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The Organization of the Insect Visual System (Light Microscopy)

I. Projections and Arrangements of Neurons in the Lamina ganglionaris of Diptera

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Summary. The structure of optic cartridges in the frontal part of the lamina ganglionaris (the outermost synaptic region of the visual system of insects) has been analysed from selective and reduced silver stained preparations. The results, obtained from studies on five different species of Diptera, confirm that six retinula cells, together situated in a single ommatidium, project to six optic cartridges in a manner no different from that described by Braitenberg (1967) from *Musca domestica*. Each optic cartridge contains five first order interneurons (monopolar cells) which project together to a single column in the second synaptic region, the medulla. The dendritic arrangement of two of these neurons (L1 and L2) indicates that they must make contact with all six retinula cell terminals of a cartridge (R1–R6). Two others (L3 and L5) have processes that reach to only some of the retinula cell endings. A fifth form of monopolar cell (L4) sometimes has an arrangement of processes which could establish contact with all six retinula cells: other cells of the same type may contact only a proportion of them. This neuron (L4) also has an arrangement of collaterals such as to allow lateral interaction between neigbouring optic cartridges. The processes of the other four monopolar cells (L1, L2, L3 and L5) are usually contained within a single cartridge. In addition to these elements there is a pair of receptor prolongations (the long visual fibres, R7 and R8) that bypasses all other elements of a cartridge, including the receptor terminals R1–R6, and finally terminates in the medulla. Four types of neurons, which are derived from perikarya lying just beneath or just above the second synaptic region, send fibres across the first optic chiasma to the lamina. Like all the other interneuronal elements of cartridges the terminals of these so-called "centrifugal" cells have characteristic topographical relationships with the cyclic arrangement of retinula cell terminals. Apart from the above mentioned neurons there is also a system of tangential fibres whose processes invade single cartridges but which together could provide a substrate for relaying information to the medulla derived from aggregates of cartridges.

Optic cartridges contain at least 15 neural elements other than retinula cells. This complex structure is discussed with respect to the receptor physiology, as it is known from electrophysiological and behavioural experiments. The arrangements of neurons in cartridges

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is tentatively interpreted as a means of providing at least 6 separate channels of information to the medulla, four of which may serve special functions such as relaying color coded information or information about the angle of polarised light at high light intensities.

Key-Words: Visual system — Insects diptera — Lamina ganglionaris — Optic cartridges — Light microscopy.

Much of our knowledge about an insects ability to perceive particular features in its visual environment can be derived from, a) electrophysiological, photometric and optical studies on the receptor layer (Reichardt, 1969a, b) some sophisiticated quantitative behavioural experiments which concern themselves with an insects capacity to track movement of objects and to discriminate patterns and colours within their visual field (von Frisch, 1965; Reichardt, 1969b; Mazokhin-Porshnyakov, 1969) and c) recordings from higher order neurons in the optic ganglia and mid-brain which respond to visual stimuli (Blest and Collett, 1965a, b; Collett and Blest, 1966; Horridge *et al.*, 1965; Bishop *et al.*, 1968; McCann and Dill, 1969; Collett, 1970; Kaiser and Bishop, 1970). Records have usually been derived from cells that have large fields and large diameter axons, and which give phasic electrical answers to movement of patterns in the visual field: it is these cells which may possibly mediate the passage of instructions to the effector organs for ambulatory or in-flight direction changes.

Investigations which reveal the anatomical organisation of the visual system (see, Vigier, 1907–1908; Cajal, 1909, 1910; Cajal and Sanchez, 1915; Braitenberg, 1967, 1970; Strausfeld, 1970a, b; Strausfeld and Blest, 1970; Strausfeld and Braitenberg, 1970) may also give some indications about what one might expect from it as a visual problem solving organ: on the one hand they will provide the kind of information which allows one to exclude certain combinations of neurons as interacting together, and on the other careful reconstructions of cell to cell arrangements will indicate which neurons are likely to have synaptic intimacy with one another.

For convenience the present account is divided into two parts. Part 1 describes the identification of those neural components which contribute to a set of elements termed an optic cartridge (Trujillo-Cenóz, 1965) and, in addition, provides a reconstruction of a cartridge thereby illustrating some possible physiological relationships between the participant neurons. Some details about the physiology of the receptor layer (considered to be particularly relevant to the lamina's structure) have also been included in this first section.

Part 2 describes the mode of projection of these elements to the second synaptic region, the medulla.

Material and Methods

The following species of Diptera have been used for these investigations. They are: *Calliphora erythrocephala*, Miegen, *C. phaenicia* L, *C. vomitoria* L, *Musca domestica* L, *Syrphus elegans*, Harris and *Eristalis tenax* L. The insects were either reared in the laboratory or caught in the wild. Only animals with phaenotypically normal wild-type eye pigmentation have been used, with the exception of those mentioned on pages 408 and 437.

It is, needless to say, vital to use both selective silver and reduced silver techniques in examining any neural architecture. Stains have been used which impregnate whole

single neurons (Golgi stains) and features of populations of neurons [Bodian (Chen and Chen, 1969), Holmes-Blest (Blest, 1961), Gros-Schultze (Meyer, 1951) and Bielschowsky[1] methods]. For insects it is necessary to modify those techniques prescribed for use on vertebrate tissue: only a brief outline of the most important of these is given here. All the methods are capricious and their success or failure is largely dependent on the alchemic propensity of the investigator.

Selective Silver Staining. Two techniques have been employed; the Golgi-Colonnier procedure (Colonnier, 1964) and a modification of the Golgi-rapid procedure (Pearson, 1971). Both techniques produce excellent results, the only differences in quality between them are that the former allows the resolution of the neuropil's texture in the unstained background (using Nomarski or Zernicke phase contrast illumination) while the latter does not. However, Pearson's modification stains more neurons in the mid-brain. There is no detectable difference between a specific neuronal type stained by one method and the same type stained by the other.

Variants of the Golgi-Colonnier Techniques. These have been described extensively elsewhere (Strausfeld and Blest, 1970) and only a brief recapitulation is needed here. The animals were killed by abdominal injection of buffered glutaraldehyde and left in the solution for up to six hours. Afterwards the opened heads, with a large area of the brain's surface exposed, were transferred to four parts 2.5% potassium dichromate and one part of 25% Kodak glutaraldehyde for as long as 6 days, in the dark, at 20° C. Afterwards they were rinsed in a 0.75% solution of $AgNO_3$ and left in a fresh solution of silver nitrate for as long as 14 days. They were then washed, dehydrated, embedded in Celloidin, and cut at 100 μm. After dehydration through alcohol the brains were cleared in terpineol or creosote, followed by Xylene and finally mounted under coverslips in Permount.

The Pearson Modification of the Golgi Rapid Procedure. The animals were killed by injection of a 2% potassium dichromate solution. Opened heads or dissected-out brains were then placed in a 2% osmium and 2% potassium dichromate solution (1 part to 3) for between 5 and 7 days in the dark at 25° C. The brains were washed in silver nitrate as above, and left in a fresh solution for between 2 and 4 days prior to dehydration and embedding.

Successfull impregnation of the smallest brains, such as those of *Musca* was achieved by adding 20 mls of a 6% sugar solution[2] to the buffered glutaraldehyde. It is noteworthy that those insects which are primarily nectar consumers are most receptive to the Golgi techniques. The very fine fibers of some mid-brain cells are more often stained when 1 or 2 drops of pyridine is added to the dichromate-glutaraldehyde solution. In these preparations the neurons stain pitch-black against a dirty yellow background.

Reduced Silver Techniques. Power's (1943) modification of the Bodian technique is capricious, but when it works it gives excellent results, as does the Chen and Chen (1968) variant of the same procedure. Immersion in a 1% solution of platinum chloride for 30 seconds, prior to toning in Gold chloride (30 minutes, at 30° C, under strong light) results in the supression of many fibers but enhances the toning of the lateral processes of some cells in the medulla and lobula (Fig. 5). These results can also be achieved in the best non-platinum treated Bodian preparations. Fibers stain blue, pink, purple and black against a clear background. Bielschowsky techniques can be used on insect tissue embedded either in celloidin or paraffin. Fixation in glutaraldehyde rather than formaldehyde seems to be essential for the successful impregnation of insect tissue. The brains were fixed in buffered glutaraldehyde[3] and after some hours they were rapidly dehydrated in equal parts acetone and ethyl alcohol followed by two changes of ten minutes each in dried ethyl alcohol. The brains were embedded in celloidin and the cooled blocks (hardened in chloroform at between −8° to −10° C) were cut at between 5 and 15 μm. Each section was individually stained as follows. 1) Sections were left in distilled water for two days and then impregnated overnight in 20% $AgNO_3$ solution. 2) Next, the sections were washed in a 1:1 mixture of formalin and tap water until the washing solution was no longer coloured. 3) The sections were then treated with ammoniacal silver nitrate and

1 See acknowledgements.

2 See also Braitenberg *et al* (1967).

3 As for the Golgi-Colonnier procedure.

subsequently reduced in 10% formalin. Further toning with gold nitrate can be employed but is not essential.

Another variant which was employed used brains fixed in a dilute glutaraldehyde solution (15% in tap water). The brains were immersed for two or three days, prior to embedding in paraffin. Deparaffinized sections were impregnated with 20° silver nitrate for between 6 to 30 hours. After washing in a formalin/tap water mixture some brains were treated with ammoniacal silver carbonate, others with ammoniacal silver nitrate and subsequently reduced in the Holmes-Blest (1961) reducing solution. They were toned in 2% gold chloride to which was added two drops of citric acid. This method is exceedingly capricious but when successful it provides excellent resolution of fine fibres throughout the brain. Its main draw-back concerns the extent of background colouration which renders these preparations least amenable for photography (Fig. 30a, b).

Excellent results have been obtained using Carnoy fixed brains treated with the Holmes-Blest procedure. The timing of impregnation in 20% $AgNO_3$ was increased to 5 hours and the use of tri-methyl pyridine instead of lutidine or pyridine constantly afforded the clearest impregnation (Fig. 1, 3).

Preparations were examined using a Leitz Orthoplan microscope equipped with Zeiss planapochromatic objectives and Leitz periplan oculars. Photographs were either taken with this combination (using a Leitz Orthomat semi-automatic camera) or with a Zeiss "Photomikroskop" equipped with Nomarski and Zernicke phase contrast, on Adox K.B.14, 36 mm film and developed in D.76. Drawings were made using a Leitz camera Lucida apparatus or (for detailed reconstructions) onto millimeter paper with reference to a calibrated eye-piece net graticule.

For the analysis of the projection patterns between the lamina and medulla (of those fibres stainable by reduced silver preparations) photographs were made of serially sectioned 10 μm sections cut tangentially through the optic lobes. Scale drawings were made of the fibres in the sections and checked against the projected photographs. It was important to relate each new section of a series to the graticule coordinates, not from "landmarks" in the chiasma itself, but from extraneous features such as cuticular patterns. The sections of a ribbon were oriented in the same way throughout a series. Thus it was possible to detect any lateral or rotational shift of a fibre bundle between one section and the next. A rotational shift of a fibre bundle between two sections was measured by rotating the graticule coordinate, aligned along the bundle in one section, so that it was again aligned to the same bundle in the succeeding one. This shift could be read off from the degree of eyepiece rotation.

The cells described from Golgi preparations were either seen in their entirety on a single section or traced through 2 or 3 sections. In all cases the whole cells, or parts of them, were stained against a clear background. Unless otherwise stated all the patterns of fibre cross-sections illustrated in this account are those seen in the upper hemisphere of the left hand eye and reconstructions of optic cartridges have been arrived at from the study of neurons situated in the frontal quadrant of the upper hemisphere of left hand eyes.

For a description of the pattern symmetry and orientation of some lamina fibres (R1–R8 and L1–L4) the reader is referred to the recent account by Braitenberg (1970).

Observations

The Identification of Cell Types in Reduced Silver Preparations. Reduced silver preparations show up particular portions of a population of neurons whereas the Golgi stains show up the entire ramifications of single neurons. For example, platinum pretoned Bodian preparations show elements in the medulla which can be matched with type 2 transmedullary cells (Strausfeld, 1970a) by virtue of the lateral extents of their branches and the projection of their perpendicular fibres through the medulla into the 2nd optic chiasma (Fig. 5, 6). Similarly there are parts of cells in the lobula stainable by Holmes-Blest procedures (Strausfeld, 1970a) and Bodian procedures (Braitenberg, 1969) which can only be correlated with type II S neurons

at that level (Fig. 1–4) in Golgi preparations. Similar correlations can be made throughout the visual system. For instance, one of the monopolar cells in the lamina, termed L3, has an outline in sections taken along the z-axis (Braitenberg, 1967) which is precisely like that of the brush monopolar cell seen in Golgi preparations (Figs. 18, 20, 26, 36, 39, 42).

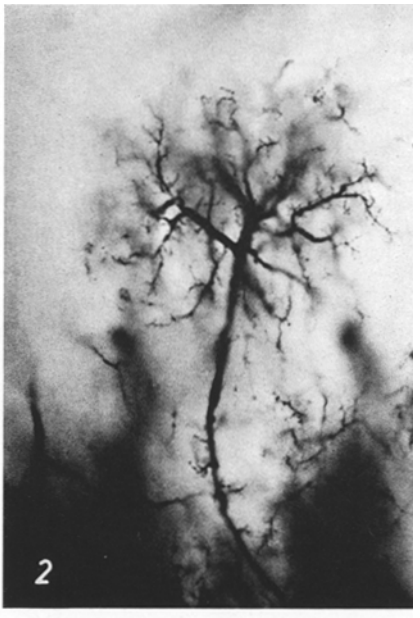
These rather obvious examples of selective and reduced silver identification of cells illustrate that it is not only possible to recognize repetitive arrays of parts of neurons in the optic lobes but that it is also possible to correlate features of them to single, wholly impregnated nerve cells visualised in Golgi preparations (see Figs. 25, 26, 31–33). In this way it is hoped eventually to relate all types of neurons with one another topographically and to estimate the relative proportions of cell types at all levels of the visual system. It is essential to realize the geometrical relationships of cells with one another before proceeding to the study of their synaptic connections. Further descriptions of these correlations are given throughout this account.

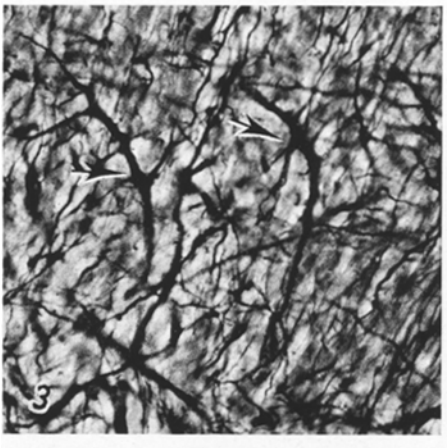
The Arrangement of Elements in the Receptor Layer (Retina)

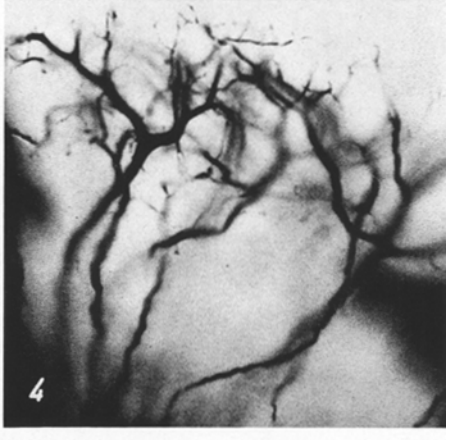
It is proposed that the neurons seen in Golgi impregnations have particular positions in the lamina with respect to the endings of receptor cells from the retina. Before describing the features of the types of interneurons that connect the lamina with the medulla it is appropriate to briefly review, and in some instances to supplement, evidence accrued by other workers which shows that the geometrical arrangement of the photoreceptors and their dioptric apparatus preserved in the lamina (Trujillo-Cenoz and Melamed, 1966; Kirschfeld, 1967; Braitenberg, 1967). This arrangement of units, such as lenses above the receptor cells or photoreceptor axons in the neuropil beneath it must be fully comprehended if we are to subsequently accept or reject any hypotheses about the visual system based on an a priori assumption that the compartmentalisation of the optic lobe neuropil into discrete columnar aggregates of neural elements represents a point for point map of the receptor level.

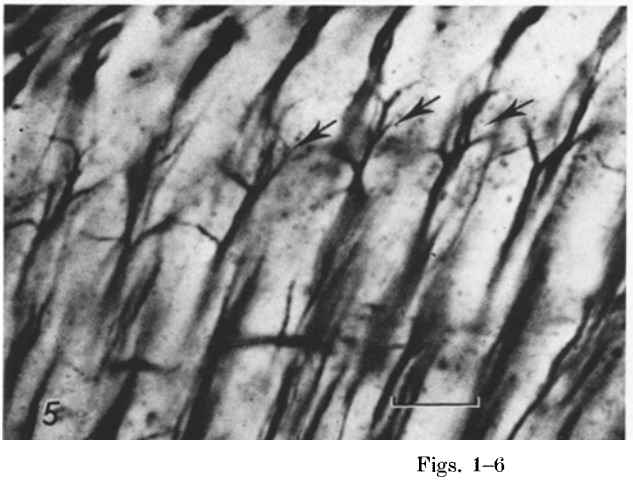
1. Lenses and Receptors. Braitenberg (1967) has shown that in spite of variations of size and form lenses of the retina (there are about 3000 per eye in *Musca*) are arranged along three coordinates (one horizontal and two oblique) termed, x, y and z; any distortion of these is merely a consequence of retinal curvature or lense size. The unfused rhabdomeres beneath the lenses and the arrangement of the individual rhabdomeres also fit into this tri-coordinate system (Fig. 7–9, 13).

The receptor arrangements in the ommatidia of Diptera have been adequately described by other authors (Trujillo-Cenoz and Melamed, 1966; Melamed and Trujillo-Cenoz, 1968). To summarize; six rhabdomeres in an ommatidium are arranged assymetrically around a central one, so that of the six one is displaced outside the circle formed by the remaining five (Fig. 9). The six cells, numbered according to Dietrich (1909), have microvilli orientations so that cells R2 and R4 share the same alignment, R1 and R5 share a second about 35° from the first, and R6 and R3 share a third, about 70° from the first. The central (seventh) rhabdomere (composed of the two ecentric cells, R7 and R8) has two sets of microvilli. That of R7 has a fourth orientation and that of R8, directly beneath and contiguous with R7, has a fifth at right angles to that above it (Melamed and Trujillo-Cenoz, 1968). The same cyclic order of cells in an ommatidium is found throughout the upper half of the eye. At the equator of the eye it is reversed so that in the lower half their order is a mirror image of that in the upper (Dietrich, 1909).









Figs. 1–6

2. The Projection of Fibres to the Lamina. Reduced and selective silver stains show the pathways of the retinula cells and long visual fibres from ommatidia into the lamina neuropil. The former technique has the singular advantage of invariably showing up all the elements from each ommatidium, the latter stains only a few, and by chance (Fig. 10, 15, 21). These pathways, which were first recognised by Vigier (1907–1908) and Cajal and Sanchez (1915), have subsequently been analysed in detail with the aid of electron microscopy (Trujillo-Cenoz and Melamed, 1966) and light microscopy techniques (Braitenberg, 1967; Horridge and Meinertzhagen, 1970). Kirschfeld's studies (1967) on the retinula cell optics (and also the studies by Kirschfeld and Franceschini, 1968, 1969) have shown that the projection patterns of the retinula cells, described by Braitenberg (1967), mean that receptor elements which share the same optical alignment, and thus "look" at the same point in the visual field, send their axons to the same optic cartridge in the lamina.

To summarize these accounts: the six retinula cells of a single optic cartridge can be followed back to six separate ommatidia arranged in a "pseudopupil" configuration. If the eye is illuminated antidromically and viewed through a narrow aperture objective a configuration of seven facets (see Fig. 8) of seven ommatidia will be seen glowing. These seven have their optical axes parallel with that of the microscope (see Kirschfeld, 1969). The prolongations of the rhabdomers from one ommatidium can be followed to a pattern of optic cartridges in the lamina which has the same asymmetric configuration of a pseudopupil arrangement except that it is twisted through 180°. In order to achieve this, the bundles of retinula cells whorl through half a circle beneath the basement membrane of the retina (see Fig. 13). No discrepancies can be seen between the projection patterns of the present species and those described from *Musca* by Braitenberg (1967).

Exceptions have rarely been detected in the retinula cell projection patterns (see appendix and Figs. 81, 82; also the account by Horridge and Meinertzhagen, 1970), and when they do occur throughout the lamina this is probably due to the fact that the lamina seems to lack first order interneurons. Even eyes whose retinula cell orientations are grossly distorted, but which contain lamina interneurons destined for the medulla, have retinula cell prolongations that project to different cartridges in a way reminiscent of the normal eye. In the wild type flies the retinula cell prolongations may undergo quite extensive morphogenic excursions around the large trachea in the fenestration layer of the lamina so as to arrive at their correct loci in the external plexiform layer. Very occasionally unusual retinula cell axon pathways have been detected between an ommatidium and a cartridge near (or at) the lamina's equator and perimeter (Fig. 80).

Fig. 1. A vertical section through the lobula of *Calliphora erythrocephala*, stained by the Holmes-Blest procedure. Features of populations of neurons can be revealed by this method; for instance the branching patterns and axis-fibres of type IIS: 2 cells (Strausfeld, 1970a), here indicated by arrows

Fig. 2. *C. erythrocephala* lobula (Golgi-Colonnier impregnation) showing the lobula component of a type IIS: 2 neuron. The Golgi stain impregnates all processes of a few neurons, whereas the reduced silver stain affords resolution of a few processes of all the cells

Fig. 3. Two reduced silver profiles of type IIS:1 cells in the lobula of *C. erythrocephala* (Holmes-Blest impregnation). The two systems of processes (arrowed) are immediately matchable with the cells impregnated by the Golgi method in Fig. 4

Fig. 4. *C. erythrocephala* lobula (vertical section, Golgi-rapid technique). A group of IIS:1 components

Fig. 5. *Musca domestica* (platinised Bodian preparation). The synperiodic arrangement of transmedullary elements in the outer stratum of the medulla (three are arrowed). Only their outermost processes are shown up by reduced silver

Fig. 6. *Musca domestica* (Golgi-Colonnier preparation). A vertical section through the outer $^1/_3$ of the medulla showing an impregnated transmedullary element whose dendritic pattern (bracketed) corresponds to the arborizations shown in Fig. 5. To the left is shown an L5 terminal and part of the medullary component of C2 (see text). Scale: Figs. 1–4: 40 μm; Figs. 5, 6: 10 μm

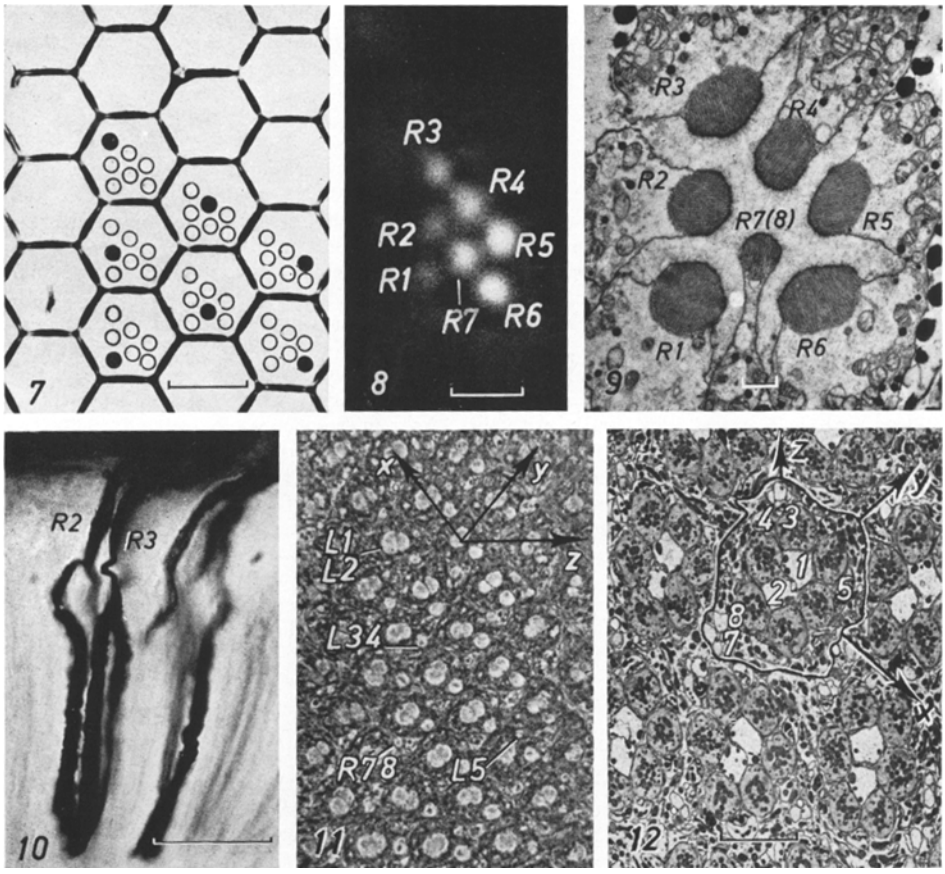


Fig. 7. Surface view of some lenses of the retina of *Eristalis tenax*. Seven rhabdomeres (indicated by open and closed circles) have a characteristic arrangement beneath each lense (after Kirschfeld, 1967). Closed circles represent retinula cells which are destined for the same optic cartridge (Braitenberg, 1966, 1967; Kirschfeld, 1967). The configuration shown here is from the upper half of the left hand eye, viewed from above (see also Fig. 9)

Fig. 8. The "pseudopupil" of *Eristalis tenax*, showing the seven rhabdomeres, beneath seven lenses, which share the same alignment to the visual environment (i.e. those elements represented by closed circles in Fig. 7) (preparation and photograph by courtesy of Dr. K. Kirschfeld, Tübingen)

Fig. 9. The open rhabdom of *Musca domestica*. Note the different orientations of the microvilli of *R1–R7*. The cross-section of *R8* lies beneath and contiguous with *R7*. (Illustration by courtesy of B. Boschek, Tübingen)

Fig. 10. Golgi impregnated retinula cells (*R2* and *R3*) in *C. erythrocephala* (vertical section of lamina, left hand eye) converging at the same optic cartridge

Fig. 11. A cross-section of the lamina (phase contrast microscopy of *C. erythrocephala*) showing the arrangement of optic cartridges in the upper left hand eye. *L1*, *L2*, *L3*, *L4*, *R7* and *R8* can be visualised as translucent sections: *x*, *y* and *z* are the three axes of the neuropil (Braitenberg, 1967)

Fig. 12. A low power electronmicrograph of the lamina (tangential section of the upper half of the right hand eye; "*z*" points posteriorly). Optic cartridges (one of which is outlined) contain other fibre cross sections in addition to those of L and R elements (L fibres numbered *1–5*, R7. R8 numbered *7*, *8*). (Illustration by courtesy of B. Boschek, Tübingen). Scale: Fig. 7: 25 μm; Fig. 8: 50 μm; Fig. 9: 1 μm; Figs. 10, 11: 25 μm; Fig. 12: 10 μm

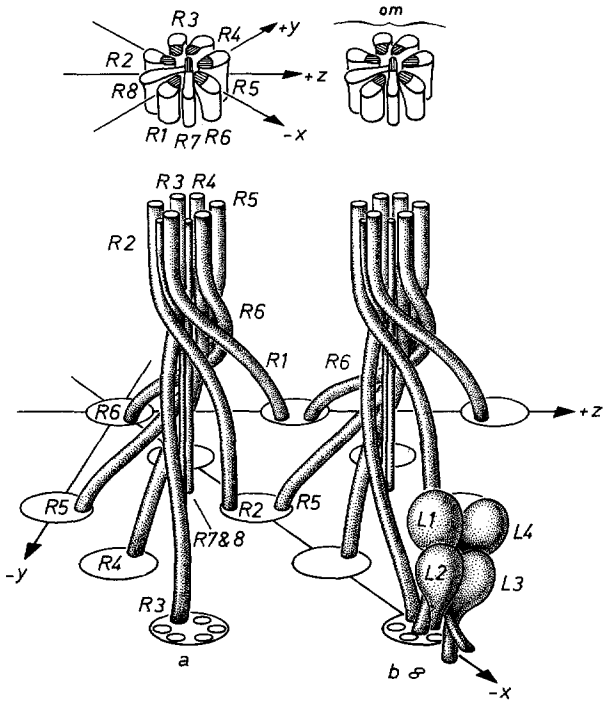


Fig. 13a and b. Summary diagram illustrating the projection of retinula cells to optic cartridges. No detectable differences can be seen between the projection patterns of R fibres in *Calliphora*, *Eristalis* and *Syrphus* and the retinula cell pathways first described by Braitenberg (1967) from *Musca* and by Trujillo-Cenóz (1966) from *Lucilia* and *Phormia*. *X*, *Y*, *Z*: the three axes of the hexagonal mosaic (— or + merely denote dorsal or ventral directions with respect to the horizontal axis, *Z*). Each rhabdomere (*R1–R8*) in an ommatidium (*om*) has its microvilli oriented along one of the three axes, *x*, *y* and *z* (Kirschfeld, 1969). The prolongations of the receptors project together as a "pseudocartridge" (Trujillo-Cenóz, 1966) as far as between 8 and 14 μm beneath the retina's basement membrane. Up to this point the R1–R8 elements keep the order that they had in an ommatidium. However, between this level and the external plexiform layer each bundle of receptor prolongations twists through 180° degrees (anticlockwise, from above, in the upper left hand eye): each prolongation projects to a separate destination in the lamina. The *R1* and *R2* fibres of the left hand bundle project to the same two cartridges (oval outlines) as the *R6* and *R5* fibres from the right hand bundle. The fibres from one ommatidium project to optic cartridges arranged in a pattern which is the reverse of the pseudopupil pattern (see Fig. 8) but the cyclic order of the terminals is the same as the cyclic order of rhabdomeres in an ommatidium. Note the projection of *R7* and *R8* from pseudocartridges straight down into the lamina (with no twist) along-side an optic cartridge which receives no retinula cell terminals from the same group (if all bundles could be drawn every cartridge would be seen to receive six *R1–R6* inputs as well as having a pair of long visual fibres (b) displaced ventrally from the retinula cell crown (a)). *L1*, *L2*, *L3* and *L4* the arrangement of four of the five monopolar cell perikarya

3. The Projection of the Long Visual Fibres. The two cells that make up the seventh central rhabdomere (and occupy the central position in a "pseudopupil" (Fig. 8) project almost directly down into the optic cartridge beneath their ommatidium of origin (Fig. 13, 29). Since they do not twist through 180° in the output bundle from an ommatidium their pathway (outside the crown of retinula

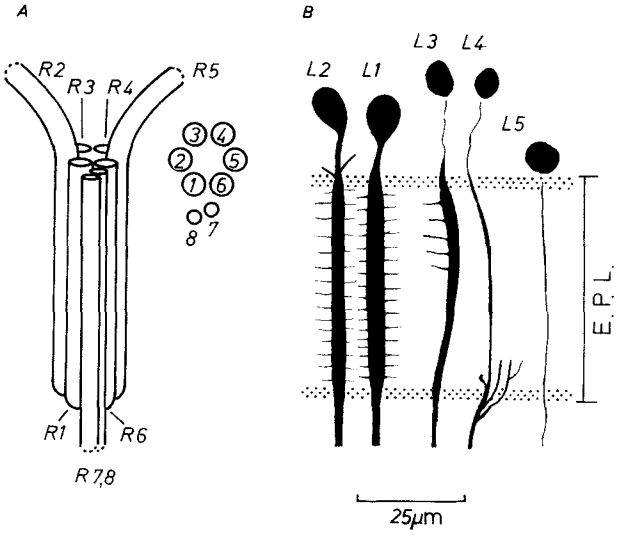


Fig. 14. A The arrangement of implicator elements of optic cartridges in the upper left hand eye (see Braitenberg, 1967, 1970; Trujillo-Cenoz, 1966). B Diagram showing the axis-fibre and cell-body silhouettes of *L1*, *L2*, *L3*, *L4* and *L5* in reduced silver preparations cut parallel to the *z* axis. *EPL* external plexiform layer

cell endings, and parallel to them through the lamina near *R1* and *R6* (Figs. 13, 14, 19) is invariably biased towards the equator (Braitenberg, 1970; Horridge and Meinertzhagen, 1970). The receptor elements of each optic cartridge in the lamina thus consists of a crown of six short visual fibre terminals (R1–R6)[4] and a pair of long visual cell fibre components (R7 and R8) which ultimately terminate deep in the medulla (see Fig. 14, 45).

4. The elementary Mosaic of the Lamina. A "mosaic arrangement" of neurons means no more than a periodic arrangement of the same sets of elements along definable axes which are natural to the neuropil structure. In the Diptera sets of cells in the visual system are usually arranged along the three axes, *x*, *y* and *z* (see Braitenberg, 1967, 1970). In the laminae of some Hymenoptera and Orthoptera sets of neural elements can be arranged along a two axis coordinate system (Strausfeld, 1970b). The first arrangement is said to form a hexagonal array or mosaic (Fig. 19) the latter a parallelogram array.

Cells are said to have a periodic arrangement in the neuropil when they are numerically associated with the subset of *implicator elements* of neuronal sets. In the case of the lamina each crown of R1–R6 endings, and its satellite R7 and R8 fibres are the *implicator elements* of a neuronal set called an optic cartridge (Fig. 14). In the medulla each pair of long visual fibre endings can be said to be the implicator elements of a set which is called a medullary column.

4 At the equator of the lamina each optic cartridge has up to eight retinula cell endings and at the lamina's perimeter there may be as few as 2 retinula cell endings per cartridge (Boschek, 1970b).

Any neurons which have a 1:1 relationship with optic cartridges (themselves having a 1:1 relationship with the ommatidia of the retina) are said to have a synperiodic arrangement in the neuropil.

More than one cell of the same type per cartridge (n:1) would have a sub-periodic arrangement. Cells that are not coincident with the set arrangement along the coordinates are a-periodic; cells which have a 1:n relationship with cartridges are said to have a supra-periodic arrangement in the neuropil.

The Arrangement of Cells in Optic Cartridges

A. Introduction

Phase contrast examination reveals that an optic cartridge (cross-section taken at the middle of the external plexiform layer) consists of 1. a crown of six retinula cells endings, 2. a pair of satellite long visual fibres, 3. a pair of fibres within the retinula cell crown (L1 + L2) and 4. a pair of fibres just outside the crown beside retinula cells Nos R5 and R6 (termed L3 and L4 Braitenberg, 1970; Strausfeld and Braitenberg, 1970). In sections taken at the outer margin of the plexiform layer this pair of fibres lies within the retinula cell crown (Fig. 11).

Electron microscopical examination of the same levels shows that there are a great many fibre cross-sections other than those already mentioned (Fig. 12). It is proposed that these must represent the various forms of neural elements, shown in Figs. 42, 43, 46, as well as the glia elements described by Boschek (1970b).

The following sections describe a) the number of centripetal fibres present in optic cartridges, b) the forms of centripetal elements in cartridges, determined from matching reduced silver profiles with Golgi stained neurons, and c) the types of centripetal relationships as surmised from selectively stained material.

The fact that the implicator elements of cartridges (namely, R1–R8) and the L1–L4 fibre cross sections have (nearly) stereotypic orientations in the lamina and the fact that they have precise and orderly arrangements across this region (Braitenberg, 1967, 1970) allows statements to be made about the lateral relationships of whole neurons (as seen in Golgi preparations) with one another within and between optic cartridges.

B. The Identification and Periodic Arrangement of Monopolar Cells

I. Introduction

There are many variations of monopolar cell form in the Diptera but only five types have been repeatedly recognised in the laminae of each of the present species. Two of them, namely the stratified radial diffuse and radial diffuse monopolar cells (Figs. 31–33, 6) are the only types of narrow field first order interneurons (Strausfeld, 1970a) whose lateral processes are disposed radially from their axis-fibres. They project perpendicularly through the external plexiform layer, enter the first optic chiasma and finally terminate in the medulla (Fig. 46). The other three types, illustrated in Figs. 34–39, 42, 43 and 47 are, namely, midget monopolar cells, tripartite monopolar cells and the brush monopolar cells. These neurons also terminate in the medulla.

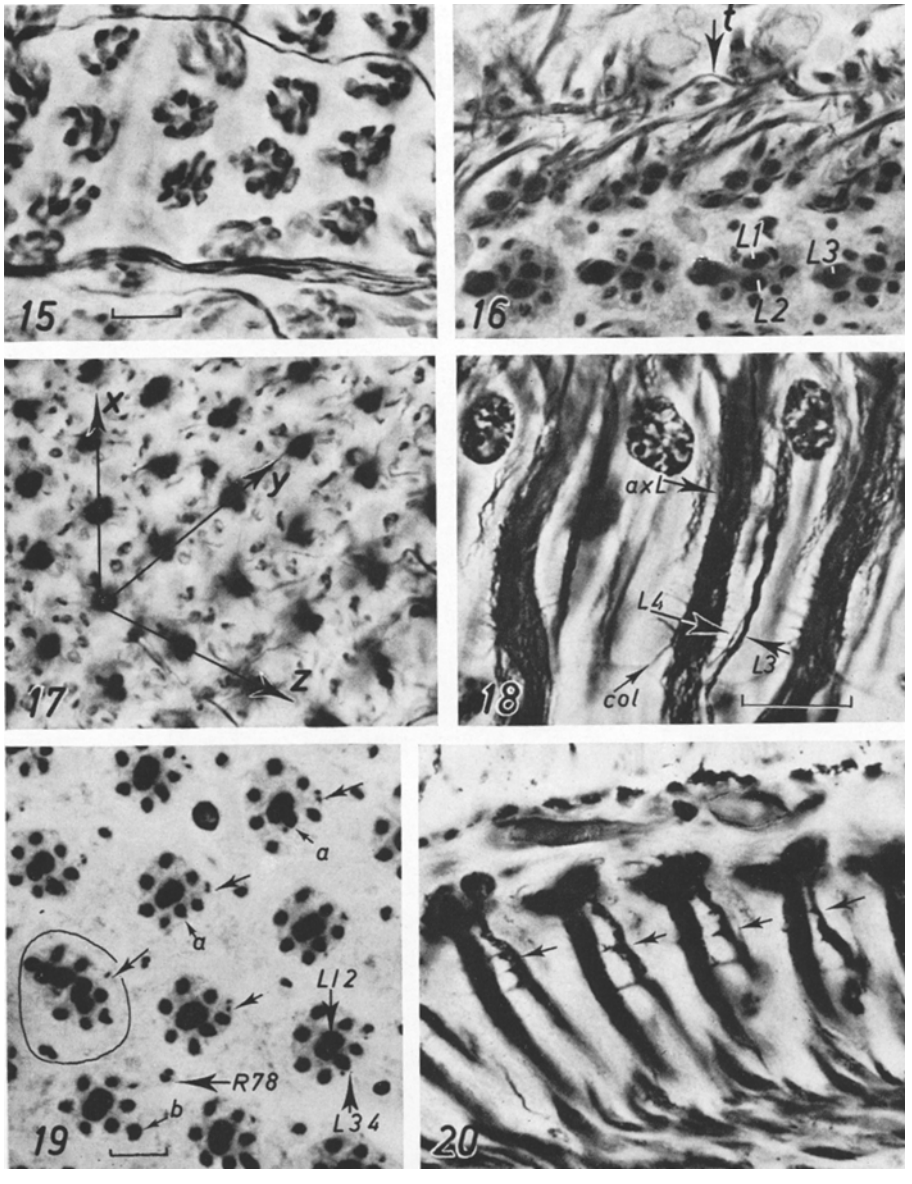


Fig. 15. Pseudocartridges in *C. erythrocephala* (Bielschowsky preparation). The longitudinal fibres running across the bundles from left to right are derived from bipolar receptor neurons situated between ommatidia and which innervate interlense receptors at the surface of the retina (Strausfeld, 1970c).

Fig. 16. *Calliphora erythrocephala* (Bielschowsky preparation). A section cut obliquely across the lower right hand lamina showing tangential processes (*t*) at the outer margin of the external plexiform layer connecting pairs of cartridges. Axial monopolar cell fibres (*L1* + *L2*) are surrounded by retinula cell fibres. At this level the L3 and L4 elements are situated between R5 and R6 (see Fig. 42). One unilateral side branch from *L3* (lower right hand cartridge) can be seen extending between two R fibres towards *L1* and *L2*

II. Cell-Bodies in the Lamina

Cresyl violet preparations show up the basophilic substance of all cells in the optic lobes irrespective of whether they are neurons, glia, or tracheoblasts. In these preparations two rows of nuclei are visible beneath the inner face of the external plexiform layer. Those nearest to it are small and elongated along the tangential plane (i.e. parallel with the lamina's inner face), while the deeper nuclei are larger and elongated perpendicularly. The outer nuclei correspond to the marginal glia cells described by Trujillo-Cenóz (1965). The deeper nuclei are located at the same level as the supposed lamina amacrine cell-bodies (Cajal and Sanchez,1915; also, Fig. 75). There is a single stratum of nuclei within the external plexiform layer itself. These are situated between optic cartridges and clearly correspond to the nuclei of epithelial cells (Trujillo-Cenóz, 1965; Boschek, 1970b). The large tangentially elongated nuclei of tracheoblasts and the smaller nuclei of supporting cells in the fenestration layer (F in Fig. 21) can be clearly discerned separately from groups of five small (between 5 and 6 μm in diameter) nuclei of five cell-bodies which lie above each group of optic cartridge implicator elements (see Table 1).

Except at the perimeter of the lamina, where they are disposed in columns (Fig. 27), these five cell-bodies are usually arranged in a characteristic configuration (Fig. 21, 42): two are situated just beneath the fenestration layer of the lamina while a third is situated to the right or left of the outer pair, about mid-way between the plexiform layer's outer surface and the fenestration layer. A fourth cell-body is situated obliquely to one side of the third at a position between 5 and 8 μm above the plexiform layer's surface. These four cells together

Fig. 17. A cross section of the upper left hand lamina of *C. erythrocephala* (Bielschowsky preparation) showing the synperiodic arrangement of *L1* cells. Their wide processes at the outer face of external plexiform layer are compressed along the x axis and stretched along the $+$ and $-$ y axis

Fig. 18. A section through the upper left hand lamina, parallel to the z axis (*C. erythrocephala*; Holmes-Blest preparation). This illustration shows the characteristic bow-like silhouettes of *L3* fibres and the exceedingly thin *L4* fibres that project alongside them through the lamina. The $-x$ axis collateral (*col*) from an *L4* fibre can be seen extending to the L1/L2 fibres at the base of a cartridge

Fig. 19. An oblique section across the upper left hand lamina of *Musca domestica* (Bodian preparation). *L1* and *L2* are surrounded by six retinula cell fibres. Peripherally in the external plexiform layer *L3* and *L4* fibres lie between R5 and R6 (arrowed *a*). Deeper in the lamina they are situated outside the retinula cell crown (arrowed *b*). Two small cross-sections (arrowed) can be seen near each cartridge, lying beside R5 and R4. One of each pair is derived from lamina tangential fibres that spread over the outer surface of the external plexiform layer, the other belongs to L5. An abnormal cartridge (ringed) demonstrates one of several kinds of morphological "errors" which can be found in almost every animal. In this instance what are presumably six retinula cell cross sections surround several other fibres in a crown; nevertheless, the tangential process, the L5 fibre, and the long visual fibre pair of this cartridge seem to have kept their normal positions

Fig. 20. The synperiodic arrangement of L3 cells in the lamina of an unidentified member of the family Drosophila. Note the serial arrangement of their unilateral side branches; these are identified with the pattern of side branches seen in Golgi preparations of brush monopolar cells. Scale: Figs. 15–17: 10 μm; Figs. 18, 20: 10 μm; Fig. 19: 10 μm

Table 1

A = Number of nuclei above cartridges: anterior upper and lower quadrants of the left hand eye (vertical sections, Nissl stained).

B = Number of nuclei above the six posterior-most cartridges of horizontal rows (sections cut parallel to z axis, Bodian stained).

C = Number of nuclei above cartridges: upper and lower quadrants of posterior half of the right hand eye (excluding posteriormost cartridges) (vertical sections, Bodian stained).

D = Number of nuclei above cartridges: frontal sections, anterior half of left hand eye (Bodian stained).

E = Number of nuclei above cartridges: frontal sections of the right hand eye (Nissl stained).

	Number of cartridges sampled	Average number of cell-bodies per cartridge
A	80	6.85
B	120	7.2
C	70	6.83
D	93	6.72
E	97	6.71

Mean number of cell bodies per cartridge including cell body layer and fenestration layer = 6.76 (excluding group B); 6,8 (including group B).

Number of nuclei arranged in discrete groups above cartridges (exclusively within the cell body layer): —

A = Upper half of left hand eye. B = Upper half of right hand eye. C = Lower half of left hand eye. D = Lower half of right hand eye.

	Number of cartridges + groups sampled	av: number of cell-bodies per group per cartridge
A	100	5.20
B	115	5.37
C	100	5.17
D	100	5.02

Mean number of cell-bodies per group per cartridge = 5,15.

comprise a compact group of perikarya whose prolongations extend together into the optic cartridge (Boschek, 1970a, b). A fifth cell-body, which is much smaller than the other four lies directly on top of the neuropil of the external plexiform layer over the terminals of R3, R4 and R5.

III. Monopolar Fibres

Serial sections cut horizontally through the lamina of *Musca* reveal that the two axial fibre cross-sections lying within a crown of retinula cell endings (Fig. 11, 12, 19) are derived from the two cell bodies that lie between 5 and 20 μm above the external plexiform layer (Fig. 14). The two smaller fibre cross sections, termed L3 and L4, that lie outside the retinula cell crown near R5 and R6 (Figs. 11, 12, 19) are derived from cell bodies situated together with and above the former pair (Fig. 42). A fifth fibre projects through the lamina beside retinula cell

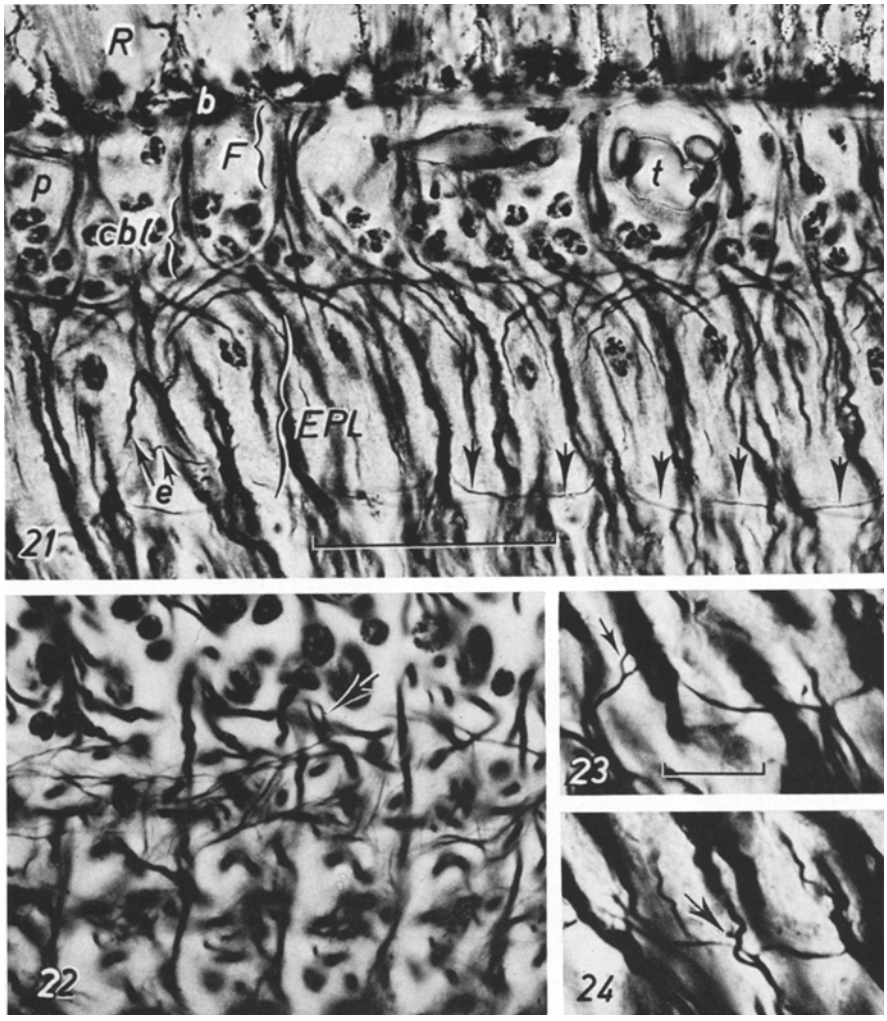


Fig. 21. Vertical section through the lamina of *M. domestica* (Bodian preparation) showing the collaterals of *L4* elements (arrowed). *R* retina, *b* basement membrane, *F* fenestration layer, *cbl* cell body layer (a group of five perikarya (*p*) lies above the extreme left hand cartridges). *t* trachea, *e* "abnormal" fibre pattern (see Appendix)

Fig. 22. *Musca domestica* (tangential section of the upper left hand lamina, Bodian preparation). The plexus of tangential fibres over the outer surface of the lamina's plexiform layer. Note the pair of thin fibres which enters each optic cartridge (arrowed): one is derived from the L5 cell body, the other from tangential processes

Figs. 23 and 24. *Musca domestica* (vertical section of lamina. Bodian preparation): collaterals of *L4*. Note the bifurcation of the collaterals against the L1/L2 components of a cartridge (arrowed) in Fig. 23, and the small side branch from the L4 axis fibre to its parent cartridge (arrowed in Fig. 24). Scale: Figs. 21, 22: 50 μm; Figs. 23, 24: 10 μm

terminals R5 and R6. This element (termed L5; Fig. 38) extends towards the smallest perikaryon which lies on top of the external plexiform layer. Next to L5, and running parallel with it through the outer $^1/_3$ of the lamina, is a sixth element which can be traced back up through the lamina, alongside the fifth cell-body and into the plexus of tangential processes that cover the external plexiform layer's outer face (Fig. 22). This element eventually joins the horizontal processes of the lamina tangential system (see Fig. 75b).

In horizontal sections cut parallel to the z-axis of the lamina these five fibres can be distinctly seen in profile (Figs. 18, 20, 30a, b, 38): they each have a characteristic course through the external plexiform layer (Fig. 14).

In the frontal and lateral parts of the lamina the two fibres surrounded by R1–R6 (L1 and L2) usually have different cross sectional diameters (L2 > L1), which are detectable both in phase contrast and in reduced silver preparations (Table 2). L1 and L2 also have different staining affinities to the reduced silver techniques (Braitenberg, 1967). In Bodian preparations the largest stains black or purple and the thinner reddish or violet. In Bielschowsky preparations the largest stains pale brown, the smallest black; or the largest pale pink and the smaller violet, depending on the variant used. However, electron microscopy shows that in cartridges where these two cross sections differ remarkably in size there is no detectable difference between the two with respect to the granular appearance of their cytoplasm (Boschek, personal communication). It should, however, be pointed out that the L2 fibre is not invariably larger than the L1 fibre. Using electron microscopy techniques, and mapping all L1 and L2 fibres across the lamina's extent, Holschuh (1970) has determined that near the posterior and anterior margins of the lamina the L1 and L2 fibres have the same cross sectional diameters. The difference between the two is maximal at about $^1/_2$ of the distance from the lamina's anterior margin (the frontal quadrant of the upper left hand eye hemisphere includes these maximum differences). In addition, this author has shown that the L3 diameter is (relatively) largest where the differences between L1 and L2 are smallest. In the upper half of the left-hand lamina the two-fibres usually, but not always, lie along the y-axis: the larger (termed L2) lies towards the equator in the upper hemisphere of the lamina and the smaller of the two (L1) lies in the polar position (Fig. 19). There is again a mirror symmetry between upper and lower lamina so that the neighbourhood relationships between R1–R6 and L1 + L2 are the same in both eye hemispheres (Braitenberg, 1970).

In Golgi preparations the axis fibres of radial monopolar cells (Strausfeld, 1970b) have the largest cross-sections whereas the brush and tripartite monopolar cells have thinner axis fibres. The fibres of the midget monopolar cells are the most slender (see Table 2). However, these differences in size do not completely satisfy as convincing correlations with the measurements of L-fibres obtained from reduced silver (Table 2) and phase contrast preparations. The reduced silver stains, especially, are not guaranteed to stain the whole fibre volume (Boycott, Gray, and Guillery, 1961) whereas the Golgi stain may fill the cell (Stell, 1967; Blackstadt, 1970) and possibly may swell it slightly. Nevertheless, there are other features of the reduced silver profiles that can be matched to the various forms of selectively stained monopolar cells.

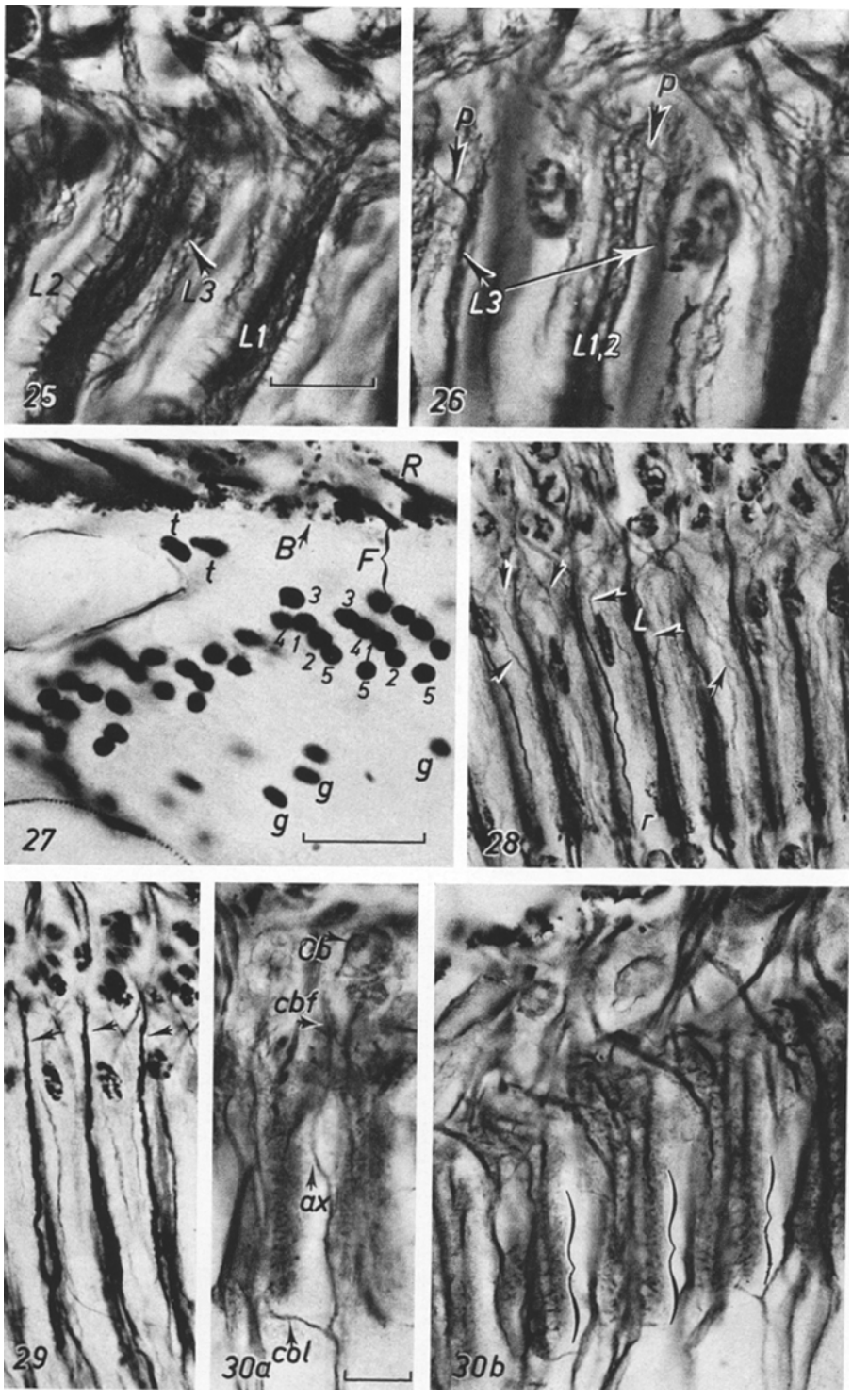
Table 2. *Fibre diameters of monopolar cells (C. erythrocephala) in the frontal quadrant of the left hand eye. Levels I, II, III are indicated in Fig. 42. The fourth set of measurements (IV) was taken just below the inner face of the external plexiform layer. Group 1 is derived from Bodian preparations, group 2 from Golgi preparations. All measurements are in μm (av. = mean diameters, s.d. = standard deviation) 25 cells of each type were measured for each group*

Group 1

	L1			L2			L3			L4			L5		
	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.
I	2.5–1.3	2.0	0.5	2.8–1.5	2.1	0.5	1.5–0.9	1.2	0.3	1.9–0.2	0.7	0.6	0.4–0.2	0.3	0.1
II	2.5–1.2	1.9	0.5	2.8–2.3	2.5	0.2	2.0–1.5	1.7	0.2	1.6–0.5	1.1	0.4	0.5–0.3	0.4	0.1
III	2.5–1.2	2.0	0.5	2.9–2.3	2.6	0.2	1.5–0.9	1.1	0.3	0.6–0.3	0.4	0.1	0.3–0.2	0.2	0.1
IV	2.5–1.2	1.5	0.4	0.2–1.5	1.8	0.2	0.8–05.	0.7	0.1	1.2–0.3	0.7	0.3	0.3–0.2	0.2	0.1

Group 2

	radial stratified diffuse			radial diffuse			brush			tripartite			midget		
	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.
I	2.7–1.5	2.2	0.4	2.7–1.5	2.2	0.4	2.0–0.6	1.2	0.5	1.8–0.4	0.9	0.4	0.8–0.2	07	0.2
II	2.8–1.5	2.1	0.4	3.0–2.3	2.6	0.2	2.8–2.0	2.4	0.3	2.0–0.8	1.5	0.4	0.6–02	0.4	01
III	2.8–1.5	2.2	0.4	32–2.5	2.9	0.2	1.0–0.3	0.8	0.2	1.2–0.3	0.6	0.3	0.5–0.2	0.4	0.1
IV	3.0–1.7	2.5	0.5	3.0–2.0	2.8	0.4	1.5–0.5	1.1	0.3	2.0–0.4	1.4	0.6	0.5–0.2	0.4	0.1


L2
L3
L1
25
P
P
L3
L1,2
26
R
t
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4
1
5
2
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g
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L
r
28
Cb
cbf
ax
30a
col
29
30b

Figs. 25–30

IV. The Match between L-Fibres and Golgi Stained Elements

1. L3 and L4. In sections taken parallel to the lamina's z-axis the L3 fibres seen in reduced silver preparations of *Calliphora* and *Musca* have outlines which are precisely the same as those of brush monopolar cells seen in golgi preparations (cf. Fig. 18, 36). The cell-bodies that give rise to L3 fibres and the perikarya of brush monopolar cells lie at equivalent positions in the cell-body layer.

Each brush monopolar cell axis-fibre gives rise to between 5 and 9 unilateral branches (in *Calliphora* and *Musca*) which lie within the outer $^1/_2$ of the external plexiform layer (Fig. 42). Reduced silver preparations show up the unilateral processes from L3 at an equivalent position in the lamina (Fig. 26). Each branch (between 0.4 and 1.0 μm long) subsequently bifurcates into two processes set at about 80° to one another. Both are arranged anteriorly from the axis fibre and project into the crown of retinula cells between R5 and R6. By virtue of their arrangements in the crown, and their geometrical relationships with R-fibres, it is supposed that the two sets of processes project to retinula cells R1, R2 and R4 (Fig. 42).

Eight out of the forty brush monopolar cells observed in *Calliphora* had an additional branch which extended posteriorly from the axis fibre towards a neighbouring cartridge along the +z axis (Fig. 47). This form variant has also

Fig. 25. Two axial monopolar cell fibres in two adjacent cartridges with rows of lateral processes (see Fig. 31). *L3* has several unilaterally arranged processes which project anteriorly towards the *L1+L2* fibres. (*Calliphora erythrocephala*: Holmes Blest preparation)

Fig. 26. The same preparation as above showing the unilateral processes (*p*) from *L3* extending towards the *L1/L2* fibres

Fig. 27. *Calliphora phaenicia* (Nissl stain). Columns of five monopolar cell nuclei above optic cartridges in the anterior margin of the lamina. Nuclei of epithelial cells (*g*) are arranged between cartridges. Nuclei situated in the fenestration layer (*F*) belong to tracheoblasts (*t*) and supporting cells. *B* basement membrane, *R* retina

Fig. 28. *C. erythrocephala* (Holmes-Blest preparation). Horizontal section of the upper left hand lamina: the axial monopolar cell fibres (*L*) can be seen as darkly stained pairs: the retinula cells appear grey, stippled black at their inner tips (*r*). This terminal difference in argyrophilia can be seen in most reduced silver stained preparations. In Golgi preparations the unimpregnated retinula cells appear deep yellow at this location. This curious staining property may indicate a special portion of the receptor ending whose physiological properties are somewhat different to those elsewhere in the plexiform layer. The fine fibres that appear to climb up the outer margin of each retinula cell (arrowed) are reminiscent of the ascendent processes of T1 and T1a basket endings (c.f. Fig. 61)

Fig. 29. The synperiodic arrangement of pairs of long visual fibres (*R7* and *R8*). Each pair (arrowed) projects into the 1st optic chiasma and terminates in the medulla (*C. erythrocephala*; Bodian preparation)

Fig. 30a. *Calliphora phaenicia* [Bielschowsky preparation (ammoniacal silver carbonate)]. Section through the lamina, cut parallel to the *y* axis (posterior to the right) showing the reduced silver profile of an *L4* neuron: *cb* cell body; *cbf* cell-body fibre, *ax* axis-fibre through the external plexiform layer, *col* collateral to adjacent cartridge along the *x* axis b *C. phaenicia* (Bielschowsky preparation). The lateral processes from *L1* cells (bracketed) to retinula cells. Scale: Figs. 25, 26: 10 μm; Figs. 27–29: 20 μm; Fig. 30a and b: 8 μm

been seen in two preparations of *Musca* (Fig. 75a). In the two species of Syrphidae all bar three of the 32 impregnated brush monopolar cell were observed to have bilateral arrangements of processes (Fig. 43) where the anterior set projected to the parent retinula cell crown and the other projected posteriorly towards the adjacent optic cartridge. It is worth remarking that as well as being a possible basis for intercartridge interaction (for example it could obtain an input from two sets of receptors "looking at" two neighbouring points in the visual field) this neuron and a second form of tangential cell (Strausfeld, unpublished observations) represent the main interspecific differences of lamina structure between species of Syrphidae and species of Calliphorinae and Muscidae.

In reduced silver preparations the L4 fibres can be resolved close to and practically parallel with L3 fibres (Fig. 18). Both the L3 and L4 cell bodies are the most peripherally placed of the five monopolar cell perikarya and lie just beneath the fenestration layer of the lamina (Figs. 30a). Their cell-body fibres project together to the outermost part of the crown of retinula cells (Fig. 13). Their subsequent prolongations (axis-fibres) take up characteristic positions within the crown, between R5 and R6, at the outermost margin of the plexiform layer (Fig. 16). They subsequently project the rest of the way through the lamina outside the crown alongside R5 + R6. These findings are summarized in Fig. 19.

Each L4 fibre gives rise to 1–4 branches into its parent cartridge at, or just below, the inner face of the lamina's plexiform layer (Fig. 24). Just beneath this level two vertically oriented collaterals extend bilaterally from each L4 fibre to the neighbouring cartridges, above and below, along the positive y- and negative x-axes (Figs. 23, 24). These collaterals (Fig. 21) form a characteristic network (Fig. 40) under the whole of the inner face of the external plexiform layer (Braitenberg, 1969; Strausfeld and Braitenberg, 1970). Each collateral extends to L1 and L2 fibres at the base of a retinula cell crown (Figs. 23, 30a).

The L4 profiles can only be matched with the Golgi stained tripartite cells (Figs. 33, 34, 42, 43, 47). These have the same lengths and orientations of processes and collaterals at the inner margin of the external plexiform layer; in addition, each cell has a peripheral set of processes just beneath its outer margin. These have not been resolved by reduced silver methods. The outer processes project forwards into the retinula cell crown of the parent cartridge at the same level as the branches of L3 (Fig. 42, 43, 47).

2. L1 and L2. Axial monopolar cell fibres, L1 and L2, are surrounded by the retinula cell terminals of a cartridge. Each of these fibres gives rise to short lateral processes throughout the depth of the external plexiform layer (Fig. 25, 30b): in the front part of the lamina one of the pair has particularly wide side branches situated at the outer surface of this layer. Frontal sections of the upper half of the left hand lamina (*Calliphora*) show these branches to be oriented along the y-axis (Fig. 17).

The L1 and L2 profiles (schematized in Fig. 14) match forms of selectively impregnated neurons; namely, those elements characterised by a) large cell-bodies situated above the external plexiform layer at a position proximal to the perikarya of L3 and L4 and, b) by thick axis fibres which give rise to radial arrangements of lateral processes through the remaining depth of the lamina

(Figs. 31, 32, 33). 40% of these neurons were observed to have bilaterally arranged processes at the outer margin of the external plexiform layer.

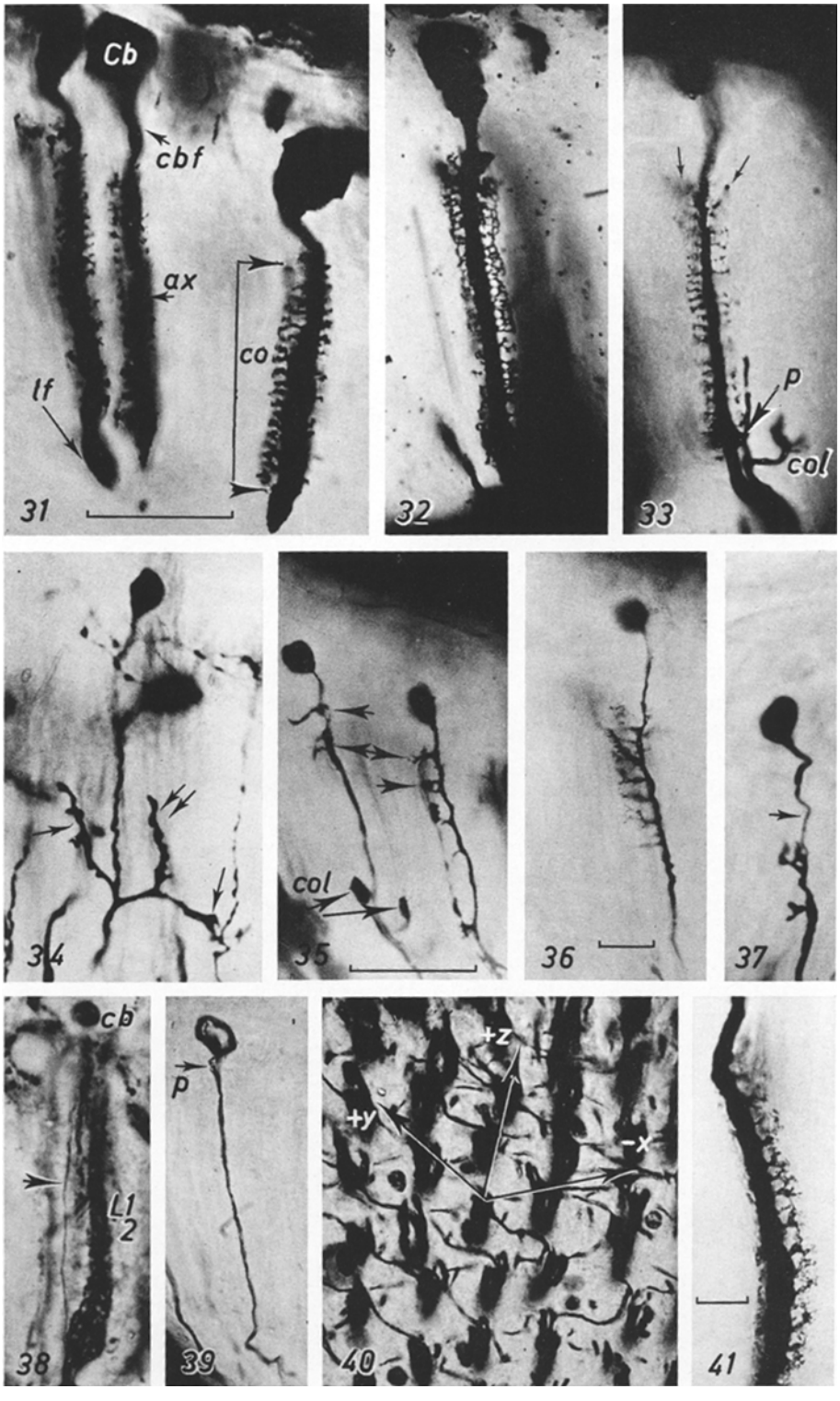
At first sight these elements appear to fall under a single category of neuron: they all lie within retinula cell crowns and their branches radiate towards the six retinula cells of their respective crown. However, a more detailed analysis of the differences between similarities of single neurons allows one to distinguish two different cell types; these have been christened "radial diffuse" and "radial stratified diffuse" monopolar cells. Features of these neurons which are particularly amenable to quantitative analysis (in the frontal part of the eye) are summarized in Table 3.

A) The Radial Diffuse Monopolar Cell. 1. Each neuron has between 170 and 155 lateral processes within the external plexiform layer (average of 2,8 processes per 1 μm depth lamina). These have a lateral spread equal to, or slightly greater than, the internal diameter of a retinula cell crown (Table 4). 2. The lengths of the processes at the outer margin of the external plexiform layer are equal to or slightly less than those within this layer. 3. Processes are irregularly arranged from around the axis fibre: their lengths and disposition allow them to contact the inwardly facing surfaces of retinula cell terminals (Fig. 43). 4. Cells having the above features (1–3) usually have short cell body fibres, up to 8 μm in length, and thick axis fibres (between 2.0 and 3.2 μm diameter).

B) The Stratified Radial Diffuse Monopolar Cell. 1. Each cell has between 108 and 126 lateral processes within the external plexiform layer (average of 2.0 per 1 μm depth of lamina) and their total lateral extent is equal to, or slightly less than, the outer diameter of a retinula cell crown (Table 4). 2. Processes at the outer surface of the external plexiform layer are longer than those within it: usually the outer processes are arranged bilaterally from the axis fibre and extend along the y-axis of the lamina (Fig. 51b). 3. The shorter processes within the external plexiform are arranged in six rows (or combs) from down the length of the fibre; each comb of processes extends between a pair of retinula cell terminals (hence the radial symmetry) and each processes bifurcates in such a way as to contact the facing and outwardly directed surfaces of the receptor terminals (Figs. 41, 43). 4. Monopolar cells with the above features (1–3) usually have cell-body fibres up to 14 μm in length and axis fibre diameters which range from between 1.5 to 2.7 μm.

It has already been remarked that these two cell types can only be clearly identified in the frontal part of the lamina where curvature is minimal. In other quadrants of this region where lamina curvature is greater (especially extreme dorsally and ventrally, as well as posteriorly) the cartridges are correspondingly very long (up to 58 μm in *Musca* and 60 μm in *Calliphora*), very narrow and are closely packed together. The retinula cell terminals are thinner than in the frontal part of the lamina and L1 + L2 fibres may have the same diameter (Holschuh, 1970).

In the lateral part of the lamina both radial diffuse and radial stratified diffuse monopolars can be separately identified; however, in the same zone there are cells (here termed "hybrid" neurons) which share features of both the above mentioned forms (Fig. 44). But posteriorly the radial monopolar cells are very elongated and it is impossible to distinguish two different forms. Rather,



Figs. 31–41

all cells have regularly arranged processes, disposed radially (and more or less symmetrically) down the length of the axis fibre. The number of processes per 1 μm depth of lamina varies between 2.4 and 3.5 and some cells have an especially dense arrangement of processes at the outer face of the external plexiform layer. However, none of them have the wide processes characteristic of the radial stratified diffuse neurons. Notwithstanding these variations across the lamina there are clearly two levels at which radial monopolar cells terminate in the medulla. The two forms of endings from the radial diffuse and radial stratified diffuse monopolars in the frontal part of the lamina (Fig. 46) look no different from the forms of radial monopolar cell endings that are derived from cells further laterally and posteriorly. Even if L1 + L2 relay the same kind of information from a single cartridge the two neurons must interact with second and third order interneurons at two discrete levels in a medullary column.

Fig. 31. *Musca domestica* (Golgi Colonnier impregnation). Three radial diffuse monopolar cell components (*L2*) in the lamina. *Cb* cell-body, *cbf* cell-body fibre, *ax* axis-fibre, *lf* linking-fibre to the medulla (here cut off near the inner face of the lamina)

Fig. 32. *Calliphora phaenicia* (Golgi-Colonnier preparation). The lamina component of a radial diffuse monopolar cell

Fig. 33. *C. phaenicia* (Golgi-Colonnier preparation). A bistratified radial diffuse monopolar neuron (*L1*) in the lamina. Note the number of lateral processes of a comb, compared with that of the diffuse radial monopolar cell in Fig. 32. The wider outer processes (arrowed) are slightly out of focus in this figure since the section has been cut parallel to the *z*-axis. Note also the small branch (*p*) from the tripartite monopolar cell (*L4*) of the same optic cartridge extending to the radial monopolar cell, and one of its two collaterals (*col*) to a neighbouring cartridge along the *x* or *y*-axis (the outer part of L4 is out of section)

Fig. 34. *Eristalis tenax* (Golgi-Colonnier preparation). A vertical section through the lamina showing the tripartite monopolar cell (*L4*). Single arrows indicate its collaterals to adjacent cartridges, double arrows indicate process to its parent cartridge. The fine, blebbed fibres to the right, and across the upper $^1/_3$ of the picture, are derived from the type 2 lamina tangential cell

Fig. 35. *Musca domestica* (Golgi-Colonnier preparation). A horizontal section through the lamina showing two L4 cells. Their processes in the outer $^1/_3$ of the lamina are indicated by arrows. *col* The vertically oriented collaterals, seen "end-on"

Fig. 36. *Musca domestica* (Golgi-Colonnier preparation). The lamina component of the brush monopolar cell

Fig. 37. *Calliphora vomitoria*. The outermost processes of the tripartite monopolar cell. Note the extremely thin cell-body fibre (arrowed) and the small oval perikaryon

Fig. 38. The L5 fibre (arrowed) and cell-body (*cb*) in the lamina of *M. domestica* (Holmes preparation: cut parallel to y axis; posterior to the left). The axial monopolar cells (*L1, 2*) are shown up as a pair of darkly stained fibres

Fig. 39. The midget monopolar cell (Golgi Colonnier preparation of *Musca domestica*). Note the single unilateral process (*p*) at the outer surface of the lamina and the extremely thin axis-fibre

Fig. 40. The network of the *L4* collaterals (tripartite monopolar cell collaterals) under the inner face of the external plexiform layer. (*M. domestica*, Bodian preparation, by courtesy of Dr. V. Braitenberg, Tübingen)

Fig. 41. The lateral processes of a bistratified radial diffuse monopolar cell in the lamina of *Eristalis tenax* (Golgi-Colonnier preparation): note their characteristic hand-shaped bifurcations. Scale: Figs. 31–33, 38, 39: 25 μm; Figs. 34, 35: 25 μm; Figs. 36, 37, 40: 10 μm; Fig. 41: 5 μm

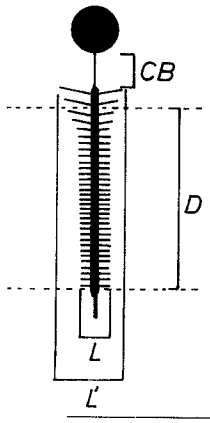


Table 3. *Comparison between radial diffuse (A) and radial stratified diffuse monopolar cells (B). 200 cells were measured. Cells were categorised as either A or B according to two parameters: neurons with cell body fibres (CB) less than 10 μm long and outer processes (L') with lateral spreads less than 8,0 μm in extent were classified as A, the other elements were classified as B.*

L = lateral spreads of processes within the external plexiform layer. P = number of processes. D = depth of lamina (external plexiform layer) (av. = mean value, s.d. = standard deviation) all measurements in μm.

A

L'			L			(L'-L)	
max.-min.	av.	s.d.	max.-min.	av.	s.d.	av.	s.d.
7.61–6.80	7.66	0.34	7.41–6.01	6.86	0.52	0.55	0.29

CB		P			D		P/D		
av.	s.d.	max.-min.	av.	s.d.	av.	s.d.	max.-min.	av.	s.d.
8.57	0.45	168–156	163.2	5.01	57.5	0.81	2.90–2.73	2.84	0.16

B

L'			L			(L'-L)	
max.-min.	av.	s.d.	max.-min.	av.	s.d.	av.	s.d.
14.01–12.30	13.14	0.60	9.40–8.30	8.78	0.45	4.11	0.55

CB		P			D		P/D		
av.	s.d.	max.-min.	av.	s.d.	av.	s.d.	max.-min.	av.	s.d.
15.09	0.55	126–108	117.6	6.80	57.5	0.81	2.21–1.86	2.05	0.14

The Student *t*-test was used to test the hypothesis that A and B cells also differed with respect to i) their lateral spreads in the external plexiform layer (L), ii) the differences between the lateral spreads at the external plexiform layer's outer surface and within this layer (L'-L) and iii) the numbers of processes through the lamina (P). L, (L'-L) and P/D values of 80 A cells and 120 B cells were compared: the differences were found to be highly significant ($p < 0{,}05$, $p < 0{,}001$ and $p < 0{,}001$, respectively).

3. L5. Up to this point we have correlated four out of the five cell-bodies and their prolongations, seen in reduced silver preparations, with four of the five types of monopolar cells resoluble in Golgi preparations. There is, however, one cell-body and a fibre (L5) that has not yet been matched with a selectively impregnated cell (the midget monopolar). It is proposed that the one does match the other and that there are five monopolar cells per cartridge.

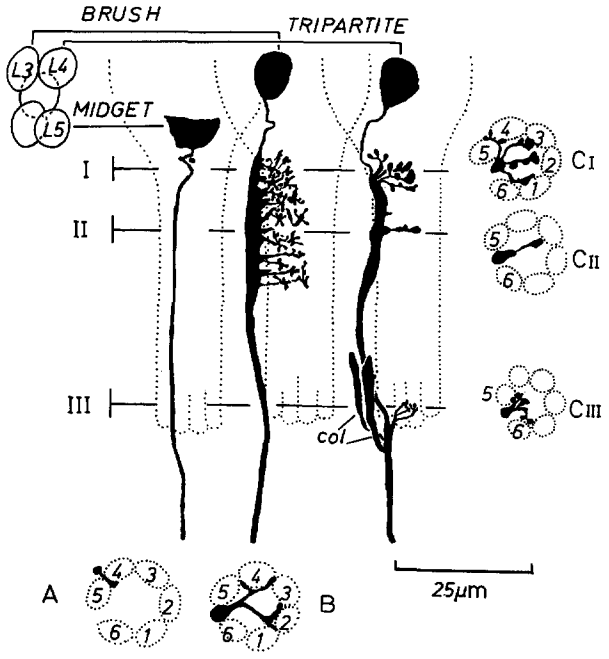


Fig. 42. Golgi impregnated midget, brush and tripartite monopolar cells, as seen in horizontal sections of *Musca domestica* (posterior to the left). Each has been drawn against the outline of a retinula cell crown. The levels at which their perikarya lie in the cell-body layer correspond to three of the five perikarya seen above each optic cartridge in reduced silver and Nissl stained preparations (*L3*, *L4* and *L5*). At level *I* in the lamina the midget monopolar cell has between one and four unilateral process from its axis fibre: inset *A* shows the insertion of one process between *R4* and *R5*. The brush monopolar cell has two sets of unilateral processes which project into the cartridge (inset *B*) and extend to R2 and R4 (or R1 and R4). The outer processes of L4 vary in number (see text page 408). They may have as fe was one process posteriorly or as many as ten processes anteriorly whose subsequent bifurcations reach to all of the retinula cell terminals. The cell illustrated here was seen in the lateral portion of the lamina: it has a group of processes at level *I* which reach to all six R terminals (inset *CI*) and a single unilateral process at level *II* which reaches to R3 (inset *CII*). Tripartite monopolar cells usually have two vertically oriented collaterals (*col*) at the base of the lamina (see text p. 396) and between one and eight short lateral processes which extend into the retinula cell crown. These contact at least one of the two axial monopolar cell fibres (*L1* and *L2*: see inset *CIII*)

The L5 fibre can rarely be followed from a cell body: usually it can be resolved through the external plexiform layer and into the first optic chiasma from a point between 2 and 5 μm beneath the fifth cell-body that lies just above the external plexiform layer's outer surface (Fig. 38). The pathway of this fibre is either directed straight through the lamina or shows a slight bend which is biased either postero-dorsally, in the upper half of the lamina, or postero-ventrally in the lower half. L5 fibres are situated outside retinula cell crowns beside R4 and R5 retinula cell terminals. Each optic cartridge contains one L5 element. In four preparations of *Musca* and two of *Calliphora* this fibre

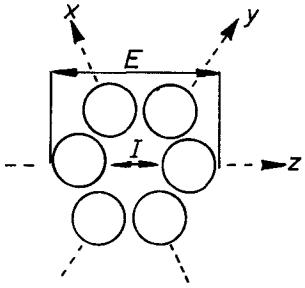
Table 4. *Mean diameters of retinula cell crowns and the lateral spreads of some lamina elements (C. erythrocephala)*

i) Mean internal diameters (I) of retinula cell crowns as measured along the x, y and z axes (all measurements in μm)

x axis	y axis	z axis	
4.5	5.5	5.0	100 cartridges sampled

ii) Mean external diameters (E) of retinula cell crowns

x axis	y axis	z axis	
7.5	9.0	7.5	100 cartridges sampled



iii) Mean lateral spreads of basket endings (T1 and T1 a) and four types of monopolar cells

cell type	y axis	z axis	o cells measured
T1, T1 a	8.5	7.8	29
Radial stratified diffuse monopolar cell	8.8	7.9	45
Radial diffuse monopolar cell	6.6	6.1	52
Brush monopolar cell	4.2	5.7	38
Tripartite monopolar cell	4.3	6.0	30

i) and ii) from Bodian preparations, iii) from Golgi preparations.

has been traced up to, and continuous with, the smallest perikaryon that lies above the terminals of R3, R4 and R5.

In Golgi preparations the midget monopolar cell is characterized by the following features. a) Its cell-body sits, so to speak, on the external plexiform layer's outer surface at a position equivalent to the centralmost of the five perikarya resoluble in reduced silver preparations, i.e. that of L5 (Fig. 42). b) The axis-fibre of the midget monopolar cell either projects straight down through the lamina into the first optic chiasma or is slightly curved posteriorly and c) in *Musca* and *Calliphora* the axis fibre gives rise to a single group of between 1 and 4 unilateral processes at the outer face of the plexiform layer (Fig. 39). In the other species of Diptera there is also a second group of unilateral processes from the axis fibre at, or just beneath, the inner face of the lamina.

Table 5. *Fibre diameters of monopolar cells (in μm) and long visual fibres in the first optic chiasma (measured between 20 and 30 μm above medulla's surface from Golgi impregnated cells)*
A = fibres from anteriormost cartridges to posteriormost medullary columns. B = fibres between lateral cartridges and medullary columns. C = fibres from posteriormost cartridges to anteriormost medullary columns.

Cell type	A			B			C			No. cells measured per group
	Mean length 155 μm			Mean length 90 μm			Mean length 220 μm			
	max.-min.	av.	s.d.	max.-min.	av.	s.d.	max.-min.	av.	s.d.	
L1	3.0–2.0	2.7	0.3	2.8–2.0	2.4	0.3	3.0–2.2	2.7	0.3	24
L2	3.2–2.3	2.8	0.3	2.8–1.9	2.5	0.3	3.1–2.3	2.7	0.3	26
L3	3.1–1.7	2.6	0.5	2.7–1.5	2.0	0.5	2.8–1.7	2.3	0.4	24
L4	1.2–0.8	1.1	0.4	1.5–0.3	0.9	0.4	1.5–0.7	1.1	0.5	25
L5	1.0–0.4	0.7	0.2	1.3–0.3	0.8	0.3	1.3–0.6	1.0	0.3	25
R7+ R8	1.5–0.8	0.9	0.3	1.2–0.4	0.9	0.3	1.5–0.6	1.0	0.3	25

The midget monopolar cell can be impregnated in any quadrant of the lamina: like the other types of monopolar cells it is not restricted to a particular portion of the lamina's horizontal or vertical extent.

Golgi preparations also show up this cell with other neural elements of the same optic cartridge. In these examples, one of which is illustrated in Fig. 75a, the axis-fibre of the midget monopolar cell always lies polar to the L1 fibre, separated from it by a distance not greater than 5 μm (in *Musca*). L3 (brush monopolar) and L4 (tripartite monopolar) are usually separated from L1 by distances exceeding 5 μm, except at the periphery of an optic cartridge. The axis-fibre of the midget monopolar cell is kinked at the periphery of a cartridge so that it approaches the L1 element: its unilateral processes are derived from the axis fibre at the kink and project between the R4 + R5 retinula cell terminals. Cross sectional maps of neurons, derived from vertical or horizontal sections of Golgi impregnated material (see page 415) indicate that the axis fibre of the midget monopolar cell is located at the same position in a cartridge as the L5 fibre seen in reduced silver preparations (see Fig. 75). Very occasional it is possible to resolve one or two minute lateral branches from the L5 fibre in reduced silver stained material which project towards the L1–L2 elements: this observation and those described above are considered valid grounds for assuming that the L5 profile is the reduced silver "Gestalt" of the midget monopolar cell.

In conclusion, there are five monopolar cells per cartridge (see Discussion) two of which lie within the crown of retinula cell endings (L1, L2) and three outside it (L3, L4, L5). The polar (and the thinner) of the two radial monopolar cells (L1) usually has wider lateral processes, oriented along the y-axis, at the interface between the plexiform layer and the cell body layer of the lamina (Fig. 17). Radial monopolar cells can have two quite distinct patterns of side branch distributions (and lengths) arranged from their axis-fibres (as in Fig. 43) or may have variations of side branch arrangements, such as the "hybrid" forms shown in Fig. 44.

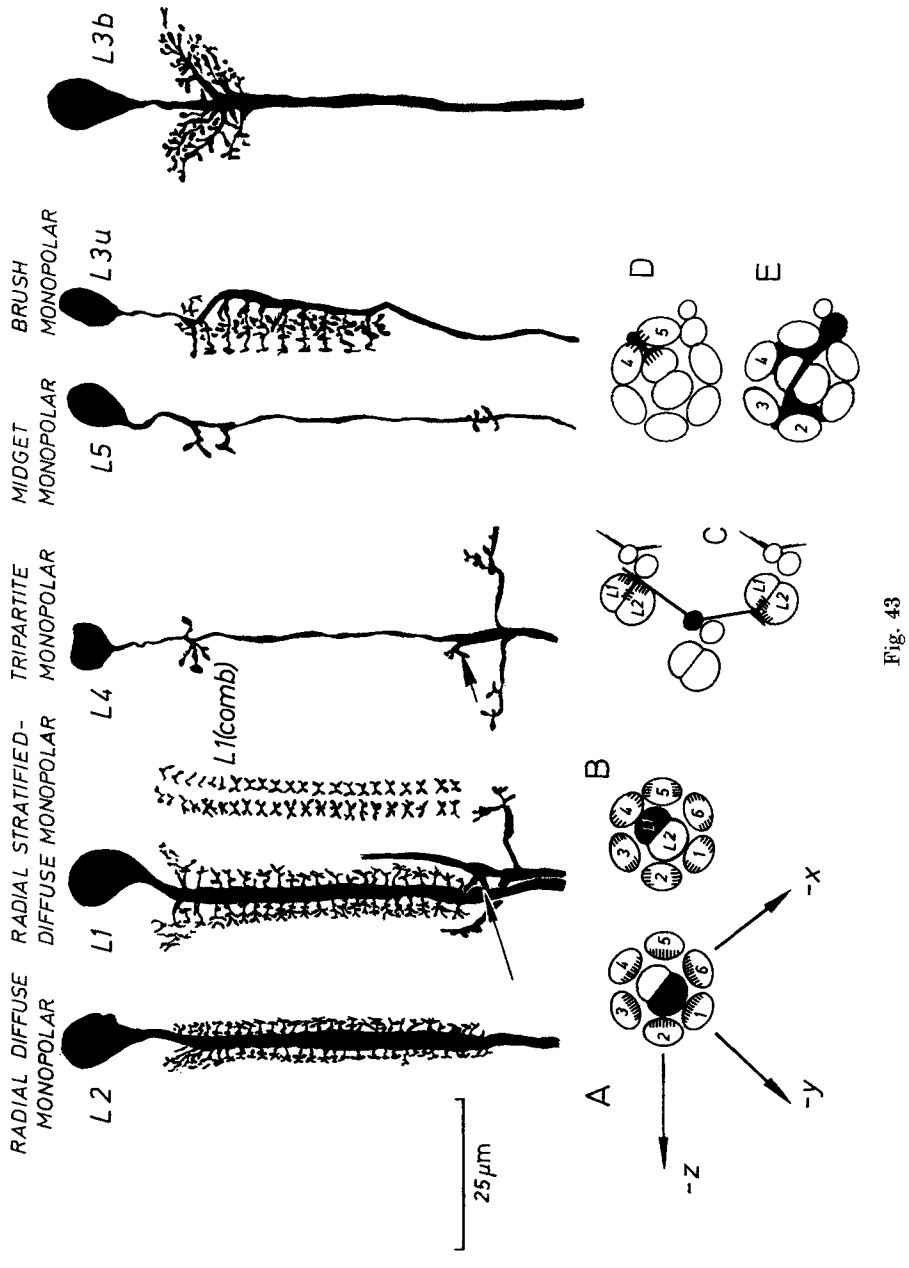


Fig. 43

Fig. 43. Monopolar cells. The monopolar cells of optic cartridges, as seen in Golgi preparations of *C. phaenicia* (*L1 L2, L3u*) and *S. elegans* (*L3b, L4, L5*). These elements are fully described in the text. Note the combs of processes of *L1*; two combs are shown as if viewed from above when the cell is lying horizontally in the field of view. This arrangement allows *L1* processes to interdigitate between the *R* terminals. They could contact the outer and facing surfaces of R1–R6 (shaded in *B*). *L2's* processes could contact the inwardly facing

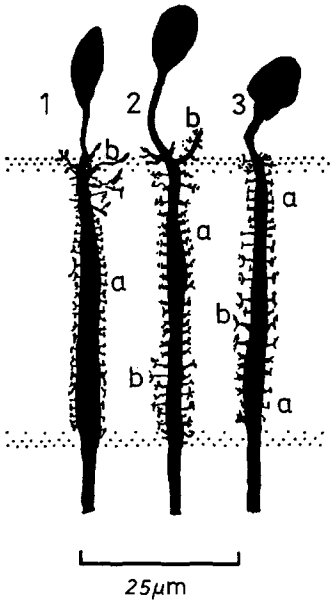


Fig. 44. Monopolar cells. Three "hybrid" monopolar cells (camera lucida drawings from *C. erythrocephala*) which share features of both the radial bistratified diffuse and stratified diffuse neurons (L1 + L2). Cell *1* has wide outer processes of the L1 type (*b*) and short processes (*a*) down its length, like L2. Cell *2* has outer and inner processes which are reminiscent of *L1* while its middle processes (*a*) are like those of L2 cells. Cell *3* has the reverse arrangement: the outer and inner processes are like those of L2 cells, the middle processes like those of L1

C. Lamina Elements Derived from the Medulla

Four forms of terminal elements, in addition to R1–R6, have been identified in the laminae of all the present species. These are derived from four types of neurons whose processes initially invade the medulla neuropil (Fig. 46). A fifth form of these elements has been tentatively identified in a preparation of *Eristalis tenax* (Fig. 45 b).

a) The T1 and T1a Neurons. The lamina components of the deep T1a and shallow T1 cells (Fig. 46) are almost identical. They consist of a "basket"

surfaces of R1–R6 (shaded in *A*). The unilateral brush monopolar cells (*L3u*) of *Calliphora* and *Musca* have two sets of processes which extend towards three of the six retinula cell terminals (shaded in *E*). Bilateral brush monopolars (*L3b*) in Syrphidae may contact receptor terminals in more than one cartridge. The *L4* neuron illustrated here has four outer processes at the inner margin of the external plexiform layer which extend to the "parent" retinula cell crown. The two collaterals extend to the *L1* and *L2* axis fibres of cartridges adjacent along the $+y$ and $-x$ axis. Their terminal branching patterns are not symmetrical (diagram *C*). In the Syrphidae *L5* elements have two sets of lateral processes disposed at the inner and outer margins of the external plexiform layer. The outer processes extend into the retinula cell crown between R5 and R4; possibly they contact two receptor terminals and the *L1* axis fibre (*D*)

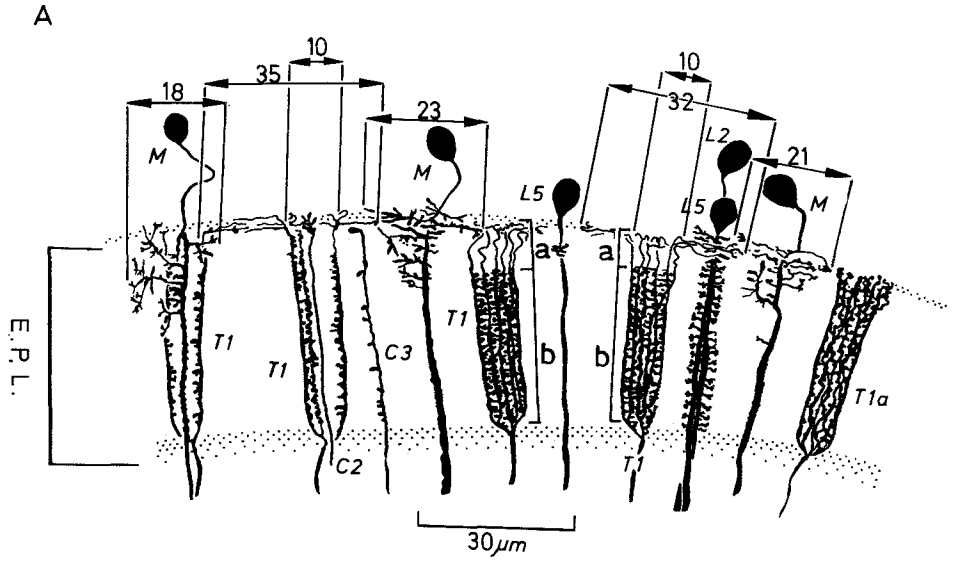


Fig. 45a. A camera-lucida drawing of the lamina, from a pupal *Calliphora vomitoria* (vertical section) fixed and stained about 24 hours pre-eclosion. Some monopolar cells (*M*) look extremely unusual, having wide outer processes whose lateral spreads vary between 18 and 23 μm (dimensions indicated between arrows) rather than 10–15 μm as in normal adult flies. The *L5* cell and a radial diffuse monopolar cell (*L2*) look no different from equivalent elements seen in adult animals. *T1* cells have outer processes whose lateral extents reach 35 μm (as opposed to 12 μm in normal adult flies) whereas the *C2* and *C3* terminals have shapes which are identical to those seen in the adult animal. Possibly this preparation shows up aberrant monopolar and T-cell forms. However, it is also possible that even in late pupae some neurons may have shapes different from those in the adult: the cells labelled *M* in this illustration could perhaps represent *L1* cells in a developmental stage where the combs of processes through the external plexiform layer (*EPL*) are not yet developed. But even if this were the case the outer processes are up to three times longer than would be expected from observations of adult *L1* neurons. Note also the lack of lateral processes from the ascendent fibres of *T1* endings at level *a*; this, and level *b*, correspond to the two main strata of the adult lamina (see Fig. 75b). b A fourth form of centrifugal terminal seen in *Eristalis tenax*. The distribution of branches is reminiscent of the L4 monopolar cell pattern

of ascendent processes whose diameter is practically the same as the external diameter of a retinula cell crown (Table 4). The only differences between the two T-endings which are detectable by light microscopy are as follows: i) the T1a endings is derived from an axis-fibre whose width is approximately half that of the T1 fibre (0,4 μm) and ii) the T1a "basket" processes appear somewhat thinner and less varicose than those of the T1 cells. Also the T1a processes

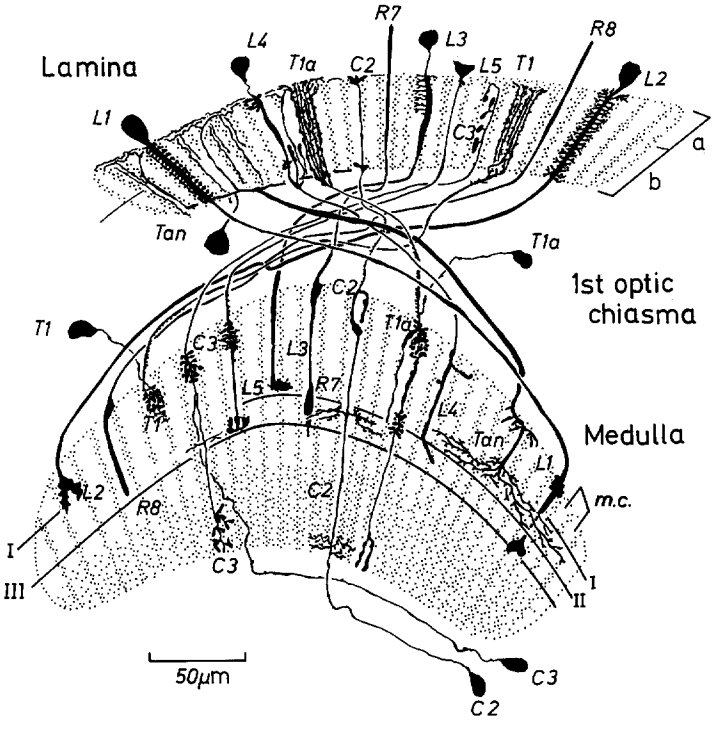


Fig. 46. Neurons shared by the lamina and medulla (*Musca domestica*). *C2* and *C3* endings in the lamina originate from perikarya situated beneath the inner face of the medulla. Their processes in the medulla are disposed at three levels; *a* in the deepest medullary stratum, *b* at the same level as the terminal swelling of *L1*, and *c* at the level of the outer swelling of *L1* and *R8* endings. *T1* and *T1a* components in the lamina look very similar to each other but their medullary components are distinctive. *T1* cell bodies, situated above the medulla's outer surface, give rise to shallow components at the same level as the *L2* terminal. *T1a* components in the medulla are more complex: in addition to processes at the medulla's surface each gives rise to a short descendent collateral which extends as far as the endings of *R8*. The descendent axis fibre of *T1a* gives rise to a group of lateral branches disposed at the same level as the inner swellings of *L1* and *L5* endings and finally terminates at the same level as the innermost arborizations of *C2* and *C3*. Each lamina tangential cell (*Tan*) has several collaterals to the medulla from the lamina (only one of which is illustrated). They end as bistratified components: a group of fibres at the medulla surface extends bilaterally through between 2 and 3 medullary columns (*m.c*=column). A second system of branches, situated at the level of *L3* terminals, spreads through an oval field whose horizontal extent is equivalent to about 14 medullary columns. Its vertical extent may reach through 20 columns. The linking-fibres of all lamina-medulla cells cross over via the first optic chiasma (see part 2). The initial components of the monopolar cells and the terminals of centrifugal cells are fully described in the text and plate figure legends (see Figs. 31–41 and 54, 62). Note the two main strata in the lamina (*a* and *b*) defined by *L3* and tangential processes. *I*, *II* and *III* denote, respectively, the levels of radial diffuse monopolar cell endings (*L2*), and *L1*, *L3*, *L4* and *L5* and long visual fibre terminals (*R7* and *R8*)

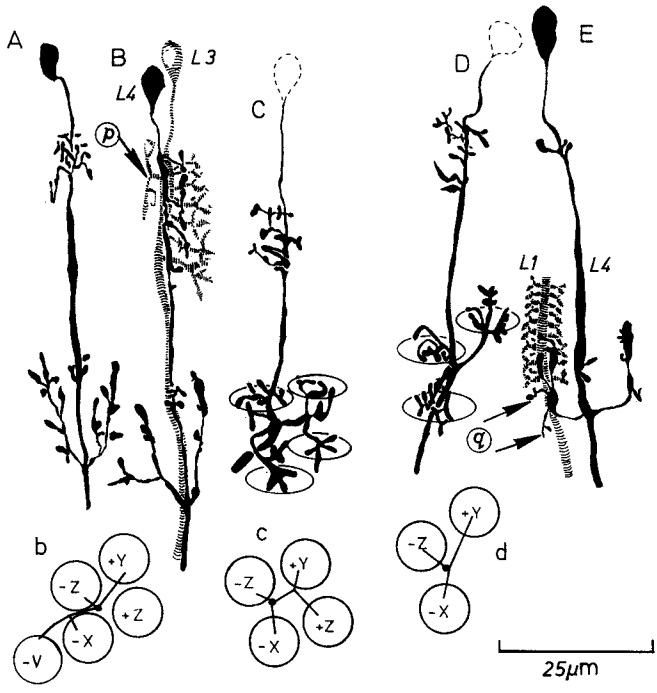
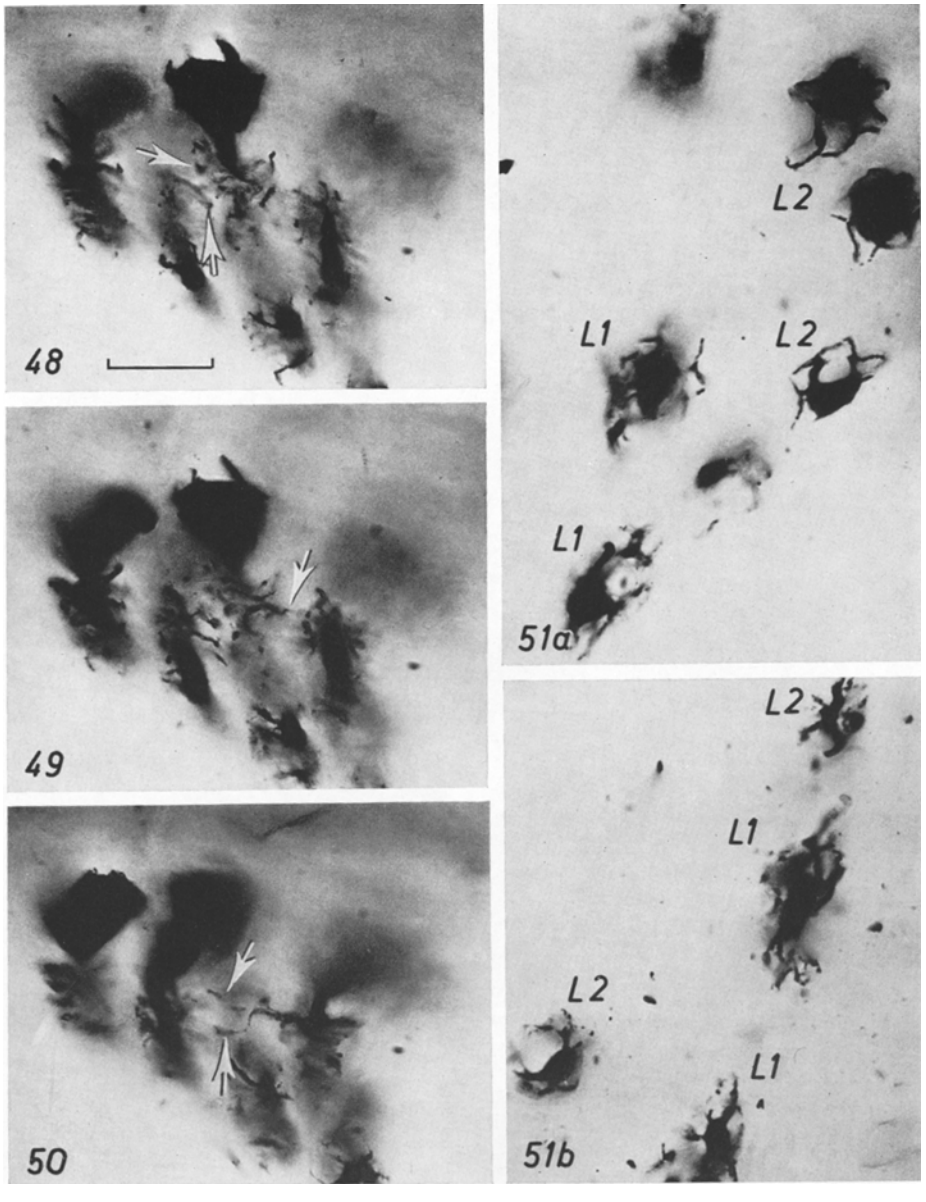
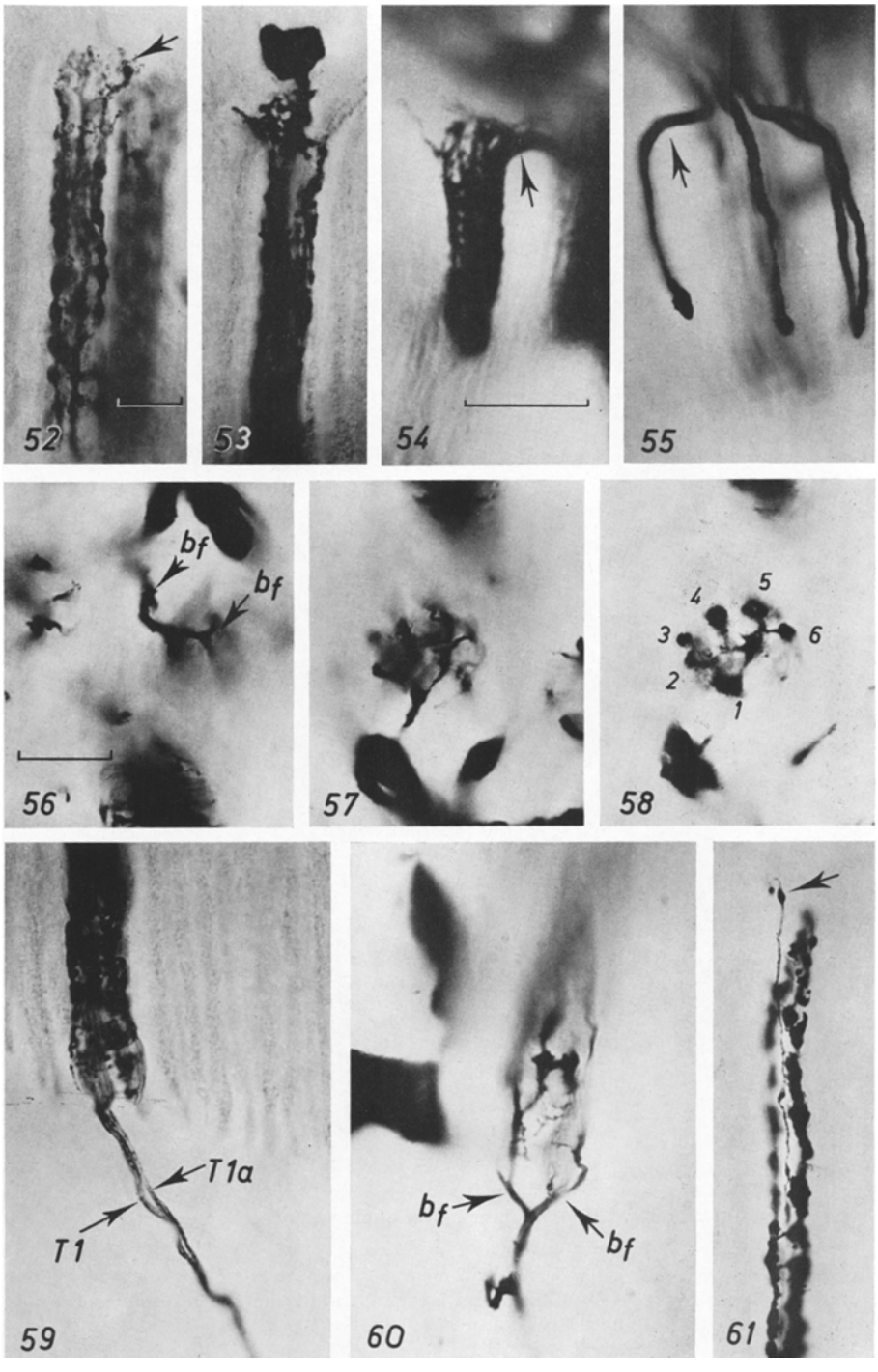


Fig. 47. Forms of tripartite cells (*C. erythrophala*, upper left hand laminae: Camera lucida drawings of neurons impregnated by the Golgi Colonnier procedure). The majority of tripartite monopolar cells (*L4*) have only two collaterals beneath the inner face of the lamina. Cell *D* illustrates their disposition from the axis fibre. This element has its three groups of inner processes (ringed) situated in the same tangential plane. Diagram d illustrates the typical tripartite arrangement where one *L4* cell links a constellation of three optic cartridges along the $+y$, $-z$ and $-x$ axes. However, exceptions to this pattern have been found (cells *B* and *C*) where *L4* neurons link constellations of four optic cartridges. In the case of *B* a fourth collateral extends to an adjacent cartridge along the "v" axis (the axis set at right angles to z: diagram b) and in the case of cell *C* two collaterals extend along the x and y axes while two others extend along the positive and negative z axes (diagram c: cell seen in white eyed mutant). The number and disposition of processes and the shapes of the *L4* collaterals vary considerably. The arrangement of the outer processes can be radial (as in *A*), unilateral (*B*) or bilateral (*C*, *D* and *E*). Possibly these differences may indicate various modes of synaptic contact between *L4* cells and the retinula cell crowns. It is also possible that the pairs of collaterals do not establish the same configurations of synaptic contact above and below their cartridge of origin. For instance cell *E* has one collateral which extends to an *L1* element in the $-x$ adjacent cartridge. Two collateral branches (arrowed q) probably extend to an L2 element (here is not impregnated). Note the difference between the terminal branches of the $-x$ and $+y$ collaterals. Variations of branching pattern are not restricted to *L4* elements: the *L3* cell stained with *B* has a single posteriorly directed branch as well as the unilaterally arranged processes directed anteriorly to its parent cartridge



Figs. 48–50. Three optical sections through a group of bistratified radial diffuse monopolar cells (*L1*) in the frontal lamina. Overlapping branches are arrowed

Fig. 51a and b. Cross sections of *L1* and *L2* neurons. Note the difference of spread between the two cell types in the external plexiform layer (a) and the marked differences at its outer face (b). Scales: Figs. 48–51b: 10 μm



Figs. 52–61

have tiny knob-like projections which point outwards away from the basket: these have not been seen on T1 processes.

b) The C2 and C3 Centrifugal Cells. Two forms of elements, derived from perikarya beneath the inner face of the medulla, send processes through all the medullary strata and finally prolongate as linking-fibres to the lamina, via the first optic chiasma, where they terminate as narrow field endings (Fig. 46). The terminal of C2 consists of a bristratified "capped" component (Fig. 67) whose processes lie at the same levels as those of L4 and L5. The ending of C3 consists of a "climbing" component (Fig. 62) composed of a single axis-fibre from which are derived a row of unilaterally arranged knob-like projections.

The Arrangement of Medulla-to-lamina Neurons in the Lamina

None of the four cell types mentioned in the preceding section can be convincingly identified in reduced silver preparations. However, some Holmes-Blest preparations show up a reticulum of fine branches at the base of, or around, each crown of receptor endings that is reminiscent of the patterns of processes formed by Golgi stained basket endings of T1 and T1a cells (compare Fig. 28, 52). Holmes-Blest and Bodian preparations also reveal some fibre cross sections at the base of a cartridge which must represent elements other than monopolar cells, retinula cells or long visual fibres (Fig. 70). One of these (Fig. 19) has been traced out in Gros-Schultze stained material; it is clearly derived from the diffuse lamina tangentials (Figs. 16, 22, 68, 75b). Two other cross-sections which are not tangential processes but which may represent C2 and C3 neurons have sometimes been detected either lying each side (polar and equatoral) of the L3 and L4 monopolar cells or near R1 and R6 or R4 and R5.

Convincing evidence that these four neurons have a synperiodic arrangement in the lamina (where one of each type is associated with each optic cartridge)

Fig. 52. T1 and T1a terminals (Golgi-Colonnier preparations of *C. erythrocephala*). A detail of the basket ending of T1 showing the bifurcations of the posterior most processes (arrowed) at the outer face of the lamina. These correspond to the bifurcations of α and β processes around L3 and L4, described by Boschek (1970a, b)

Fig. 53. A T1 ending stained with an axial monopolar cell (L1 or L2) of the same cartridge. Note how the monopolar cell projects down into the centre of the basket ending

Fig. 54. A T1 ending. An R3 fibre (arrowed) can be seen entering the same cartridge (posterior to the left)

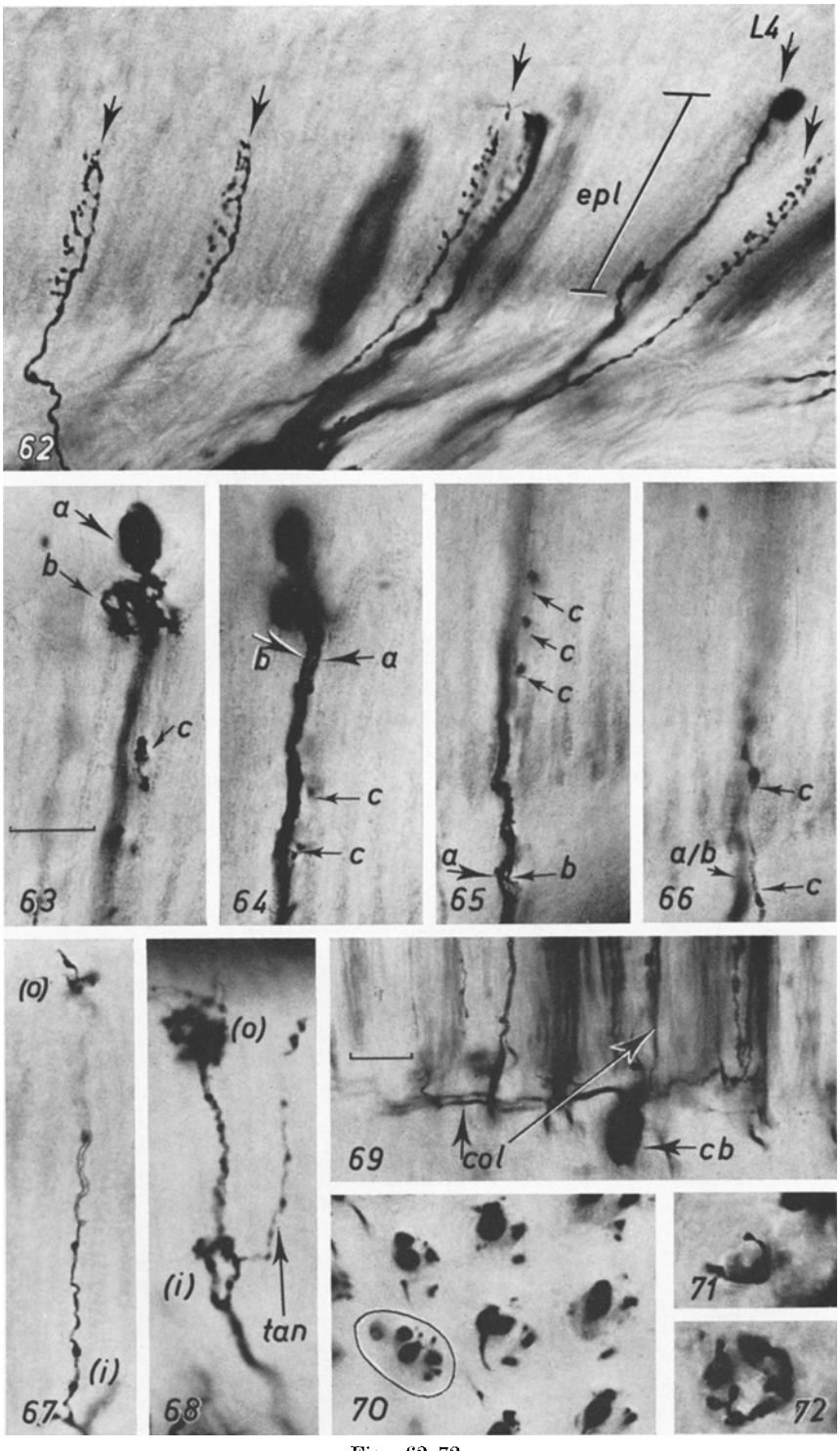
Fig. 55. Four retinula cells from a single ommatidium: R3 arrowed (posterior to the left)

Figs. 56–58. Serial optical sections of a T1 ending through a depth of 3 μm (taken tangentially across the lamina) showing the single fibre derivation of two branches (*bf*, in Fig. 56) which, in turn, give rise to six ascendent processes (Figs. 57, 58)

Fig. 59. *T1a* and *T1* endings stained together at the same optic cartridge. Note the dense appearance of the two basket endings together compared with the single *T1* ending in Fig. 52

Fig. 60. The first bifurcations a T1a component (*bf*). Note the arrangement lateral of spines from the irregularly swollen ascendent processes

Fig. 61. Two processes of a T1 ending stained with one of the descendent fibres from a lamina tangential cell (arrowed). Scales: Figs. 52, 53, 59: 10 μm; Figs. 54, 55: 25 μm; Figs. 56–58, 60, 61: 10 μm


L4
epl
62
a
b
c
63
b
a
c
c
64
c
c
c
a
b
65
c
a/b
c
66
(o)
(i)
67
(o)
(i)
tan
68
col
cb
69
70
71
72

Figs. 62–72

comes from geometrical reconstructions of cartridges from Golgi stained material where several different cell types are stained together within a narrow portion of the lamina's curvature (Fig. 62). Brains which exhibit "en masse" impregnation of neurons both in the lamina and medulla have also provided data for these reconstructions. In these preparations the minimum distance separating any one type of cell-components from another (of the same type) is equal to an inter-medullary column interval (Fig. 46). Two, and sometimes three, of the four cell types have been seen together within a medullary column, and there is an equal chance of impregnating any one of the four types anywhere along the vertical or horizontal extent of this region. It must be assumed therefore, that each medullary column contains at least one of each of these four cell types. Since each type sends a linking-fibre to the lamina it must also be assumed that their respective lamina components are associated with each optic cartridge if, and only if, there is no lateral permutation of neighbourhood relationships during their crossover from the second synaptic region to the first. That this is so is borne out from preparations in which monopolar cells and these so-called "centrifugal cells" (Strausfeld and Blest, 1970) are stained together between the lamina and the

Fig. 62. *Calliphora vomitoria* (horizontal section, Golgi-Colonnier impregnation). Four terminals (arrowed) of the C3 centrifugal cell. Note the characteristic bow shaped contour of each ascendent fibre, similar to that of the L3 neuron axis-fibre. *L4* the tripartite monopolar cell. *epl* external plexiform layer

Figs. 63–66. Four successive optical sections of the lamina (cut parallel to the z-axis) of *C. vomitoria* (Golgi-Colonnier impregnation) showing the C2, C3 and L5 fibres situated close together in an optic cartridge. Fig. 63 shows the cell-body of an L5 neuron (*a*) situated above the lateral processes of an C2 ending (*b*). Further down the axis fibre, to the right, are swellings of the unilateral processes of a C3 ending (*c*). Fig. 64 illustrates the axis-fibre of L5 (*a*) with C2 (*b*), the two elements are separated by a distance less than 0.5 μm. C3 processes (*c*) can also be seen deeper in the lamina. Fig. 65 shows C3 processes (*c*) in the lamina with the linking-fibres of L5 and C2 (*a* and *b*) beneath the inner face of the external plexiform layer. The unimpregnated retinula cell terminals are clearly visible. Finally, Fig. 66 shows the extremely thin axis-fibre of C3 (*c*) and some of its blebbed specialisations; the L3 and C2 fibres are slightly out of focus. The distance between the C3 fibre (Fig. 66) and L3 and C2 fibres (Fig. 65) is approximately equal to 2.7 μm

Fig. 67. *C. erythrocephala* (Golgi-Colonnier impregnation). A capped terminal of the C2 ending showing the two layers of lateral processes (*i* and *o*)

Fig. 68. *Eristalis tenax* (Golgi-Colonnier impregnation). The capped terminal of C2. Note the course of the tangential fibre (*tan*): this is another substrate for inter-cartridge interaction

Fig. 69. A wide-field amacrine cell process in *E. tenax* (Golgi-rapid impregnation). This neuron may also mediate inter-cartridge interaction. Amacrine cells are invariably stained amongst many other elements, hence the lack of clarity in this photograph

Fig. 70. *M. domestica* (tangential section taken just beneath the lamina: Bodian preparation). This preparation provides one of the rare instances in which a reduced silver staining shows up ten fibre cross-sections in a fibre bundle (ringed) from a optic cartridge some of which probably correspond to centrifugal elements

Figs. 71 and 72. (*E. tenax*; Golgi-Colonnier). Tangential sections of two optic cartridges showing the unilateral arrangement of processes from the C3 (Fig. 71) ending and the radial disposition of the outermost processes of the capped terminal of C2 (Fig. 72). Scales: Figs. 63–66, 70–72: 10 μm; Fig. 62, epl. depth: 55 μm; Fig. 67 (o) — (i): 50 μm; Fig. 68 (o) — (i): 55 μm

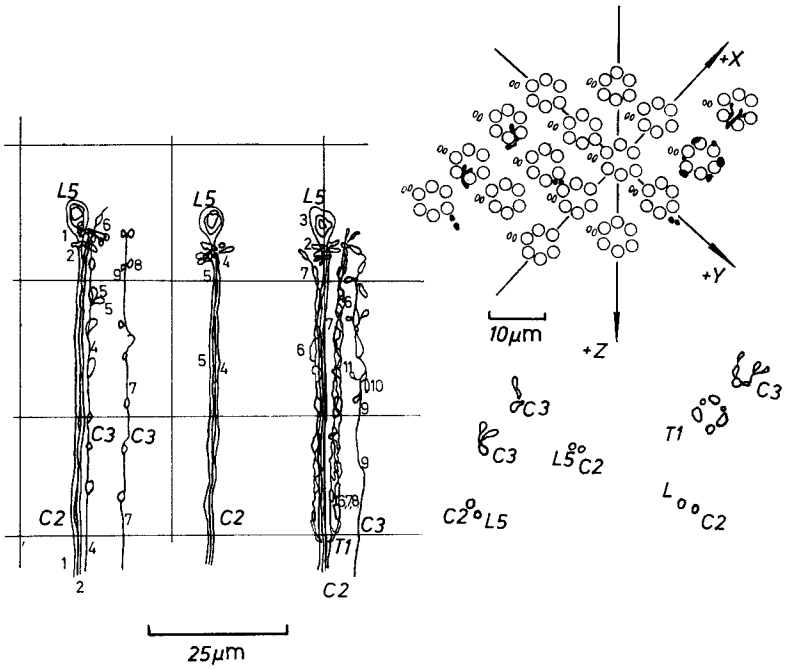


Fig. 73. One method for determining the positions of elements which cannot otherwise be adequately stained by reduced silver. The orientation of the section and the locations of the *T1* and *L5* cells with respect to *R1–R6* and *L1*, *L2*, *L3* and *L4* are already known from reduced silver studies as well as in preparations where *T1* and *R* fibres have been selectively impregnated at the same cartridge (Figs. 53, 54). The locations of the *C2* and *C3* elements, shown to the left, were measured laterally and in depth and subsequently mapped in cross section (middle diagram). This map was then fitted to the hexagonal mosaic of the lamina (right hand diagram)

medulla. Since we know that the monopolar cell arrangements are preserved *via* the first optic chiasma from lamina to medulla (see part 2) then elements stained with one of these centripetal fibres, from or to the same column or cartridge, must likewise preserve their lateral relationships between themselves and other elements between the two regions.

Phase contrast microscopy indicates that each basket cell ending embraces a crown of retinula cell endings. This is further substantiated in preparations where a basket ending and retinula cells or monopolar cells have been stained together at the same cartridge (Fig. 53–55). Both the T1 and T1a ascendent processes invest a single retinula cell crown and send short spiny lateral branches into the crown (Fig. 56–61). Although their ascendent processes cannot be individually resolved when both the T1 and T1a elements are stained together at the same locus the two axis-fibres can be clearly distinguished (Fig. 59). The disposition of the ascendent fibres of T1 and T1a elements correlate well with the pairs of α and β fibres that are disposed around a retinula cell crown and which were previously described by Trujillo-Cenóz and Melamed (1966). The two T-cell types (T1 + T1a), both of which project separately to the medulla

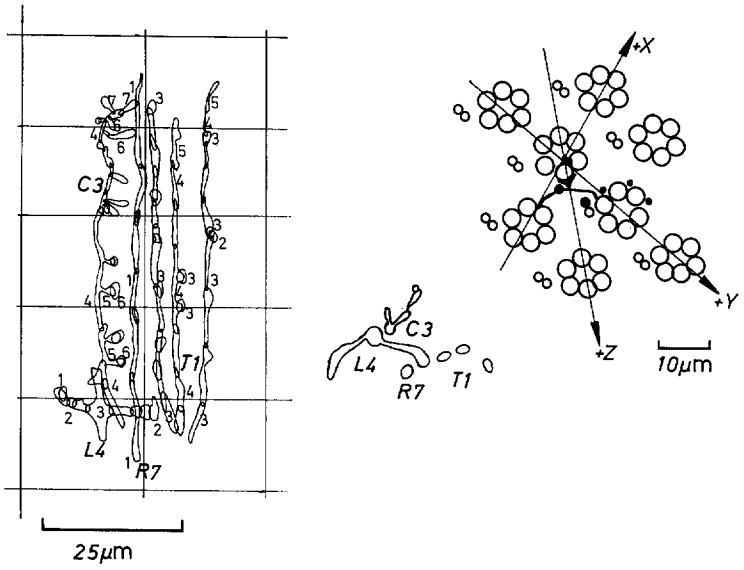


Fig. 74. Location of elements in Golgi preparations. Again the orientation of the section is known (and therefore the orientation of retinula cell crowns) as are the positions of *L4* and *T1*, with respect to *L1*, *L2*, *R1–R6*, *R7* and *R8*. Tracings (to the left) were made of *C3*, *L4*, *R7* and *T1* components and their vertical aspect was subsequently translated into a cross-sectional plan (middle diagram). The right hand diagram shows this plan fitted into the hexagonal mosaic of the lamina

(Fig. 45), would also explain the separate α–β fibre origins beneath the external plexiform layer of the lamina.

When several different types of elements are stained together within a fraction of the lamina's arc they provide valuable data about the topographic relationships of elements within the lamina as a whole. Careful measurements, laterally and in depth, allow one to piece together the distances of the C2 and C3 elements from other neurons whose axis-orientations, and whose precise positions in the optic cartridges, have already been determined by other methods. Several elements stained together in a single section (whose orientations to either the x, y or z axis are known) give the kind of 3-dimensional jig-saw puzzle solution shown in Fig. 73–75a. These drawings illustrate serial camera lucida transcriptions of neurons (obtained by calibrated stepped-focussing through a thick section and plotting each optical section by means of a grid graticule) which have subsequently been translated into a cross-sectional plan and mapped onto the known hexagonal pattern of the lamina's set arrangement. The positions of the L3 and L4 elements have been reconstructed by this method and checked against their known positions from reduced silver preparations. This technique finds them in their expected locations. The positions of the C2 and C3 fibres derived by this procedure indicate that the former element usually lies equatorially to L5, separated from it by a distance not exceeding 5 μm (*Calliphora*). The C3 elements usually lies adjacent to R5 or R4, polar to L4. Of all the

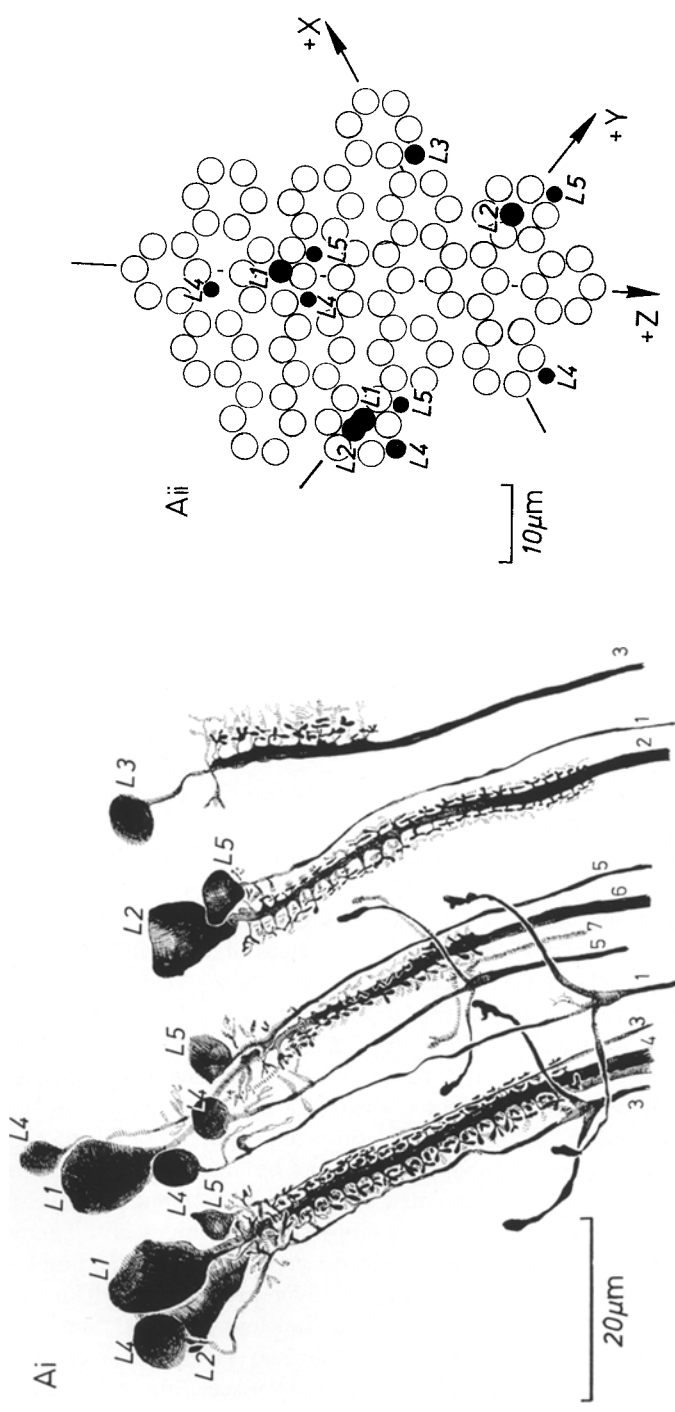


Fig. 75. A i) A camera lucida drawing of monopolar cells in the lamina of *Musca* (vertical section) showing the lateral relationships of *L1*, *L2*, *L4* and *L5* cells in cartridges (the numbers denote the five depths of focus at which these cell were resolved). ii) Elements mapped onto the hexagonal mosaic arrangement

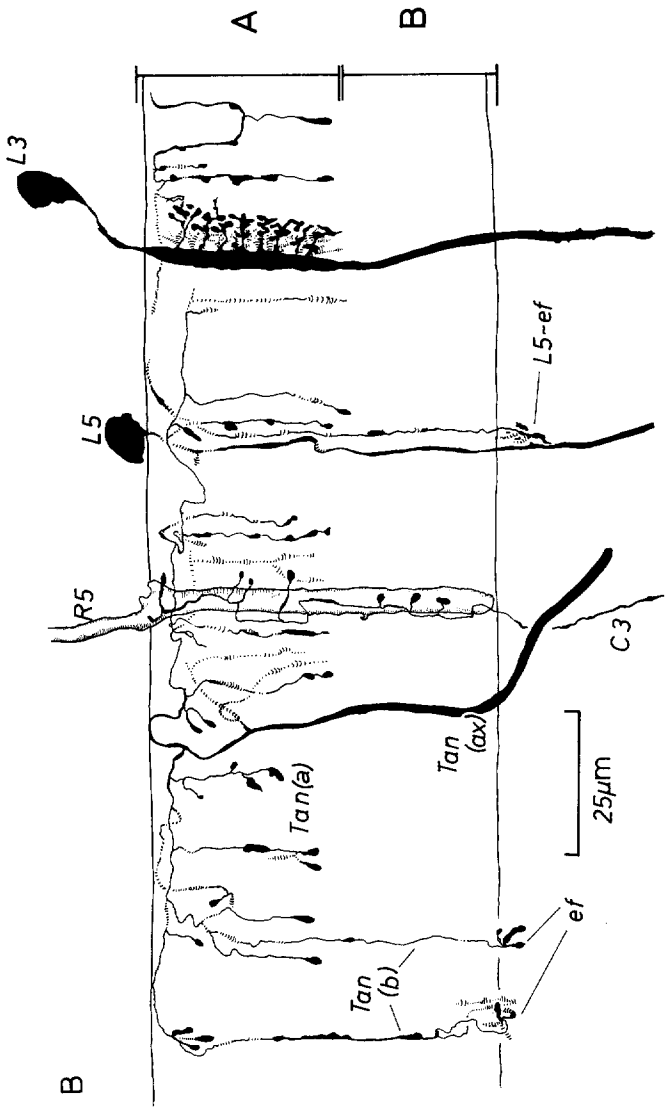


Fig. 75. B Camera lucida drawing of a portion of the lamina tangential element of *C. erythrocephala*. Note the two levels of descendent processes: the deepest (*Tan b*) terminates against the *L5* axis fibre (*L5-ef*) via a characteristic flowerlike specialisation (*ef*). The shallow processes (*Tan a*) extend through a depth of lamina equal to that invaded by lateral processes of the L3 cells (Stratum *A*). A *C3* terminal is shown "climbing" up a receptor terminal (*R5*)

elements of an optic cartridge the C2 and C3 elements show the most spatial variation[5].

The processes of C2 and C3 extent anteriorly from their terminal axis-fibres (along the $-z$ or $-x$ axes) and insert into the crown of retinula cell endings. The previous suggestion that C2 was arranged close to the R7 and R8 fibres (Strausfeld, 1970a) is disproven by these more sophisticated measurements. In fact, the various locations of both C elements seem to be identical to two tiny fibre cross-sections sometimes seen in Bodian preparations either side of L3 + L4 (Fig. 70).

A detailed reconstruction of optic cartridges in the antero-lateral portion of the upper left hand lamina is shown in Fig. 76. Further substantiations, concerning the locations of the various lamina elements, have been obtained from plotting the perpendicular curvature of neuronal components at the outer margin of the plexiform layer, and then matching these against the known curvatures of the inputs to optic cartridges from the retina (Fig. 75b).

Incerta Sedis. A sixth form of monopolar cell has been detected by the Golgi method at the equator of one lamina (where retinula cell crowns are composed of up to eight terminals). This neuron, illustrated in Fig. 45, is positioned at the L1 or L2 locus in a cartridge or located posteriorly down beside a retinula cell crown: it is characterised by wide-field processes, disposed at the outer margin of the external plexiform layer, which are compressed along the x or z axes and which have a total lateral extent equivalent to between 2 and 3 optic cartridge diameters. Also the T1 components shown in figure 45 are peculiar in that they possess wide lateral processes (ending in claw-shaped terminals) which extend to adjacent cartridges along the *z*-axis. These features have been seen in one late pupa of *Calliphora*, but not in *Musca*. Whether these elements represent standard local differences in lamina construction or merely variants of the L1/L2 cells and T1 components (which have been stained by chance equatorially) is not yet known. Since some mistakes in the projection patterns of retinula cells may occur, albeit very occasionally[6] it is conceivable that some interneurons may, during embryogenesis, compromise their shape so as to compensate for the errors of others. So far such interneuron variations have not been convincingly demonstrated by reduced silver techniques.

Discussion

Introduction

At least fifteen elements and a crown of six retinula cell endings constitute an optic cartridge in *Calliphora* and *Musca*. In the Syrphidae there are some additional neuronal components in the lamina that have not yet been related to the periodic structure of this synaptic region (Strausfeld, in preparation) and it is conceivable that an optic cartridge in *Eristalis tenax* could contain up to 16 neuronal elements with each crown of retinula cell endings.

The structure of optic cartridges (as seen in the upper left hand lamina of *Musca*) is summarized in Fig. 76. The elements are as follows:

1 and 2. Two long visual fibres which by-pass the crown of retinula cell endings and all the other elements listed below. Both long visual fibres, which are derived from the central rhabdomeres of an ommatidium (R7 and R8), terminate deeply in the outer layer of the medulla (Fig. 46).

5 The descendent tangential process (Figs. 19, 61) is usually located beside R4 and L5 but it has also occasionally be seen at a position nearby R à and R4

6 See appendix 1, and Figs. 19, 80–83.

3 and 4. L1 and L2. These consists of a pair of radial monopolar cells (the stratified radial diffuse and radial diffuse monopolars (Fig. 43) which project through the lamina down through the centre of a retinula cell crown. They terminate together, at two levels, in the outer strata of the medulla (Fig. 46).

5 and 6. L3 and L4: these are the brush and tripartite monopolar cells, respectively (Figs. 42, 43), both of which project through the lamina alongside retinula cells R5 and R6.

7. The midget monopolar cell, L5: this neuron projects down the outside of the retinula cell crown beside R5 and R4.

The L3 element has a unilateral arrangement of processes which project into the retinula cell crown between R5 and R6. Each process subsequently bifurcates and the resulting fibres, set roughly at right angles to each other, project towards R1, R2 and R4.

The L4 element has two sets of processes which project into the parent retinula cell crown. The outer set could contact all of the R1–R6 terminals. This neuron is also remarkable in that it serves to connect neighbouring pairs of cartridges one above and one below, posteriorly along the $+y$ and $-x$ axes. The majority of tripartite cells therefore connect triplets of cartridges (Fig. 40) in a precise and orderly manner under the whole of the lamina's inner face (Strausfeld and Braitenberg, 1970).

The L5 element of *Musca* is unistratified. In other species it is bistratified. There are between 1 and 4 outer processes which extend towards L1 between R5 and R4. In *Calliphora* and in the Syrphidae L5 elements have a second set of processes at or just beneath the lamina: some have been seen enclosed by terminal specialisations of the descendent processes of lamina tangential cells (see 14, 15, below). Tangential processes have the same relationships with L5 of *Musca*; however they are not indicated by inner specialisations from the L5 axis-fibres.

The terminals of L1 and L5 end at the same level in the medulla (Figs. 46, 78). The L3 endings are situated between the terminals of L2 and L1/L5 (Fig. 46).

8 and 9. The basket "endings" of the T1 and T1a cells from the medulla: these elements are arranged in pairs so that each pairs surrounds the crown of retinula cells and its axial L1 and L2 components. The basket fibres only surround the L3 and L4 axis-fibres at the extreme outer limit of the external plexiform layer (Fig. 52). The unistratified initial processes of T1 cells are situated in the outer strata of the medulla at the same level as the L2 terminals. The initial component of each T1a cell is tristratified (Fig. 46): an outer group of processes is situated at the same level as the T1 and L2 components in the medulla. A second group of processes is situated at the same level as the R7 terminals, and a third group is located in the deepest medullary strata at the same level as the first (inner) processes of the C2 and C3 "centrifugal" cells (Fig. 46).

10 and 11. C2 and C3 "centrifugal" cells (Figs. 46, 62, 67): both types of neurons have a tristratified arrangement of initial processes in the medulla at a) the levels of the T1a processes, b) the levels of R8, L5 and L1 terminals and c) at the surface of the medulla. At this last location the characteristic

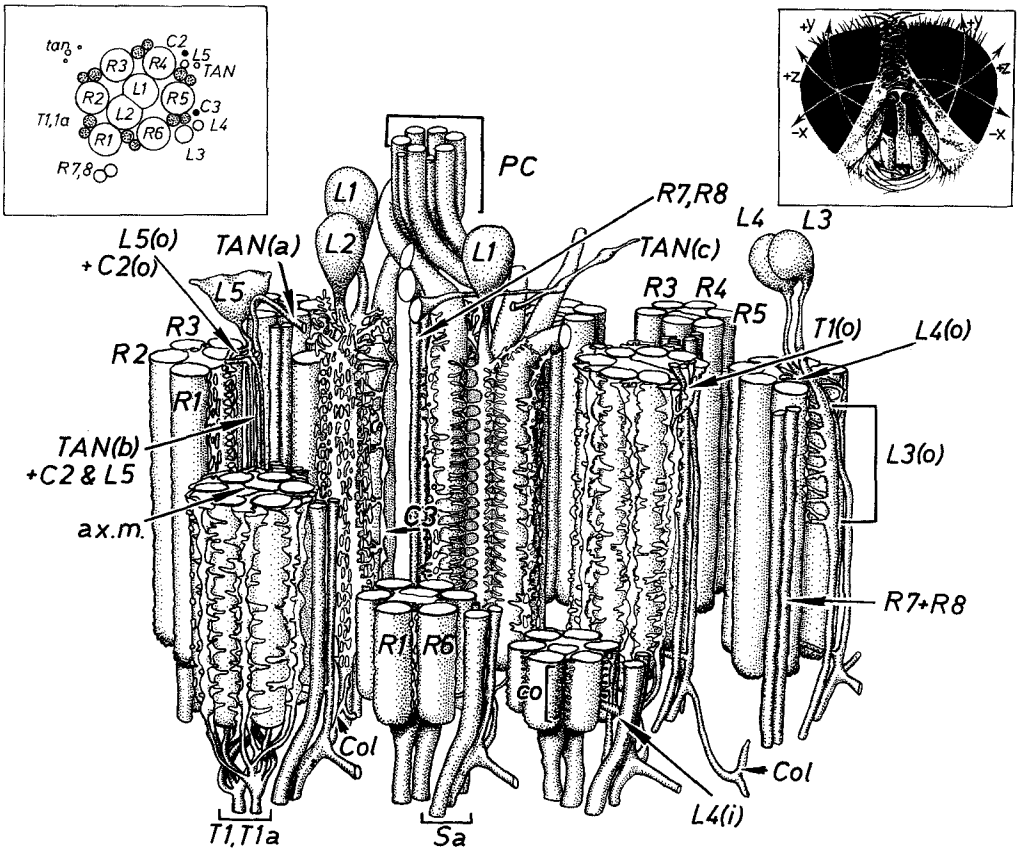


Fig. 76. Summary diagram of the structure of optic cartridges. The location of the cartridges portrayed in this figure are shown as eleven white spots in the frontal portion of the upper left hand eye of *Musca domestica* (right hand inset). The axis of the eyes are shown from front to back as $+y$, $+z$ and $-x$ (the long axis of the main diagram, from left to right, is oriented along the $+z$ axis). The eleven cartridges have been drawn in such a way as to represent a lamina "dissection". All bar the first, second and third cartridges of the second row (counting from the left) show the crowns of six retinula cell terminal (*R1–R6*). *L1* is characterized by radially arranged combs (*co*) of processes which interdigitate between the retinula cells whereas *L2's* processes are rather irregularly arranged from the axis-fibre and are usually contained within the confines of the crown (*L1* and *L2* cross sections are labelled *ax.m.*: axial monopolar cells). *R7* and *R8* have been drawn for only three cartridges. They project separately through the lamina apparently without coming into contact with any of the other cell types. *L3* and *L4* send their fibres together to the outer margin of a retinula cell crown (see Boschek, 1970a, b). Processes extend into the crown between *R5* and *R6* from the outer half of the *L3* axis fibre (their topographical relationships are shown in Fig. 42). One *L5* cell has been drawn: it sends a single process [*L5(o)*] into the crown between *R5* and *R4*. Tangential cell fibres [(*TAN (a)*] send processes [*TAN (b)*] down beside each crown, alongside *L5's* axis fibre. The *C2* centrifugal terminal is presumed to lie near the *L5* fibre whereas *C3* extends up beside the retinula cell crown near R5, polar to *L3*. *C3* processes invade the confines of each crown. Other tangential fibres [*TAN(c)*] project between retinula cells and monopolar cell bodies above the outer face of the external plexiform layer. *T1* and *T1a* invest the outer surfaces of retinula cells and also send spiny prolongations between them into the crown (see descriptions of

inverted U process of each C2 (Fig. 46) has dimensions and patterns such as which would allow it to fit precisely against the outer swelling of the R8 and/or the L1 terminal in the same medullary column. The linking-fibres of the two centrifugal cells extend to the lamina. Each C2 neuron terminates as a bistratified extension of its linking-fibre; it ascends through the external plexiform layer near R4 and R5. Each C3 ending ascends through the external plexiform layer up alongside R5 or R4 at a position polar to L4 (Fig. 76).

12 and 13. The terminals of two collaterals, each of which is derived from an L4 neuron from two neighbouring cartridges (anteriorly placed along the $+x$ and $-y$ axes), press close against the L1 and L2 axis fibres at the base of each cartridge.

14 and 15. The tangential fibres. The processes of tangential cells in the lamina (Fig. 21) have a complex distribution (Strausfeld, 1970a; and in preparation). However, at least two descendent processes from two separate tangential origins project down beside each cartridge (Fig. 61) from the cell body layer of the lamina. In *Calliphora* one passes completely through the lamina alongside R4, R5 and L5 as far as the inner face of the external plexiform layer. The other tangential process projects part of the way down in the lamina, near L3. This, and L3's lateral processes define the outer stratum of the plexiform layer (Fig. 75b). In *Calliphora* and Syrphidae the longest tangential fibre terminates against the axis fibre of L5 (Fig. 75b).

16. The amacrine cells (Figs. 69, 77) are at present something of a mystery. They stain infrequently and our knowledge of them in *Musca* is mainly derived from the studies of Cajal and Sanchez (1915). In *E. tenax* they most probably have a supra-periodic arrangement with respect to the cartridges. However, we

α and β fibres by Trujillo-Cenóz and Melamed, 1967; and by Boschek, 1970b). T1a lies against the retinula cell terminal and *T1* climbs up the processes of *T1a*: it also overlaps the *T1a* processes, so that it comes directly into contact with the *R* fibres. The inset in the upper left hand corner of this picture is a summary diagram of the cross sectional plan of a cartridge which includes the findings of Braitenberg (1970), Trujillo-Cenóz and Melamed (1966) and Boschek (1970a, b) as well as the results described in this account. Apart from the most marginal cartridges at the edge of the eye which receive inputs from less than six ommatidia (Boschek, 1970b), and equatorial cartridges which receive as many as 8 receptor cell terminals (Horridge and Meinertzhagen, 1970), it seems that all cartridges contain the elements shown in this diagram[7]. *T1* and *T1a* fibres are shown as stippled cross sections, *C2* and *C3* as black cross sections. *PC* pseudocartridge i.e. retinula cells (R1–R8) from one ommatidium before their decussation to seven optic cartridges. *R1–R6* short retinula cell terminals. *R7*, *R8* long visual fibres components, derived from the central rhabdomeres of each ommatidium (see Fig. 9). *L1* the stratified radial diffuse monopolar cell. *L2* the radial diffuse monopolar cell. *L3* the brush monopolar cell. *L3* (*o*) the outer processes of the brush monopolar. *L4* the tripartite monopolar cell. *L4*(*o*) outer processes of L4, *L4* (*i*) inner processes of L4 to parent retinula cell crown. *Col* L4 collaterals. *L5* the midget monopolar cell. *L5* (*o*) outer processes of L5. *C2* the type 2 centrifugal cell "capped ending". *C2* (*o*) outer processes of C2. *C3* the type 3 centrifugal cell "climbing ending". *T1* and *T1a* the type 1 centrifugal cells ("basket endings"). *Sa* the satellite fibres of each cartridge; i.e. those elements whose axis fibres are separate from the crown of retinula cells but which give rise to processes that extend to, or into, the crown

7 This does not exclude the possibility of further cell types, hitherto unimpregnated.

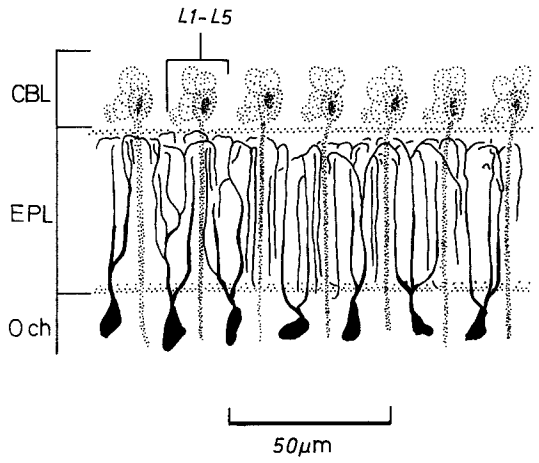


Fig. 77. The arrangement of amacrine cells in the lamina (adapted from Cajal and Sanchez, 1915). In *Musca* amacrine cells most probably invade the spaces between sets of three or four optic cartridges. Cajal and Sanchez figure pairs of monopolar cell axis fibres, and groups of monopolar cell perikarya situated between the arborizations of amacrine cells

know too little about these elements to make further comments on them except to mention that they, as well as the L4 cells and tangential fibres (Fig. 68), could mediate lateral interaction between sets of optic cartridges arranged in certain particular geometrical configurations (Table 9). Another possible mode of lateral interaction between cartridges could be mediated by the wide outermost branches of L1 cells (see page 397).

The glia cells in the external plexiform layer are intriguing[8]. We know that they give rise to the peculiar "capitate projections" that insert into the R1 to R6 fibres (Trujillo-Cenóz and Melamed, 1966) and Boschek (1970a) has detected what appear to be presynaptic specialisations in nerve cell processes that are apposed to glia convolutions. However, up to the present time, no synaptic vesicles have been found with these ribbons, and the glia cell seems devoid of postsynaptic membrane thickenings (Boschek, 1970b). Nevertheless, these cells cannot be discounted as possible integrative units.

Lamina Structure and Retinal Physiology

Some details about the synaptology of optic cartridges are known from recent studies by Trujillo-Cenóz and Melamed (1966) on *Lucilia* and *Phormia*, and by Boschek (1970a, b) on *Musca*. Their findings are summarized in Table 6. All three authors agree on one important point, namely that the monopolar cells L1 and L2 are postsynaptic to all the receptor terminals of a crown. Presumably both relay summed information from the six receptors to the medulla.

However, the observations of these authors are somewhat conflicting, with respect to the relationships between α–β fibres (the ascendent processes of T1 and T1a "basket endings"; Figs. 56–58, 60) and other elements. According to Boschek the six pairs of α and β processes are postsynaptic to retinula cell terminals, presynaptic to the L3 and L4 fibres (peripherally in the external plexiform

8 Boschek (1970b) has shown that they are periodically arranged in the lamina. Each invades a third of the surrounding three cartridges.

Table 6. *Synapses in the lamina*

From Boschek (1970a, b)

L1 and L2 postsynaptic to R1–R6
α–β fibres pre- and postsynaptic with each other
α–β fibres postsynaptic to R1–R6
α–β fibres presynaptic to epithelial cells
R1–R6 postsynaptic to epithelial cells
α–β fibres presynaptic to fibre cross-sections at L3 and L4 locus

From Trujillo-Cenóz (1965)

L1 and L2 postsynaptic to R1–R6
α–β fibres presynaptic to R1–R6
(also mentioned but not illustrated, α–β fibres presynaptic to L1 and L2)

Braitenberg (1970, unpublished observations)

L4 collaterals presynaptic to L1 and L2 axis fibres of adjacent cartridges along the $+y$ and $-x$ axes.

layer) and pre- and postsynaptic to each other. Trujillo-Cenóz and Melamed describe α and β fibres as being presynaptic to the receptor terminals and the two monopolar fibres within the retinula cell crown.

Thus it seems that although the forms of the T1 and T1a cells are very similar between different species these elements may serve a function in *Musca* different from that in *Lucilia* and *Phormia*. On the one hand the α–β fibres of *Musca* seem to constitute a system of apical dendrites whereas on the other the fibres in *Lucilia* and *Phormia* appear to be centrifugal terminals of pathways from the medulla.

But it is possible that there are pre- and postsynaptic components in α–β fibres that have not yet been resolved. If both sets of fibres contain both pre- and postsynaptic specialisations like those described by Boschek *and* Trujillo-Cenóz and Melamed then it could be speculated that both the T1 and T1a cells provide a system for local interaction between different elements of an optic cartridge as well as constituting a paired centrifugal pathway.

The forms of the T1 and T1a processes in the lamina are relevant to this argument. Both types of elements have "spiny" and "tuberous" components (Strausfeld and Blest, 1970) and it seems that both in vertebrates and insects the swellings and spines from some fibres are indicative of pre- and postsynaptic specialisations. Rather nice example of this are to be found in the cerebellum (presynaptic swellings of climbing fibres, presynaptic thickenings of parallel fibres and postsynaptic spines of Golgi cells and purkinje cells, to name but a few) (Eccles *et al.*, 1967). In insects there are similar arrangements of spiny purkinje-like cells and a system of parallel fibres in the corpora pedunculata (Pearson, 1970; Strausfeld, 1970b). Spines and swellings are, respectively, post- and presynaptic sites (Steiger, 1967). In the case of the lamina the spines from the L1 and L2 neurons are postsynaptic to the swollen terminals of receptor cells (Trujillo-Cenóz and Melamed, 1966; Melamed and Trujillo-Cenóz, 1967; Boschek, 1970a, b). Likewise, the swollen terminals of one of the axial monopolar cells (L1 or L2) have been described by Trujillo-Cenóz and Melamed (1970) as being presynaptic to the spiny processes of T1 components in the medulla.

There is also evidence that the swollen terminals of L4 collaterals are presynaptic to the L1 and L2 fibres at the base of an optic cartridge (Braitenberg; Boschek; personal communication): this implies that L4 cells provide local channels for interactions between interneurons in pairs of adjacent cartridges. Whether or not both collaterals exert the same kind of influence on the L1 + L2 cells, above and below L4's cartridge of origin, is an

Table 7. *The elements*
The crosses indicate where synaptic contact between elements is supposed to be

	R1	R2	R3	R4	R5	R6	R7	R8	L1p	L1f	L2p	L2f
R1			×	×	×		×	×		×		
R2				×	×	×	×	×		×		
R3	×				×	×	×	×				
R4	×	×				×	×	×				×
R5	×	×	×				×	×				×
R6		×	×	×			×	×				
R7	×	×	×	×	×	×	×	×	×	×	×	×
R8	×	×	×	×	×	×	×	×	×	×	×	×
L1p							×	×				
L1f	×	×					×	×				
L2p							×	×				
L2f				×	×		×	×				
L3p							×	×				
L3f	×	×	×	×			×	×		×	×	×
L4op							×	×				
L4ip							×	×				
L4c						×	×	×				
L4f	×	×	×	×			×	×			×	×
L5p	×	×	×			×	×	×				×
L5f	×	×	×			×	×	×			×	×
T1							×	×				
T1a							×	×				
C2	×	×	×				×	×				×
C3							×	×				
Tan												
E												

open question: in Golgi preparations of *Calliphora* the terminal branching patterns of the two collaterals appear dissimilar (Fig, 47).

At present our knowledge about the synaptic relationships between elements in the lamina is still rudimentary. However, at least it is possible to exclude some combinations of connections simply on the basis of the geometrical relationships between neurons in a cartridge. A list of possible synaptic connections can be drawn up using the same criteria. Table 7 indicates which synaptic contacts could not occur by virtue of elements being separate from each other down the length of a cartridge and Table 8 indicates synaptic contacts that might be expected between adjacent neurons. Finally, Table 9 lists elements whose fields spread through two or more cartridges and which could provide interaction between them.

Schemata such as that outlined in Table 8 are liable to be biased in favour of one functional hypothesis or another. Of the many attitudes that could be taken the simplest is to speculate about the lamina's organisation with respect to the following considerations:

1. the kind of information that arrives at the external plexiform layer from the retina. 2. the perceptual capabilities of Diptera, especially with regard to the kinds of information derived from optomotor experiments on *Musca*. 3. the geo-

of an optic cartridge
impossible by virtue of their positions within the cartridge (see Fig. 76)

L3p	L3f	L4op	L4ip	L4c	L4f	L5p	L5f	T1	T1a	C2	C3	Tan	E
	×				×	×	×			×			
	×				×	×	×			×			
	×				×	×	×			×			
	×				×								
				×		×	×						
×	×	×	×	×	×	×	×	×	×	×	×		
×	×	×	×	×	×	×	×	×	×	×	×		
	×												
	×				×		×						
	×				×	×	×			×			
			×	×									
						×	×						
			×	×									
×		×				×	×						
×		×			×	×					×	×	
				×		×	×						
	×		×	×	×								
	×		×		×								
				×									
				×									

metry, synaptology, projection patterns and levels of endings of optic cartridge elements.

1. a) By virtue of the divergence angles ($\Delta\varphi$) of receptor elements in adjoining ommatidia (Autrum and Wiedemann, 1962; Wiedemann, 1965; DeVries and Kuiper, 1958), the acceptance angles of retinula cells within ommatidia ($\Delta\varrho$) and their neural superposition arrangement (Kirschfeld, 1967; Braitenberg, 1967) the retinula cells (R1–R6) to an optic cartridge are apparently arranged for the reception of light at especially low intensities (Kirschfeld and Franceschini, 1969).

b) The central rhabdomeres in an ommatidium (R7 and R8) have a narrower acceptance angle than the retinula cells (R1–R6) and are apparently specialized for reception of light at high light intensities (Kirschfeld and Franceschini, 1968, 1969).

c) Cells R7 and R8 remain unshielded by pigment granules at high light intensities whereas R1–R6 become shielded (Kirschfeld and Franceschini, 1968).

d) R1–R6 have two peak sensitivities, namely at 360 nm and at 520 nm whereas R7 and R8 have a peak sensitivity at about 480 nm (Langer, 1967; Eckert, 1970)[9]. Neither that of R8 nor of R7 alone, has been measured.

9 This author's measurements are the most precise, taking into account the different effects of absorption and light scattering. He has shown the following peak sensitivities: R7/8 = 462 nm; R1–R6 = 362 nm and 486 nm.

Table 8. *The elements*

Circles indicate where synaptic contact is thought to be a likely possibility on the basis of cell positions within the cartridge (Fig. 76). P indicates which elements in the left hand column are postsynaptic to elements shown in the top row. Pr indicates which elements

Boschek,

	R1	R2	R3	R4	R5	R6	R7	R8	L1p	L1f	L2p	L2f
R1									Pr		Pr	Pr
R2									Pr		Pr	Pr
R3									Pr	Pr	Pr	Pr
R4									Pr	Pr	Pr	
R5									Pr	Pr	Pr	
R6									Pr	Pr	Pr	Pr
R7												
R8												
L1p	P	P	P	P	P	P						
L1f			P	P	P	P						
L2p	P	P	P	P	P	P						
L2f	P	P	P			P						
L3p	○	○	○			○						
L3f					○[a]	○[a]						
L4op	○[b]	○[b]	○[b]	○[b]	○[b]	○[b]						
L4ip	○	○	○	○	○	○				○		○
L4c										○[c]		○[c]
L4f					○							
L5p				○	○							
L5f												
α–β T1	P	P	P	P	P	P			○		○	
α–β T1a	P	P	P	P	P	P			○		○	
C2												
C3												
Tan	○	○	○	○	○	○						
E												

[a] At the outer $^1/_3$ of the external plexiform layer.
[b] Some L4 cells have more processes than others and may have various arrangement:
[c] At least one L4 collateral has been shown to be presynaptic to an L1 or L2 fibre

e) Dichroic extinction exists in Dipteran rhabdomeres in retinula cells R1–R6 as well as in R7 (Langer, 1967; Kirschfeld, 1970). The receptor potential amplitude of these elements to stimuli of linearly polarised light varies with the angular position of the E-vector (Burkhardt and Wendler, 1960). Kirschfeld (1970) has demonstrated that more light is absorbed by R1 to R6 when the E-vector is parallel to their microvilli whereas most light is absorbed by R7 when it is arranged at right angles to the microvilli.

2. Behavioural experiments, using the optomotor response as an assay, have shown that:

a) There is good evidence for suggesting that only R7 and R8 can assimilate polarised light information to mediate an optomotor response (Kirschfeld and Reichardt, 1970).

of an optic cartridge

are known to be presynaptic (P and Pr from Boschek, 1970a, b). p = processes, f = axis fibre, ip = inner processes (of L4), op = outer processes (of L4), c = collaterals of L4. Tan = descendent tangential fibres. E = epithelial cells (Trujillo-Cenóz and Melamed, 1967; 1970b).

								α–β					
L3p	L3f	L4op	L4ip	L4c	L4f	L5p	L5f	T1	T1a	C2	C3	Tan	E
○		○[b]	○					Pr	Pr			○	
○		○[b]	○					Pr	Pr			○	
○		○[b]	○					Pr	Pr			○	
		○[b]	○			○		Pr	Pr			○	
	○[a]	○[b]	○		○	○		Pr	Pr			○	
○	○[a]	○[b]	○					Pr	Pr			○	
								○	○				
			○	○[c]									
								○	○				
			○	○[c]									
											○		
								P	P				
										○	○		
								○	○				
										○		○	
												○	
	Pr				○			PPr	PPr			○	Pr
	Pr				○			PPr	PPr			○	Pr
		○				○						○	
○		○											
						○	○	○	○	○			
								P	P				

in different optic cartridges (see page 408).
of an adjacent $+y$ or $-x$ cartridge (see Table 6).

b) R1 to R6 do not mediate a discernable reaction to linearly polarised light. Since they are individually polarisation sensitive this information must presumably be lost somewhere in the succeeding neuropil (Kirschfeld and Reichardt, 1970). Possibly this might occur at the convergence of R1–R6 at the radial monopolar cells L1 and L2.

c) Experiments suggest that R7 and R8 cannot mediate an optomotor response below a certain light intensity, whereas the R1–R6 system can mediate a response to luminance as low as 10^{-4} apostilbs (Eckert, 1970). However, both the R1–R6 system and R7 and R8 contribute to optomotor responses in normal conditions of illumination at and above that necessary to elicit a response from R7 and R8 alone. There seem to be then, two systems in the retina. On the

one hand R1 to R6, which have high absolute sensitivity, two spectral sensitivity maxima (at 362 and about 490 nm) and low contrast transfer capability; and on the other, R7 and R8 which have a low absolute sensitivity, a single spectral sensitivity maximum (for R7) at about 460 nm and a high contrast transfer capability. This second system apparently is also sensitive to polarised light, with respect to the optomotor response (Kirschfeld and Reichardt, 1970). Both systems are synperiodic; hence they are equal with respect to resolving power, given by the angular density of the visual elements.

3. It seems amazing that only the R1 to R6 system demands the enormously complicated structure of the retinula cell crown and its associated elements (monopolar cells, tangential fibres, centrifugal elements and T1 and T1a components). The subset R7 and R8 of an optic cartridge passes directly through the lamina and does not appear to have any synaptic contacts until it reaches the medulla (Trujillo-Cenóz and Melamed, 1966; Trujillo-Cenóz, 1969; Boschek, 1970a, b and personal communication). These facts demand a closer look at the kinds of information that could be extracted from the R1 to R6 system.

The two axial monopolar cells, namely L1 and L2, are surrounded by the retinula cell endings of a cartridge. Their side branches reach to all the receptor terminals and there are between 90 and 150 lateral processes from each cell; in other words the two cells together give out about 250 side branches, each of which bifurcates at least once. If each bifurcation has one postsynaptic site with respect to retinula cell endings then there should be at least 500 synapses from the R1 to R6 system onto L1 and L2 in each cartridge.

The three satellite monopolar cells, namely L3, L4 and L5, also have lateral processes which project to retinula celle endings of their parent cartridge. But it is not yet possible to be certain whether or not they exclusively make contact with receptor terminals. There are, at most, 15 processes from L4 and only 4 processes from L5. Whereas some L4 elements could contact all the receptor terminals of a crown L5 could, at most, contact only two of them. It would be a liberal estimate indeed if we are to reckon with more than 10 synaptic sites onto the processes of L5 from R5 and R4 (see Fig. 42). Even L4 must have only a fraction of the number of synapses which are supposed to be present in the R1 to R6+L1/L2 system. L3 also has fewer processes than L1 and L2, though more than either L4 or L5. Their disposition from the axis fibre is indicative of a special relationship between L3 and R1, R2 and R4 (see Fig. 42).

It is fair to assume that the quantal release of transmitter substances by R1–R6 monotonically increases with the light intensity. Here it is supposed that the fewer the presynaptic sites of the R1–R6 endings onto an interneuron the less this neuron's chance, at low light intensities, of receiving enough transmitter substance from the R1–R6 system in order to evoke an effective potential change for a signal to the medulla. Thus the plausibility of separate photopic and scotopic pathways from the R1 to R6 system must be seriously considered. On the one hand L3, L4 and L5 might possibly represent up to three parallel photopic pathways to the medulla, whereas on the other L1 and L2 could represent a duplicate pathway from one cartridge to one column in the medulla (Fig. 78) and could be receptive to luminances ranging from 10^{-4} apostilbs up to and in-

cluding intensities which evoke responses from R7 and R8. In the case of L4 it should be borne in mind that its vertically oriented collaterals to adjacent cartridges (see page 396) imply that it has a more complex possible photopic activity than that of relaying information from a single cartridge to a single column in the medulla.

It is possible to weave several speculative schemes about the lamin's function from the kind of anatomical data that has been presented here. But only a few alternatives are considered in any detail: namely that there is an anatomical substrate in the present species of Diptera for separate functions which might perhaps be as follows.

On the basis of the proposals that have already been made it is possible that L5 could respond only at high light intensities. This element, if it is post-synaptic to R4 and R5, could provide a blue green-U.V. pathway to the medulla in parallel with the R7 and R8 system. However, the L5 elements could have a more complex role by virtue of their probable relationship with the lamina's tangential system (see Fig. 75b). This arborization of fibres spreads through groups of many optic cartridges and also sends collaterals to the medulla. It may serve to register the activity of groups of cartridges via the L5 elements.

The R7 and R8 receptors have not been separately analysed: all we know about them is that together they have a peak sensitivity at 462 nm and that they mediate optomotor reactions to patterns of polarised light at high light intensities. It is not absolutely certain that R7 has a spectral sensitivity maximum which is the same as that of R8.

Another photopic pathway could be mediated by L3. This neuron most probably has synaptic intimacy with only some of the retinula cell endings of a retinula cell crown. If this is found to be the case then it might serve to relay information about the angle of polarised light in, say, R4 and R2, to the medulla. If it were found that the six receptor cells that lead to one cartridge had different spectral sensitivity maxima then this cell might also be a candidate for relaying colour coded information to the second synaptic region.

The L4 neuron is enigmatic. It probably provides a pathway between its own cartridge and L1 and L2 of adjacent cartridges (Fig. 40). Its collaterals appear to be presynaptic to the L1 + L2 cells of adjacent cartridges (Braitenberg; Boschek personal communication), but as in the case of all the synapses in the lamina we have no idea about their excitatory or inhibitory nature. Possibly L4 cells could modify the outputs of the L1 + L2 cells in the adjacent cartridges in response to sudden intensity fluctuations mediated by the R1–R6 elements of its own cartridge. Another suggestion that has recently been made (Braitenberg, 1969, 1970; Strausfeld and Braitenberg, 1970) is that the extremely orderly arrangement of L4 collaterals complies with one model of a motion detector recently put forward by Götz (1968). However, it should be pointed out that there are similar orderly patterns of connections in the medulla (see Fig. 10, part 2) which link surrounds of 4 medullary columns (representing four cartridges) and so provide a symmetrical pattern from front to back and vice versa. Also these cells have their processes at a level at which the R1–R6 system and R7 and R8 system could converge.

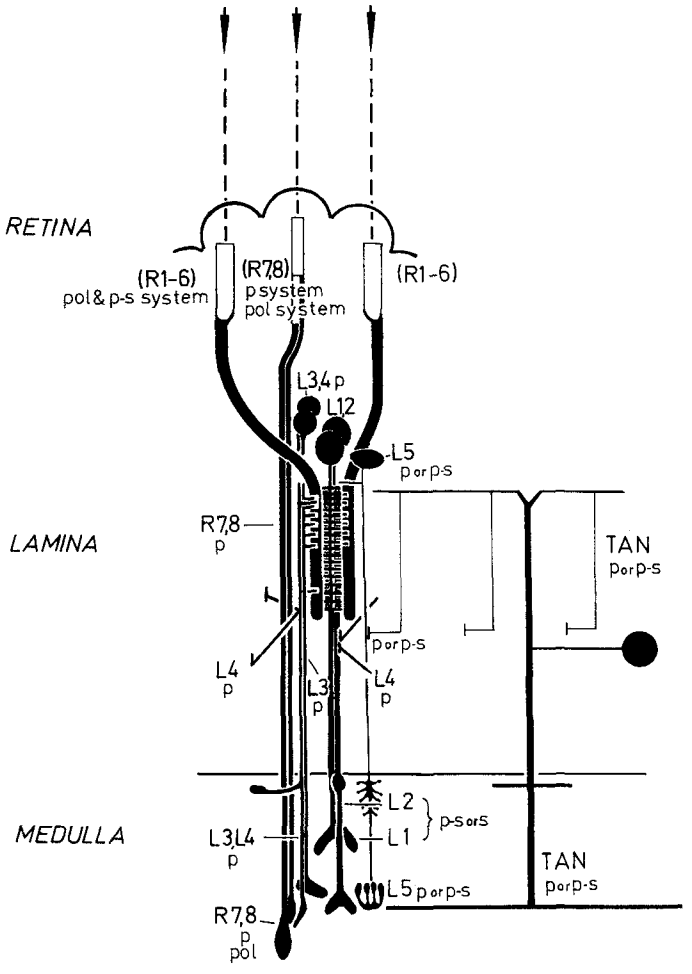


Fig. 78. The seven centripetal pathways to the medulla. Each cartridge in the lamina receives an input from six retinula cells, distributed in six different ommatidia (see Fig. 7), which "look" at the same point in the visual field. In addition, two other fibres, *R7* and *R8* (whose optical alignment is the same as the *R1–R6* input to a cartridge) are derived from a seventh ommatidium and pass through the lamina outside the crown formed by the other six terminals. *R7* and *R8* terminate in the medulla. There is good evidence that a) *R1–R6* are susceptible to excitation through a large intensity range (*s* scotopic range, *p* photopic range), b) each *R* fibre is sensitive to the angle of polarised light (*pol*) and that c) *R7* and *R8* are susceptible to excitation in the "photopic" range (see text p. 425). The two systems, *R1–R6* and *R7 + R8*, are here termed, respectively, the *pol + p − s* system and the *pol + p* system. The former presumably makes contact with five interneuron pathways to the medulla (*L1–L5*). The *pol* components of *R1–R6* cells would be effectively lost in the *L1* and *L2* cells by virtue of the convergence of all six retinula cell terminals onto these two neurons. However, *L3* might possibly relay information about the angle of polarised in the *pol + p − s* system if its processes extended to only two or three of the receptor endings of a crown (see text p. 395 and Fig. 42). It is speculated that the *L1* and *L2* neurons relay information at *p* and *s* intensities (or exclusively at *s* if the *L4* neuron inhibits the *L1 + L2* output at high light intensities). *L3* and *L4* are suggested to have a photopic role (see text p. 429). Depending on whether *L5* is postsynaptic to *L1* or to *R4 + R5* this

Further Anatomical Considerations

Objections will certainly be raised against these speculations: they are unrefined and lean heavily on anatomical data obtained by light microscopy alone. Indeed, some of the results of this account provide many more questions about the functional organisation of the system than the few which have been considered here. The centrifugal fibres have hardly been commented on at all but they must, needless to say, play an important rôle with respect to the L1–L5 output.

One feature that must also be borne in mind concerns the different fibre diameters of the L1–L5 cells in the lamina and in the first optic chiasma. Golgi studies on *Calliphora* indicate that the L1 and L2 fibres have cross sections twice as large as the L4 fibres and are about $2^1/_2$ times as large as L5. The L-fibres also have different widths in the first optic chiasma (Table 5). Possibly these differences may have a significant importance with respect to sequences and intensities of transmission from the lamina to the medulla (although we have no knowledge about the electrical characteristics of L-fibre membrane and cytoplasm). It should not be assumed that when all L-fibres of a cartridge are stimulated simultaneously their terminals in the medulla are simultaneously de-(or hyper-) polarised.

From the comparative anatomical point of view it is worth mentioning that most types of L, C and T lamina elements in *Musca* have forms which are hardly different from the L, C and T elements in *Calliphora* and Syrphidae. T and C elements look, respectively, similar in all the present species, notwithstanding differences of size (Figs. 67, 68). There are also interspecific similarities between the different species of L-neurons. There is little to distinguish between L1 and L2 cells in *Musca* and the same kinds of elements in *Calliphora* and the Syrphidae, although the branching patterns of these cells vary across the lamina (page 399). Such intra-laminar differences may be a geometrical consequence of lamina curvature and the consequent packing and lengths of retinula cell crowns. For example, the steeper the angle at which retinula cell axons enter the external plexiform layer the shorter L1's outermost lateral processes. But in the front part of the eye of *Eristalis* and *Calliphora* L1 processes appear to overlap and intermingle with those from adjacent cartridges along the y-axis rather than follow the pathways of retinula cells part of the way back towards the retina (Fig. 48–50).

L5 cells are unistratified in *Musca* and bistratified in *Eristalis*. *Calliphora* and *Syrphus*. Brush monopolars (L3) of *Musca* and *Calliphora* have unilaterally arranged processes whereas in the Syrphidae their arrangement is bilateral. In

element could possibly have a *p-s* or exclusively a *p* function. *R7* and *R8* provide a paired photopic pathway to the medulla which most probably relays information about the angle of polarised light. The tangential cell is, at present, enigmatic: it may possibly have a functional relationship with the *L5* elements as well as other elements such as the *R1–R6* terminals. It might possibly relay information to the medulla throughout the sensitivity range of the *p* and *p-s* system. Apart from the tangential cell, which must obviously interact with aggregates of optic cartridges, all the seven channels (*R7*, *R8* + *L1* — *L5*) from one cartridge arrive at the same column in the medulla

Table 9. *Wide field elements in the lamina*

Species	Cell type	No. of cartridges invaded by processes			Level of processes in lamina	Level of terminals in medulla
		x axis	*y* axis	*z* axis		
E. tenax	tangential fibres (G)[a]	25	15	26	outer face of external plexiform layer through external plexiform layer	all process derived from same cell-body. Two levels in outer stratum of medulla
	descendent tangential fibres (G)	1	1	2		
	tangential fibres (G)	10	6	10	inner face of external plexiform layer	?
C. erythrocephala *C. vomitoria*	tangential fibres (G)	wide spread through pseudocartridges (15–20 along all three axes)			cell body layer of lamina	axis fibre projects towards mid-brain. No medullary component identified
C. erythrocephala *C. vomitoria* *C. phaenicia*	tangential fibres (G)	20	8	10	outer face of external plexiform layer. 1 descendent fibre/cartr. through this layer	
M. domestica	tangential fibres (G, R)[a]	25	10	10	outer face of external plexiform layer. 1 descendent fibre/cartr. through $^1/_3$ this layer	

Table 9 (continued)

E. tenax	amacrine cell (G)	5	5	4	under and through external plexiform layer	none. These cells are intrinsic to the lamina
M. domestica[c]	amacrine cell (G)	2–3 cartridges along x, y and z axes			under and through the external plexiform layer	None
E. tenax *S. elegans* *M. domestica* *C. erythrocephala* *C. vomitoria* *C. phaenicia*	tripartite monopolar cell (G + R) L4	$2(-x)$[b]	$2(+y)$[b]	0	beneath the inner face of the external plexiform layer	Bistratified terminal. Two components at level of L1 (see figure 47)
E. tenax *C. erythrocephala* *M. domestica*	some bistratified radial diffuse monopolar cells (G + R) L1	—	2	—	at the outer face of the external plexiform layer	Bistratified terminal. (see figure 47)
E. tenax	L3 (G)	—	2	—	at the outer face of the external plexiform layer	Terminal not identified

[a] G = from Golgi stained material. R = from reduced silver stained material.
[b] In upper half of left hand lamina.
[c] From Cajal & Sanchez (1915)

the Syrphidae the diffuse lamina tangential cells have two forms of descendent processes through the external plexiform layer, one of which contacts the L5 element. The other has a recurrent process that extends to an adjacent cartridge (Fig. 68). This second component has not been detected in either *Calliphora* or *Musca*. In addition, there is a second form of tangential in *Eristalis* which invests the inner face of the lamina (Table 9).

L4 cells are identified by their collaterals at the base of the lamina (page 396). But though their arrangement usually corresponds to the pattern outlined by Braitenberg (1969) exceptions have been found in Golgi and reduced silver stained material (see appendix and Fig. 47). Observations on *Musca* and *Calliphora* have determined that L4 cells have different arrangements as well as having different numbers of processes in the outer $^1/_3$ of the external plexiform layer. Whether these variations are restricted to different horizontal or vertical zones, or whether they have a graded arrangement (like the L1, L2 and L3 fibre diameters: Holschuh, 1970), is not yet known.

One further anatomical feature of the lamina concerns its bistratified nature. There are two layers, the outer $^1/_3$ of the region being defined by the processes of L3 and L4 as well as the short descendent processes of the diffuse lamina tangential (Fig. 75b).

This account has by no means provided a definitive description of the lamina. It has though cleared up some of the ambiguous anatomical data of earlier accounts (Cajal and Sanchez, 1915; Strausfeld, 1970a). The Spanish authors showed figures of monopolar cells in *Musca* and *Calliphora* which are clearly those elements here listed as L1, L2, L3 and L5. However, neither they nor Strausfeld (1970a) identified the tripartite monopolar cell, L4. In the 1970a account its partially impregnated collaterals and axis-fibre were wrongly supposed to be a centrifugal terminal and its outer segment in the lamina was also erroneously classified as a unistratified radial monopolar cell element. The classification of monopolar cells must therefore be revised. There are five types, namely radial monopolar cells (either stratified or diffuse: L1 and L2), midget monopolar cells (either uni-or bistratified: L5), brush monopolar cells (L3) and tripartite monopolar cells (L4). Two further errors in the 1970a account concern the identification of the L5 and L1 terminals in the medulla. These are not situated shallowly in the outer stratum, as was previously supposed, but extend at least as far as the deepest sub-field of the lamina tangential ending (Fig. 46).

Appendix

Throughout this account mention has been made of morphological errors in the lamina. An error means simply that a fibre projection (or position) does not correspond to the overall schemes of projections or positions as described by Braitenberg (1967, 1970), or in the present account. These normal features are as follows

a) the projection patterns of six retinula cells from one ommatidium to six optic cartridges (see Braitenberg, 1967 and Fig. 13).

b) The characteristic orientations of R1–R6, R7 and R8, L1–L5 along the x, y, z axes (Braitenberg, 1970; Strausfeld and Braitenberg, 1970 and the present account (see Fig. 76)).

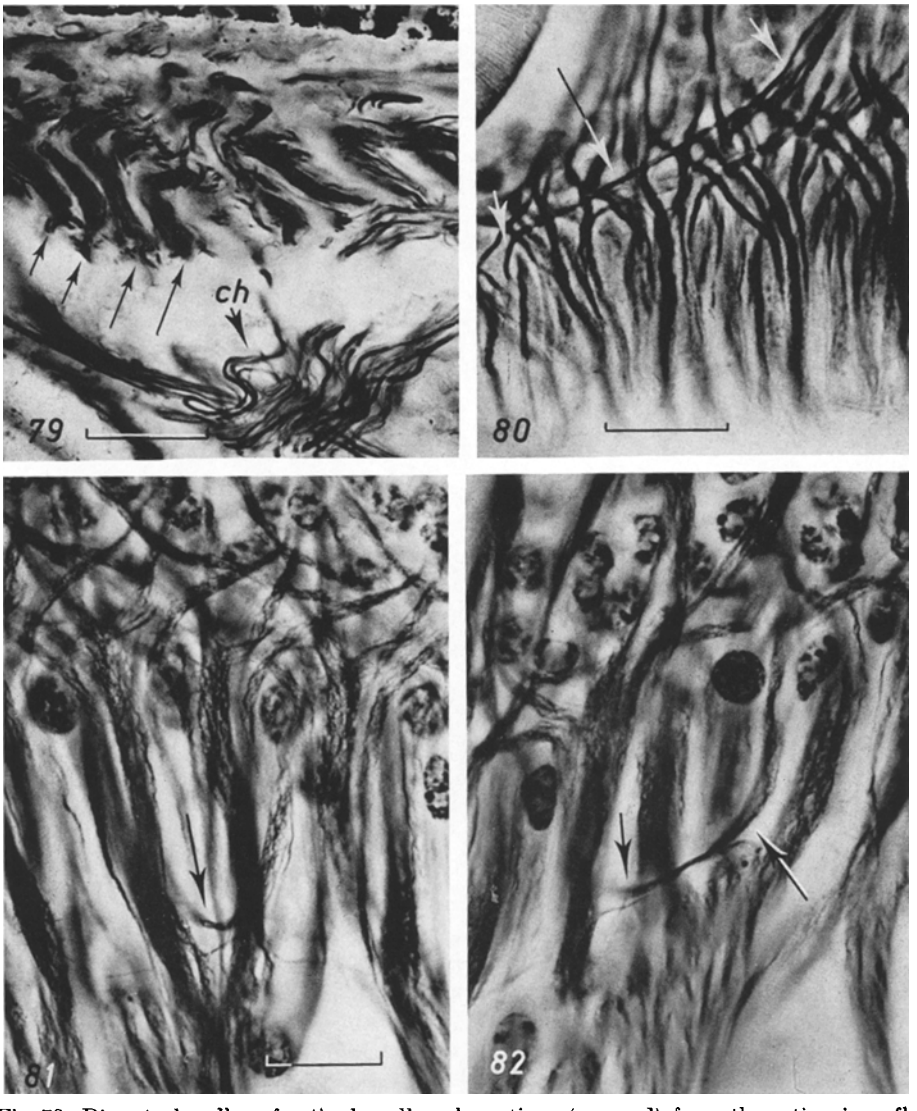


Fig. 79. Discrete bundles of retinula cell prolongations (arrowed) from the retina in a fly (*C. erythrocephala*) which lacks a lamina, medulla and lobula complex (Holmes-Blest preparation). Note the "chiasma" (*ch*) lying at a position equivalent to that of the first optic chiasma in the normal fly. There is no decussation between retinula cell fibres at all reminiscent of the projection patterns illustrated in Fig. 13

Fig. 80. An unusually long retinula cell prolongation (*R3*) to a cartridge which apparently lies outside the lamina domain normally invaded by the *R1–R6* fibres (*C. erythrocephala*, Bielschowsky preparation)

Fig. 81. A recurrent retinula cell prolongation (arrowed) to an adjacent cartridge (*C. erythrocephala*, Holmes-Blest preparation)

Fig. 82. An aberrant retinula cell which projects to a subadjacent cartridge along the v axis (*C. erythrocephala*. Holmes-Blest preparation). Scale: Fig. 79: 25 μm; Fig. 80: 25 μm; Figs. 81, 82: 10 μm

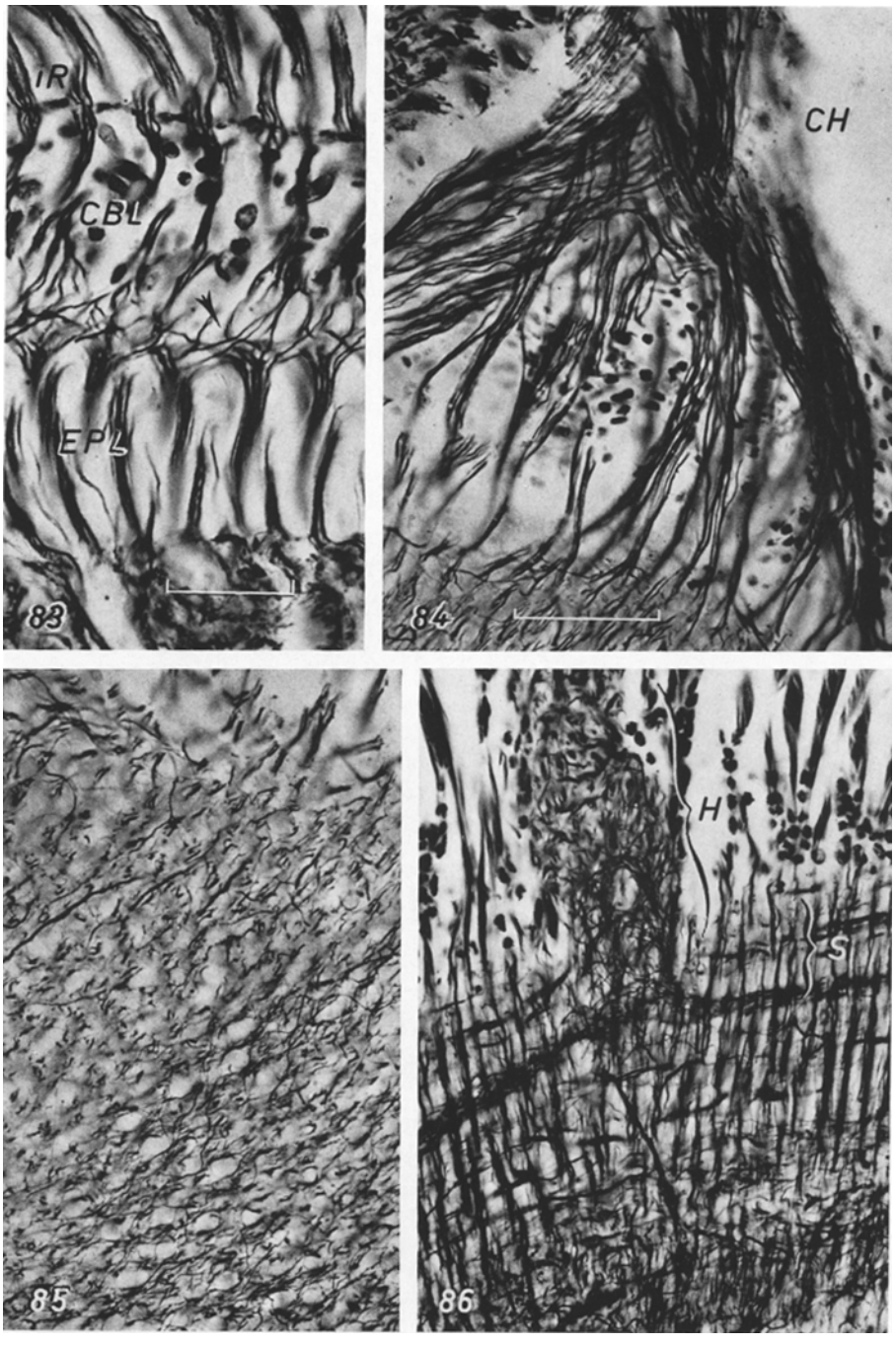


Fig. 83. Cross over patterns between retinula cells from a distorted retina (*iR*) to the lamina (see text). Some of the patterns of cross over (arrowed) and the positions of monopolar cell bodies (*CBL*) above the external plexiform layer (*EPL*) appear hardly different from those of normal wild type animals (*C. erythrocephala*, Holmes-Blest preparation)

c) The alignment of sets of elements (optic cartridges) along the three axes x, y and z.

d) The projection patterns of fibre bundles from cartridges to the medulla (Horridge and Meinertzhagen (1970b) and Part 2 of the present account).

There are technical difficulties which prevent examination of a complete lamina for exceptions to the features described in the works cited above. Only frontal sections best show the cross sections of cartridges and the patterns of fibres from retina to lamina. Vertical and horizontal sections show lateral relationships between cartridges and the pathways of retinula cells and interneuron fibres through the plexiform layer. Notwithstanding these limitations it is clear that mistakes in the lamina do occur and may possible be less rare amongst interneurons than amongst the receptor cell population (see Horridge and Meinertzhagen, 1970b). Some of these mistakes have already been mentioned in the text and figure legends (see Figs. 19, 22, 45). The commonest (and probably unimportant) error concerns the orientation of L1 and L2 which though normally aligned along the y-axis in the upper left hand eye often appear to be oriented along the z or x axes. Only if the disposition of processes from these elements is radially symmetric then such a variation would not change their topographical relationships with R1–R6 of a crown. One interesting feature is that although L1 and L2 may be misaligned L3, L4 and L5 retain their usual positions.

The following is a list of errors that have been seen in laminae of the present species (normal flies in so far that they contained the full complement of ommatidia, a lamina, medulla and lobula complex, and a bilaterally symmetrical brain).

1. Cross-sections of cartridges containing more than two axial fibres (in addition to fibre cross sections at the L3, L4 and L5 loci) surrounded by a distorted arrangement of what are presumably retinula cell terminals (Fig. 19).

2. Cartridges containing a single axial fibre within the retinula cell crown. It has not been possible to detect the absence of an L3, L4 or L5 fibre in a cartridge amongst an array of many cartridges in frontal sections of the eye.

3. Ommatidia which give rise to unusually long retinula cell fibres (Fig. 80). In vertical sections their pathways do not seem to correspond to the vertical aspect of the normal retinula cell pathways described by Braitenberg from *Musca*.

4. Retinula cell endings that invade two optic cartridges (Fig. 81, 82).

5. Patterns of L4 collaterals which connect constellations of 4 or 5 optic cartridges rather than the usual number of 3. This error is usually detectable at least 10 times per red eyed wild-type animal (frontal sections of the front part of the lamina). The laminae of some white eyed mutants may contain as many as

Fig. 84. The first optic chiasma, beneath the "distorted retina". Although fibres cross over their projection patterns appear imprecise (c.f. Figs. 5, 6, part 2)

Fig. 85. The surface of the medulla of the "distorted retina" fly. There is little evidence of a mosaic pattern of input bundles from the lamina (see Fig. 9, part 2)

Fig. 86. A medulla "hernia" (*H*). A portion of the medulla neuropil, comprising about six column diameters along the vertical axis (and 20 column diameters along the z axis) has grown out into the space occupied by fibres of the first optic chiasma. Even so, the columnar arrangement beneath level *S* appears normal: the topographical relationships of axis fibres are apparently little affected. Scale: Fig. 83: 25 μm; Figs. 84–86: 50 μm

60 quadripartite L4 neurons which link constellations of four cartridges (Strausfeld and Braitenberg, 1970).

6. Collateral arrangements in the lamina (excluding the posterior 8 cartridges as seen in horizontal sections) which neither correspond to tripartite monopolar cell collaterals nor to tangential cell processes (Fig. 22). They do not occur in each individual and the patterns appear very different in different animals.

Lacking further evidence it is too early to suggest the percentage of error per lamina or, for that matter, per chiasma and medulla (see Fig. 2, part 2). The study of lamina "monstrosities" may be a valuable tool for investigating neurogenesis in insects and for determining to what degree neurons must grow to precise topographical locations in order to establish connections with subsequent interneurons. Fig. 77–86 illustrate some examples of gross morphological errors. Up to the present time the most interesting of these resided in a single *Calliphora* whose retinulae were so queerly distorted that some light guides were directed into the head capsule and others were bent and twisted in such a way that their receptive fields most probably had arrangements other than those expected in the normal eye. Notwithstanding these distortions the retinula cell prolongations showed decussation patterns, some of which were remarkably like those seen in normal eyes (Fig. 83). The fibres terminated as crowns and fibre cross sections at the L1–L5 loci were associated with many of the groups of endings. However, the chiasma was distorted (Fig. 84) and the medulla neuropil did not exhibit the precise mosaic structure of normal animals (Fig. 85). Another fly which lacked lamina, medulla and lobula complex, but which had a normal retina, showed no evidence of decussation patterns of retinula cells from ommatidia (Fig. 71). Instead, groups of receptor prolongations from a single ommatidium extended together as pseudocartridges some distance beneath the retina (see Eichenbaum and Goldsmith, 1968). Ultimately all the groups crossed over as a kind of first optic chiasma at a location equivalent to the chiasmal cross over in a normal fly. It seems that, at first sight, the presence of lamina and monopolar cells (but not the exact alignment of the retinula cells in the retina) is a requisite for retinula cell axon decussations of some kind which are similar if not the same as those described from *Musca* (Braitenberg, 1967). The presence of a chiasmal cross over of fibres may be independant of the presence of lamina or medulla but the precision of cross-over presumably depends on the existance of these two regions as well as their correct alignment with each other (part 2) and the presence of interneurons. Errors in the normal eye, even where a portion of neuropil has undergone a morphogenic distortion (Fig. 86), do not seem to effect the mosaic organisation of neuropil lying central to the error.

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