both the distal and proximal part of a cartridge is represented diagrammatically in figure 9. These reconstructions were each derived from 25 serial sections.

The network of glial tissue that surrounds each cartridge is primarily recognised by its highly convoluted shape. The glial cell has a relatively clear, non-filamentous cytoplasm which contains scattered mitochondria. Its processes infiltrate the spaces between the cartridge elements, the glial membranes following closely those of the adjacent neurones (figures 6–8).

Description of plate 4

Electronmicrographs of synapses and the ultrastructure of their elements in the dragon-fly lamina. Calibration bars 0.5 μm.

Figure 10. Transverse section through a synapse showing the pleomorphic synaptic vesicles clustered near the button-shaped presynaptic density. Membrane specializations are seen on the median postsynaptic element (M II) and on the lateral unidentified element (u) but not on the glial element (gl) which has assumed the position of the second lateral element in the synapse. Filamentous material is clearly seen in the synaptic cleft.

Figure 11. Longitudinal section through a synapse. The vesicles are aligned along the rod-like presynaptic density. Profiles (*) may be lateral elements which are revealed at either end of the synapse in this plane of section (see text).

Figure 12. Longitudinal section through a synapse. The section is cut beyond the presynaptic density and although not including it contains many of the vesicles that cluster presynaptically. A clear membrane specialization is seen as well as filamentous material in the synaptic cleft.

Figures 13 and 14. Transverse sections through the cartridge showing synapses cut transversely (solid arrows) and longitudinally (open arrows). *A*, *C* next to the synapses indicate their configuration type. In figure 14 both synapses converge upon a process of M II whereas in figure 13 both synapses converge upon the axis fibre of M II. Figure 14 also shows that adjacent synapses of the same type are not necessarily orientated in one direction.

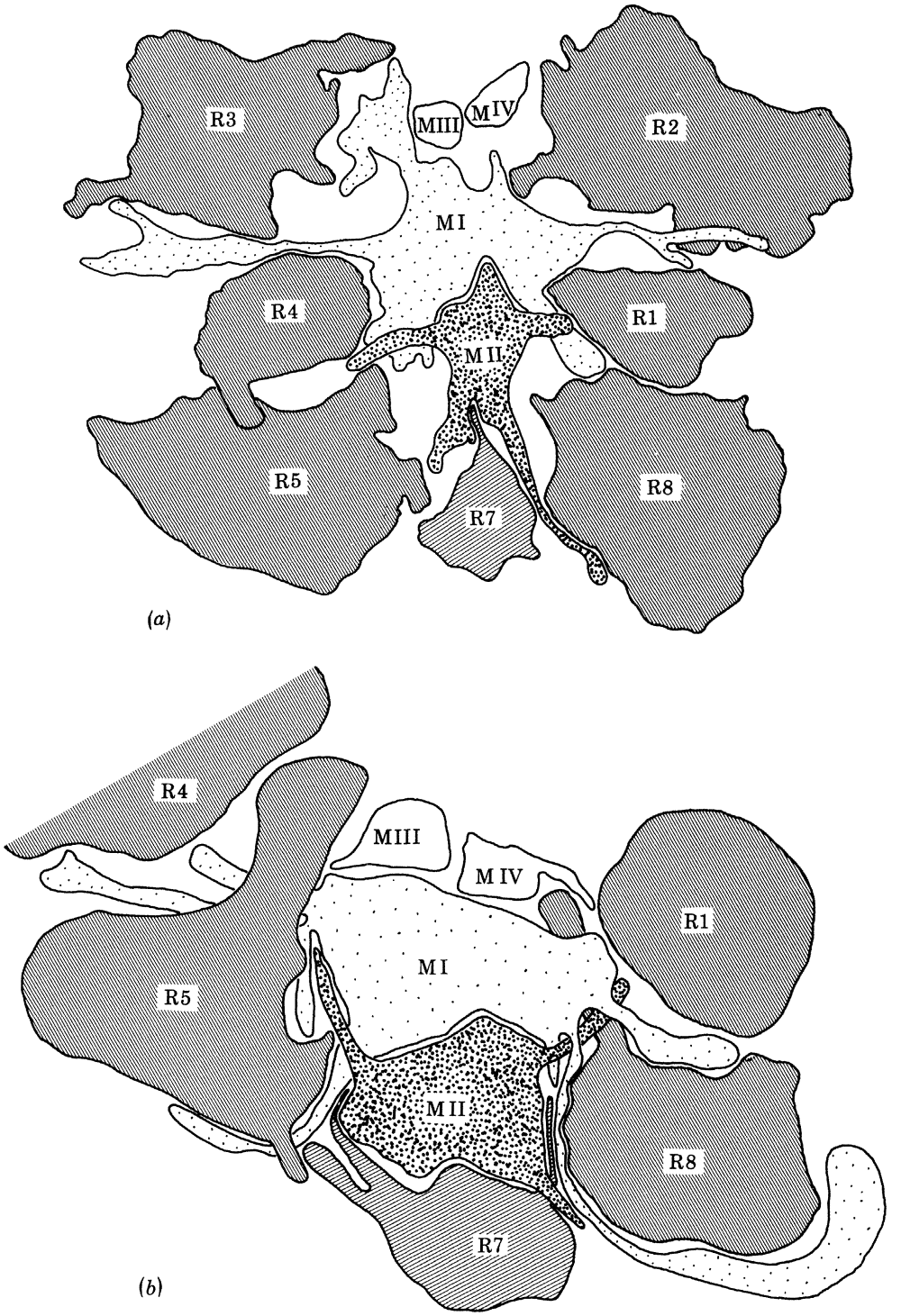


FIGURE 9. Diagrammatic reconstructions from 25 consecutive electronmicrographs of cartridge cross-sections taken from (*a*) the distal and (*b*) the proximal lamina of the dragon-fly ventral eye. Six retinular terminals are seen in (*a*) and four in (*b*).

Ultrastructure of synapses

The synapses of the lamina are of the type described as 'button synapses' by Dowling & Chappell (1972) in the median ocellus of the dragon-fly. In transverse section an electron-dense presynaptic structure is associated with a cluster of synaptic vesicles and separated from the presynaptic membrane by a distance of 10 nm. A cleft of 20 nm between pre- and postsynaptic elements is typically observed and, at clearly resolved synapses, evidence of an electron-dense web within the cleft and on the postsynaptic membrane is seen. These features are best demonstrated in the micrograph of figure 10, plate 4, but may also be seen in synapses of figures 13, 15, 19–22, plates 4–6 (solid arrows).

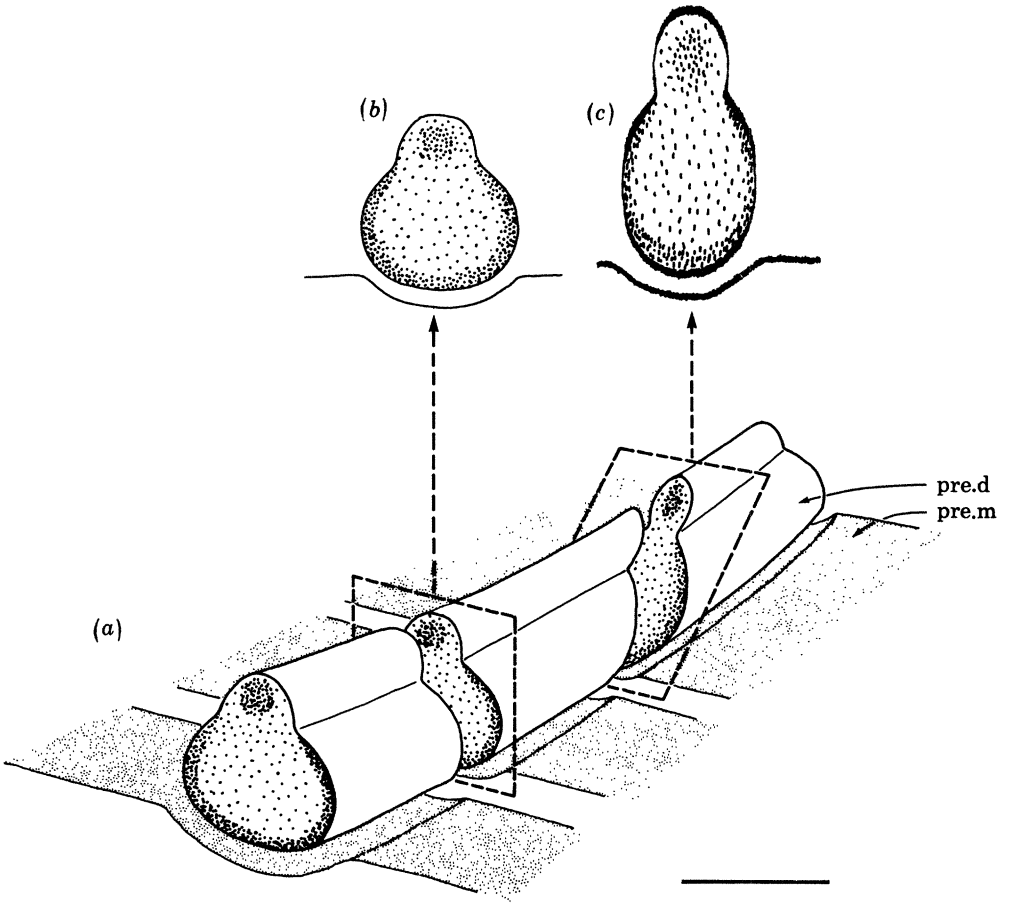


FIGURE 16. Schematic drawings of the elongate presynaptic density (pre. d) found in the dragon-fly lamina. (*a*) Three-dimensional view of the density seen in relation to the presynaptic membrane (pre. m). The more darkly shaded areas on the cut surfaces illustrate the division into subelements described in the text. (*b*, *c*) Drawings of presynaptic densities sectioned transversely (*b*) and obliquely (*c*) along the planes indicated by the broken lines of (*a*). Calibration bar approximately 0.05 μm.

At high magnification the presynaptic density can be resolved into two sub-elements: a curved peripheral element (with a diameter of 60 nm) which follows the curvature of the presynaptic ridge and surrounds a second round density of smaller diameter (figure 10). Examination of serial sections reveals that the presynaptic density is elongate rather than spherical. A histogram of the frequency distribution of length of the presynaptic density shows a normal distribution about a mean of 0.35 μm with a standard deviation of 0.14 μm (assuming a thickness of 60 nm per silver-grey section). Elongate presynaptic densities were also occasionally observed in both single and serial sections and are interpreted as longitudinal sections through the presynaptic structure, the form of which is believed similar at all synapses in the dragon-fly lamina. This structure is shown diagrammatically in figure 16. Variations in shape of presynaptic densities (figures 10–13) are presumably the consequence of the plane of section through the synapse (figure 16).

Very occasionally in single sections no presynaptic density is observed at a contact site and such junctions appear to represent a second type of synapse (figure 12). Such contacts were observed in the dragon-fly ocellus and were termed conventional synapses (Dowling & Chappell 1972). Serial sections show that in the lamina these are not a second class of synapse, but a longitudinal section through the periphery of a synapse beyond the presynaptic organelle. Thus, our evidence suggests that with respect to presynaptic specializations the dragon-fly lamina has only a single class of synapse.

Two main types of postsynaptic arrangement are observed. These have been distinguished in synapses cut transversely since only in this configuration are the postsynaptic elements seen clearly in relation to the presynaptic element. In the first, the presynaptic element is juxtaposed to three postsynaptic profiles in a triadic arrangement. At such synapses a median process is flanked by two lateral processes (figures 11, 14, 15, 19–22, plates 4–6). In the second arrangement the presynaptic element is directed toward two symmetrically placed postsynaptic elements in a dyadic configuration (figures 18–20, plate 6). The preceding distinctions are easily made in selected examples of synapses. However, in certain instances the distinctions were somewhat equivocal because of plane of section.

Description of plate 5

Figure 15. Transverse section of a lamina cartridge taken from a series of consecutive sections. Differences in the cytoplasmic appearance of the axis fibres (R 7, M I, M II) and retinular terminals are obvious and described in the text. gl, glial element. Septate junctions (↑) are seen between M II and the glial cell separating M II and M I (Staehelin, 1974). All three types of synaptic configuration (*A–C*) are represented and in addition a dyadic synapse (*) with an unknown element presynaptic to R 4 is indicated. Inset: shows configuration *B* at the level where it is clearest, two sections distal to the rest of the figure. The inset figure confirms the reciprocity of connections between R 5 and M I suggested in the corresponding region of the rest of the figure. Electron-micrograph, calibration bar 0.5 μm.

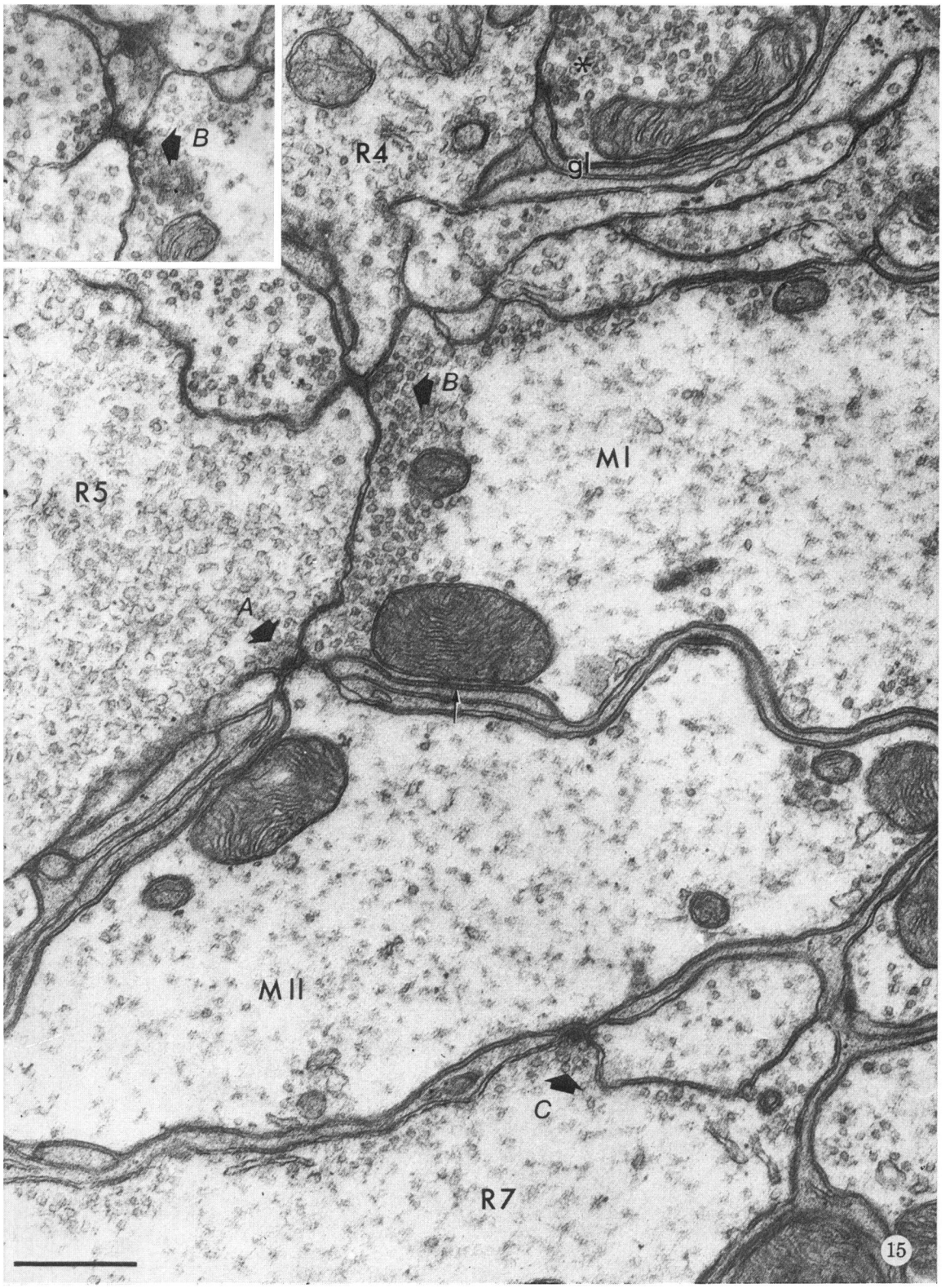
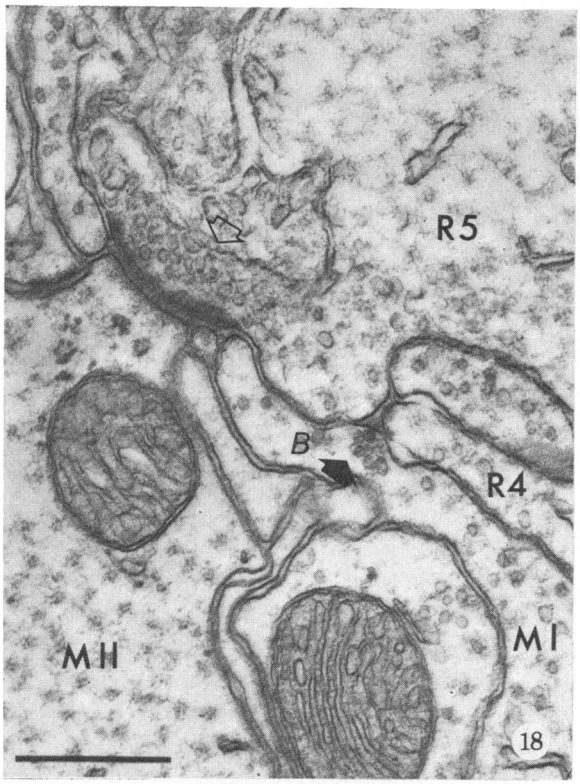
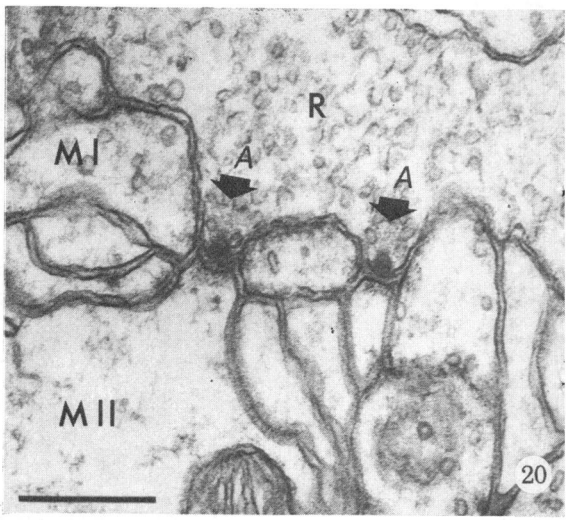
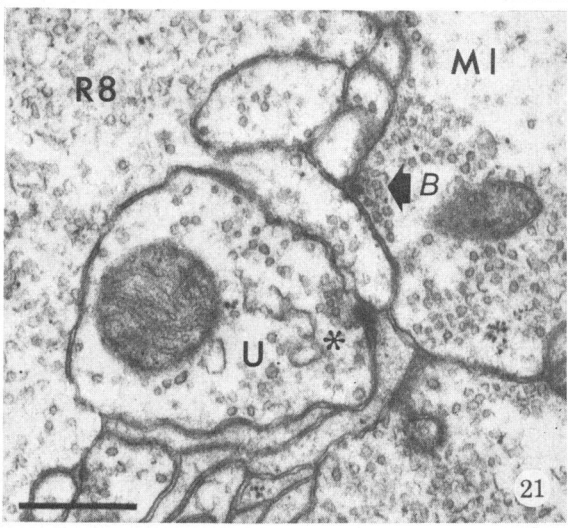


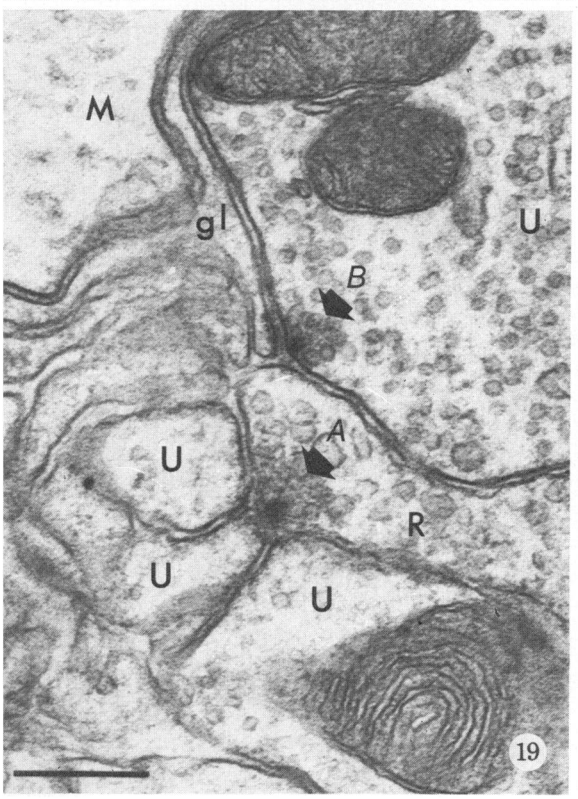
FIGURE 15. For description see opposite.

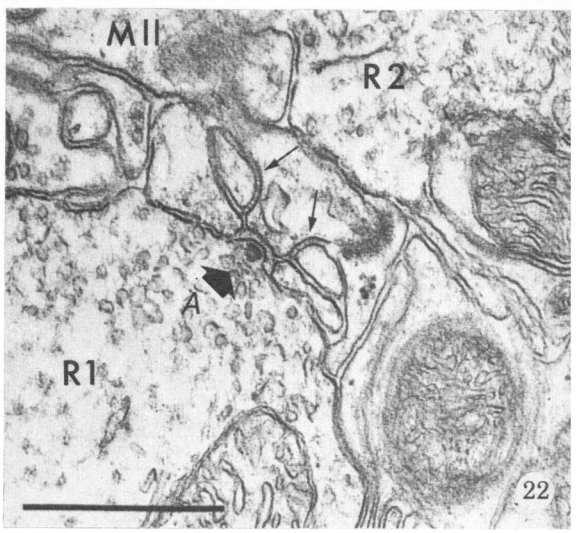
(*Facing p.* 398)











FIGURES 18–22. For description see opposite.

Infrequently in serial sections, a dyadic arrangement with three postsynaptic structures was encountered. They were termed a 'running dyad' because in any one location only two of the structures were postsynaptic, one changing place with another along the length of the presynaptic ridge. It is possible that there are also 'running triads' although these have not been seen in serial sections. Occasionally more than three elements converge towards one presynaptic location allowing for this possibility (figure 22).

Elements were considered postsynaptic if membrane specializations were observed. These specializations included a distinctness of the membrane at the synaptic site and filamentous material (subsynaptic web) extending from the membrane into the cytoplasm of the cell, which gave the postsynaptic membrane a thickened appearance. In addition filamentous material between the membranes could often be observed. A difficulty encountered was that glial processes occasionally were positioned at a postsynaptic site. Glial elements, however, do not in general show evidence of membrane specialization (with one exception, figure 19) and filamentous material is lacking between presynaptic and glial membrane. Figure 10 shows this especially clearly. Thus we conclude that although glial processes may be located in postsynaptic positions, their lack of membrane specializations may mean that they are not involved in the process of synaptic transmission (see below). Since glial elements have on occasion been seen in every postsynaptic location we will throughout use the terms dyadic and triadic synaptic arrangement even though not all postsynaptic elements may be functional.

Description of plate 6

Electronmicrographs to illustrate synapse configuration types in the dragon-fly lamina. Calibration bars 0.5 μm.

Figure 18. A reciprocal arrangement of synapses between R 5 and M I is suggested by a longitudinally sectioned synapse probably of configuration *A* (open arrows) and an adjacent dyad configuration *B* synapse, assuming the incorporation of M I as the lateral postsynaptic element at both.

Figure 19. Two transversely sectioned synapses, a dyad and a triad, have a serial arrangement. This micrograph is the only example encountered with a clear postsynaptic specialization on a glial process.

Figure 20. Two examples of configuration *A* synapses, oriented in parallel, reveal clear postsynaptic membrane specializations for all the participating elements of the triads. Parallel, adjacent configuration *A* synapses are frequently seen and have sometimes been shown to share the same postsynaptic processes.

Figure 21. This micrograph was from one of the series of proximal lamina and shows a particularly clear example of a dyadic configuration *B* synapse feeding back upon a retinular element. An additional synapse (*) has an unidentified presynaptic element and therefore was one of the synapses which could not be dealt with in our numerical analysis.

Figure 22. A clear example of a configuration *A* synapse. Septate junctions (↑) are seen separating monopolar elements from neighbouring processes, probably glial.

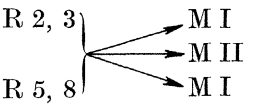
The dyadic and triadic synaptic arrangements may also be distinguished by the vesicle morphology in the presynaptic element. The synaptic vesicles at dyads are typically round and very regular in shape, while the vesicles at triadic synapses are pleomorphic and generally less distinctive. This difference is shown particularly clearly in figure 15. The possible significance of these observations is discussed below.

Types of synaptic interconnection

The ensuing analysis of synaptic connections is taken mainly from series of consecutive micrographs from the proximal (left eye) and distal (right eye) part of the lamina. The cartridges were all from the central portion of the ventral lamina, that is, equidistant from both the periphery and the dorso-ventral boundary. Connections were analysed mainly from synapses cut transversely and for each synapse studied, pre- and postsynaptic elements were scored. Synapses cut more nearly longitudinally did not include all postsynaptic elements and were not scored. Their relatively low frequency of observation did not greatly reduce the sample size upon which the analysis of connectivity was based. Many synaptic configurations incorporate one or more unidentified elements which could not be identified by their ultrastructure or traced to their cell of origin.

Synaptic connections have been observed most commonly from (1) retinular terminals to monopolar axons, (2) monopolar axons to retinular terminals, and (3) long visual fibres to monopolar axons. Occasionally synapses between retinular terminals and long visual fibres have been seen, and once a synapse between monopolar axons was observed. Sometimes within the plane of a single section, reciprocal connections between a retinular terminal and a monopolar cell axon were observed (figure 15). Our results further suggest that the connectivity of the individual elements is quite specific, and that there are three basic types of synaptic configuration in the lamina.

Configuration A is a triad, presynaptic from retinular terminals to monopolar cells, usually from R 5 & R 8 and R 2 & R 3 to a triad of postsynaptic elements comprising M II as the median and M I as both lateral elements. This configuration in its most common form is illustrated below, and the data on which this conclusion is based is shown in table 1.



(*A*)

MII is the median element in 52 of the 58 synapses in which the central element was identified, but a lateral element, questionably, only once. By contrast, M I is an identified lateral element 72 times and a median element only 6 times. Occasionally triads with postsynaptic elements which are inconsistent with configuration *A* were observed. In 6 instances M I was a median element and in 2 instances R 7 was a lateral element (these last 2 synapses are the only examples of connections between identified retinular elements). The synapses of configuration

A have either a process or the axis cylinder as the median postsynaptic element (compare figure 14 with figure 15) while it is invariably processes from M I, either one or two per synapse, that form the lateral postsynaptic elements of the configuration.

Retinular terminal pairs R 2 & R 3 and R 5 & R 8 are matched in frequency of synaptic engagement in the lamina and specificity of postsynaptic configuration. R 1 and R 4 appear to have much lower synaptic frequencies than the other two pairs, and too few synapses from these terminals have been observed to allow firm generalization about the nature of the postsynaptic processes. In the distal

TABLE 1. THE POSTSYNAPTIC ELEMENTS IN TRIADIC CONFIGURATION A

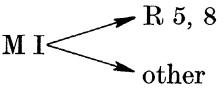
presynaptic		*a*	*b*	*c*	*d*	*e*	total
distal lamina	R 3		4	2	2 5		13
	R 2		1 2		1 3		7†
	R 1			1	3 1	1?	5+1?
	R 4	1?	2		2 1		5+1?
	R 5	2 1	1	4	5 2	1	16
	R 8			2 5	5 3		15
	R → M	M I u M I	M I M I	M I M I	u u	R 7 M I M II	
	R → M	M I M I M I	u u	MII MII	M II u	M II M II M II	
	R → M	M IV u u	u M I	M I u	u u	R 7 R 7 u	
proximal lamina	R 8		2 3	3 5	1 6		20
	R 5		1 3	5 4	1	1	15
	R 4	1 1	1		3		6
	R 1				1		1
	total	1 3+1? 1	12 8	10 21	19 26	1 1 1?	105

The frequencies of postsynaptic combinations are shown for each retinular terminal in a triadic configuration. Included are *all* the synapses observed in approximately 400 serial sections in which a retinular terminal was identified as the presynaptic element. The generalized configuration is illustrated on the left. Horizontal rows show the numbers of synapses formed by individual retinular terminals arranged as matched pairs and according to their distal and proximal position in the lamina. Vertical columns give the numbers of synapses for which the particular combination of postsynaptic elements shown has been scored. u, unidentified element. Columns of postsynaptic combination are combined in five sets *a*–*e*. Synapses of sets *b*–*d* are most numerous and all are consistent with the preferred configuration of set *c* which has M II as the medial element and M I as the lateral elements. Sets *a* and *e* are incompatible with this preferred configuration; set *a* has M I as the median elements, set *e* has lateral elements other than M I. Sets *b* and *d* are wholly compatible with sets *a* and *e* respectively but not exclusively so; the combinations in which there are two or more unidentified postsynaptic elements are compatible with most other examples of all sets. †, this number includes only part of the synaptic population of R 2, since for one series part of the cell had been trimmed from the block face. ?, identification of elements was uncertain. For further details see text.

lamina the triads of R 1 and R 4 appear to conform to configuration *A*. By contrast, in the few observed synapses of R 1 and R 4 in the proximal lamina, the only identified median element was M I and the only identified lateral elements were M I and M IV.

Examples of synapses consistent with configuration *A* are marked in figures 14, 15 and 19–22. Those longitudinally sectioned synapses marked with open arrows in figures 13, 14 and 18 may well be of this type also since they have a retinular terminal as the presynaptic element and M II as the postsynaptic element.

Configuration B, found almost exclusively in the proximal lamina, is a dyad in which monopolar cell M I is presynaptic and one of the postsynaptic elements is a retinular terminal, either R 5 or R 8. This is shown below:



(*B*)

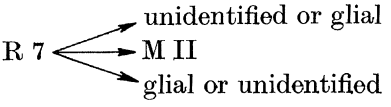
Examples are labelled *B* in figures 15, 18, 19 and 21, and the data upon which this conclusion is based are shown in table 2. In the proximal lamina, configurations *A* and *B* result in a reciprocal arrangement of synaptic connections mainly between retinular terminals R 5 or R 8 and monopolar cell M I (figure 15). On occasion M I forms triadic synapses which, however, always include as postsynaptic elements retinular cell terminals.

TABLE 2. THE POSTSYNAPTIC ELEMENTS IN DYADIC CONFIGURATION B

	proximal R 5				proximal R 8				distal
M I → R 5 or R 8	R 5	R 5	R 5	u	R 8	R 8	R 8	R 8	R 8
M I → other	R 4	g	u	u	R 1	g	u	M III	u
	1	1	7	1	1	1	2	1	1

The frequencies of postsynaptic combinations in dyadic configuration *B*. Included are all the dyads photographed in the series of sections in which M I was the presynaptic element ($n = 16$); an additional 5 triads were also observed. Configuration *B* is illustrated at the left and the combinations of postsynaptic elements observed are arranged in vertical columns. g, glial element; u, unidentified element.

Configuration C is a triad in which R 7, the long visual fibre, is presynaptic to monopolar cell M II as the median process and unidentified or glial processes as the lateral elements:



(*C*)

Examples are labelled *C* in figures 10, 13 and 20 and table 3 provides the

numerical data. In addition to synapses scored from serial sections, unequivocal examples of configuration *C* are frequently seen in single sections, since of the three synaptic configurations this one invariably occurs directly between the parent elements, i.e. between a retinular cell axon and the axis fibre of M II.

TABLE 3. THE POSTSYNAPTIC ELEMENTS IN TRIADIC CONFIGURATION C

	proximal		distal	
g/u	g	g	u	g
R 7 → M II	M II	M II	M II	M II
g/u	g	u	u	g
	2	2	1	4

The frequencies of postsynaptic configuration in triadic configuration *C*. Included are all those synapses ($n = 9$) in the series of sections in which R 7 was presynaptic to M II. Configuration *C* is shown at the left and the combinations of postsynaptic elements observed are arranged in vertical columns. g, glial element; u, unidentified element. It is unlikely that the glial elements are functionally postsynaptic in these triads (see text).

Configuration *C* is of special interest because in most instances one or even both of the lateral elements is a glial process. A total of 18 such synapses were studied (10 were from the proximal lamina and 8 from the distal lamina). In all 18 synapses the central element, M II, had a clear postsynaptic membrane specialization. All but 3 of these synaptic complexes were triads, and they engaged a total of 33 lateral postsynaptic structures. Of these, 21 were glial processes with no obvious postsynaptic specialization, 2 were glial processes with questionable postsynaptic specializations, 4 were unidentified lateral elements with questionable postsynaptic specializations, 3 were unidentified lateral elements with questionable postsynaptic specializations, and 3 were not clearly distinguishable as glial or non-glial processes.

Consideration of these three synaptic configurations indicates that the connectivities of the monopolar neurones M I and M II are clearly different. M II has been observed only as a postsynaptic element and, with one doubtful exception, it is also always the median element at a triadic synapse. It receives input from all the retinular terminals, R 1–5 and R 8, and from the long visual fibre R 7. M I, on the other hand, receives input only from the retinular terminals ending in the lamina and it is postsynaptic usually as the lateral element in the triadic arrangement. It is also presynaptic, usually at a dyad, feeding back to either R 5 or R 8. The extent of the reciprocal interaction between M I and R 5 and R 8 is revealed only by serial sections, although examples in which they are contained in the same plane provide visually the most compelling evidence.

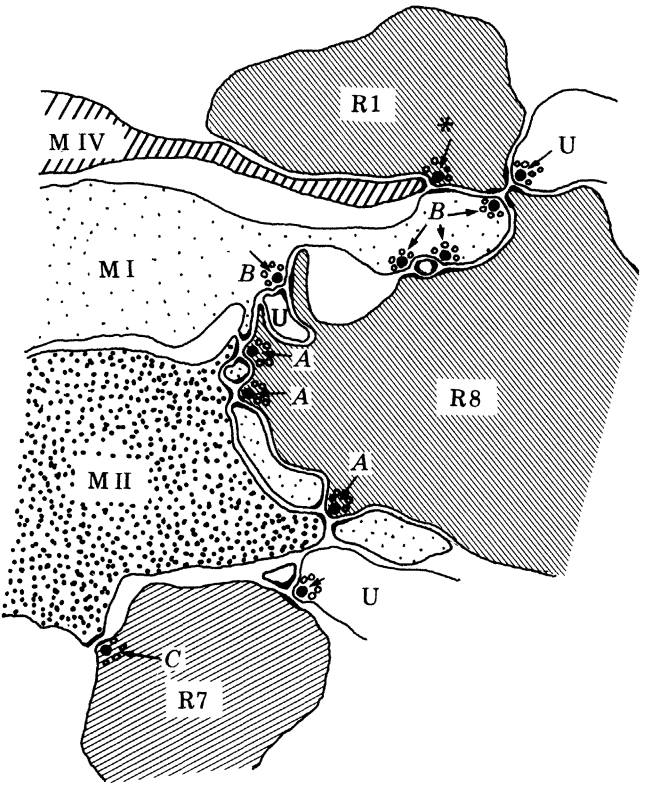


FIGURE 17. Reconstruction from 25 consecutive sections in the proximal lamina of the dragon-fly to show the synaptic connectivity pattern between the retinular and monopolar elements. (*A–C*) indicate the type of synaptic configuration. * One of the few examples of a retinular cell making a dyadic synapse. Glial elements are not included.

DISCUSSION

The focus of the present study was to identify the synaptic connections between the retinular and monopolar cells in the dragon-fly lamina and to deduce the synaptic organization of the individual cartridges. A necessary preliminary was to elucidate the organization (number and arrangement) of the principal elements in a cartridge between which these synapses form and to relate this to the pattern of overlying receptors. A summary diagram of the synaptic connectivity observed in a single cartridge is shown in figure 17. This drawing, based on a reconstruction from 25 serial sections, illustrates the three basic types of synaptic configurations observed in the dragon-fly lamina. These are labelled *A*, *B* or *C* according to the nomenclature designated above. The three most interesting new findings from this study are that: (1) the two principal monopolar elements, M I and M II, have strikingly different synaptic connections, (2) monopolar cell M I forms numerous reciprocal synapses with one pair of retinular cell terminals, R 5 and R 8, and (3) the long visual fibre, R 7, makes synapses within

the lamina upon monopolar cell M II. In the following discussion aspects of the cellular and synaptic organization of the dragon-fly lamina will be discussed and related to earlier studies.

Cellular organization of the retina and lamina

(i) *The retinula of Odonata*

The patterns of retinular axon projection found in *Sympetrum* conform to those found in all other species with fused-rhabdome ommatidia (Meinertzhagen 1976). The arrangement of elements within a cartridge clearly relates to the arrangements of receptors in the retinula, and it is a problem that existing accounts of the structural organization of odonate ommatidia indicate either inconsistencies or species differences.

TABLE 4. ODONATE RETINULAR CELL NUMBERING CONVENTIONS

Sympetrum ...	1	2	3	4	5	6	7	8
Cersion, Ishunura	6	3	4	7	5	8	1	2
Aeschna	5	4	3	2	1	8	7	6

The common features in three contemporary accounts of ommatidial organization of the damsel-flies *Ishunura* and *Cersion* (Ninomiya *et al.* 1969) and on the dragon-flies *Sympetrum* (Horridge 1969) and *Aeschna* (Eguchi 1971) are twofold. Firstly, a tripartite rhabdome of four rhabdomeres (with R 2 and R 3 contributing one limb, R 5 and R 8 each of the other two) extends most of the length of the ommatidium. In the damsel-flies and *Aeschna* it is joined apically by the rhabdomere of cell 7 although this was not observed by Horridge (1969) and may therefore be doubtful for *Sympetrum*. Secondly, in the damsel-flies and *Aeschna* a paired basal rhabdome is contributed by the rhabdomeres from the third cell pair R 1 and R 4, although this also was not directly observed by Horridge in *Sympetrum*. A rhabdomere of R 6 has not been described in any account.

Assumed correspondences between the cell numbering conventions adopted here for *Sympetrum* with those for the damsel-fly (Ninomiya *et al.* 1969) and for *Aeschna* (Eguchi 1971) are shown in table 4. These cross-identifications between species may be made by criteria of cell position in the retinula with respect to the posterior unmatched pair of retinular cells, R 6 and R 7, and also between *Sympetrum* and *Aeschna* with respect to the positions of four crystalline cone processes. Strict correspondence between individual cells of the different species, i.e. deciding whether the cell numbering sequence proceeds clockwise or anti-clockwise around the retinula depends, of course, upon the correct identification of the asymmetries between R 6 and R 7, which, although clear in *Sympetrum* (R 6 ventral to R 7), is not in the accounts of other species. Nevertheless correspondence between matched pairs of cells is fairly clear and coincides broadly with the tiering of rhabdomeres. It may also coincide with tiering in the positions of retinular cell nuclei, since in *Aeschna* Zimmerman (1914) found 5 nuclei

apically, tentatively identified here with R 5 & R 8, R 2 and R 3 & R 7, and 3 nuclei approximately equidistant between cornea and basement membrane (i.e. R 1 & R 4 and R 6).

(ii) *Stratification of the lamina*

A feature of the dragon-fly lamina is the stratification of neuronal processes into proximal and distal zones (Strausfeld 1976*a*), an arrangement which has been reported in many accounts of Golgi-impregnated insect laminae (Strausfeld 1970, 1971, 1976*a*; Strausfeld & Blest 1970; Ohly 1974; Ribi 1975*a*, *b*). This stratification is especially well developed in the dragon-fly where it is most obvious for retinular terminals R 2 and R 3 *vis à vis* the remaining two pairs. Theoretical analyses have suggested that rhabdome tiering may affect photoreceptor sensitivities (see Gribakin 1975; Snyder *et al.* 1973). We might suspect that importance is also attached to the stratification of terminals in the lamina. Connectivity analyses so far reveal merely subtle differences in the proximal and distal lamina. These are (1) the absence of feedback synapses from M I to R 5 and R 8 in the distal lamina, and (2) the suspicion that, for the triads of R 1 and R 4 terminals in the proximal lamina, M I rather than M II is the median element and M I and M IV are the lateral elements. Synaptic analysis of the many unidentified cells of the cartridge may reveal further differences in synaptic connectivity between proximal and distal lamina. It is significant that the differences in synaptic connectivity which have been encountered are between elements which are juxtaposed at all depths in the lamina. That is to say, differences in proximal and distal synaptic patterns are not solely contingent upon the failure of elements to overlap in the depth of the lamina. This implies some more fundamental basis for the stratification of the lamina than the mere depths at which retinular elements are represented.

Synaptic structure

(i) *Presynaptic specialization*

Our observations indicate that in the dragon-fly lamina all synapses are characterized by a single type of presynaptic structure which is rod-shaped and has dimensions approximately 40 nm × 60 nm × 0.35 μm. A similar presynaptic density elongated in the plane of the presynaptic membrane has also recently been described in retinular terminals of the crayfish (Hafner 1974) and in sensory terminals of the leech ventral ganglion (Muller & McMahan 1976). As the result of its elongate shape, the appearance of the presynaptic density depends upon the section plane and variants are often seemingly convincing evidence for alternative shapes. In transverse section the presynaptic densities observed here resemble closely those termed button synapses in the dragon-fly ocellus (Dowling & Chappell 1972). Possibly the presynaptic density of the ocellar button synapses is also elongate.

In transverse sections the presynaptic density can be resolved into two sub-

elements. This has also been seen in both the crayfish (Hafner 1974) and the dragon-fly ocellus and it is also true for another type of presynaptic density found in the lamina of the optic lobe of the fly (Trujillo-Cenóz 1965; Smith 1967; Boschek 1971). In the fly, however, although most recent evidence (Braitenberg & Burkhardt 1976) also interprets the presynaptic specialization to be elongate (albeit less so than in the dragon-fly) the morphologies of the two subelements are different; i.e. in the fly the presynaptic specialization comprises a plate surmounting a cruciform peduncle (Trujillo-Cenóz 1969). It is unclear why this difference exists but such a T-shaped presynaptic structure appears to be characteristic of a number of species of fly. For example, T-shaped presynaptic densities with cruciform peduncles are found in the ocellar receptor axons of the flesh-fly *Boettscherisca* (Toh, Tominaga & Kuwabara 1971; Toh & Kuwabara 1975) as well as in other non-visual areas, e.g. those areas of the thoracic ganglion of *Phormia* where tarsal afferents are known from degenerative studies to terminate (Geisert & Altner 1974).

(ii) *Postsynaptic elements*

The presumed synapses encountered in this study almost always show multiple postsynaptic elements (the '*Mehrfachkontakten*' of Lamparter *et al.* 1969) orientated parallel to the long axis of the presynaptic density. The few synapses which appear to involve a single postsynaptic process and resemble the conventional synapse of the ocellus (Dowling & Chappell 1972) or the '*Simplex-form*' synapse of the ant CNS (Lamparter *et al.* 1969), are interpreted to be sections cut parallel to the presynaptic density and hence cut through only a single postsynaptic process. At synapses cut in transverse section usually two or three postsynaptic elements are present. Despite difficulties in comparing postsynaptic arrangement of elements in different invertebrates where somewhat different criteria for synaptic involvement may have been used, it seems clear that in the fly, as in the dragon-fly, both dyads and triads are seen (Trujillo-Cenóz 1965; Boschek 1971; Strausfeld 1976*b*). In crayfish, on the other hand, three postsynaptic elements are generally present (Hafner 1974) whereas in the dragon-fly ocellus two postsynaptic processes are the rule (Dowling & Chappell 1972). In the eye of the crustacean *Daphnia* only one element is situated postsynaptically at each synapse (Macagno, Lopresti & Levinthal 1973). Although in the compound eye of the dragon-fly a dyadic arrangement might result from the detachment of one of the three postsynaptic elements of a triad (the only circumstance in which they are found in the crayfish (Hafner 1974)) serial sections show that the dyads almost never have a third postsynaptic process. Thus in the dragon-fly lamina, two specific arrangements of postsynaptic processes are suggested, i.e. either a dyad or triad. That these two arrangements are found usually associated with different pre- and postsynaptic elements lends further support to this notion (see above).

It is also interesting that the morphology of the synaptic vesicles differs at triadic and dyadic synapses in the dragon-fly lamina. The vesicles in the

presynaptic terminal at triads tend to be pleomorphic, while presynaptically at dyads round vesicles are the rule. Perhaps different transmitters are employed at these different synaptic configurations, since in a number of studies differing morphologies of synaptic vesicles have been correlated with specific transmitters (Nadol & de Lorenzo 1968; Atwood & Lang 1972; Tisdale & Nakajima 1976). In those instances where the presynaptic elements are identified in the dragon-fly lamina, the triadic synapses are made predominantly by retinular elements whereas the dyadic synapses are made generally by the monopolar elements. The majority of unidentified processes in the lamina also make dyadic synapses, which suggests perhaps that centrifugal and amacrine elements are involved in junctions of this type.

Glial processes are occasionally positioned at postsynaptic sites but no convincing morphological evidence has been obtained to include them as a synaptic communicant. Such glial elements are found in all possible postsynaptic positions, including the median process of a triad (two cases) and either, or even both, lateral processes of a dyad or triad. Although all glial elements that appear to be postsynaptic are closely apposed to the presynaptic area, only one postsynaptic density has ever been observed on any of these processes. Boschek (1971) also observed glial elements postsynaptically at synapses in *Musca* and he also had difficulty in visualizing glial postsynaptic specializations. We do not believe that the evidence in this study substantially challenges the conventional wisdom that neural elements alone show ultrastructural evidence of involvement is synaptic transmission.

The inclusion of glial processes at synapses may satisfy a requirement to fill a set number of postsynaptic sites at synapses or a need to maintain a rather precise spatial orientation of postsynaptic processes at synapses. Support for these ideas is most suggestive in the fly lamina; glial processes take the place of the L 3 monopolar cell processes at synapses of the proximal region where this monopolar cell is not represented (Braitenberg & Burkhardt 1976). This would seem to imply that it is the presynaptic element that determines the number of postsynaptic elements, i.e. whether there are two or three, and perhaps this explains why all retinular elements in the dragon-fly lamina form triads including R 7 whose synapses often have glial processes as lateral postsynaptic elements. Why it should be glial processes that move to the synapse is not clear but the insinuative behaviour of glia is well known in vertebrates. For instance it has been recently observed in the inner plexiform layer of the vertebrate retina that glial processes take the place of one of the postsynaptic elements at a bipolar cell dyad when that element has degenerated after treatment with certain neurotransmitter analogues (Ehinger & Dowling, in preparation).

Specificity of synaptic connections

The elements in the cartridge are not indiscriminate in their synaptic connections. This is apparent in spite of the qualifications already discussed above

concerning postsynaptic glial processes and unidentified pre- and postsynaptic processes. In view of the spread of the lateral processes of M I and M II we presume that many unidentified processes are simply extensions into the reconstructed portion of the cartridge of additional M I and M II processes. The remainder of the unidentified postsynaptic processes may include centrifugal, amacrine, tangential or other monopolar cell processes.

The observed patterns of specificity are not merely the result of synapses occurring between elements whose axis cylinders happen to be situated close to each other in the cartridge. Indeed some juxtaposed elements, notably retinular terminals, fail to connect synaptically *inter se*; others do so only infrequently in spite of large areas of apposed membrane (e.g. M II and R 7), while yet others which are widely separated connect synaptically with high frequency through intervening processes (e.g. R 5 & R 8 and M I). Nor do elements fail to form synaptic connections because of the interpolation of the glial sheath, since there are juxtaposed elements with an interrupted glial investment (e.g. retinular terminals) which are not synaptically connected. Highly specific connections have also been found in two other studies on arthropod optic laminae: connectivity analyses of the total synaptic population among cartridge elements of *Daphnia* (Macagno *et al.* 1973) and similar analyses on selected samples of synapses in the cartridges of *Musca* (Boschek 1971; Strausfeld 1976*b*).

The receptors of many fused-rhabdome ommatidia are matched in pairs of similar size, position (Meinertzhagen 1976) and spectral sensitivity (Menzel & Blakers 1975). Usually the arrangement of these pairs has bilateral symmetry and they remain matched in size and position in the axon bundles and underlying cartridges (Meinertzhagen 1976). In the dragon-fly not only are the retinular axons matched in size and position but also in the depths at which they terminate in the lamina and in the pattern of their connectivities. There are some disparities in the numbers of synapses formed by different pairs of terminals, but to some extent these differences may relate to variations in terminal size and surface area. In both distal and proximal lamina the larger terminals, R 2 & R 3 and R 5 & R 8, all have between 11.5 and 13.5 configuration *A* synapses per 100 μm^2 membrane, whereas the smaller terminals, R 1 and R 4, have approximately half this number (7–7.5 configuration *A* synapses per 100 μm^2 membrane).

Matched retinular cell connectivities have yet to be revealed in synaptic analyses of other species; these are none the less to be expected in those forms where previous studies have already shown receptor cell bodies to exist as matched pairs. The matched pairs of terminals do not invariably demonstrate a single synaptic configuration and it is unclear whether the variations in configuration *A* which we observe are the product of somewhat loose constraints upon the formation of synaptic connections or of some – as yet – obscure strategy of biological design.

As already noted M I and M II of *Sympetrum* have unique connectivities.

M II is totally postsynaptic (in configuration *A* and *C*) whereas M I is both postsynaptic (configuration *A*) and presynaptic (configuration *B*). An additional characteristic of M I is the synaptic convergence upon R 5 and R 8 which provides the substrate for reciprocity between these three elements in the proximal lamina. This is strikingly similar to the situation in the fly for which a contemporaneous study (Strausfeld 1976*b*) has revealed that L 2 and L 1 appear homologous with M I and M II respectively. Another interesting difference between M I and M II is the ability of M II to contribute any part of its membrane area, process or parent element, postsynaptically at configuration *A* while for M I this role is apparently restricted to the processes.

Long visual fibres are clearly present in the dragon-fly optic lobe. They are recently described from Golgi-impregnation in *Coenagrion* by Strausfeld (1976*a*); we confirm his observations of a stout fibre which we show to be R 7 and implicate R 6 as its slender partner. Their existence in dragon-flies, long denied, (Zawarzin 1913, and discussed at length by Cajal & Sánchez 1915) is now affirmed. Moreover R 7 has been shown to be synaptic in the lamina, in configuration *C*, and is unlike both long visual fibres in the fly which are so far described without synapses in this region (Boschek 1971).

Functional significance of the synaptic connectivity patterns

The importance of specific connectivities for each of the matched pairs of retinular cells can be intuitively recognized. Each pair of terminals is a continuation of a matched pair of cells in the retinula and the information that is contained in the pattern of retinular cell activity appears preserved in the first optic neuropile by the stratification of terminal depths and the specificity of retinular connections. To understand how this specificity of connections functions it will be necessary to relate the structural findings with the physiology of the monopolar cells, the output neurones of the lamina.

Laughlin (1973), recording from large monopolar cells of the dragon-fly *Hemicordulia tau*, reported only one type of response, a sustained hyperpolarization with transient 'on' and 'off' responses at high intensity. Although some of the cells were identified by injection of procion yellow dye, it is not known whether one or both of the central monopolar elements was impaled in the study. It is conceivable that his electrodes preferentially impaled only one of the cells or, on the other hand, he may have recorded from both types of cells and his stimulus parameters were such that he could not differentiate between the two types of monopolar elements. Our anatomical results suggest that under appropriate conditions it may be possible to differentiate M I and M II responses physiologically because of their differing synaptic connections.

Other results of this study are more consistent with his findings. For example, our findings provide morphological evidence for the amplification of the retinular voltage signal and the improved signal-to-noise ratio found in the monopolar neurones. Convergence of several retinular terminals upon a single cell is seen

and multiple synaptic inputs from single retinular terminals onto monopolar axons have been confirmed. Assuming an even synaptic distribution, one may calculate from the results of table 1 that, as they course through the lamina, R 8 and R 5 each form synapses with M II between 75 and 100 times which, according to the estimates of Laughlin (1973), is more than adequate to account for the improved signal/noise ratio in the monopolar cell. Absence of connections between retinular terminals (save for a few upon the long visual fibre R 7) indicates that no summation of retinular cell activity takes place within the lamina.

The effect of the reciprocal synapses between M I and retinular terminals R 5 and 8 remains obscure, although it is possible that the feedback loop functions to produce the 'on' and 'off' transients in the monopolar cells in a manner similar to that suggested by Chappell & Dowling (1972) for the second-order cells in the median ocellus of dragon-fly. Before further significance can be attached to the complex synaptic patterns revealed by this study, more information about activity of specific cell types in the dragon-fly lamina will be required.

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