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A Golgi-Electron-Microscopical Study of the Structure and Development of the Lamina ganglionaris of the Locust Optic Lobe

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Summary. The gross structure as well as the neuronal and non-neuronal components of the *lamina ganglionaris* of the locust *Schistocerca gregaria* are described on the basis of light- and electron-microscopical preparations of Golgi (selective silver) and ordinary histological preparations. The array of optic cartridges within the lamina neuropile – their order and arrangement – and the composition of the cartridges are described. There are six types of monopolar neurons: three whose branches reach to other cartridges and three whose branches are confined to their own cartridges. Retinula axons terminate either in the lamina or the medulla neuropiles. There are three types of centrifugal neurons, two types of horizontal neuron, as well as glia and trachea in the lamina neuropile. The development of the lamina neuropile is described in terms of developing monopolar and centrifugal axons, growing retinula fibres, and composition of the developing optic cartridges.

Key words: Lamina ganglionaris – Locust – Neurons – Development – Light and electron microscopy – Golgi study

The repetitive nature of the insect compound eye and visual ganglia (Figs. 1 and 2) makes this system ideal for various experimental studies. The locust, with its large compound eyes and relatively short period of larval development, has been an animal suitable for studies on behaviour (Wallace 1959; Thorson 1964; Burtt and Catton 1966), optics (Palka 1965; Tunstall and Horridge 1967; Palka and Pinter 1975), physiology of the retina (Horridge and Barnard 1965; Shaw 1967; Bennett et al. 1967) and the optic lobe (Kien 1975; Horridge et al. 1965) and on development.

As a consequence of the studies conducted in our laboratory on the development of the compound eye of the locust and the development of neuronal connections in the retina-lamina projection (Anderson 1976, 1978a, b; Shelton 1976; Eley and Shelton 1976; Shelton and Nowel, in prep.), we now require a clear idea of the three-dimensional structure of this system. Horridge (1966) and Wilson

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et al. (1978) have provided descriptions of locust ommatidia, and Meinertzhagen (1976) has investigated the anatomy of the locust retina-lamina projection. We now provide a description of the *lamina ganglionaris* (hereafter referred to as the lamina) neuropile itself.

The light- and electron-microscopical investigations reported herein examine the type and arrangements of neural and non-neural elements of the lamina neuropile through silver impregnation techniques and conventional histological preparations. Both mature and developing neurons are examined.

Materials and Methods

Compound eyes and optic lobes of 4th instar larvae of the desert locust *Schistocerca gregaria* were dissected in phosphate-buffered (Hayat 1970) Karnovsky's (1965) fixative. As much of the surrounding tissue was removed as was possible (including the dorsal and ventral tips of the eyes) to facilitate penetration of the fixing and staining solutions. The tissue was fixed at 4° C for a least 4 h, after which it was transferred to a Karnovsky-based fixative which contained potassium dichromate at a concentration of 2%, and left in this solution in the dark for 2–3 days. After being rinsed several times in a 0.75% silver nitrate solution (until the washings ran clear and not red), the tissue was left in the silver nitrate staining solution for 3 days in the dark, renewing the solution every 24 h. After staining, the material was dehydrated in an acetone series and embedded in Araldite.

The embedded eyes and optic lobes were then cut into 150 µm thick horizontal sections with a sledge microtome following superficial warming of the plastic block to 85–95° C (West 1972). Sections were serially placed on siliconized (Repelcote) slides and left to flatten on a hotplate. A quantity of Araldite embedding mixture sufficient to surround the sections was poured onto the slide, and a second Repelcoted slide was placed over the first. After curing at 70° C for 24 h, the resulting film of Araldite containing the sections of retina and optic lobe was removed from between the slides, remounted on an ordinary slide using immersion oil as an adhesive, and covered with a coverslip to prevent scratching. Over 350 preparations of eye/optic lobe were processed in this way.

The sections were examined with a light microscope. Elements with components in the lamina neuropile (i.e., retinula cell and monopolar cell axons of the lamina ganglion, centrifugal terminals, horizontal neurons, glia and trachea) were examined and classified with respect to cell type, geographical location, size and branching pattern. Examples of each distinct cell type were selected for further anatomical examination. Drawings were made with a Zeiss *camera lucida,* and photographs were taken of individual neurons or glia using a Zeiss Photomicroscope II. Areas selected for resectioning were cut out of the film, reembedded in Araldite, and resectioned perpendicular to the axes of their lamina neuropile fields and prepared for light and/or electron microscopy.

Alternatively, tissue was fixed for 4 h in Karnovsky's (1965) fixative, post-fixed for 1–12 h in buffered 1% osmium tetroxide, dehydrated and embedded in Araldite for ordinary light and electron microscopy.

Semithin (1 µm) sections were cut on a Huxley Ultramicotome with glass knives, and stained with 1% toluidine blue in 1% borax; 80–120 nm sections were collected on 0.5% collodion or 0.25% formvar (Pease 1964) films to be subsequently placed onto slot grids. These were stained for 10 min in a saturated solution of uranyl acetate in 50% alcohol (washed in 50% alcohol) followed by 4 min in Reynold's (1963) lead citrate (washed in boiled distilled water). The sections were examined using an AEI-802 electron microscope to determine positions of the particular cell type in question within the sub-structure of the lamina, general relationships between major cell types, extent of branching, axes of branching, and so on.

Finally, Golgi-impregnated cells with axons at the most anterior (i.e., the youngest) border of the lamina neuropile were examined, classified and subsequently resectioned to determine the neuronal composition of the youngest cartridges.

Histological preparations were prepared by fixing half heads in alcoholic Bouin (Dubosq-Brasil) (Pantin 1969) and then left in 70% isopropyl alcohol for a period of several days to several weeks (to soften the cuticle). After dehydration in ethanol, clearing in methyl benzoate and benzene, the tissue was embedded in paraffin wax and sectioned horizontally at 10 µm on a Cambridge rocking microtome. Material was stained in haematoxylin and eosin (Pantin 1969).

Scanning-electron microscope preparations were made of the cast exoskeletons of 4th instar nymphs. These were cleaned in a dilute solution of sodium hypochlorite, dried, mounted on aluminium stubs, gold coated in a Polaron Sputter Coater to a thickness of 13 nm ± 2 nm and examined with an ISI-60 Scanning Electron Microscope.

Results

General Morphology

The insect optic lobe contains three visual ganglia or "geographical areas" (Strausfeld 1976) which underly the compound eye. Moving centrally from the retina, these ganglia are the lamina, the medulla, and the lobula. Connections between the lamina and the medulla, and between the medulla and the lobula, pass through the outer and inner chiasmata, respectively (Fig. 1 c).

The lamina, the most peripheral of the visual ganglia, shows three main zones in histological sections (Fig. 1 c). These are: a) an external zone composed of tracheae and bundles of photoreceptor axons (the fenestration zone), b) an intermediate zone including the cell bodies of the second order monopolar neurons, and c) a neuropile or plexiform region, where the synaptic relay between efferent and afferent neurons occurs. The lamina neuropile, the external plexiform layer, can be further divided into two strata based on analysis of the branching patterns of constituent neurons in Golgi preparations: a more distal region designated EP-1 and a more central region designated EP-2.

In the locust, axons of the eight light receptor cells in each ommatidium pass from the retina through the basement membrane in discrete units called pseudocartridges. Within a short distance, the individual bundles join and are incorporated into larger tracts of retinula axons from many ommatidia, where there is no visible distinction between axons from different ommatidia (Meinertzhagen 1976). The distance from the basement membrane to the external surface of the lamina neuropile is about 300 μm (Meinertzhagen 1976).

The lamina is composed of many units called neuro-ommatidia or optic cartridges (Trujillo-Cenoz 1965) (Fig. 2). Each optic cartridge corresponds to a single ommatidium, and there is an exact topographical projection of visual space upon the array of both lamina and medulla cartridges, though, because of the chiasma, there is an inversion of the antero-posterior axis in the medulla (Meinertzhagen 1976). The arrangement of the cartridges within the lamina neuropile corresponds to the hexagonal packing arrangement of the ommatidia in the retina. Thus, rows of cartridges can be assigned to the X, Y and Z axes (Braitenberg 1970) (compare Figs. 1 b, 2 a).

Neuronal Elements of the Lamina Neuropile

Monopolar Neurons

Golgi preparations reveal six classes of monopolar cells (Fig. 3). The cell bodies of each of the six classes lie proximal to the basement membrane of the retina and distal to the lamina neuropile, into which each monopolar axon projects. Of these

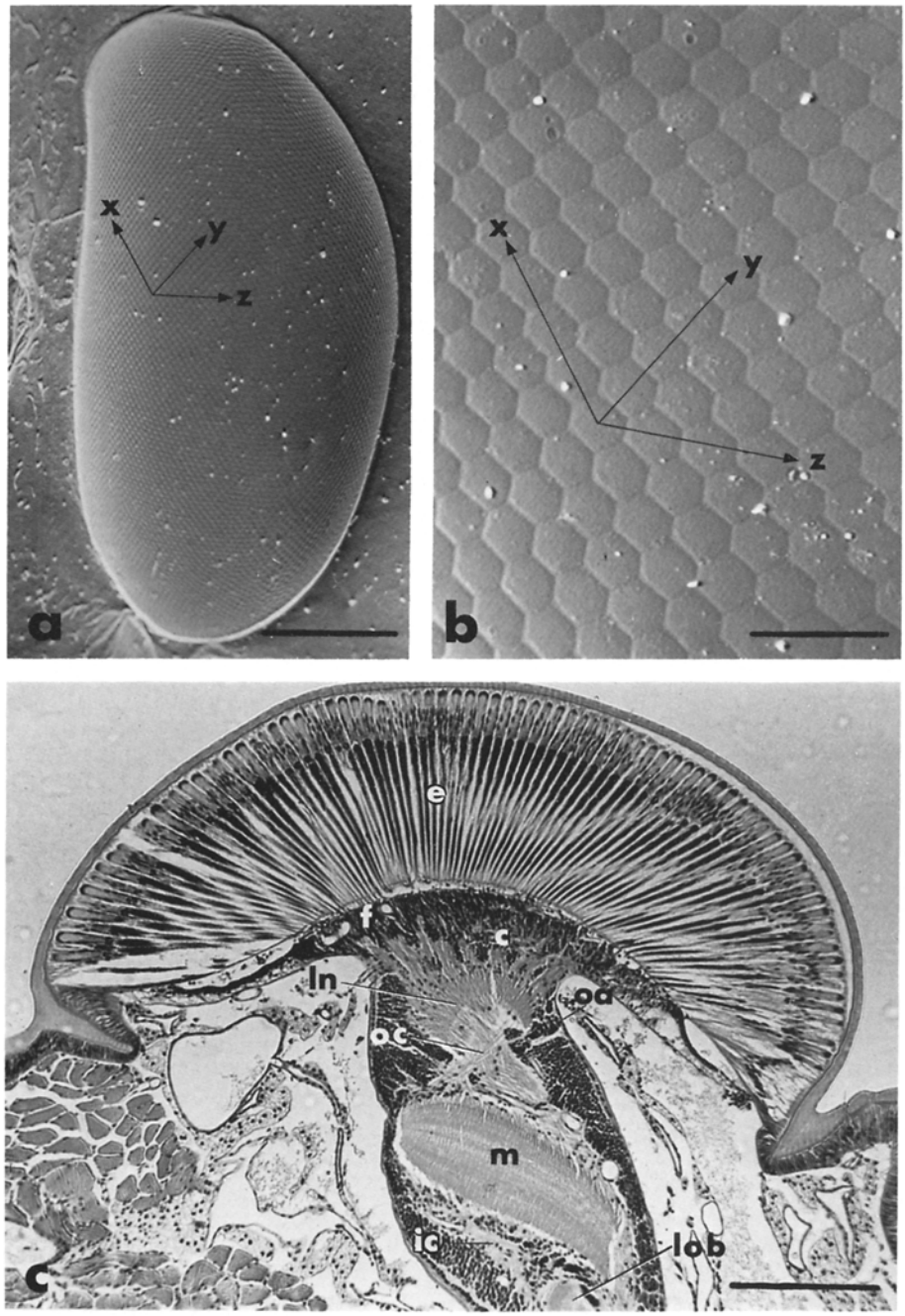


Fig. 1a–c. A low (**a**) and high (**b**) power scanning-electron micrograph of a 4th instar locust eye showing the arrangement of the facets and the X, Y and Z axes. Bars represent (**a**) 0.5 mm; (**b**) 50 μm. **c** Horizontal section through the compound eye (*e*) and optic lobe of an adult locust, showing the cell body layer (*c*), fenestration zone (*f*), and neuropile (*ln*) of the lamina. Medulla neuropile (*m*); lobula (*lob*), outer and inner chiasmata (*oc, ic*); outer optic anlage (*oa*). Bar represents 200 μm

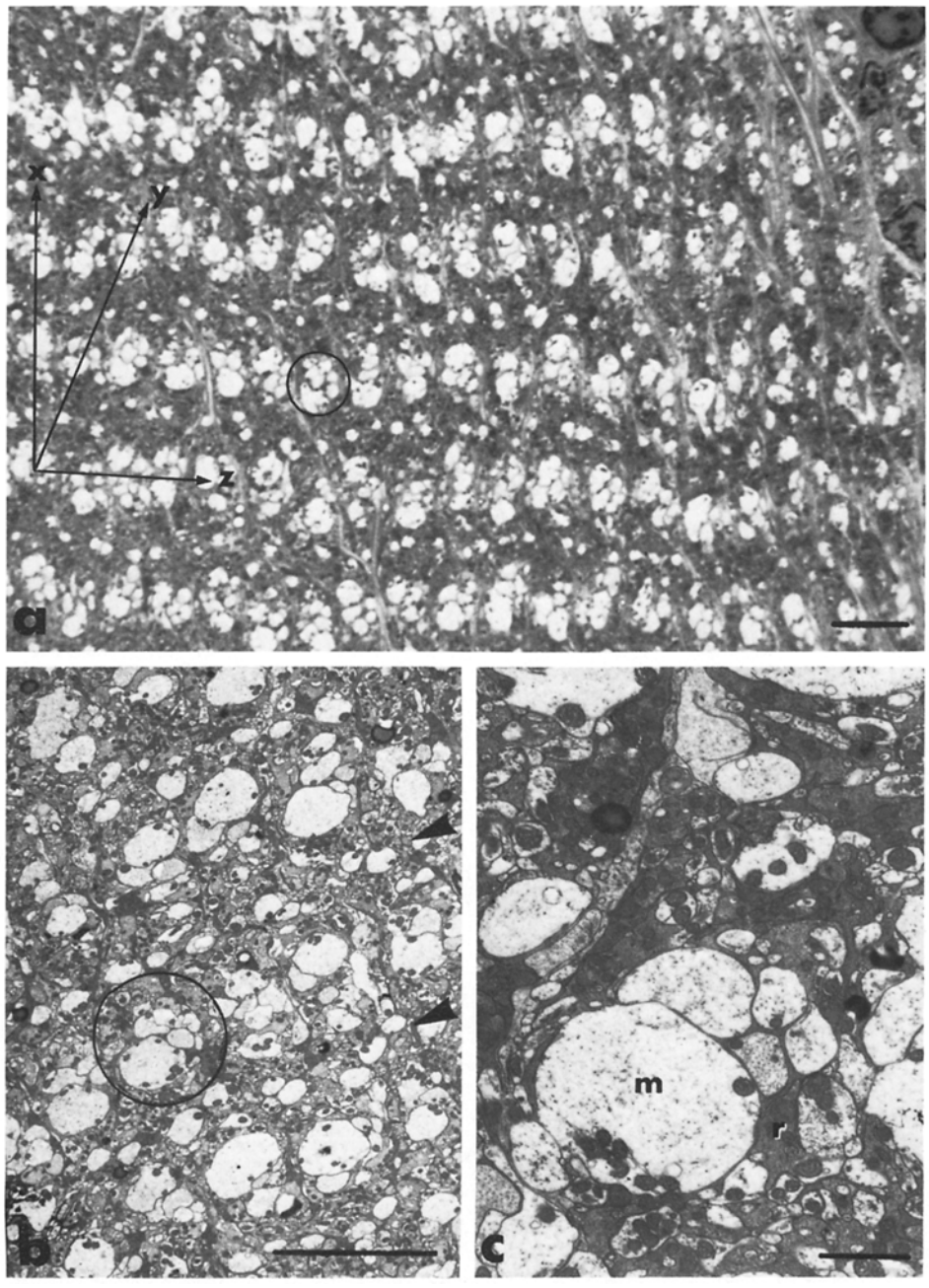


Fig. 2a–c. Light (**a**) and electron micrographs (**b** and **c**) of transverse sections through the lamina neuropile of 4th instar locusts. X, Y and Z axes are indicated. The regularity of the array is obvious, with rows of cartridges (examples *circled*) alternating with rows of single monopolar axons (*arrows*) in the horizontal (*Z*) axis. Cartridges are composed of 5 monopolar axons (*m*) surrounded by retinula axons (*r*). Bars represent (a), (b): 10 μm; (c): 2 μm

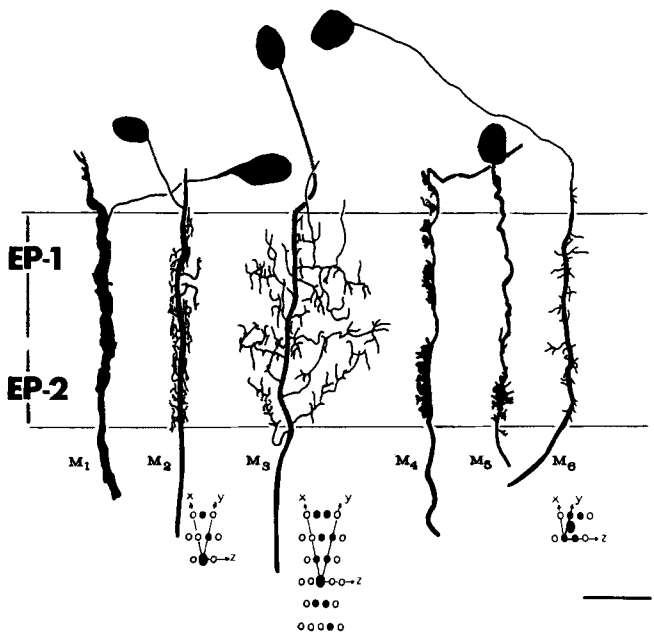


Fig. 3. *Camera-lucida* drawing of Golgi preparations of monopolar axons M_1–M_6 showing the patterns of branching in the external plexiform layers (*EP*) *1* and *2* of the lamina neuropile. Diagrams beneath the giant monopolars (M_2, M_3 and M_6) represent the cartridge array, with filled circles representing cartridges within the field of the neuron. Branching is in a general D-V orientation. X, Y and Z axes are indicated. Bar represents 25 μm

six cell types, the axons of five are grouped within each optic cartridge while the sixth appears only between the horizontal rows of cartridges along the Z axis (Fig. 2a, b). In electron micrographs, the axoplasm of these neurons appears electron lucent, and contains neurofilaments evenly distributed in cross section (Fig. 2b, c).

Although there is a certain amount of variability in size of neurons between specimens and in different parts of the neuropile, two of the six monopolar cells have axons of relatively large diameters – that is, roughly twice the size of those labelled "small-diameter fibres". These two "large-diameter fibres" are often seen as a pair within the cartridge.

a) M_1 – the "Pipecleaner" Monopolar Neuron (Figs. 3, 4a). The larger of the two large-diameter fibres (its axon is approximately 4–5 μm in diameter), the "pipe-cleaner"-shaped fibre penetrates the synaptic plexiform region of the lamina centrally and passes through the outer optic chiasma to terminate in the medulla neuropile. A spur is often seen projecting from the distal face of the lamina neuropile into the plexiform stratum (Figs. 3, 4a), presumably developing along the projection of retinula axons that innervate the cartridge to which this monopolar cell belongs. The spur can be of considerable length (equal to $^1/_3$ the depth of the neuropile itself). The oval-shaped cell body (approximately 12 μm by 15 μm) is connected to the axon by a fine process called the "neurite" or cell body fibre (Strausfeld 1976) – the "*segmento intercalar indifferente*" of Cajal (1909).

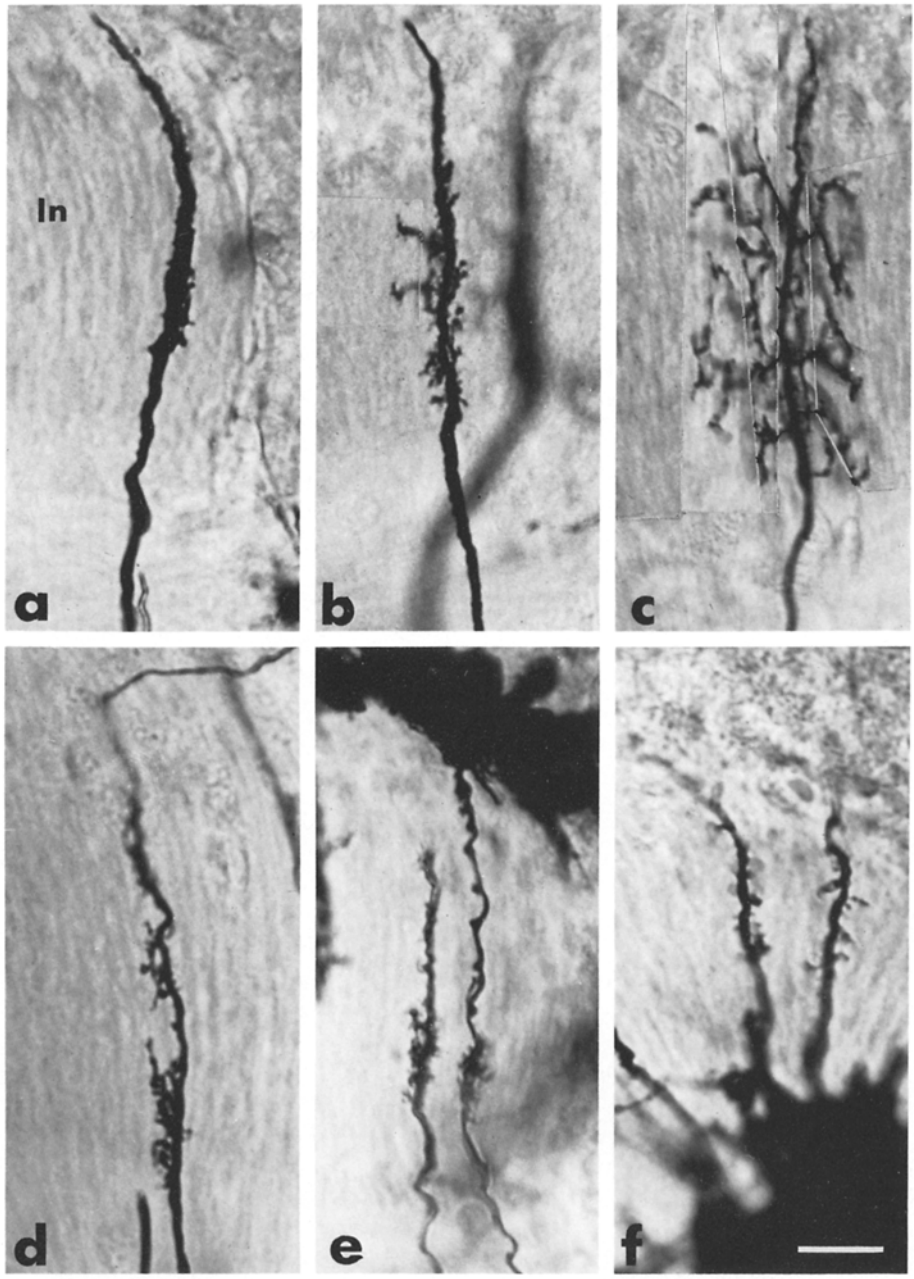


Fig. 4a–f. Micrographs of Golgi preparations of monopolar axons in the lamina neuropile (*ln*). Bar represents 20 µm for (**a–f**). **a** M_1, pipe-cleaner monopolar neuron; **b** M_2, side-arm monopolar; **c** M_3, widefield monopolar; **d** M_4, unilateral monopolar; **e** M_5, narrow-field monopolar; **f** M_6, bean-stalk monopolar

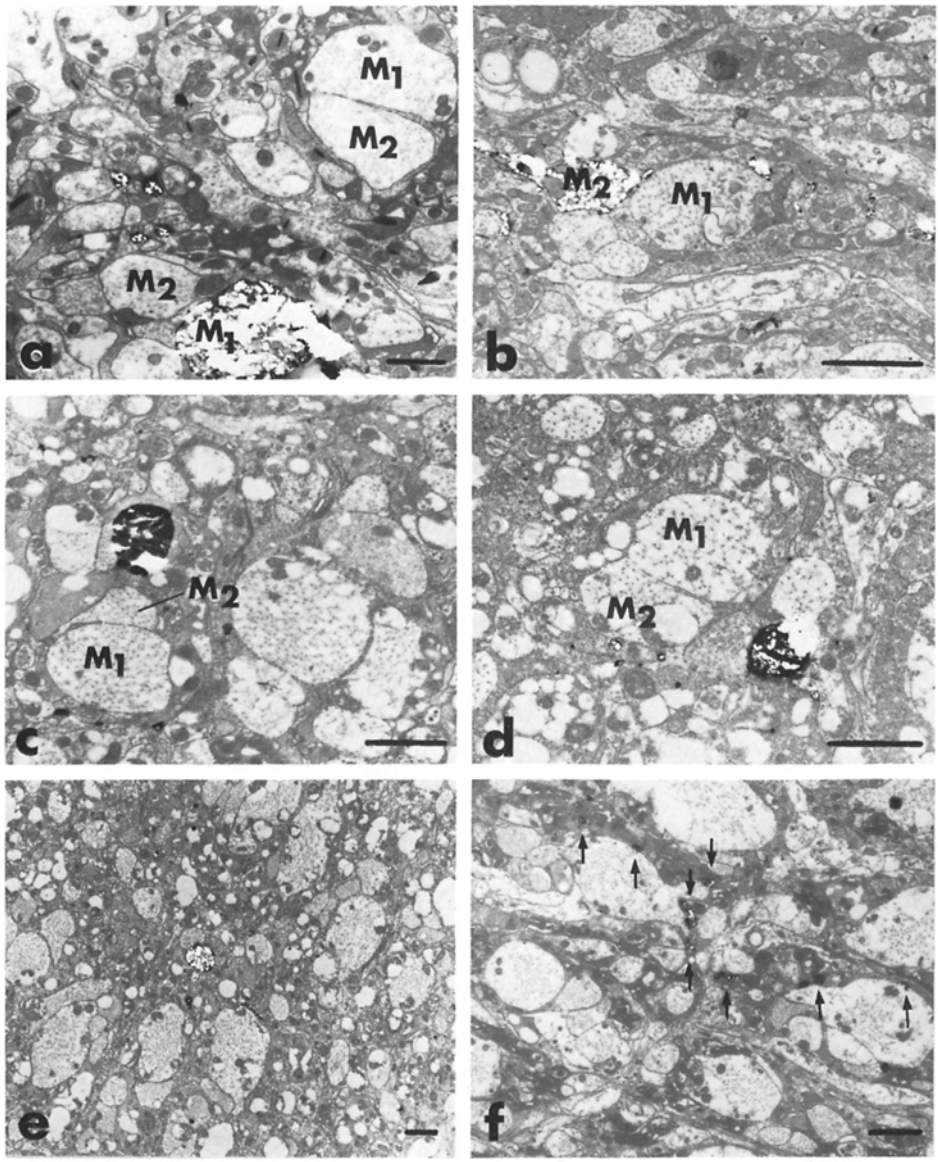


Fig. 5a–f. Electron micrographs of Golgi preparations of transverse sections of lamina cartridges including a filled neuron. Bars represent 2 μm. **a** M_1, pipe-cleaner monopolar neuron; **b** M_2, side-arm monopolar; **c** M_3, wide-field monopolar; **d** M_4, unilateral monopolar; **e** M_6, bean-stalk monopolar lying in the neuropile between two rows of cartridges; **f** C_3, candelabra centrifugal neuron. *Arrows* show fine branches in two separate cartridges

Within the synaptic layer, the "pipe-cleaner" axon is clearly the largest single element in the cartridge. This is especially apparent in the older cartridges, where the pipe-cleaner fibre is very prominent. In Golgi preparations, the axon occassionally exhibits a few short side branches within the chiasma. Most of the dense, short branches are found both in the EP-1 portion of the lamina neuropile

and along the spur. None of these side branches extends laterally to other cartridges but they are confined within their own cartridges (Fig. 4a).

b) M_2 – the "Side-arm" Monopolar Neuron (Figs. 3, 4b). The "side-arm" fibre is the narrower of the two thick fibres (2–3 μm in diameter), these having longer side branches which rarely (but occassionally) extend into adjacent cartridges. Beside these branches, there are several (usually two) longer branches or "side-arms" that extend further still – in some cases across two rows of cartridges in the vertical axis. The side-arms are found in the distal (EP-1) region of the lamina neuropile.

The shorter branches occur along the entire length of the axon within the plexiform layer. These appear to radiate from the central axon and turn through 90° towards the distal surface of the lamina (Fig. 3). The distally-running parts of the side branches are located at the periphery of the cartridges (Fig. 5b). Both branches and side-arms have very short, spiny projections along their lengths (Fig. 4b).

The cell bodies of these fibres are oval-shaped, approximately 12 μm by 15 μm. They are connected to the main trunk of the axon by a neurite from which a spur or several fine processes are occassionally seen to have developed distally (Figs. 3 and 4b). The axon is devoid of branches within the chiasma and its termination in the medulla is thick and has spiny projections.

There are three thinner fibres of monopolar neurons within the cartridge units:

c) M_3 – the "Wide-field" Monopolar Neuron (Figs. 3, 4c). Sidebranches of these fibres extend to cartridges three rows away. These sidebranches themselves branch, and the secondary branches often lie in a proximo-distal orientation, along the lengths of individual optic cartridges. Cell bodies of these neurons are about 10–12 μm in diameter. The central axon is approximately 1–2 μm in diameter.

d) M_4 – the "Unilateral" Monopolar Neuron (Figs. 3 and 4d). Short (4–6 μm) side branches of these fibres occur predominantly along a single side of the axon (Fig. 5d), invariably on the anterior face of the fibre – that is, on the side nearest the developing edge of the lamina. Along the fibre, these branches usually occur in two distinct strata: in the distal portion of EP-1 and throughout EP-2, separated by a short length of central axon devoid of branches. Branching in the EP-2 stratum is the more dense of the two regions. Cell bodies of these fibres are approximately 12 μm by 15 μm. The axon itself has a diameter of about 1–2 μm.

e) M_5 – the "Narrow-field" Monopolar Neuron (Figs. 3 and 4e). Cell bodies of these neurons are approximately 12 μm by 17 μm and are characteristically found directly above the cartridge in which the fibre is located. The axon (1–2 μm in diameter) passes through the EP-1 region of the neuropile usually without showing any side branches, although occasionally a very few short (1–2 μm side branches can be seen in this stratum. Through this level the fibre often appears wavy, as if the axon were twisted within the optic cartridge. Side branches are normally limited to the EP-2 stratum, where they form a dense tuft.

f) M_6 – the "Bean-stalk" Monopolar Neuron (Figs. 3 and 4f). The central axons of this sixth type of monopolar neuron lie between each horizontal row of cartridges along the Z axis (Fig. 5e). Axons of these neurons are narrow (1–2 μm), and the

perikarya are small (10 μm). Side branches, range from 3 to 12 μm in length. They extend to the two Z rows of cartridges between which these bean stalk axons are found. The sparse side branches, which exhibit an "opposite" and "alternate" branching pattern, occur mainly within the EP-1.

In characterizing neuron types, both Cajal and Sánchez (1915) and Strausfeld and Blest (1970) have used the terms "giant monopolar" and "small monopolar". Each group, however, has applied different criteria: Cajal and Sánchez (1915) call "giant monopolars" those second order neurons with lateral processes extending the full length of the axon within the lamina neuropile; other monopolar cells were termed "small". Strausfeld and Blest (1970) prefer to use "giant monopolars" for those cells whose axon branches extend beyond the limits of the optic cartridge to which it belongs, and "small monopolars" for those second order neurons whose lateral processes are limited to the one optic cartridge.

Of the two sets of criteria, the latter is thought to be less ambiguous for *S. gregaria,* though these terms must not be thought to describe actual size or bulk of the axon. Applying the terms as defined by Strausfeld and Blest (1970), small monopolar neurons include the "piepe-cleaner" neuron (M_1), "unilateral" monopolar (M_4) and "narrow-field" monopolar (M_5). Giant monopolars include M_2, the "side-arm" monopolar, M_3, the "wide-field" monopolar, and technically also M_6, the "bean-stalk" monopolar (as the lateral processes of this fibre reach more than one cartridge, although the axon is not itself a component of any single cartridge).

Centrifugal Neurons

The term "centrifugal" fibre is used here in the structural sense, and not necessarily in a functional sense. Although the exact location of the cell bodies of these neurons has not been precisely determined, they are thought to lie central to the medulla neuropile.

Three separate categories of centrifugal fibre contribute to the lamina neuropile (Fig. 6):

a) C_1 *– the "Wide-field" Centrifugal Neuron* (Figs. 6, 7a). The single axon entering the neuropile from its proximal aspect (i.e., from the outer optic chiasma) bifurcates repeatedly in the EP-1 region of the lamina neuropile, and the branches from a single neuron reach cartridges as many as ten rows apart in the vertical axis. Most of the fine branches extend centrifugally and pass out of the plexiform region a short distance into the peripheral cell body stratum.

One preparation (Fig. 14b) shows a bifurcation of the axon immediately distal to the medulla neuropile. Thus, two fibres from a single neuron cross the outer optic chiasma and enter the lamina neuropile to terminate there in their characteristic, wide-field network.

Within the medulla neuropile, the axons of the wide-field centrifugal neuron has short, spiny projections with a region of wider-ranging branches (Fig. 14b).

b) C_2 *– the "Inferior Plexus" Centrifugal Neuron* (Figs. 6, 7b). The appearance of this fibre within the medulla neuropile is similar to that of the wide-field centrifugal

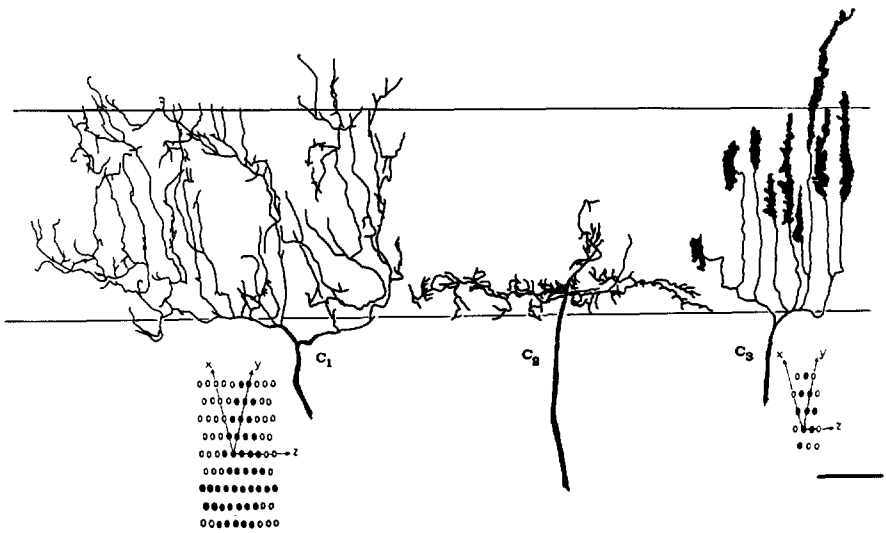


Fig. 6. *Camera-lucida* drawings of Golgi preparations of centrifugal neurons C_1, C_2, C_3 within the lamina neuropile. Cartridges lying within the neurons' fields are indicated beneath C_1 and C_3. Bar represents 25 μm

neuron (C_1). Within the lamina neuropile, however, the terminals are confined almost exclusively to the EP-2 stratum, and are very wide-ranging within this level.

c) C_3 – the "Candelabra" Centrifugal Neuron (Figs. 6, 7c). This class of fibre enters the lamina neuropile as a single very fine axon that ramifies into a relatively small number of branches. Each branch, terminating in a dense tuft of lateral projections, is apparently associated with a single optic cartridge (Fig. 5f). Branches terminate at different levels within the neuropile, occasionally entering the cell body layer distal to the neuropile region (Fig. 7c).

Retinula Cells

As in most insects, ommatidia in the locust *S. gregaria* have eight retinula cells. Each optic cartridge in the lamina of *S. gregaria* (and presumably in all insects having ommatidia with fused rhabdoms) contains the retinula axons of a single ommatidium.

Six of the retinula axons (Rs_1–Rs_6) are "short fibres" – i.e., all six terminate within the lamina neuropile. From the Golgi preparations, at least six anatomically distinguishable types of short retinula terminals can be seen (Figs. 8, 9a–d). They are distinguishable on the basis of their branching patterns and their depth of penetration into the lamina neuropile. Whether or not these six types correspond to each of the endings Rs_1–Rs_6 is not certain.

The remaining two (long) retinula axons (Rl_1 and Rl_2) (Figs. 8, 9e, f) pass through the lamina and terminate within the neuropile of the medulla. Rl_1 has a spiny appearance owing to its very short side branches within the lamina neuropile (both in EP-1 and EP-2), and is called a "spiny" long visual fibre. Rl_2 has longer side

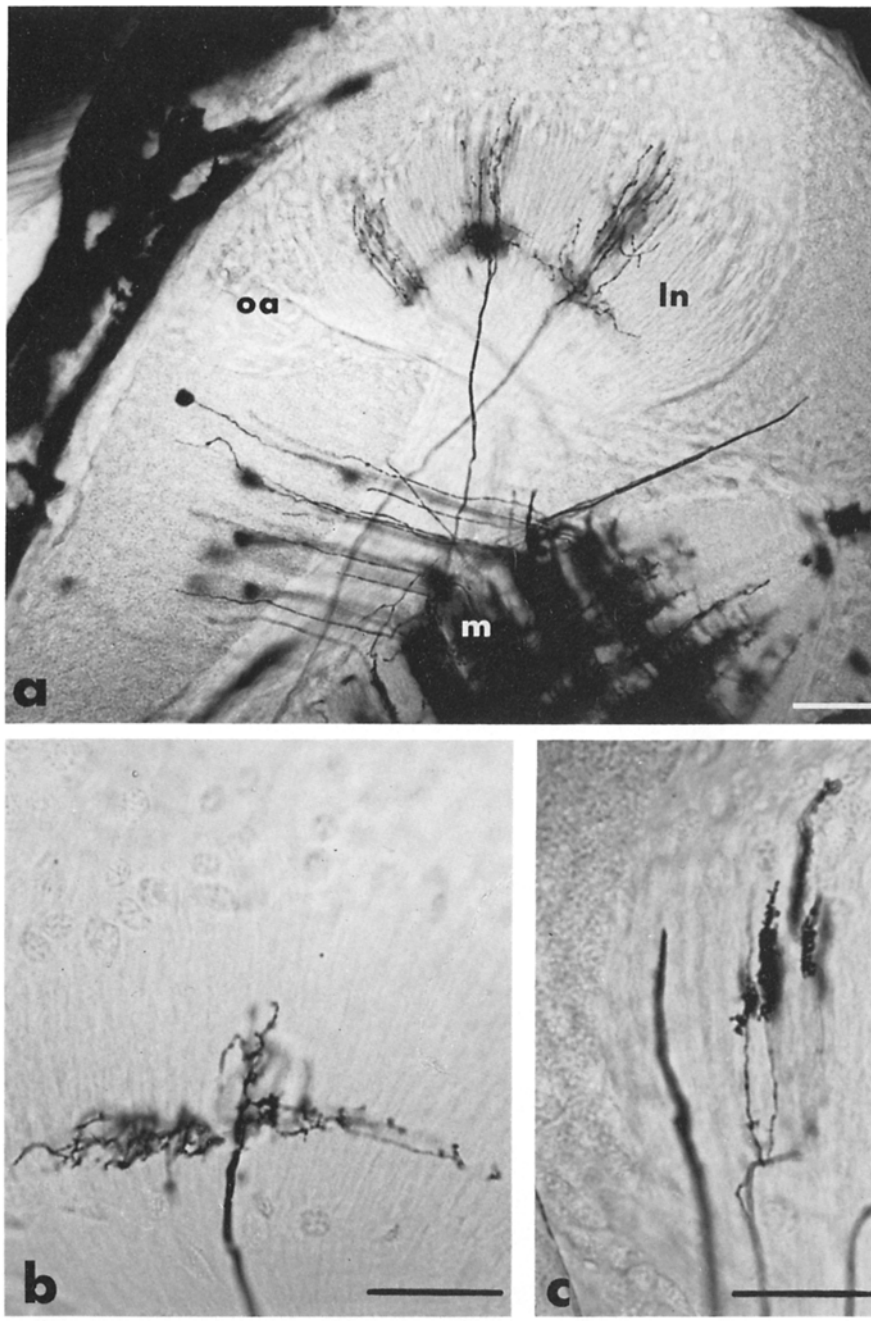


Fig. 7a–c. Golgi preparations of **a** three wide-field centrifugal neurons (C_1); **b** centrifugal neuron of inferior plexus (C_2); and **c** candelabra centrifugal neuron (C_3) in the lamina neuropile (*ln*). Medulla neuropile (*m*) outer optic anlage (*oa*). Bars represent 50 μm

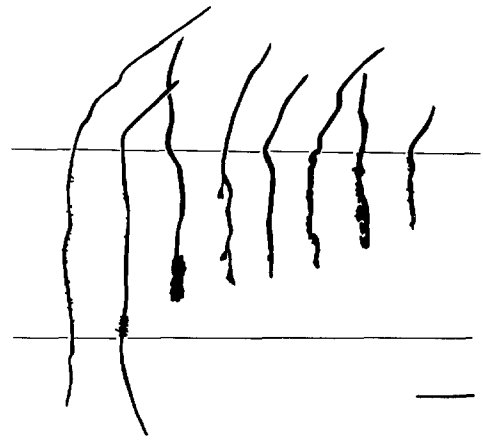


Fig. 8. *Camera-lucida* drawings of retinula axons within the lamina neuropile: two long retinula fibres (Rl_1 and Rl_2) which terminate in the medulla neuropile, and six short fibres, terminating in the lamina. Bar represents 25 μm

branches which are restricted to the EP-2 stratum of the lamina neuropile. Each of these long visual fibres has a narrower diameter within the outer optic chiasma (0.3 μm) than in the lamina neuropile (0.5 μm) (Fig. 9g, h).

Horizontal Neurons

Two types of horizontal neuronal elements have been found in the lamina. The perikarya of these cells have not been located, but they are presumed to lie proximal to the lamina neuropile. The orientation of the dendritic fields of these cells is parallel to the surface of the lamina and is horizontal to the sheet of neuropile as opposed to the other "perpendicular" elements which penetrate the neuropile at right angles. However, whether they are "tangential" neurons (i.e., ones which link one "geographical region" or neuropile complex with another) or are "amacrine" neurons (which do not, but are restricted to a single layer of neuropile) (Strausfeld and Blest 1970) remains uncertain.

a) H_1 *– the Horizontal Neuron of the "Intermediate lamina"* (Figs. 10a, 11a). A main linking fibre (presumably connected directly or via a neurite to the elusive perikaryon) extends dorso-ventrally through the neuropile, midway between its proximal and distal faces. In the examples of this neuron examined, numerous extremely fine processes extend horizontally and anteriorly along the Z axis between adjacent rows of cartridges (Fig. 11a, b). It appears that a single neuron could encompass a quarter of the area of the lamina neuropile with its fine net.

b) H_2 *– the "Inferior plexus" Horizontal Neuron* (Figs. 10b, 15c). In Golgi preparations, H_2 appears as a tangle of fibres ranging along the entire antero-posterior extent of the lamina neuropile. The field of this horizontal neuron is restricted to EP-2, the inferior region of the lamina neuropile. Unlike H_1, there is no single main fibre within this field. The extent of the field of H_2 in the dorso-ventral axis is unknown.

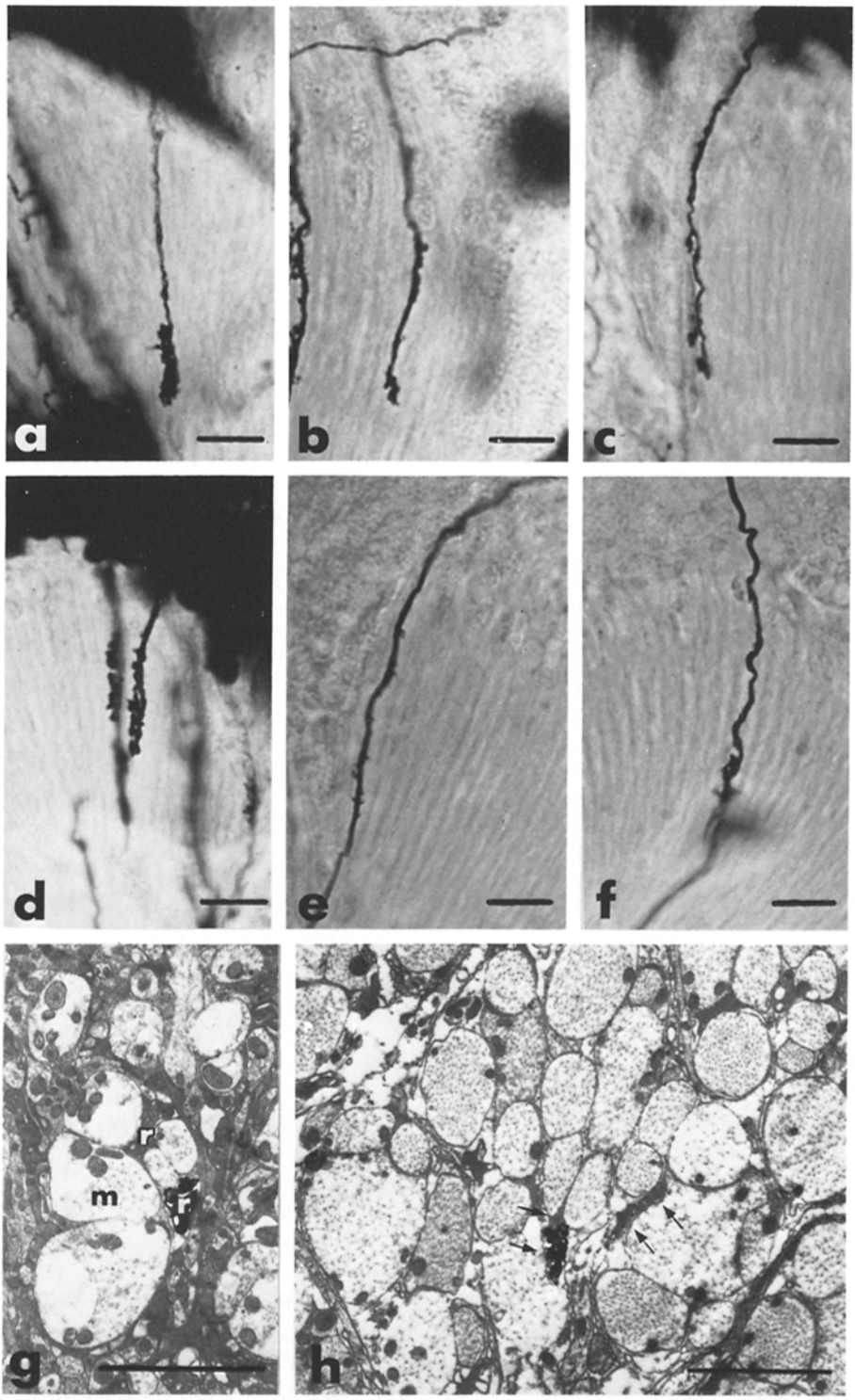


Fig. 9a–d. Golgi preparations of four short retinula axons in the lamina neuropile. **e** and **f** Long retinula fibres. Bars **a–f** represent 20 μm. **g** Electron micrograph in EP-1 of a transverse section of an optic cartridge including a filled long retinula axon (*r*) peripheral to the monopolar axons (*m*). An unfilled retinula axon is also indicated (*r*). **h** Electron micrograph immediately proximal to EP-2. Bundles of fibres are made up of 6 or 7 axons of centrifugal and monopolar cells and the 2 long retinula axons (*arrows*). Bars (g) and (h) represent 4 μm

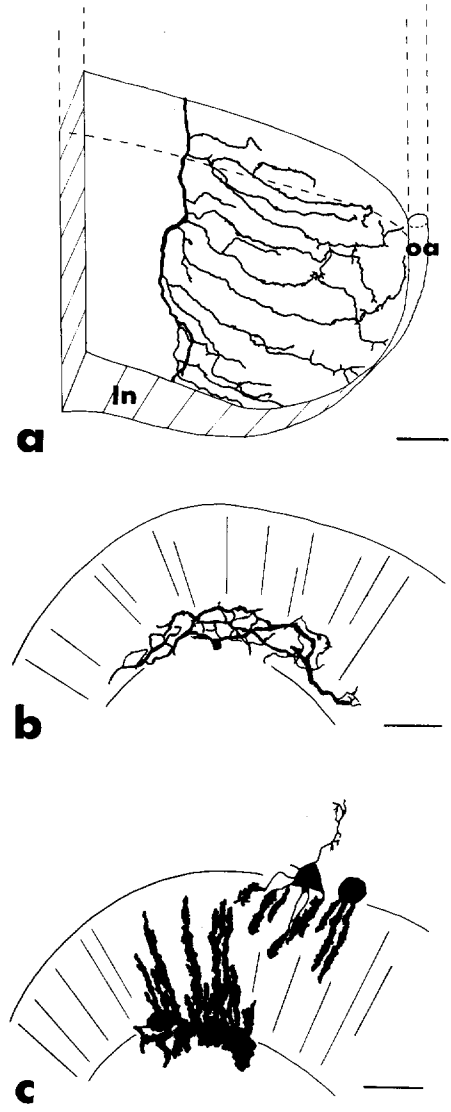


Fig. 10. **a** *Camera-lucida* drawing of H_1, the intermediate lamina horizontal neuron which lies halfway between the proximal and distal faces of the sheet of lamina neuropile (*ln*). The main fibre is orientated dorso-ventrally, and the fine branches extend horizontally towards the outer optic anlage (*oa*).
b *Camera-lucida* drawing of H_2, the inferior plexus horizontal neuron.
c *Camera-lucida* drawings of glial cells at the proximal and distal faces of the lamina neuropile. Cell processes into the neuropile are visible. Bars represent 25 µm

Non-neural Elements of the Lamina Neuropile

Glia

Non-neural elements of the lamina, which appear to have a role in structurally sequestering bundles of axons, occur at both the proximal and distal faces of the neuropile (Figs. 10c, 11c, d). Glia whose somata are situated at the superior edge send their processes centripetally while those at the inferior border have centrifugal projections. The cell bodies are approximately 12 µm in diameter and are arranged in a discrete layer at the neuropile surface (Fig. 10c) while the small number of

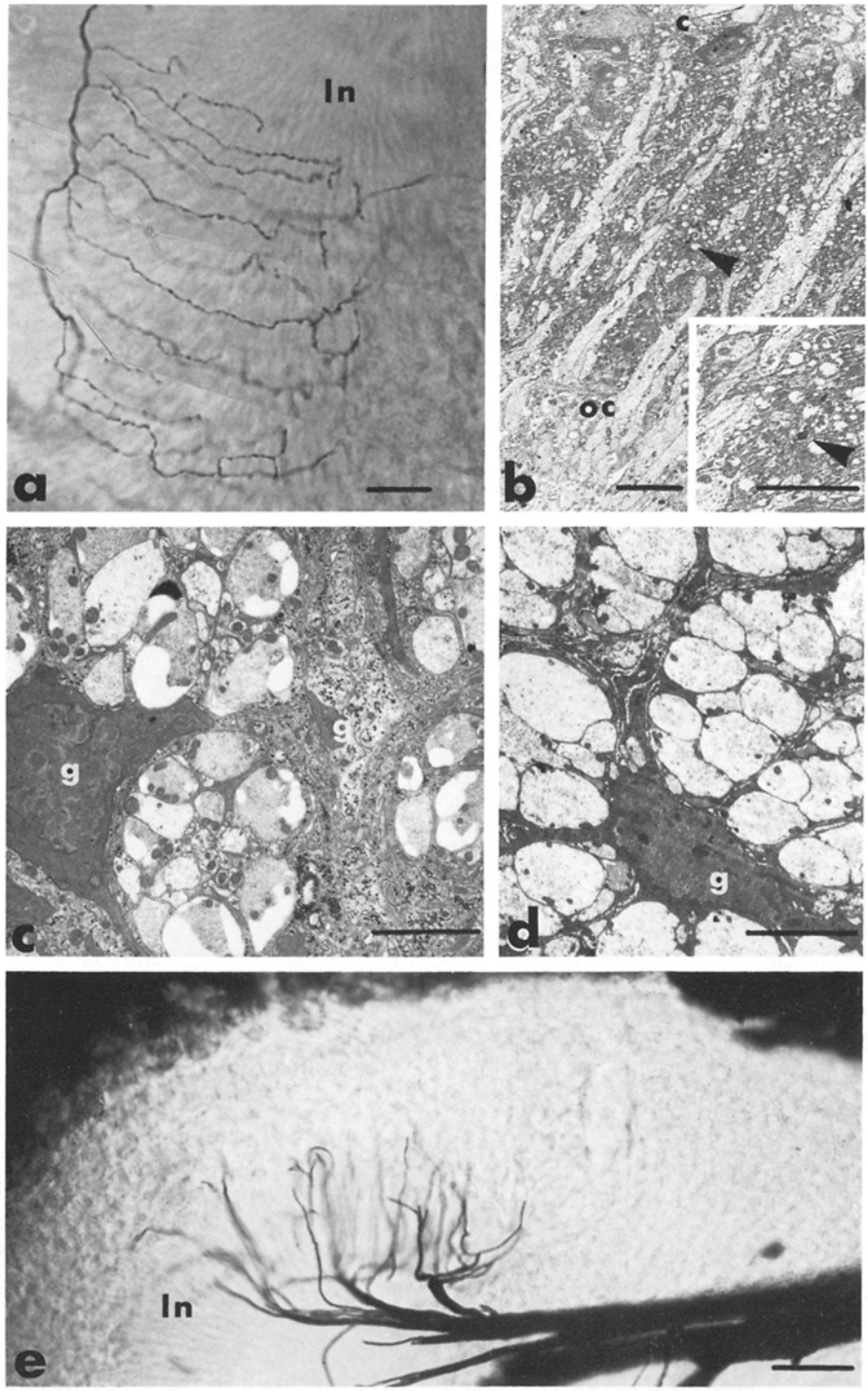


Fig. 11. **a** Golgi preparation of H_1, in a surface view of the lamina neuropile (*ln*). Bar represents 25 μm. **b** Electron micrograph of a longitudinal section through the lamina neuropile to show the location of one of the fine branches of H_1 (*arrow*) midway between the cell body layer (*c*) and the outer optic chiasma (*oc*). *Inset* shows H_1 at a higher magnification. Bars represent 10 μm. **c** and **d** Glial cells (*g*) at the distal (**c**) and proximal (**d**) faces of the lamina neuropile separate the optic cartridges. Bars represent 5 μm. **e** Tracheae are occasionally filled during Golgi-staining. The lamina neuropile appears well tracheated. Bar represents 45 μm

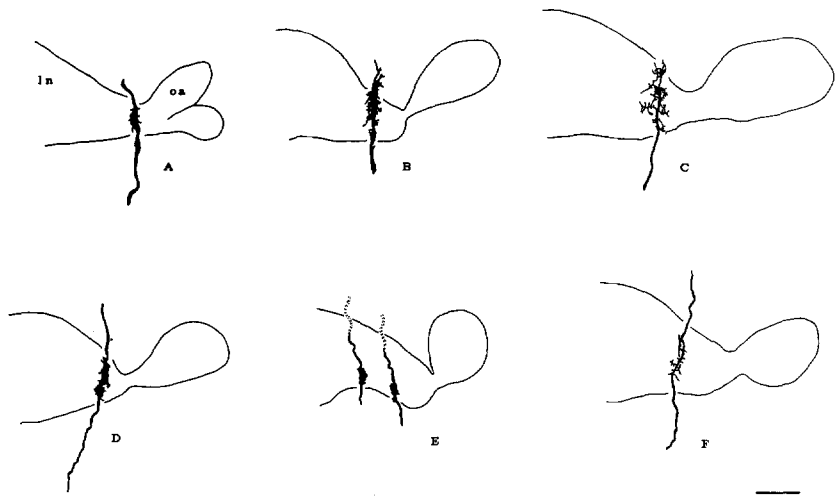


Fig. 12A–F. *Camera-lucida* drawings of developing monopolar neurons showing their branching patterns and their position in the lamina neuropile (*ln*) with respect to the outer optic anlage (*oa*). Bar represents 25 μm. **A** developing M_1, pipe-cleaner monopolar neuron; **B** developing M_2, side-arm monopolar; **C** developing M_3, wide-field monopolar; **D** developing M_4, unilateral monopolar; **E** developing M_5, narrow-field monopolar; **F** developing M_6, bean-stalk monopolar

bushy projections from each cell penetrates the neuropile perpendicularly, the extent to which the glia maintain isolation of the cartridge is unclear.

Tracheae

The lamina neuropile is richly supplied with elements of the tracheal system (Fig. 11e). Endings lie parallel to and alongside cartridges, and join main trunks of the tracheal system proximal to the neuropile.

Aspects of the Developing Lamina

Golgi-impregnated axons are randomly distributed throughout the lamina neuropile, and the specimens described and illustrated in the previous sections were selected from the more mature regions as examples of lamina components that are well developed in fourth instar animals. The following examples of impregnated axons lie along the most anterior border of the growing lamina neuropile, and are therefore among the most recently produced (and thus, differentiating) lamina neurons.

Developing Monopolar Neurons (Figs. 12, 13)

Examples of each type of lamina ganglion (monopolar) neurons are found in the developing region of the lamina neuropile, both those forms that comprise the optic cartridges (Figs. 12a–e, 13a–e) and that do not contribute to the cartridge (Figs. 12f, 13f). Even at this early stage, they are unambiguously defined as

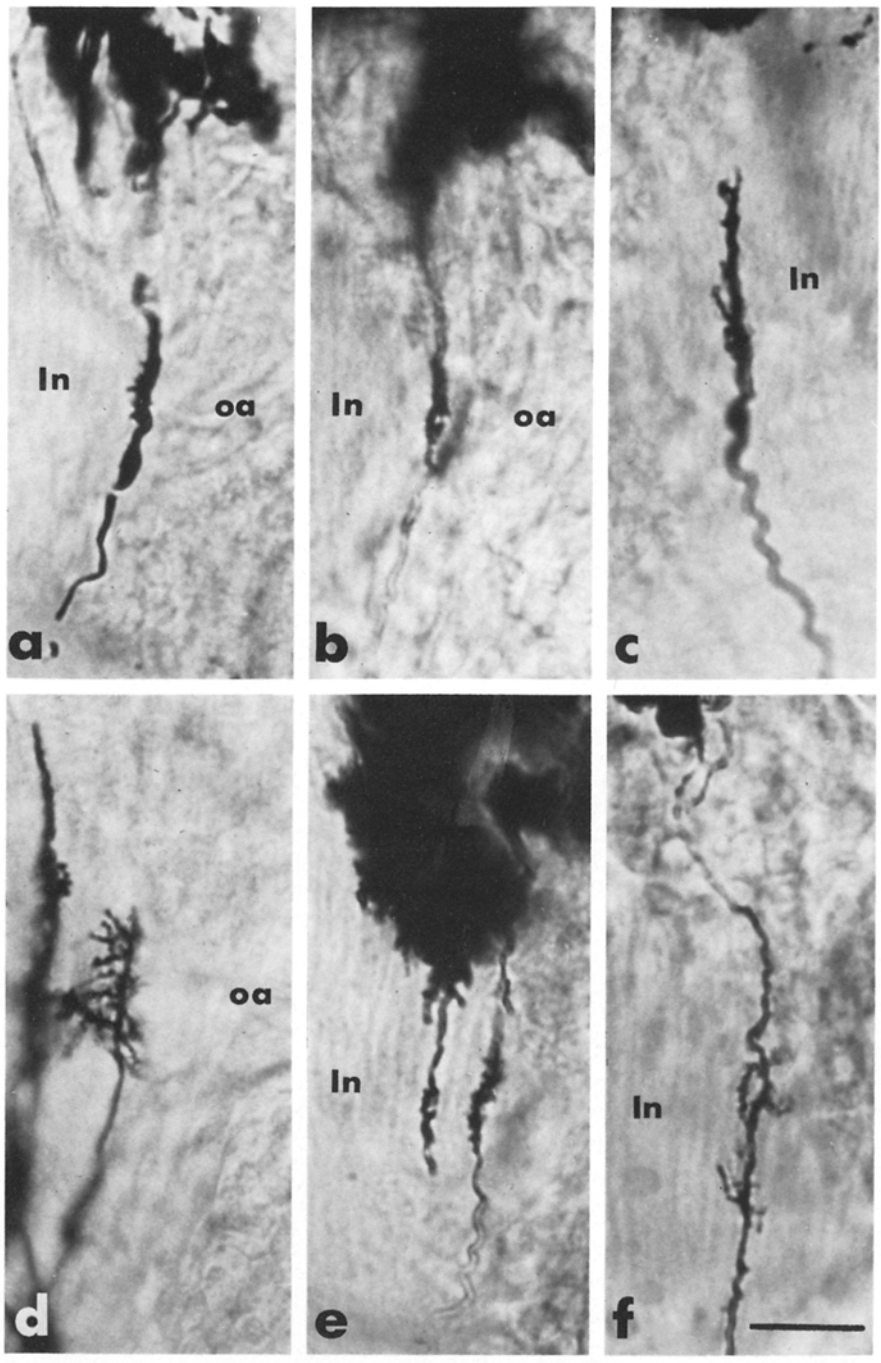


Fig. 13a–f. Golgi preparations of developing monopolar neurons; *ln*, lamina neuropile; *oa* outer optic anlage. Bar represents 25 μm for (**a–f**). **a** developing M_1; **b** developing M_4; **c** developing M_2; **d** developing M_3; **e** developing M_5; **f** developing M_6

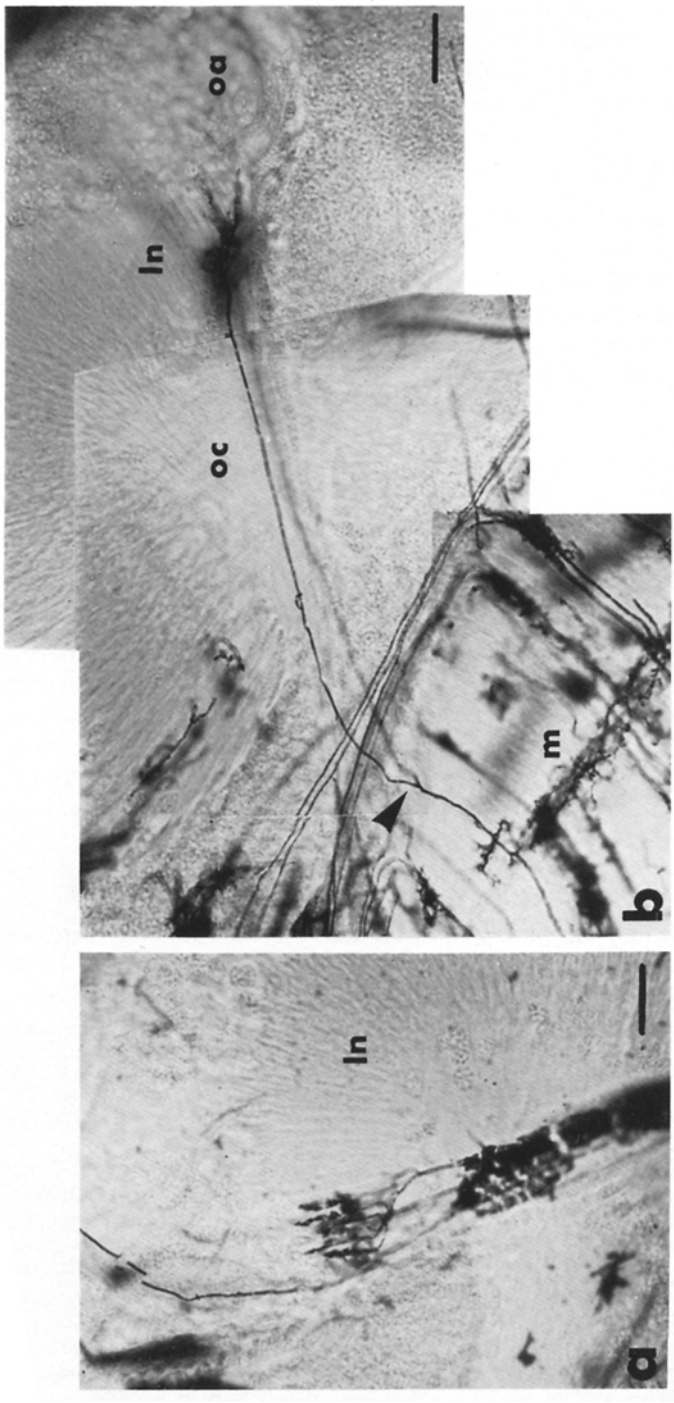


Fig. 14. a Developing C_3, candelabra centrifugal neuron. **b** Developing C_1, wide-field centrifugal neuron. Note that at the distal face of the medulla neuropile (*m*), the axon bifurcates (*arrow*). Branches project through the outer optic chiasma (*oc*) to adjacent regions of the lamina neuropile (*ln*) next to the outer optic anlage (*oa*). Bars represent 30 μm

particular monopolar types, and not diverging or evolving forms of an intermediate prototype. However, the extent of the branching of two "giant" monopolars – those whose branches extend to other cartridges (Strausfeld and Blest 1970): M_2 (Fig. 12b) and M_3 (Fig. 12c) – appears reduced during their early development.

Developing Centrifugal Neurons (Fig. 14)

Two types of centrifugal fibres terminate among the youngest, most anterior cartridges of the lamina neuropile. As with the developing monopolar neurons, although the extent of the branching of these young fibres is reduced, the developing centrifugal cells observed belonged unambiguously to particular classes of neurons and not to an ambiguous intermediate type. In the earliest stages, C_1 and C_2 might be indistinguishable because the full branching pattern is not yet developed; however, these fibres were always distinguishable from the candelabra centrifugal neuron, C_3.

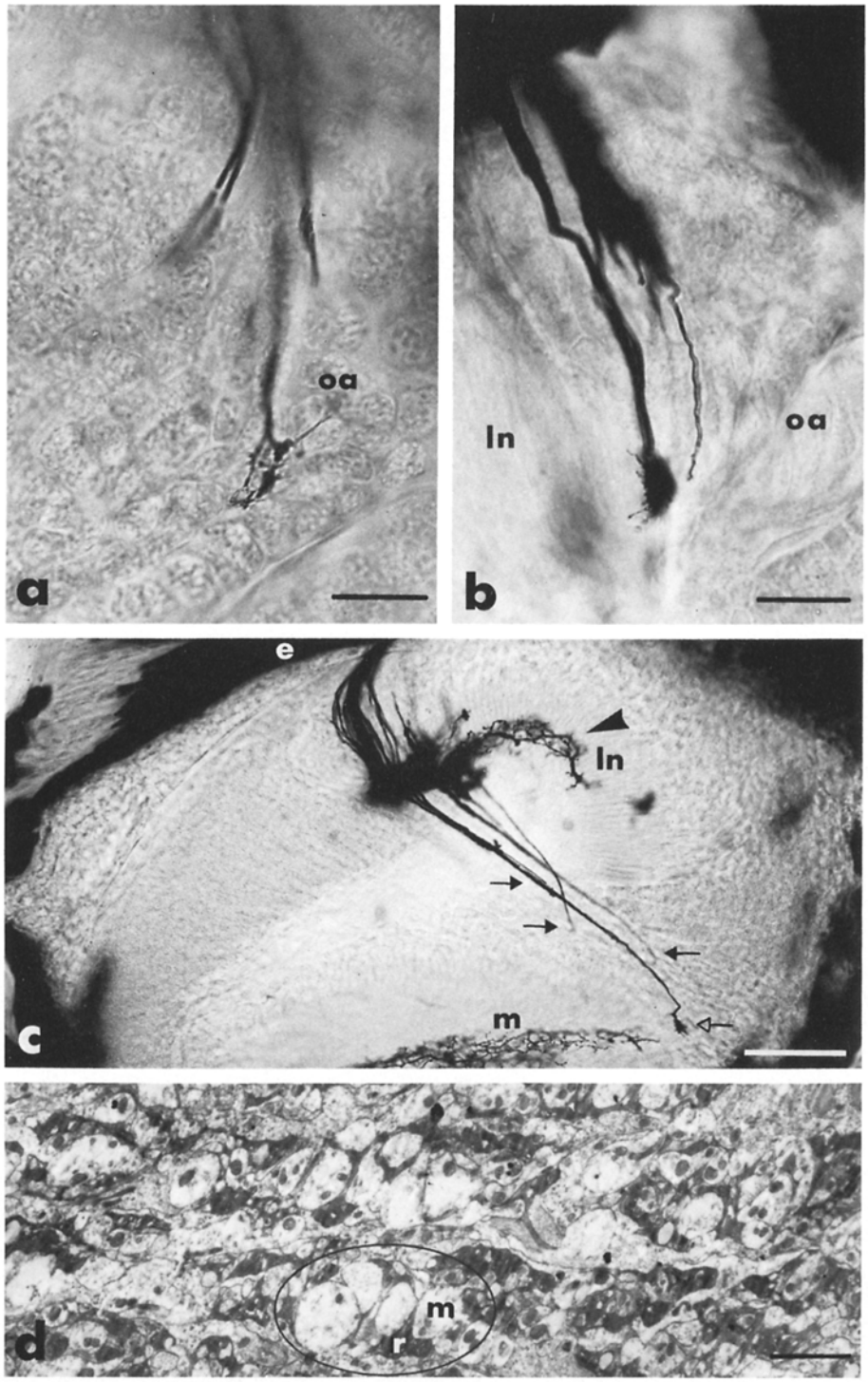
Developing Retinula Cells (Fig. 15)

Newly-formed retinula axons project from the retina to the outer optic anlage, where they become associated with sets of lamina ganglion cells. In the visual tracts of the butterfly *Pieris brassicae* (Sánchez 1919) and in the locust *S. gregaria* (Shelton 1976), the projecting tips of retinula axons are smooth and streamlined (Fig. 15a–c). When the axons have reached the outer optic anlage, their tips become greatly expanded and are covered with filopodia (Cajal and Sánchez 1915; Shelton 1976; Meinertzhagen 1976) (Fig. 15a). It is significant that these so-called "growth cones" are not visible between the retina and the lamina neuropile, or between the lamina and medulla neuropiles, but appear only in the outer optic anlage (Fig. 15a) or in the neuropiles themselves (Fig. 15b, c).

Although in the amphibian tectum, terminals of retinal ganglion cells can branch over as wide an area as 750 μm across (see Hunt and Jacobson 1974), retinula axon terminals in *S. gregaria* do not branch outside their own cartridge area. Therefore they appear either club-shaped or slender in Golgi preparation, and although in mature cartridges variations in depths of terminations are discernible, in the very shallow youngest cartridges these variations are not apparent. However, long visual fibres are easily distinguishable from short ones even at this early stage.

Cartridges in the youngest region of the lamina neuropile are certainly the shortest (compare the depth of the neuropile of the young anterior end to that of the

Fig. 15. a The tip of a developing retinula axon, dilated and covered with filopodia in the outer optic anlage (*oa*). **b** Extensive filopodia of two adjacent retinula axons in the youngest portion of the lamina neuropile (*ln*). Bars **a** and **b** represent 20 μm. **c** A large bundle of developing retinula axons projecting from the compound eye (*e*). Axons extending between the lamina and medulla (*m*) neuropiles are thought to be long retinula fibres. Note the filopodia-covered tip of the axon (*open arrow*) in the youngest posterior edge of the medulla neuropile. Note in **b** and **c** growing fibres not in a neuropile or the optic anlage have streamlined tips with no filopodia (*filled arrows*). Note H_2 neuron (*arrowhead*) in the lamina neuropile of (**c**). Bar represents 100 μm. **d** Electron micrograph of a transverse section of the youngest region of the lamina neuropile showing developing cartridges (*one circled*). Electron-lucent monopolar axons (*m*) are in a central position, and electron-dense retinula axons (*r*) have a peripheral position. Bar represents 2 μm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older, posterior end, Fig. 7a) and least organised (compare the electron micrographs of the developing cartridges in Fig. 15d with those of more mature cartridges in Fig. 9g). But, as all the monopolar cells, which form the core of the optic cartridges as well as the row of inter-cartridge "bean-stalk" fibres; the centrifugal neurons, and the retinula axons, which appear to initiate the development of the lamina ganglion (Meinertzhagen 1973, 1976; Mouze 1978a, b); are present in the most newly formed region of the lamina neuropile, it becomes clear that maturation of the optic cartridges involves elongation and development of the correct quantity and quality (specificity) of connections rather than the sequential addition of new classes of neuron.

Discussion

In *S. gregaria* axons of the eight retinula cells comprising an ommatidium project to the outer optic anlage in the optic lobe. Developing retinula axons occur both with and without dilated tips and filopodia; those with growing tips that have reached the outer optic anlage or the target (lamina or medulla) neuropiles have such an appearance. Those with tips that lie between the retina and lamina neuropile, or between the lamina and medulla neuropiles lack such structures. Apparently the function of these expanded tips is not to aid elongation, to maintain adjacent axon distance, or to "feel the way" to the target, the outer optic anlage. Rather, they may function at the target tissue a) to select particular second order cells or cell clusters (see Sperry 1963), b) to maintain or reestablish retinula axon order and spacing (see Dunn 1971), or c) to organise undetermined lamina ganglion cells in the outer optic anlage into optic cartridges by a process of induction (see LoPresti et al. 1973).

The outer optic anlage in the locust *S. gregaria* appears as a folded tissue lying in a dorso-ventral strip anterior to and parallel with the lamina neuropile (see Figs. 1c, 7a, 10a, 15a). Ganglion cells of both the lamina and the medulla cortices are derived from the outer optic anlage, while cells of the lobula are derived from an inner optic anlage (Nordlander and Edwards 1969). Medulla neuropile originates from both the outer and inner optic anlagen, the outer two thirds having a common origin with the lobula (Strausfeld 1976).

As the lamina ganglion cells are produced, they displace the older cells further and further from the anlage (Nordlander and Edwards 1969; Anderson 1976, 1978a). Autoradiographic studies have confirmed that the lamina ganglion cells are laid down in definite chronological order, the oldest elements being furthest posterior and furthest from the outer optic anlage (Nordlander and Edwards 1969).

As cells in the retina and lamina continue to proliferate and as these tissues grow, terminals of retinula axons from each ommatidium must assume their peripheral positions around the five second order cells which form the core of each optic cartridge. The terminal dilatations or "growth cones" develop into the terminals typical of more mature retinula axons (Figs. 2b, c, 8).

The observation that particular classes of monopolar neurons are established and grouped together very early in the development of the lamina neuropile does not necessarily suggest cell lineage wherein by monopolar cell type is determined as a result of a particular sequence of cell divisions of a ganglion mother cell in the outer optic anlage. This remains unknown. A number of other mechanisms for

determination of neuron types are possible: a) by the order of contact by the ingrowing retinula lead fibre, b) by position within a bundle of ganglion cell bodies,[1] or c) by the order of outgrowth of axons from these second order cell bodies.

The present description of lamina cell dendritic fields suggests that the hexagonal packing arrangement of the retina and neuropile is of little functional consequence. We agree with Anderson (1976) who cites several reaons why considering two axes – the horizontal (the equivalent of Braitenberg's (1970) Z axis) and the vertical (rather than the diagonal X and Y axes) – would be more valuable and informative when describing the retina (Figs. 1a, b) and lamina neuropile (Fig. 2a). The behavioural investigations of Goetz (1968, 1972) and the electrophysiological experiments of McCann and Dill (1969) and McCann and Foster (1971) demonstrate motion-detector sensitivity of flies to be most acute in the horizontal and vertical axes. In addition, some systems, for example, the eye of the cockroach (see Butler 1973) cannot be assigned the XYZ coordinate system. Anderson's (1976, 1978a) own studies on growth of retina and lamina in the locust show that the growing fronts of these tissues lie in a vertical axis that advances along the horizontal axis. Furthermore, in *S. gregaria,* in which a strikingly regular retina (Fig. 1a, b) exhibits the XYZ axes of an hexagonal lattice, the fields of several neurons contributing to the lamina neuropile lie either in the vertical (M_2, M_3 and M_6: see Fig. 3; C_1: see Fig. 6) or the horizontal (H_1: see Figs. 11a, b) axis.

The present study provides an anatomical framework for future electrophysiological and developmental studies on the insect lamina. In addition, our observations on the shapes of growing retinula fibres approaching and within the optic anlagen and neuropiles confirms our earlier doubts (Shelton 1976) on the terms "lead" and "follower" fibres applied in another arthropodan optic system, that of *Daphnia magna.* LoPresti et al. (1973) have suggested that it is only the lead fibre (the first of the ommatidial bundle to grow out) that has a "growth cone," the other seven having streamlined tips. It is possible that all eight develop these dilated tips on reaching the optic anlage, in which case the term "growth cone" is inappropriate in this system. At the time that the axon tips dilate, growth has either temporarily or permanently stopped, and cell-to-cell contacts are being established.

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1 It should be noted here that in *S. gregaria* unlike the house fly (Strausfeld 1976), discrete bundles of lamina ganglion cell bodies corresponding to axon bundles within mature optic cartridges are thought unlikely to exist. Perikarya of the narrow field monopolar neurons are invariably found directly overlying the cartridge in which their axons are constituents, while the somata of other monopolars are often widely displaced

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