Cell Tissue Res (1989) 258:441–475

Cell and Tissue Research

The optic lobe of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structure

K.-F. Fischbach[1] **and A.P.M. Dittrich**[2]

[1] Institut für Biologie III, Freiburg, Federal Republic of Germany;
[2] Institut für Genetik und Mikrobiologie, Würzburg, Federal Republic of Germany

Summary. Golgi studies of the neurons in the optic lobes of *Drosophila melanogaster* reveal a large number of neuronal cell types. These can be classified as either columnar or tangential. Columnar elements establish the retinotopic maps of the lamina, medulla, and lobula-complex neuropiles. They are classified according to the position of their cell bodies, the number, width, and level of their arborizations, and their projection areas. Tangential elements are oriented perpendicularly to the columns. The arborizations of different tangential neurons are restricted to different layers of the optic neuropiles, within such layers their dendritic fields may span the entire retinotopic field or only part of it. The abundance of cell types inside each of the columnar units of the optic lobe is discussed with regard to its possible functional significance. By means of their stratified arborizations the columnar neurons form what appear to be multiple sets of retinotopically organized parallel information processing networks. It is suggested that these parallel networks filter different kinds of visual information and thus represent structurally separated functional subunits of the optic lobe. Such a parallel organization of visual functions increases the sites for function-specific gene actions and may explain the behavioral phenotypes of recently isolated structural mutants of the optic lobe.

Key words: Optic lobe – Neurons – Cell types – Structurae mutanto – *Drosophila melanogaster* (Insecta)

It is often tacitly assumed that the extensive anatomical descriptions of the brains of several dipteran species (e.g., Cajal and Sánchez 1915; Strausfeld 1970; Campos-Ortega and Strausfeld 1972a, b, 1973; Strausfeld and Campos-Ortega 1972, 1973a, b, 1977; Strausfeld 1976; Hausen 1981) can simply be extrapolated to the fruit fly *Drosophila melanogaster*. Although these studies can be safely generalized with regard to the overall organization of the nervous system, the assumption of perfect identity between cells of different species is not justified. Behavioral differences between species are often correlated with connectivity differences of homologous neurons (Shaw and Meinertzhagen 1986), and this might show up in an interspecies variation of neuronal shapes (Fischbach 1983b). Thus, considering the evolutionary relationships between recent families of flies, comparison of the brain structure of different species at the level of single neurons might reveal how brains have been modified in the course of evolution. This is one of our justifications for presenting the shapes of neurons derived from Golgi impregnation in yet another dipteran visual system. Furthermore, the importance of the fruit fly as a model biological system continues to grow. It is the genetically best understood animal with a highly developed nervous system. Accordingly, a large number of neurological mutants has accumulated (Hall 1982; Fischbach and Heisenberg 1984). Behavioral analyses have also reached a high level, including research on vision (Heisenberg and Wolf 1984), learning (Aceves-Piña et al. 1983; Tully 1988), courtship, and biological rhythms (Konopka et al. 1983; Hall 1984; Ewer et al. 1988). By contrast since the classical study of Power (1943), the structural analysis of the nervous system of adult *Drosophila* has progressed rather slowly and relatively few details, often connected with the phenotype of structural mutants, have been added (e.g., Heisenberg et al. 1978; Tanouye and Wyman 1980; Heisenberg 1980; Fischbach and Lyly-Hünerberg 1983; Technau and Heisenberg 1982; Stocker et al. 1983; Garen and Kankel 1983; Fischbach 1983a, b). A comprehensive reference catalogue of wild-type neurons is still lacking.

Knowledge of the wild-type structure of the brain of *D. melanogaster* is necessary not only for a meaningful interpretation of mutant phenotypes but also for a proper evaluation of the distribution of immunoreactivity (Buchner et al. 1986, 1988; Nässel 1988a, b) and of activity label in the brain (Buchner et al. 1984; Bülthoff and Buchner 1985; Bausenwein 1988). This paper presents new observations about the neuronal organization of the *Drosophila* visual system. The obvious organization of its optic lobe into multiple sets of retinotopically organized pathways is discussed as being the result of the selective advantage of independent genetic modifiability of different visual functions. The actual degree of this independent genetic modifiability of functions should be testable in *Drosophila* by the study of mutants.

Materials and methods

Stocks

Young (1- to 7-days-old) female and male flies of the wild-type strains *Kapelle* (Heisenberg and Buchner 1977) and *Berlin* (Jacob et al. 1977) were used. At the single-neuron level no obvious structural differences between sexes and

Send offprint requests to: Prof. K.-F. Fischbach, Institut für Biologie III, Schänzlestraße 1, D-7800 Freiburg, Federal Republic of Germany

strains were detected. This may, of course, merely reflect our observational insufficiencies and the search for such differences should not be discouraged.

Flies were reared on standard cornmeal-agar-molasses medium at 21° C and 60%–65% relative humidity.

Histological techniques

For neuropile staining we applied the reduced-silver procedure of Holmes and Blest (Blest 1961) after fixation with Carnoy's solution and paraffin embedding.

Golgi impregnations were carried out following the slightly modified procedure of Colonnier (1964) as described in detail by Fischbach and Götz (1981). Very important for a high yield of impregnated material was the vigorous shaking, using a vortex mixer, of narcotized flies together with fine glass splinters in a test tube prior to fixation. The procedure resulted in small, randomly distributed penetrations of the cuticle allowing rapid entry of the solutions. In addition, the antennae and the proboscis of most flies were cut prior to fixation. Fixation took place in a mixture of 25% glutaraldehyde and 2.5% potassium dichromate for 7 days in the dark at 20° C. Afterwards the heads were submerged in 0.75% $AgNO_3$ for 7 days in the dark. Most preparations were so-called mass-Golgi preparations in which the whole procedure was repeated once. Specimens were then embedded in Araldite and cut at 35 µm. The preferred plane of sectioning was horizontal (from anterior to posterior), because the projections of most visual neurons are oriented in this plane. However, vertical sections (from dorsal to ventral) were analyzed as well. Sections of more than 300 successfully impregnated wild-type brains were evaluated. Supporting evidence from about 1000 preparations of flies of various mutant genotypes was also available but is not documented in this paper. Golgi shapes were drawn using a camera lucida. For the construction of composite pictures of camera lucida drawings, individual columnar cell types were placed at the exact position of the neuropile at which they had been impregnated.

Results

1. The overall organization of the optic lobe

Fig. 1 shows the overall organization of the optic lobe of adult *Drosophila melanogaster* in a series of silver-stained horizontal sections. The lobe is subdivided into four neuropiles, called lamina, medulla, lobula, and lobula plate. At all levels, the columnar organization of these is obvious, especially in the lamina and the medulla. The number of columns in each neuropile corresponds to the number of ommatidia in the compound eye. As the consequence of the neural superposition principle, which has firmly been established anatomically in *Muscidae* and *Calliphoridae* (Braitenberg 1967; Kirschfeld 1967, 1973), one visual column (neuro-ommatidium) receives direct projections from eight retinula cells with identical optical axes recruited from seven neighboring facets. These then form a single visual unit, sampling a single point in visual space. The columns of the lamina (called cartridges) are linked to the columns of the medulla by fibers that retain their relative spatial relationships, but cross in a horizontal plane such that anterior cartridges are connected to posterior medulla columns and vice versa. Their trajectories constitute the outer optic chiasm. The posterior medulla therefore subserves the anterior visual field, and the importance of the frontal visual field is reflected in the increased thickness of this part of the medulla.

The fibers of the inner chiasm retinotopically connect the columns of medulla, lobula, and lobula plate. Lobula and lobula plate are oriented face to face. Fibers from the posterior medulla project into nearby columns of the distal lobula-complex, while fibers from the anterior medulla terminate in columns of the proximal lobula-complex (the terms 'distal' and 'proximal' in this paper refer to the distance of a structure along the visual pathway from the center of the brain).

In addition to its columnar organization the silver-stained optic lobe reveals a second feature, the existence of stratifications running perpendicular to the columns. This is especially obvious in the medulla, and its importance has already been recognized in large flies (Campos-Ortega and Strausfeld 1972a). Most striking is the serpentine layer, which is formed by tangential neurons entering the medulla anteriorly via Cuccatti's bundle. The cell bodies of these tangential neurons lie clustered in front of the medulla neuropile. Some send their axons via the posterior optic tract into the contralateral medulla.

In wild-type flies the serpentine layer marks the border between the distal (outer) and the proximal (inner) medulla. The distal medulla shows further stratifications in reduced-silver-stained preparations, while the proximal medulla appears to be more homogeneous (see Fig. 1). According to Campos-Ortega and Strausfeld (1972a) a particular stratum is formed by the discrete concentration of synaptic specializations extending through the medulla as a shallow network at a particular depth perpendicular to the columns. The neurons involved in the formation of a stratum may be tangential or columnar, and may also have arborizations in other strata. Campos-Ortega and Strausfeld (1972a) assume that information may spread across a stratum perpendicular to the columns. Deoxyglucose activity staining of only parts of the visual field demonstrates, however, that at least the main stimulus-driven information flow takes place in the centripetal direction (Buchner et al. 1984).

Various tracts connect the optic lobe with the central brain. The axons of the medulla tangentials in Cuccatti's bundle form the posterior optic tract (Figs. 1C, 2A). The axons of the lobula plate tangentials run close to it (Fig. 1C, D). Of the visual neuropiles, the lobula is most intimately connected to the central brain. Various sets of isomorphic lobula columnar neurons project via various routes (e.g., arrowheads in Fig. 1A, B) into different regions of the central brain (optic foci; Strausfeld 1976). An isomorphic set contains all columnar neurons of one type, which together scan a large part or all of the visual field. Some of them are the main constituents of the anterior optic tract (Fischbach and Lyly-Hünerberg 1983), others use the giant commissure to reach the contralateral lobula (Fischbach 1983a). In addition, the lobula contains many tangential neurons projecting into the central brain (e.g., Figs. 2, 4, 5, 18, 22).

2. Subdivision of the optic lobe neuropiles into layers

The diagrams representing the optic lobe neuropiles (Figs. 3–19) are subdivided into tangentially oriented layers. This division is not derived from the stratifications seen in reduced-silver-impregnated sections (Fig. 1; note the shrinkage of the neuropiles due to the fixation procedure

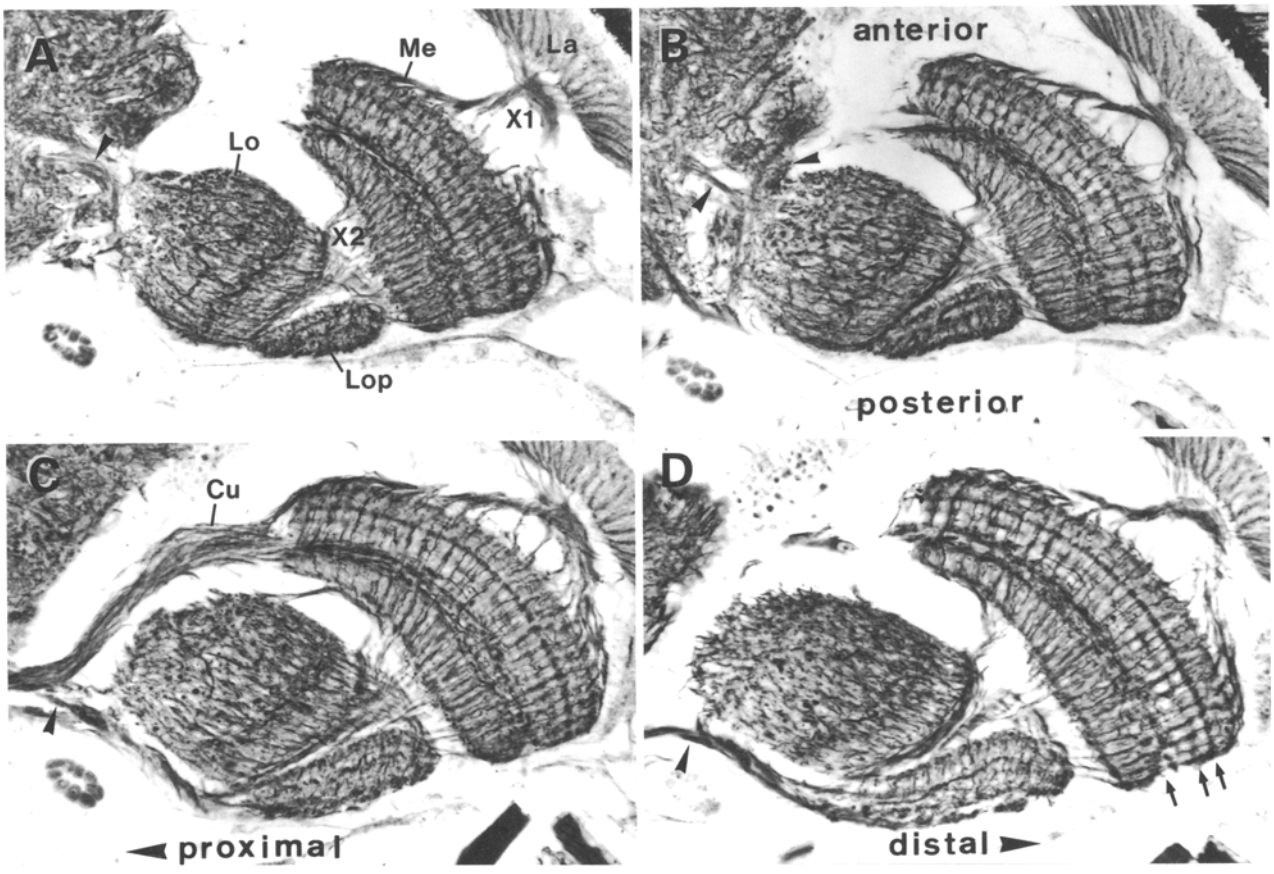


Fig. 1 A–D. Holmes-Blest silver stained, serial horizontal sections (7 μm) through the optic lobe's neuropile of a wild-type male fly (*Drosophila melanogaster*) starting dorsally (**A**). Fixation in Carnoy's solution is responsible for some shrinkage of the neuropile. Cell bodies have not been stained. Note the partitioning of the optic lobe into lamina (*La*), medulla (*Me*), lobula (*Lo*), and lobula plate (*Lop*). Lamina and medulla are connected by the first (outer) optic chiasm (*X1*) and medulla, lobula, and lobula plate by the second (inner) optic chiasm (*X2*). The arrowheads in **A** and **B** mark some of the fiber bundles connecting the lobula with the central brain. The small arrowheads in **C** and **D** point to fibers connecting the lobula plate with the central brain. Cuccatti's bundle (*Cu*) contains the axons of medulla and lamina tangential neurons, a significant proportion of which are of contralateral origin. The organization of the optic lobe neuropiles into repeating columns and stacked layers is especially obvious in the medulla. The 3 arrows in **D** point to strata, which are even visible in unstained brains, e.g., in the unstained background of Golgi preparations. The most proximal *arrow* indicates the serpentine layer (=M7, see Fig. 3A); the next *arrow* points to a weaker stratum, which is at the level of M4. The most distal *arrow* points to the layer of line amacrine cells (see Fig. 5), which marks the border between M2 and M3. ×470

as opposed to the Golgi preparations), but rather relies on the stratifications of Golgi-impregnated single neurons. Cross-reference to the reduced-silver layers, however, is easy (see below). In the course of our study it became evident that neurons of the same kind showed dendritic or terminal arborizations at the same depth in the neuropile. Furthermore, it was apparent – especially at the level of the distal medulla – that the arborizations of two different kinds of neurons tended to overlap each other either totally or not at all. For example, in the medulla the *T1* specializations completely overlap the *L2* terminals in depth, while the terminal specializations of *L1* and *L2* are mutually exclusive. Using these and similar data we subdivided the medulla neuropile into ten layers, the lobula plate into four layers, and the lobula into six layers. The layers have been numbered, but in addition a common name may be given, which refers to a characteristic cell type that branches in this layer. The approximate position of a layer relative to the surface of the respective neuropile is indicated by the percentage depth in front of its label.

Lamina. We did not explicitly subdivide the lamina into different layers. However, the Golgi shapes of some neurons (e.g., *C2, La wf, L4, L5*) clearly suggest that such a subdivision is possible (see below).

Medulla (for subdivision see Fig. 3A)

0%–10%: layer M1, (distal *L1* layer) is defined by the extent of the distal *L1* arborization.
10%–20%: layer M2, (*L2* layer) is defined by the extent of the *L2* arborization and also contains the distal *L4* and the *T1* arborizations. The proximal border of layer M2 is also defined by the distal expansion of the *L5* terminal, which fills layers 1 and 2.
20%–30%: layer M3, (*L3* layer) extends from the proximal border of the *L2* arborization to that of the *L3* terminal and contains the main medulla arborization of the lamina wide-field neuron and most *R8* terminals. The border between *M2* and *M3* is marked by the line amacrines (*Dm3*;

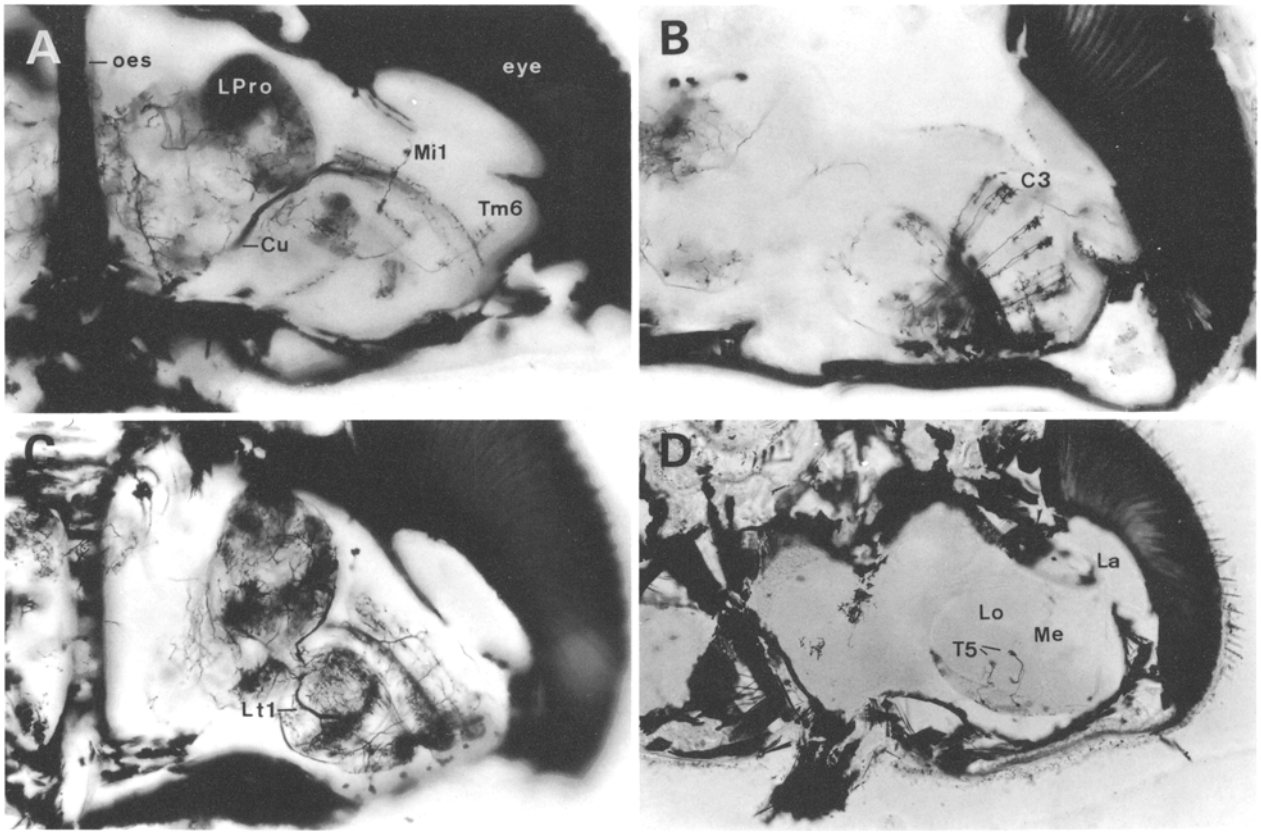


Fig. 2A–D. Horizontal sections through the heads of different adult *Drosophila melanogaster* showing typical examples of neuronal impregnation after the application of the mass Golgi-Colonnier procedure. One purpose of these low magnification pictures is to show the spatial arrangement of the optic lobe in the head capsule. Note that each brain shows an individual pattern of impregnation revealing only certain aspects of its neuronal organization. Furthermore, although in most brains many neurons are impregnated, it is often easy to identify the shape of single neurons (e.g., in **A** and **D**). In **A** a giant medulla tangential element projecting its axon via Cuccatti's bundle into the medulla is impregnated, as well as two medulla columnar neurons (*Mi1* and *Tm6*; see Fig. 27F for higher magnification); **B** shows the arborizations of several *C3* neurons in the medulla (see Fig. 26F for higher magnification); in **C** tangential elements of the medulla and lobula-complex are impregnated (see also Fig. 28A); in **D** two *T5* neurons terminating at different levels of the lobula plate can be seen; *oes* oesophagus. ×180

Fig. 5) visible as a narrow band in reduced-silver preparations (most distally placed arrow in Fig. 1D).
30%–35%: layer M4, (*L4* layer) this rather slim layer lies between the *L3* terminal and the proximal *L1* arborization. It contains the proximal *L4* arborization, and the long terminals of R8.
35%–43%: layer M5, (proximal *L1* layer) is defined by the proximal *L1* arborization, and also contains the proximal *L5* terminal.
43%–54%: layer M6, (*R7* layer) this layer lies between the *L1* arborization and the serpentine layer. It contains the terminal enlargements of *R7* and may tentatively be further divided into layer M6a and layer M6b according to the two types of *R7* terminals.
54%–66%: layer M7 (serpentine layer). This layer contains tangentially oriented axons of many medulla tangential neurons (Figs. 5–7, 14–17). In reduced-silver-impregnated sections it is easily identified as the extension of Cuccatti's bundle into the medulla neuropile (Fig. 1).
66%–73%: layer M8 is defined by stratified arborizations of certain medulla intrinsic neurons (e.g., *Mi3–7*, Fig. 4), by *Tm* and *TmY* neurons (Figs. 8–13), and by the lamina efferent *C2*.
73%–91%: layer M9 houses several types of medulla amacrine cells (e.g. *Pm1*, Fig. 5).
91%–100%: layer M10 (*T4* layer) is the innermost layer of the medulla. It is defined by the height of the bushes of *T4* neurons (Figs. 14, 26F).

Lobula plate (for subdivision see Fig. 6). The lobula plate can be subdivided into different layers according to stratified arborizations of axonal and dendritic terminals as well as by functional criteria (Buchner et al. 1984). At least four thin layers have to be distinguished.
0%–25%: layer Lop1 (*HS* layer) is the innermost layer of the lobula plate, abutting the inner chiasm. The dendrites of the *HS* cells (Fig. 20) arborize exclusively in this layer. According to Buchner et al. (1984) this layer contains elements that are sensitive to movement from front to back (progressive movement).
25%–50%: layer Lop2 contains the terminals of *T5b* cells (see Table 1; Fig. 14). According to Buchner et al. (1984) this layer contains neurons that are sensitive to movement from back to front (regressive movement).
50%–75%: layer Lop3 contains the terminals of *T5c* cells (see Table 1; Fig. 14). According to Buchner et al. (1984) this layer is activity labeled by upward movement.

75%–100%: layer Lop4 (*VS* layer) contains most of the dendritic arborizations of the *VS* neurons (Fig. 18) and *T4d* and *T5d* terminals (Fig. 14). This layer is activity labeled by downward movement (Buchner et al. 1984).

Lobula (Fig. 4)

0%–10%: layer Lo1 (*T5* layer) is defined by the dendritic specializations of *T5* neurons (Fig. 14) and by the terminal arborizations of *Tm1* and *Tm9* (Fig. 8).
10%–16%: layer Lo2 is occupied by the lobula tangential *Lt5* (Fig. 7).
16%–23%: layer Lo3 contains specializations of the lobula tangential *Lt6* (Fig. 18).
23–52%: layer Lo4, 52%–75%: layer Lo5, 75%–100%: layer Lo6. These layers are not as clearly defined as the other layers. They mark limits, however, within which several cell types tend to restrict their arborizations, e.g., *Lt7* in layer 6 (Fig. 4), *Lcn1* in layers Lo5 and Lo6 (Fig. 5) and *Lcn5* in layers Lo4 and Lo5 (Fig. 15). A further subdivision of Lo4 seems possible (e.g., the stratified terminals of *Tlp* cells in Fig. 4).

3. The Golgi shapes of neurons in the optic lobe

For Lepidoptera (Strausfeld and Blest 1970) and large Diptera like *Eristalis, Calliphora, Syrphus* (Strausfeld 1970), and *Musca* (Strausfeld 1971, 1976), detailed descriptions of neuronal shapes inside the optic lobe are already available. The present documentation shows a very similar cellular organization for *Drosophila*. The principles of optic lobe constructions are comparable. The shapes of neurons are, however, variable species-specific and in many cases the altered shapes suggest changes in neuronal connectivity.

3.1. Terminology and the classification of neuronal types into "columnar" and "tangenital"

Columnar neurons establish multiple and stacked retinotopic maps in the optic lobe. They connect the distinct cellular regions: retina, lamina, distal medulla, proximal medulla, lobula, lobula plate, and optic foci in the central brain. Retinotopy requires a mapping of one level upon another by fibers that strictly maintain their topographical relationships. The projection of retinal fibres *R1–6* is a special case in which the projection pattern corrects for the optics of the retina according to the rules of the neural superposition eye (Braitenberg 1967; Kirschfeld 1967).

A neuron is classified as columnar irrespective of the lateral extent of its arborizations if its axon is oriented parallel to the main axis of the visual columns. This classification, therefore, requires unambiguous recognition of the axon, which may be difficult if impregnation is incomplete. The periodicity of columnar neurons may be the same as or some multiple of that of the visual columns. The columnar neurons are further classified according to the position of their cell bodies, the shape and position of their arborizations, and the projection areas of their terminals.

It should be noted that our definition of columnar neurons leads to a reclassification of some neuronal types that were formerly described as "tangenital" neurons because of the wide spread of their collaterals. One practical justification to use the orientation of the axon as the main criterion is that there is a continuous transition from small- to large-field columnar neurons. Another, scientifically more compelling, reason is that from a developmental point of view the position of cell bodies and the initial direction of axonal outgrowth is similar for the two kinds of cell types. The distinction between them rests upon the later secondary development of patterns of dendrite arborizations.

Most basic cell types that are described from the optic lobe of *Musca* (Strausfeld 1976) also have been found in *Drosophila*. In many cases identification of homologous neurons in the two species is straightforward. This is especially true for lamina monopolar neurons, T- and C-cells, and lobula plate tangentials. In these cases it is clearly justified to use in *Drosophila* the same designations as in *Musca* (Strausfeld 1976). For many other neurons only educated guesses about their homologous counterparts are possible or a basis for such guesses may even be missing due to the lack of intermediate phenotypes. Whenever plausible, we name possible homologous counterparts of the *Drosophila* neurons in other Diptera. With regard to future comparative studies, labeling of neurons in our account is not based on features that are likely to vary in the course of evolution like the number of stratifications and the shape of dendritic fields.

In the following we document the Golgi-impregnated forms of most cell types found so far in the optic lobe of wild-type *Drosophila*. Not all neurons drawn by camera lucida are shown in the micrographs and vice versa. Since there are so many neurons, we have tried to avoid a verbal description of all of them, but rather prefer to point to those neurons and features which we consider especially interesting.

3.2. Retinula cell terminals

3.2.1. In the lamina. The thick axonal terminals of retinula cells *R1–6* extend through the entire length of a cartridge in the lamina (Fig. 3A). They have recently been shown to be most probably histaminergic in larger flies (Hardie 1987; Nässel et al. 1988). Electron microscopic cross sections show that in each cartridge six such electron-dense terminals, which are filled with vesicles, surround lighter profiles of presumed lamina monopolar neurons (Hauser-Holschuh 1975). It is interesting that in *Drosophila* the receptor terminals often possess spinelike extensions that have not been described in larger flies (e.g., Strausfeld 1976). This possibly is a direct consequence of the small size of *Drosophila*. The size of single synapses is approximately the same in *Musca* and *Drosophila* even though the terminals are much smaller in *Drosophila* (Hauser-Holschuh 1975). A certain number of synapses per terminal may thus require a relative enlargement of the smaller cell surface in *Drosophila*.

3.2.2. In the medulla. The axons of receptor cells *R7* and *R8* of the same ommatidium project in close proximity through the underlying cartridge of the lamina without any signs of synapse formation. They reach the medulla via the first optic chiasm and penetrate deeply into the distal medulla (Figs. 3A, 25A, B). They differ in the depth of their termination and display different immunoreactivities. *R7* neurons are possibly GABAergic (Datum et al. 1986), while *R8* seems to be histaminergic (Nässel et al. 1988), like the receptor terminals of *R1–6* in the lamina. Campos-Ortega and Strausfeld (1972a, b) found that in *Musca R7*

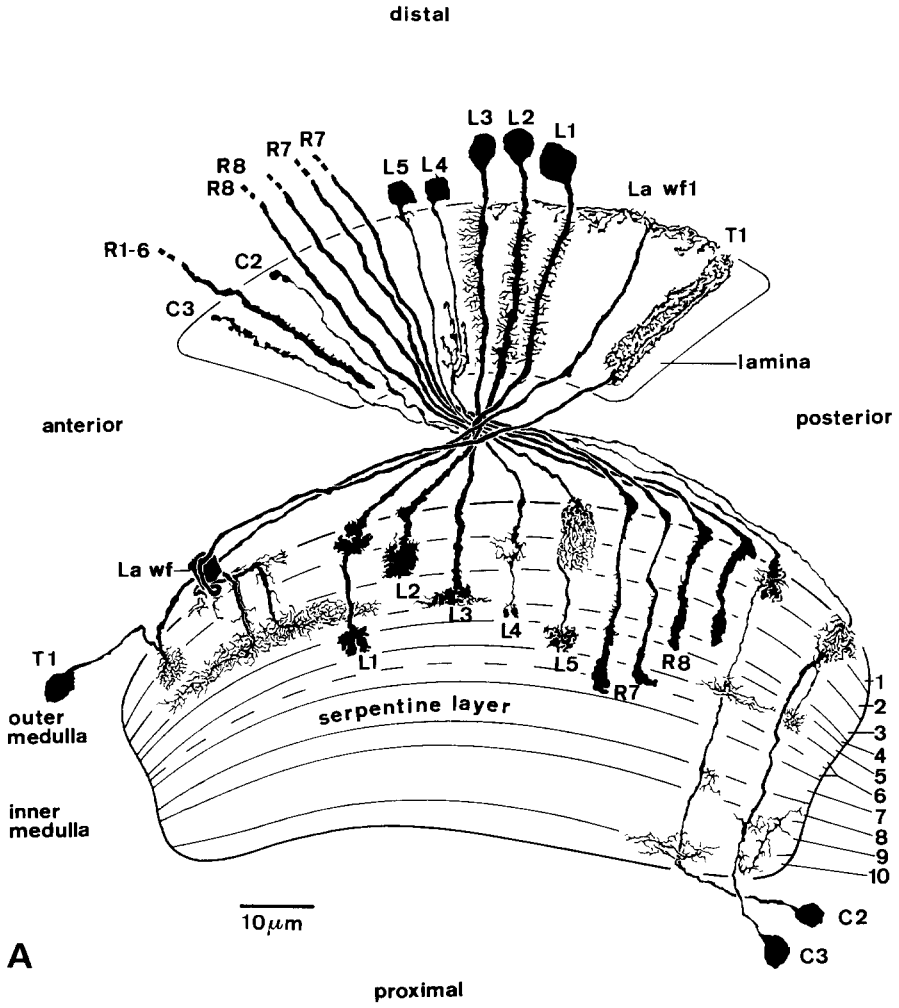
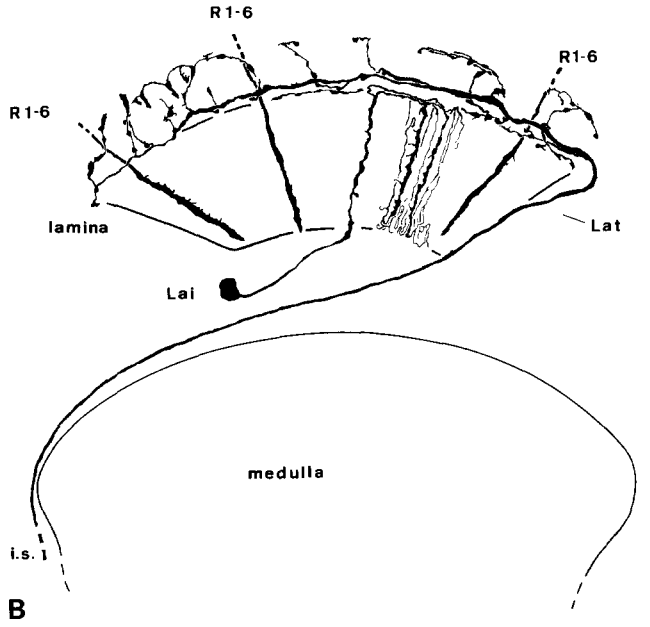
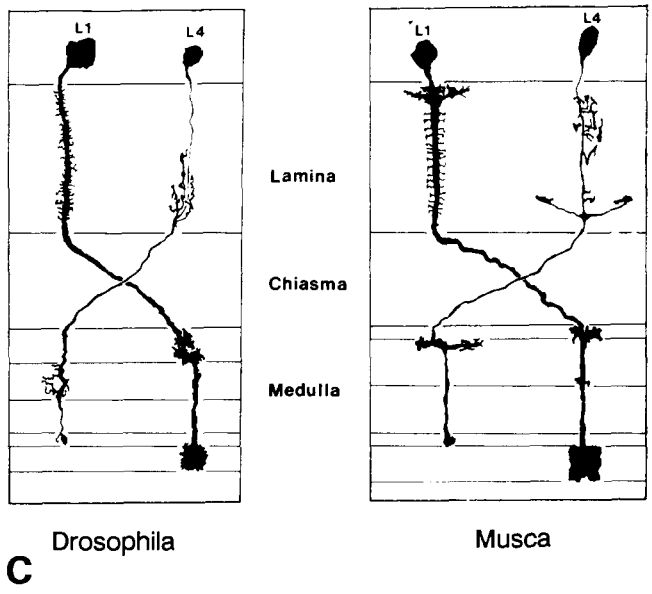


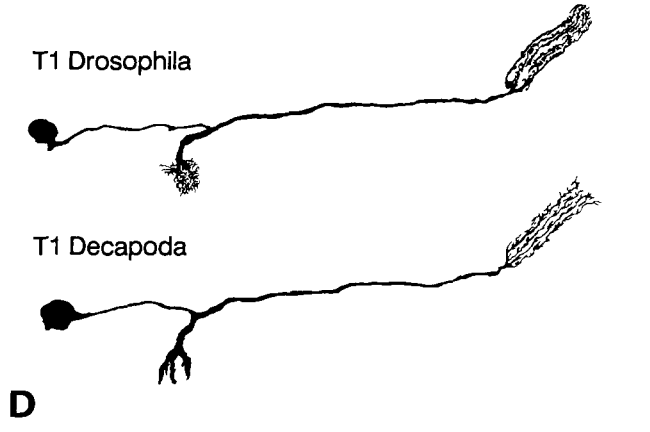
Fig. 3A–D. Camera lucida drawings of receptor cell axons and of neurons connecting the lamina with the medulla. The composite picture represents the view of a horizontal section through the neuropiles and the first optic chiasm at the level of Cuccatti's bundle. The parallel curved lines drawn across the medulla mark the borders of the 10 layers, which are defined by the arborizations of neurons as explained in the text. The serpentine layer (M7) separates the distal from the proximal medulla. Receptor axons of the retinula cells *R1–R6* terminate in the lamina, while the axons of the retinula cells *R7* and *R8* project into the medulla. Two variants of each of the 2 types of long visual fibers have been drawn. The long types of *R7* and *R8* terminals are rare. Nine interneurons are depicted, which arborize in lamina and medulla. Their stratifications define the borders of the 6 layers of the distal medulla. The 5 lamina monopolar neurons (*L1–L5*) have their cell bodies in the cell body rind distal to the lamina neuropile. The cell bodies of 2 interneurons (*T1* and lamina wide field *Lawf1*) lie in the cell body rind of the distal medulla; *C2* and *C3* cell bodies are located between the posterior rim of the proximal medulla and the lobula plate. With the exception of the *Lawf1* neuron one representative of depicted neurons occurs per column. The number of *Lawf1* neurons and their distribution amongst cartridges is still unclear for *Drosophila*. The *scale bar* applies to Figs. 3–20; **B** composite of camera lucida drawings showing the lamina tangential neuron (*Lat*) with arborizations in the lamina cell body rind, a lamina intrinsic neuron (*Lai*), and terminals of short receptor axons (*R1–R6*). The *Lat* neuron was impregnated only in one fly. Unfortunately its axonal projections could not be traced; **C** comparison of the shapes of *L1* and *L4* neurons of *Drosophila melanogaster* and *Musca domestica*. The *Musca* neurons have been redrawn from Strausfeld and Campos-Ortega (1972) and adjusted to the size of the *Drosophila* neurons. Please note the much more regular structure of the *L1* dendrites in *Musca* as compared to *Drosophila*. The different pattern of arborization suggests species-specific connectivity of both neuronal cell types; **D** comparison of the shapes of *T1* neurons of *Drosophila melanogaster* and of a decapod (Hanström 1928). The similarity of the neurons is remarkable and may suggest that they perform a basic and conserved visual function

and *R8* axons terminate in different medulla layers without branching. This is true as well in *Drosophila* (Fig. 3A).

Just before they enter the medulla neuropile, *R7* terminals have a characteristic swelling. The axons are rather slim in layers M1–3 before they form a club-, foot- or head-shaped terminal in the distal half of layer M6. A rare, but consistently occurring variant terminates more deeply in the proximal half of this layer (Fig. 25B, arrows). There also may be two types of *R8* terminals, some ending in layer M3, others in layer M4 (Fig. 3A). The long variants of *R7* and *R8* seem to be too rare to be correlated with the distribution of the different kinds of pigments in the pale and yellow *R7* rhabdomeres of *Drosophila* (N. Franceschini, as cited in Heisenberg and Wolf 1984; review for







Musca: Hardie 1986). In *Calliphora erythrocephala* Strausfeld and Wunderer (1985), confirmed by Nässel et al. (1988), have found that dorsal marginal long receptor endings project more deeply into the medulla than the majority of *R7/8* axons. The variability described here by us is a different phenomenon, which is not restricted to marginal columns. It may correspond to the variability of "normal" *R8* receptor terminals, which is also apparent in *Musca* and *Calliphora* (Nässel et al. 1988). Whether it has some functional significance is still unclear. Specialized dorsal rim receptors in *Drosophila* have been seen in electron microscopical (EM)-sections at the level of the retina (unpublished results). They were, however, not Golgi impregnated.

Comparison of the shapes and the different termination strata of *R7* and *R8* clearly suggest that their terminals are connected to different (albeit probably overlapping) sets of postsynaptic neurons. Inside the medulla columns *R7* and *R8* axons are close together (Campos-Ortega and Strausfeld 1972b). Their different immunoreactivity has provoked the postulation of an antagonistic interaction of *R7* and *R8* (Nässel et al. 1988).

3.3. Intrinsic columnar neurons of the optic lobe

3.3.1. Neurons connecting the lamina with the distal medulla and lamina amacrine cells. The layer between the basement membrane of the compound eye and the lamina neuropile contains the cell bodies of five types of lamina monopolar neurons (*L1–5*). These neurons differ in shape and connectivity. The latter has been shown by electron microscopy in *Musca domestica* (Boschek 1971; Strausfeld and Campos-Ortega 1977; Shaw 1981). Some data about the synaptology of lamina neurons in *Drosophila* are reported by Hauser-Holschuh (1975). A more detailed investigation on *Drosophila*, that indicates some differences with respect to the connections in *Musca* is in preparation (S. O'Neil and J.A. Meinertzhagen, in preparation). The differences in the shapes of the neurons in *Musca* (Strausfeld and Campos-Ortega 1972; Strausfeld and Nässel 1981) and *Drosophila* (Fig. 3A, C) should justify further comparison between these two species.

L1 and L2 neurons. Judged from the size of their axons *L1* and *L2* seem to be the most important relay neurons of the fly's lamina. Their radially arranged dendrites (Fig. 3A) are postsynaptic to all six receptor terminals of the cartridge in a tetrad type of synapse (Burkhardt and Braitenberg 1976; Nicol and Meinertzhagen 1982). At the EM level *L2* can be distinguished from *L1* due to its feedback synapses onto receptor terminals (Meinertzhagen and O'Neil 1988). In addition to the regularly arranged dendrites along the length of a cartridge, *L1* neurons (Strausfeld and Campos-Ortega 1972) and *L2* neurons (Strausfeld and Nässel 1981) in *Musca* may possess a garland of short collaterals near the distal surface of the lamina neuropile. This collar seems to be an optional feature as both cell types are sometimes shown without it. According to the data of Strausfeld and Campos-Ortega (1977) summarized in Shaw (1981), *C2* neurons in *Musca* may synapse with *L1* neurons in the collar region. The *L1* and *L2* neurons of *Drosophila* differ from their counterparts in *Musca* (Fig. 3C), argued elsewhere to be homologous (Shaw and Meinertzhagen 1986), in that collars are always absent and

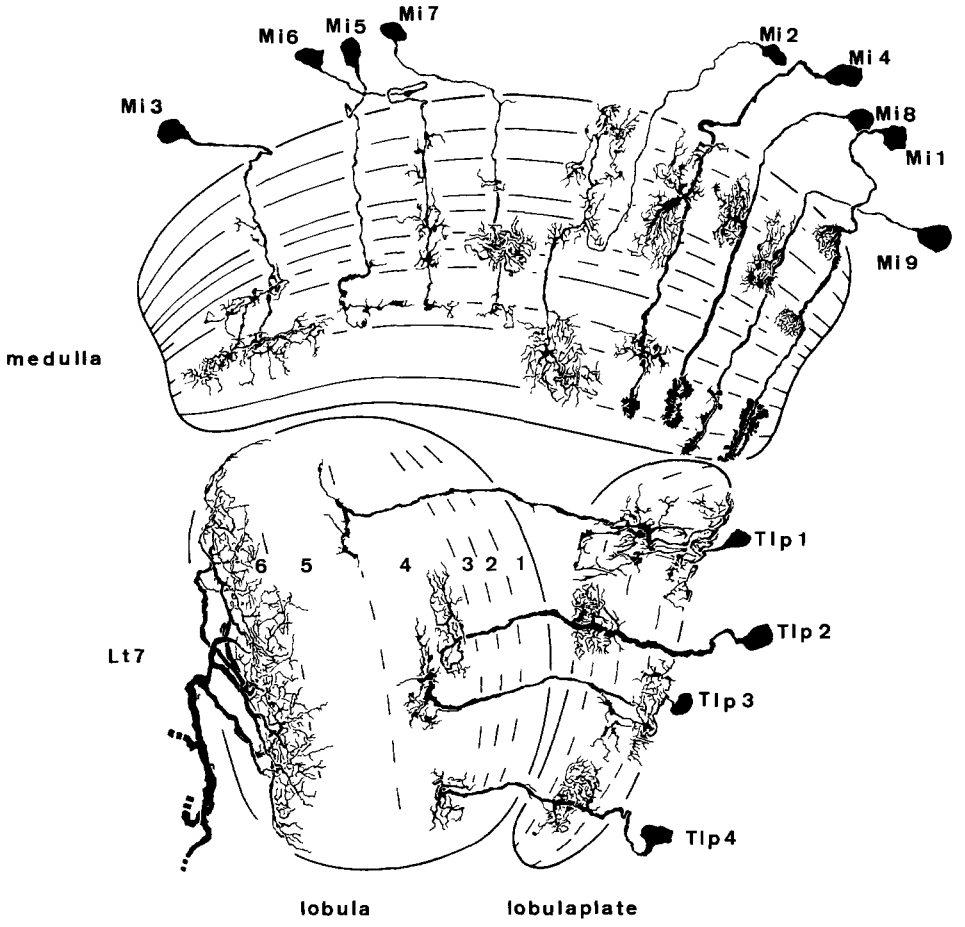


Fig. 4. Composite of camera lucida drawings showing medulla intrinsic neurons (*Mi*), translobula-plate neurons (*Tlp*) and the arborizations of the lobula tangential 7 (*Lt7*) in the most anterior layer of the lobula. Medulla intrinsic neurons connect the distal with the proximal medulla. Their cell bodies are located in the medulla cortex. *Mi1* is very frequently stained. It has been called *Sut* (small field unilateral tristratified neuron) in earlier work (Fischbach 1983a). *Tlp* neurons have their cell bodies in the cortex of the lobula plate. They connect different layers of the lobula plate with layer Lo4. Their narrow stratifications suggest that this layer of the lobula could be further subdivided

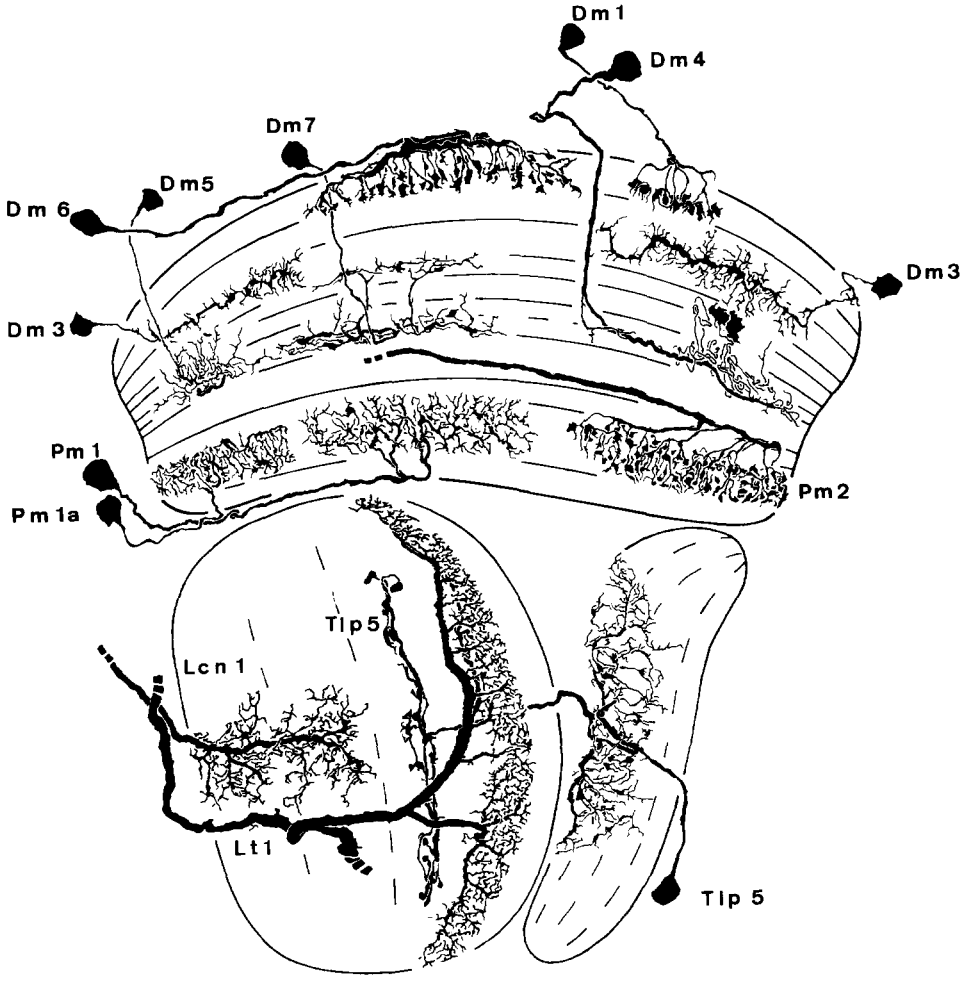


Fig. 5. Composite of camera lucida drawings showing amacrine cells of the distal (*Dm*) and proximal medulla (*Pm*), as well as the wide-field translobula-plate neuron *Tlp5*, the lobula tangential *Lt1* and the lobula columnar neuron *Lcn1*. The assembly of *Dm3* line amacrine cells forms a thin network of fibers at the border between layers M2 and M3. This network is visible in silver-stained preparations as a thin, intensely stained stratification of the distal medulla (see arrow in Fig. 1D). It is noteworthy that the *Pm1* and *Pm2* neurons arborize only in layer M9. This is one of the criteria for separating layers M8 and M9. The arborizations of the lobula tangential *Lt1* in the lateral protocerebrum can be seen in Fig. 22A

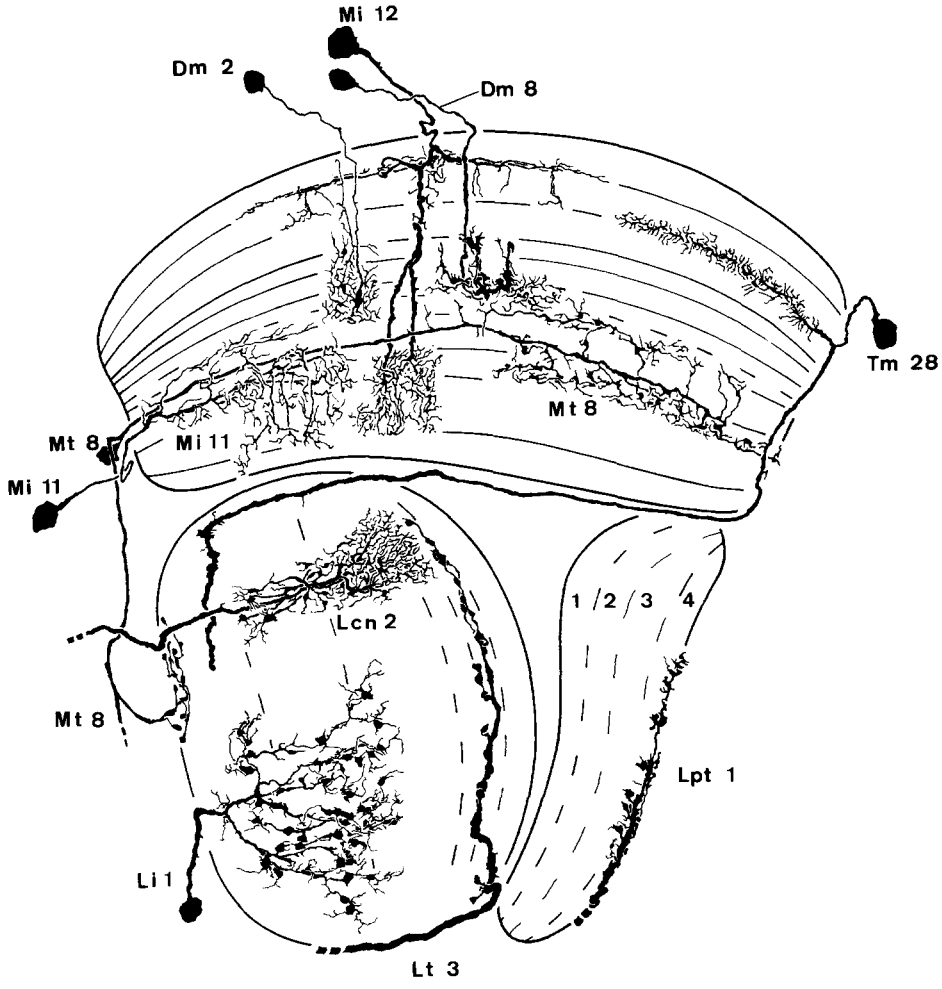


Fig. 6. Composite of camera lucida drawings showing various cell types in the optic lobe. Most interesting is the transmedullary neuron *Tm28*, which has a long unilateral arborization in the layer of line amacrines (see Fig. 5) and a retinotopically orientated terminal in layer 6 of the lobula. This neuron has so far only been found in parts of the neuropile that subserve the frontal visual field. The same is also true for *Mt8*, a tangential neuron innervating the lateral protocerebrum and the innermost layer of the lobula. The terminal arborizations of *Mt8* are depicted in Fig. 21 B

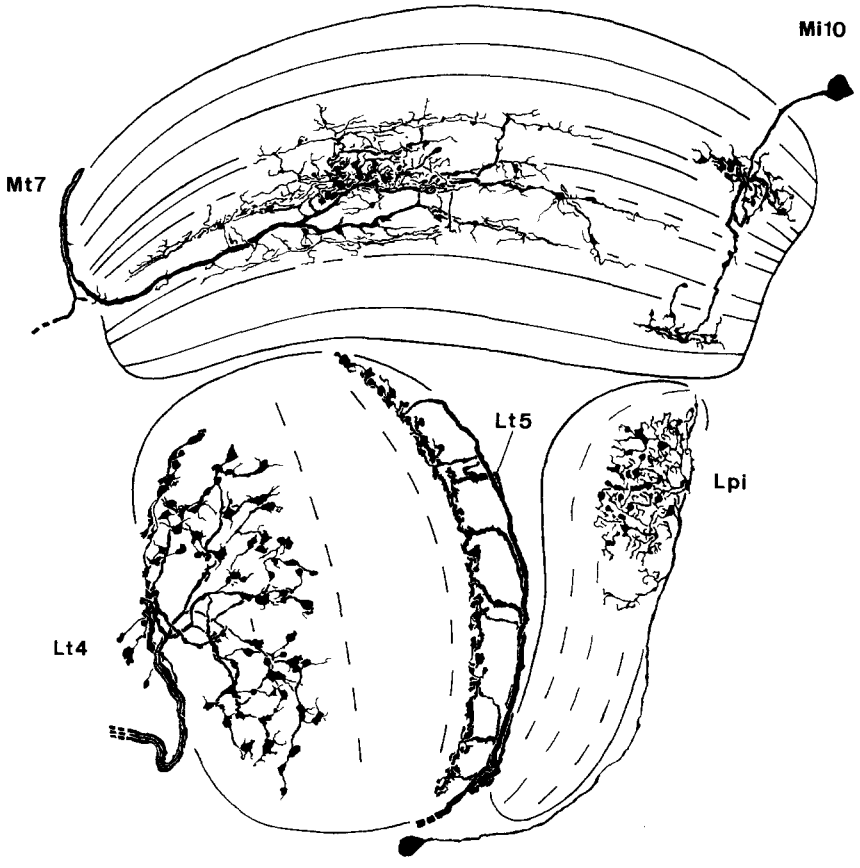


Fig. 7. Composite of camera lucida drawings showing various cell types in the optic lobe. the central brain projections of the medulla tangential neuron *Mt7* are depicted in Fig. 21 A. *Mi10* connects the *L3* layer (M3) with M9. *Lt5* is a beautiful, stripfield tangential element of the lobula in layer Lo2, while *Lt4* arborizes more diffusely in layers Lo5 and 6. The lobula plate element shown (*Lpi*) is the only lobula plate amacrine encountered in this study

Table 1. The relative depth of Golgi-impregnated T4 and T5 terminals in the lobula plate

Depth[a]	Number of T4 terminals		Number of T5 terminals		T5 neuron terminating
	Absolute	%	Absolute	%	
0–10	1	1.3	3	2.5	
10–20	10	12.5	24	20	T5a
20–30	6	7.5	2	1.7	
30–40	9	11.3	11	9.2	
40–50	7	8.8	19	15.8	T5b
50–60	0	0	3	2.5	
60–70	10	12.5	18	15.0	T5c
70–80	10	12.5	9	7.5	
80–90	19	23.8	21	17.5	T5d
90–100	8	10.0	10	8.3	

[a] The depth of the deepest terminal arborization of a cell is given in percent of total thickness of the lobula plate at that position where the cell was impregnated. Selective impregnation of cells terminating at certain positions cannot be ruled out completely

the arrangement of spines along the length of a cartridge is less orderly.

We were not able to distinguish *L1* and *L2* reliably in *Drosophila* merely from their Golgi-impregnated shape in the lamina. *L1* and *L2* neurons in *Musca* and *Drosophila* can most easily be identified by tracing their axons into the medulla (see Fig. 3A). The pattern of terminal arborizations of *L1* neurons in the medulla of *Musca* and *Drosophila* define two medulla layers by the location of their distal and proximal terminals (in layers M1 and M5, respectively). However, the distal *L1* specialization is more pronounced in *Drosophila*, while in *Musca* the neuron shows a small additional arborization in the proximal part of M2 (see Fig. 3c). The *L2* terminal defines the second layer of the medulla, immediately proximal to the distal *L1* arborization. The L2 layer is shared with the *T1* medulla specialization.

The L3 neuron. *L3* is visibly distinct from *L1* and *L2* at the level of the lamina. The spines are arranged to one side like bristles on a toothbrush (see Fig. 3A; please note that no preferential orientation of the *L3* spines was found). In *Drosophila* the *L3* axon is spiny along its entire extent in the lamina neuropile, and the spine length decreases proximally. In *Musca* the *L3* spines are entirely restricted to the distal two-thirds of this neuropile (Strausfeld and Campos-Ortega 1972, 1973b). *L3* axons terminate in the third layer of the medulla. In contrast to the *L1* and *L2* specializations, *L3* terminals may invade more than one medulla column. Compare the lateral extent of the terminal arborizations of these neurons in Fig. 3A.

The L4 neuron. The differences between *L4* neurons of *Drosophila* and *Musca* are most striking (Fig. 3C). In the proximal lamina of *Musca*, *L4* neurons have two to three collaterals, which form reciprocal synapses with *L4* collaterals of two neighboring cartridges and are presynaptic to *L1* and *L2* neurons of their own and two neighboring cartridges (Braitenberg and Strausfeld 1970; Braitenberg and Debbage 1974). Distally located dendrites that are postsynaptic to lamina amacrine cells have to be considered as the input device for this *L4* network (Strausfeld and Campos-Ortega 1973a). In *Drosophila* the distal dendrites are absent. Furthermore, the shape of the proximal collaterals is different from that in *Musca*. They originate at the proximal neuropile border of the lamina and extend distally being confined to the inner one-third of the neuropile. Their lateral extension is surprisingly narrow compared to that of their *Musca* homologues, and comparable to that of the T1 basket (Fig. 3A, D). They synapse on the *L4* collaterals of neighboring cartridges (S. O'Neil and I.A. Meinertzhagen, in preparation), as in *Musca*, but do so with a different morphology.

In the medulla, the *L4* arborizations also look distinctly different in the two species (Fig. 3C). It is therefore possible that evaluation of serial electron micrographs will reveal connectivity differences between *L4* neurons in *Musca* and *Drosophila*.

The L5 neuron. *L5* neurons hardly arborize in the lamina. They have zero to two small spines near the distal surface of the lamina neuropile. In *Musca L5* is said to be postsynaptic to the lamina wide-field neuron and to the amacrine cells (Strausfeld and Campos-Ortega 1977). It, therefore, seems possible that the activity of the *L5* neuron represents a measure of the activity in several neighboring cartridges.

At first glance the *L5* terminals in the medulla resemble *L1* terminals (Fig. 3A). The arborizations, however, show a much finer branching pattern and the distal specialization is not restricted to the *L1* layer but reaches into the *L2* layer.

Amacrine cells. Lamina intrinsic cells (amacrine cells) did not stain frequently. The best examples are from mutant flies (no shown). Fig. 3B contains a reconstruction of a wild-type intrinsic neuron (*Lai*). Its shape is similar to that already described in *Musca* (Campos-Ortega and Strausfeld 1973). The α-type climbing fibers described by Trujillo-Cenóz (1965) derive from this intrinsic neuron in *Musca* (Campos-Ortega and Strausfeld 1973). In this species, single cartridges may derive their α-fibers from one to six different amacrine cells (Campos-Ortega and Strausfeld 1973). Whether this is true in *Drosophila* as well has to be shown by Golgi electron microscopy. Lamina amacrine cells have been compared with horizontal cells in vertebrate retinas and their possible role in neural adaptation and lateral inhibition has been discussed (Strausfeld and Campos-Ortega 1977).

3.3.2. Neurons connecting the distal or proximal medulla with the lamina (T1, lamina wide-field and C-cells). The medulla neurons *T1*, lamina wide-field (*La wf*), and centrifugal cells, *C2* and *C3*, project into the lamina (Fig. 3A). The cell bodies of *T1* neurons are located in the outer layer of the distal cell body rind of the medulla (medulla cortex), which as a whole is formed by the outer optic anlage (Meinertzhagen 1973; Hofbauer 1979). The fiber of the *T1* cell body branches at the medulla surface in a T shape to form the linking fiber between a bushlike arborization in the distal medulla and a bundle of climbing fibers in the lamina. The latter form a characteristic basket, and have been shown in *Musca* to contribute β profiles to the cartridge cross section (Campos-Ortega and Strausfeld 1973); these are closely associated with α profiles of amacrine cells (see above).

In *Musca* and *Lucilia*, T1 neurons, like *L4* and *L5*, are third-order interneurons. Despite earlier reports in the literature they do not seem to obtain direct input from photoreceptors (Nicol and Meinertzhagen 1982; Shaw 1984). In *Lucilia* T1 neurons are instead said to be postsynaptic to *L2* neurons at tetrads, which include also the feedback synapse of *L2* to photoreceptors (Shaw 1984). In *Drosophila* T1 is replaced by *L4* at this synapse (Meinertzhagen 1989). In *Lucilia* (Shaw 1984) and *Musca* (Boschek 1971; Burkhard and Braitenberg 1976) *T1* cells also receive α input at complex glial invaginations called gnarls, and there are reciprocal T1 inputs upon α. In *Drosophila* the interface between α- and β-fibers differs from its *Musca* homologue at the ultrastructural level (Hauser-Holschuh 1975). The gnarls are non-invaginating and there are no β inputs upon α (Meinertzhagen 1989).

T1 neurons have been impregnated very often in our preparations. By comparing *T1* cells from different regions of the retinotopic array it is apparent that there are no obvious variations of *T1* shape in the antero-posterior or dorso-ventral plane. Recent labeling of the entire *T1* array using a specific antibody also shows its homogeneity (Buchner et al. 1988).

T1 cells are remarkable insofar as quite similar cell types have already been described to connect the first and second optic neuropiles underlying compound eyes of so distantly related arthropods as Chelicerata and Decapoda (Hanström 1928; Strausfeld and Nässel 1981). In Fig. 3D the *T1* neuron of a fly is compared with that of a decapod.

The cell bodies of lamina wide-field neurons are part of the medulla cortex. It is possible that two distinct types exist in *Drosophila*. The tangential *La wf1* is depicted in Fig. 3A, and part of the tangential *La wf2*, in Fig. 24F. In the lamina the arborizations of *La wf2* are restricted to the most distal layer occupying about 20–25 cartridges (Fig. 24F). The specializations of *La wf1* are more widely spread and seem to cover about 60 cartridges (Fig. 3A). In *Musca* two different kinds of lamina wide-field neurons have been described as well (named lamina tangentials *Tan1* and *Tan2;* Strausfeld 1970; Strausfeld and Nässel 1981). One (*Tan1*) is said to be presynaptic to *R1–6* and *L1–3*, the other (*Tan2*) is presynaptic to *L5* (Strausfeld and Campos-Ortega 1977).

At the level of the medulla we did not detect differences between the two types of lamina wide-field neurons of *Drosophila*. They arborize at the distal surface of the medulla and seem to cover a circular area of about 20 columns; several linking fibers project into the M3 layer, where a rich arborization enters 30–40 columns (Fig. 3A). The main fiber of the lamina wide-field neurons ascends via the first optic chiasm into the lamina. The similarity of the medulla specializations of *la wf1* and *la wf2* leave some doubt whether the two forms really represent two different cell types or only two variants of one. So far both elements have not been seen together in the same preparation.

C2 and *C3* neurons are derived from the inner optic anlage because their cell bodies lie posteriorly between the proximal face of the medulla and the lobula plate (Fig. 3A). In the medulla neuropile the arborizations of *C3* and especially *C2* cells extend into neighboring columns at several levels (Fig. 3A). The layers and extent of *C2* and *C3* arborizations are very similar in *Drosophila* and *Musca*, although *Drosophila* C2 neurons show an additional arborization just proximal to the serpentine layer. So far, the synaptic connections of *C2* and *C3* cells have been described in the lamina of *Musca* only (Strausfeld and Campos-Ortega 1977). In that species, their synaptic specializations within the lamina are each restricted to a single cartridge. *C2* is presynaptic to *R1–6* and *L1–3*, while *C3* is presynaptic to *L1* and *L2*. *C2* fibers contain GABA and glutamic acid decarboxylase in the blowfly (Datum et al. 1986). Therefore, it is reasonable to speculate that *C2* is part of a negative feedback loop to the lamina.

3.3.3. Neurons connecting the distal and proximal medulla and medulla amacrine cells. The medulla appears to be the most complex of the optic neuropiles with regard to number of cell types. Campos-Ortega and Strausfeld (1972a) estimated that in *Musca* 120 different cell types participate in the neuropile formation of any single column. Among these are the receptor terminals *R7* and *R8*, 10 lamina-medulla neurons, 34 columnar neurons occurring in every column, and 74 neurons of less frequent periodicity (Campos-Ortega and Strausfeld 1972a). There is no evidence for any less complexity in *Drosophila*. So far, this fact has deterred the electron microscopists from analyzing medulla synaptology. The analysis of neuronal cell types participating in the formation of the medulla neuropile from Golgi impregnation is a necessary first step for subsequent studies at the ultrastructural level.

The medulla neuropile of insects and of crustaceans consists of a distal and proximal part, which are separated by the serpentine layer formed by the axons of large medulla tangential neurons (Strausfeld and Nässel 1981). Cells participating in the formation of the distal neuropile are mainly derived from the outer optic anlage (Meinertzhagen 1973) and their cell bodies are situated in the distal medulla cortex. The neuropile of the proximal medulla is formed in part by neurons derived from the inner optic anlage, and many of its cell bodies are situated in the rind between the proximal face of the medulla and the lobula plate. Therefore, neurons connecting the distal and proximal medulla connect distinctly different neuropiles and are here considered separately from those neurons that arborize exclusively in one or the other.

In Figs. 4 and 6, 10 types of medulla intrinsic neurons (*Mi* cells) are shown that connect the distal with the proximal medulla neuropile. Note the small-field unilateral tristratified *Mi1* neuron (Fig. 4). Its two fine-branched, densely packed dendritic stratifications in the distal medulla coincide with those of the *L1* terminal. This suggests that *Mi1* gets direct input from the lamina. The axonal terminals of *Mi1* in the proximal medulla are very characteristic. The

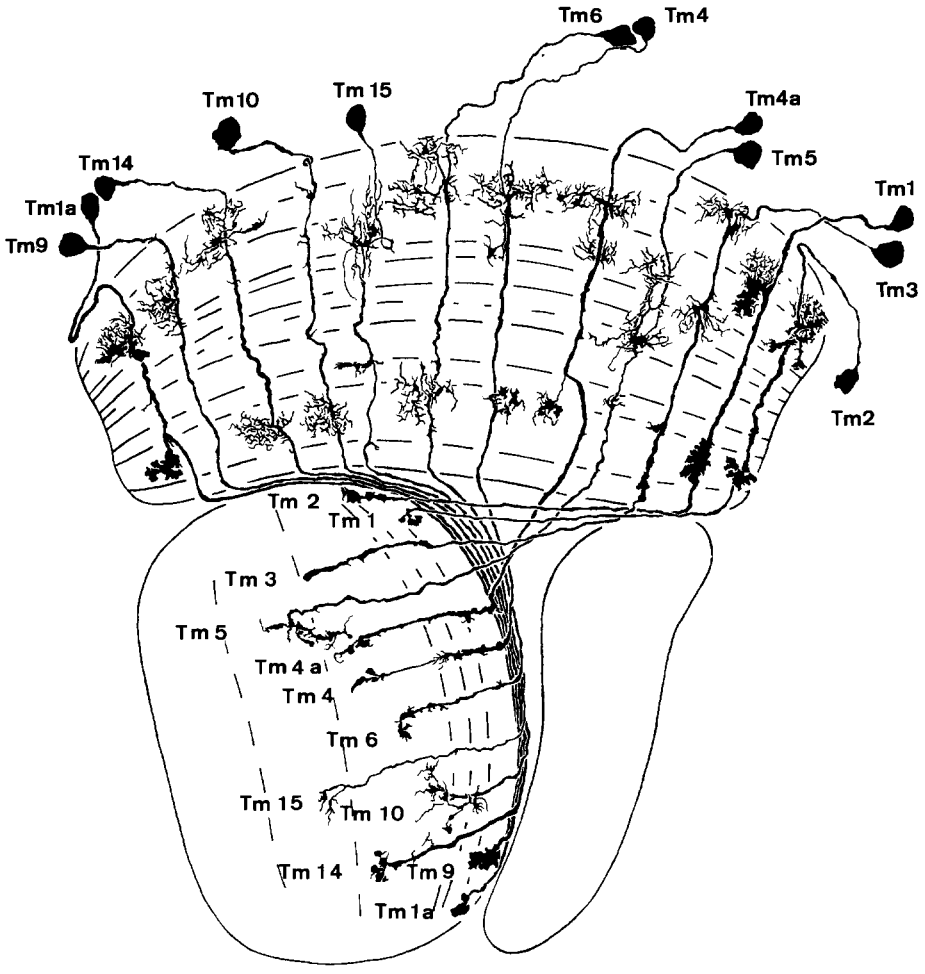


Fig. 8. Composite of camera lucida drawings showing various types of transmedullary neurons. These neurons have their cell body distal to the medulla neuropile, arborize in one or several layers of the medulla and their axons project into the lobula. Some *Tm* variants may send short branches into the lobula plate (e.g., *Tm3Y* in Fig. 13 is a variant of *Tm3*, and *Tm5Y* in Fig. 11 a variant of *Tm5*), but neurons with a cell body in the distal medulla cortex terminating exclusively in the lobula plate do not exist. It is instructive to compare variants of the same type, e.g. *Tm1* with *Tm1a*, and *Tm18* with *Tm18a*. In these cases the arborizations in the proximal medulla either sprout directly from the main fiber or they are connected to it by a stalk. It is remarkable that most *Tm* neurons do not have any specializations in M10 (the T4 layer). *Tm1*, *Tm2*, and *Tm9* are the only *Tm* neurons that terminate superficially in the lobula

axon branches at the inner face of the medulla to form two to three varicose recurrent terminal specializations, which extend backwards up to the inner border of layer M8. Due to its high frequency of impregnation *Mi1* is well suited to serve as a natural marker of medulla layers in Golgi preparations (see Figs. 26A, C–E; 27D, F). We found examples of this cell type in all parts of the retinotopic field. As in the case of *T1* neurons and long visual fibers, no obvious position-dependent variation of their shape was found (e.g., Fig. 26E). *Mi1* may be homologous to the small field unilateral bistratified (*Sub*) neuron of the blow fly (Strausfeld 1984).

The structures of the terminals of *Mi8* and *Mi9* in the proximal medulla are *Mi1*-like, although that of *Mi8* resides in layer M8 only. These two cells possess only one stratification in the distal medulla, which does not reach into the *L1*-layers M1 or M5. They may contact the other lamina monopolar neurons. *Mi9* is reminiscent of the small-field unilateral bistratified neuron described in *Musca* (Strausfeld 1976; his Plat 7.12D).

Noteworthy is also *Mi3* (Fig. 4). This neuron has radial specializations in layers M6 and M8 with small extensions into layer M9. The specializations in M6 arise due to two or more recurrent fibers originating from layer M8. A neuron of very similar shape and position has been described in *Calliphora* (Strausfeld 1970; called *Lfbb1* in his Fig. 79).

Typical examples of amacrine cells are shown in Figs. 5 and 6. They have been called *Dm*, if they aborize exclusively in the distal medulla, or *Pm*, if they branch exclusively in the proximal medulla. Amacrine cells are often restricted to one layer (e.g., *Pm1*, *Dm1*). Others connect different layers but these do not necessarily abut each other (e.g., *Dm7*). Comparison of the amacrine cells with those of *Musca* (Strausfeld 1976; unlabeled cells in his plate 7.12D, E) suggest that related types seem to exist in both species, but interspecific variability seems to be pronounced, especially among *Dm* cells. A possible exception are the unilateral line amacrine cells (*Dm3*, forming a stratum in reduced-silver preparations; see distal arrow in Fig. 1D), which have been reported to occur in *Musca* (Strausfeld 1976), *Calliphora* and *Eristalis* (Strausfeld 1970). The *Pm* cells shown in Fig. 5 also seem to have their homologous counterparts in *Musca* (Strausfeld 1970).

For reasons explained below, the neuron called *Mt14* in Fig. 27E, which resides entirely inside the medulla, is not listed under this heading.

3.3.4. Neurons connecting the distal medulla with the lobula (Tm cells). Figs. 8–11 depict columnar cell types with a cell body in the distal rind of the medulla that penetrate the medulla neuropile into the lobula (transmedullary neurons, *Tm* cells). A common characteristic of these neurons is that they arborize only at certain levels of the medulla neuropile, different types choosing different layers. They are also distinguished by the lateral extent of their arborizations and by the depth of their projections into the lobula.

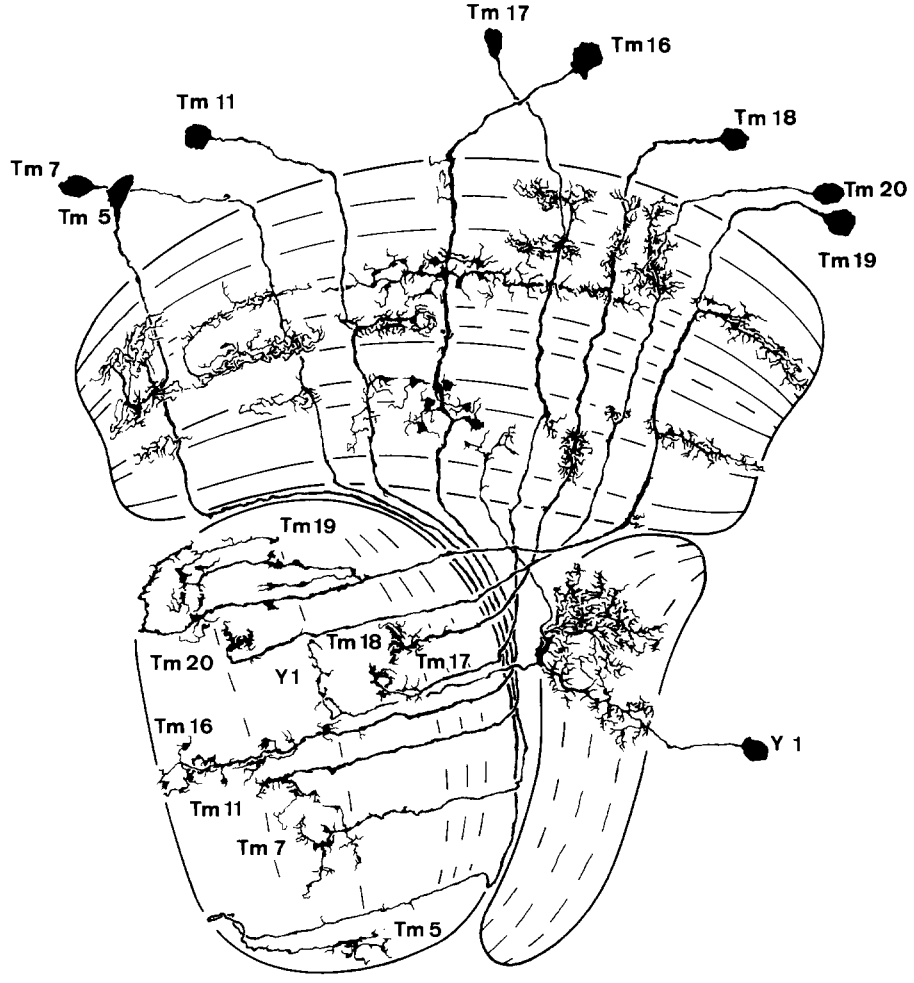


Fig. 9. Composite of camera lucida drawings showing various types of *Tm* cells and the *Y1* cell. The *Y1* cell has its cell body in the rind of the lobula plate and arborizes extensively, in an asymmetrical manner, in this neuropile. It sends one relatively small branch into the medulla, another into the lobula. It is apparent that the unilateral lobula terminal occupies the same retinotopic region as the bushy lobula plate arborization. The unusual appearance of the terminal of the *Tm5* neuron shown is noteworthy. The cell terminates in the lobula in the same layer in which other *Tm5*(Y) neurons arborize (Figs. 8, 10, 13), but during its growth the axon apparently missed its target neurons in that layer at the first encounter and had to grow back again. Another remarkable cell type is *Tm19*, which has two unilateral dendritic extensions, one in M3, the other in M8. *Tm19* terminates in Lo4–6 again in a unilateral fashion. The axonal terminals arborize in that region of the lobula that subserves the same visual field as the posterior medulla. Please note that the *Tm* neurons hardly have any specializations in M10

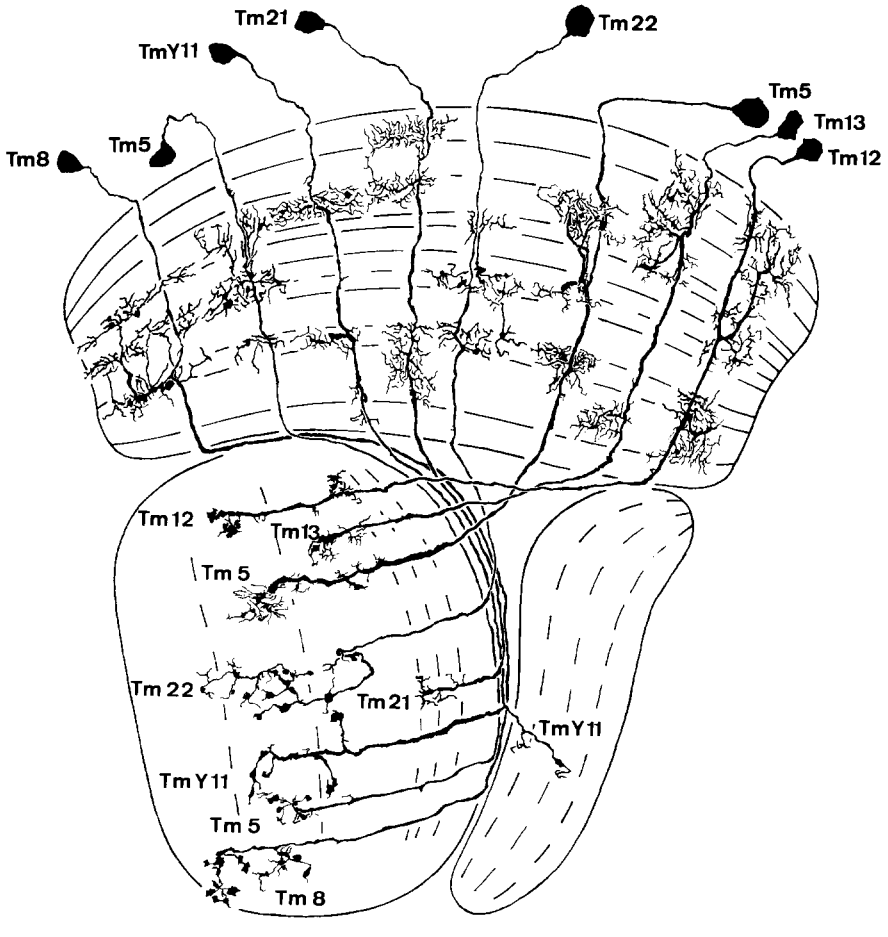


Fig. 10. Composite of camera lucida drawings showing *Tm* cells and *TmY11*. Two additional *Tm5* neurons have been included to give an impression of the variability between cells of the same type (see also Figs. 8, 9, 13). *Tm8, Tm12, Tm13, Tm21,* and *Tm22* also spare the M10 layer. *Tm22* and *Tm8* have several characteristic features in common (e.g. the recurrent linking fibers from the arborizations in M8 to M6 and M4), and might be different variants of one *Tm* type, although the widths of their dendritic arborizations are different (see Discussion)

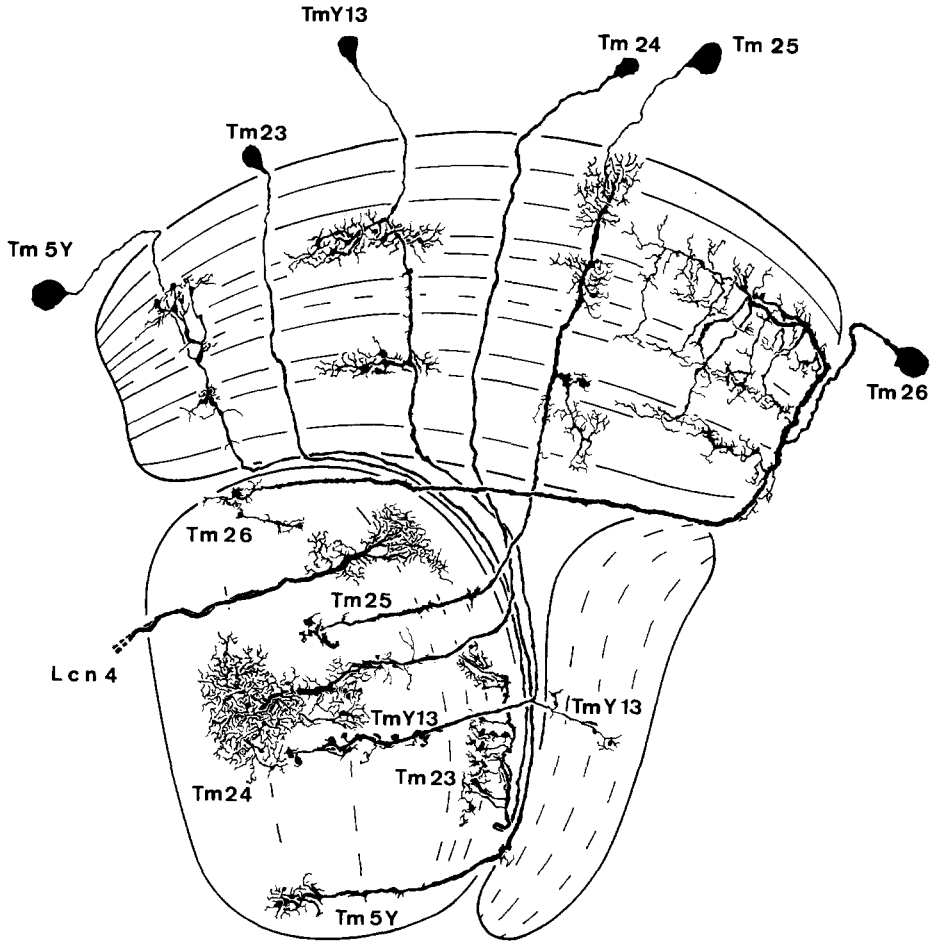


Fig. 11. Composite of camera lucida drawings showing various types of *Tm* cells, *TmY13*, and the lobula columnar neuron *Lcn4*. *Tm23* and *Tm24* are strange insofar as they do show no branching in the medulla. These neurons seem to function as local interneurons of the lobula. *Tm26* has been identified only in those parts of the optic neuropiles that subserve the frontal visual field. Its long unilateral arborizations display an interesting feature, i.e. the fine branches in M4, M6, and partially also in M8 are derived from linking fibers descending from the dendrites at the interface of layers M2 and M3

In most cases the shape of their specializations in the medulla and lobula are very different, showing extensive fine branching in the medulla only. They thus have a comparable architecture to lamina monopolar cells between the lamina and medulla.

Due to the selective stratification of *Tm* neurons as well as of lamina monopolar neurons in the medulla, synaptic contacts between certain neuronal pairs are more likely than between others. The consequence of this is the obvious existence of different pathways for the afferent flux of information. Specifically, the most prominent lamina monopolar neurons (*L1–3*) seem to be connected to different *Tm* neurons. This can nicely be exemplified by pointing to the implications of *Tm1 and Tm3* structure (a quantitative analysis of this kind of data using all columnar cell types documented in this report is in preparation). As seen in Fig. 8 *Tm3* will hardly receive input from *L2* (Fig. 3A); its stratifications overlap selectively the two *L1* specializations as well as those of *Mi1* (Fig. 4) in M1 and M5. Therefore, *L1 Tm3*, and *Mi1* qualify to interact. The arborizations of *Tm3* and *Mi1* in the proximal medulla further allow for their direct connection to the *T4* system (see Fig. 14).

Unlike most *Tm* neurons, which project deeply into the the lobula (like *Tm3*), *Tm1, Tm2* and *Tm9* (Fig. 8) terminate superficially in the lobula. As the latter neuron was seen much less frequently impregnated than *Tm1* and differs only by its lack of arborizations in the proximal medulla, the possibility exists that *Tm9* is a rare variant of *Tm1*. This may be true for *Tm2* as well. Due to the stratification of *Tm1*-like neurons in the distal medulla (which avoid the *L1* layers) it is probable that their main input is not from *L1*. Their arborizations overlap with other lamina monopolar neurons, namely *L2* and *L3*. In the proximal medulla *Tm1*-like neurons arborize in M9. In contrast to *Tm3*, they have no specializations in M10 and it is, therefore, unlikely that they represent a major input onto *T4* neurons. The *Tm1*-like cells arborize superficially in the lobula, which means they are in a position to mediate visual input directly onto the *T5* system (see below). Hence, the visual pathways leading to the lobula plate's fine-grained visual input via *T4* and *T5* cells (see Fig. 14) are already separated at the level of the lamina. Using a structural analysis of the blow fly's visual system and a homologous set of neurons, Strausfeld (1984) reached the same conclusion (the homologous counterpart of *Drosophila*'s *Tm3* neuron is called in his account *Tm5*, that of *Mi1* is called *Sub*; see section 3.3.3.). He hypothesizes that in the lobula plate the convergence of *T5* and *T4* terminals onto dendrites of the giant fiber system allows for computation of movement.

While *Tm1* and *Tm3*, due to their structural characteristics, immediately stimulate the above functional speculations, not much can be said about possible roles of other *Tm* neurons. However, their large number implies that flies are able to filter different sets of visual data with different sets of retinotopically organized neuronal networks.

Two special cases of *Tm* neurons shown in Fig. 11 need mentioning. These neurons (*Tm23, Tm24*) do not seem to

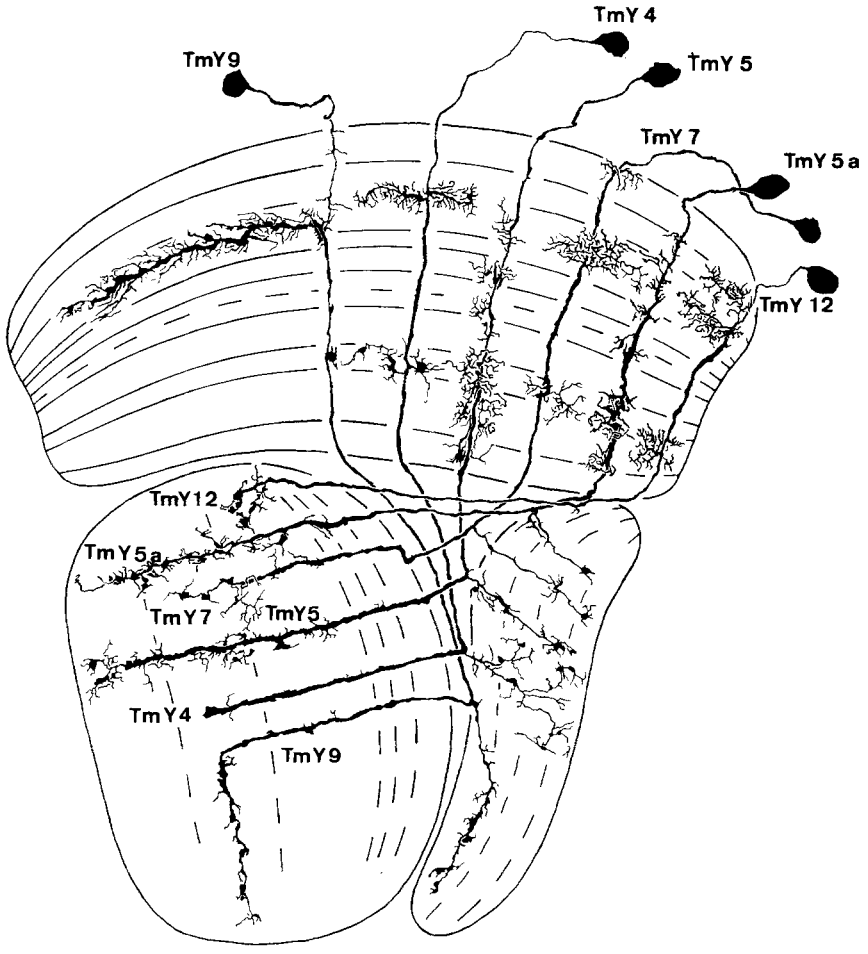


Fig. 12. Composite of camera lucida drawings showing various types of *TmY* cells. *TmY9* is a remarkable cell with some resemblance to *Tm28*. However, its retinotopically oriented terminal in the lobula is in layer Lo5 and a parallel branch projects anteriorly along the border of layer Lop1 and Lop2 of the lobula plate. *TmY5* is one of the *TmY* cells that does not spare M10

arborize in the medulla. Functionally they may therefore represent two kinds of lobula amacrine cells. Alternatively, like monopolar neuron *L5* in the lamina (Figs. 3a, 24I), they may have rare synaptic contacts in the medulla. One of these *Tm* types has been seen in optic lobe rudiments of eyeless *sine oculis* flies as well (Fischbach 1983a). In view of their lack of medulla spines it is perhaps not surprising that they do not depend upon innervation from retina and lamina neurons for survival which is, however, the case for most other *Tm* neurons (Fischbach 1983a).

3.3.5. Neurons connecting the distal medulla with both the lobula and the lobula plate (TmY cells). Transmedullary *Y* cells have many features in common with the *Tm* neurons. Their cell bodies are located in the distal cell body rind of the medulla and they project through the medulla neuropile into the lobula. However, they differ from *Tm* neurons in that they branch within the inner optic chiasm and send a collateral into the lobula plate (see Figs. 10–13). In Figs. 11, 13 it can be seen that this arborization is sometimes tiny. The overall shape differences between *Tm* and *TmY* neurons are therefore graded, and in the extreme cases very small. Some individual members of a retinotopic set of transmedullary neurons may or may not form the branch into the lobula plate. We believe this to be the case in the sets of *Tm5* and *Tm3* neurons. Variants with a branch into the lobula plate have been labeled by a *Y* after their *Tm* number (see different *Tm5* neurons in Figs. 8, 9, 10 and the variants *Tm5Y* in Figs. 11, 13, or *Tm3* in Fig. 8 and *Tm3Y* in Fig. 13). Other neuronal types, however, always branch into the lobula plate. These have been classified as *TmY* cells, irrespective of the size of this branch (e.g., *TmY2* and *TmY10* in Fig. 13). As in most *Tm* neurons (one exception is *Tm9*, Fig. 8) all *TmY* cells arborize in both the distal and proximal medulla.

Our terminology differs from that of Strausfeld and Blest (1970) and Strausfeld (1970, 1976). These authors call all columnar neurons that arborize in the medulla, the lobula, and the lobula plate *Y* cells, irrespective of the position of the cell bodies, while we distinguish between *TmY* with their cell bodies in the medulla cortex and *Y* cells with their cell bodies behind the lobula plate neuropile (see section 3.3.8. and Figs. 9, 15). Our justification for a distinction between these neuronal types is that all *TmY* cells arborize in the distal medulla and in the proximal medulla, whereas the *Y* cells we have seen in *Drosophila* do not project into the distal medulla. Therefore, there is a clear difference in connectivity. Moreover, the different positions of cell bodies indicate a different developmental origin. *TmY* neurons are obviously produced by the outer optic anlage while the *Y* cells are most likely descendants of the inner optic anlage.

3.3.6. Neurons connecting the proximal medulla exclusively with the lobula plate (T4 cells). *T4* neurons (Fig. 14) stain very frequently with the Golgi method. Their cell bodies lie in the cell body rind posterior to the lobula plate. The cell body fiber passes through the lobula plate neuropile

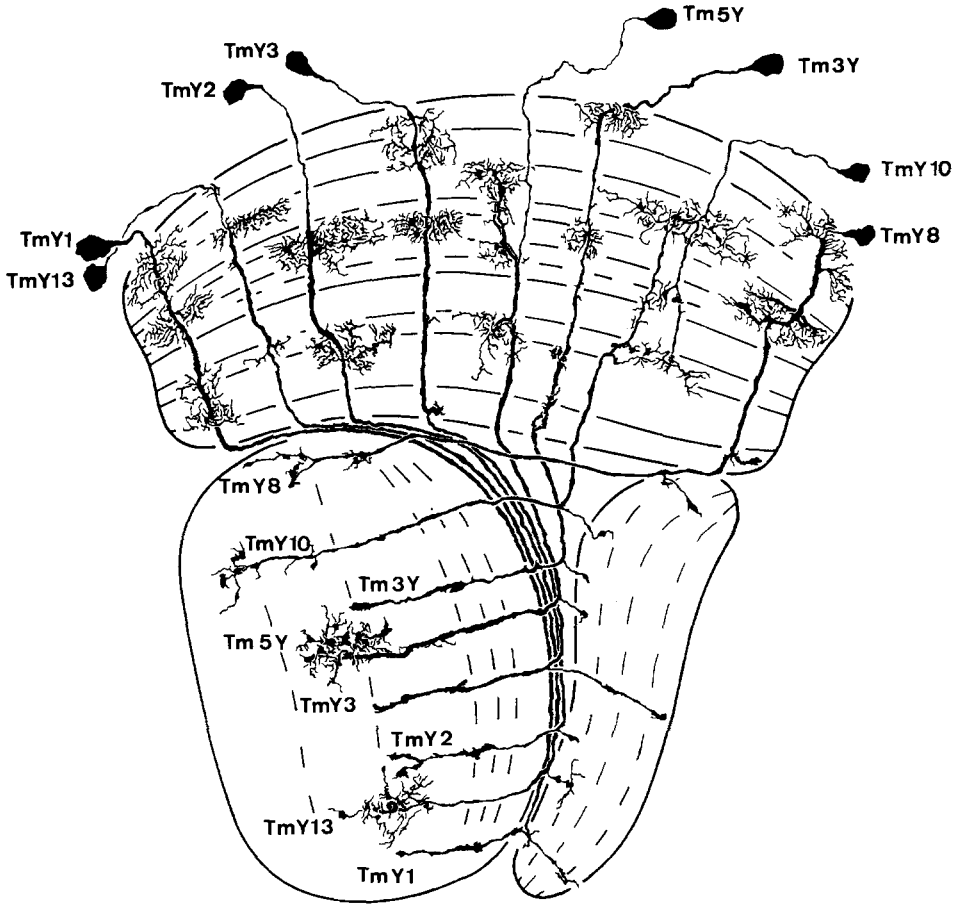


Fig. 13. Composite of camera lucida drawings showing various types of transmedullary neurons that send branches into the lobula plate. Some of these cannot be regarded as independent cell types as they differ from certain types of *Tm* neurons only by a tiny branch into the first layer of the lobula plate. They therefore have been labeled *Tm5Y* or *Tm3Y* to designate their assumed membership in the respective class of *Tm* neurons. For the other *TmY* cells such relationships have not been found. Notice that *TmY3* and *TmY8* have short varicose axonal collaterals in M10. This is true for *Tm3Y* (and for *Tm3*, Fig. 8, as well) which underlines the exceptional position of *Tm3* cells among the *Tm* cells (see section 3.3.4)

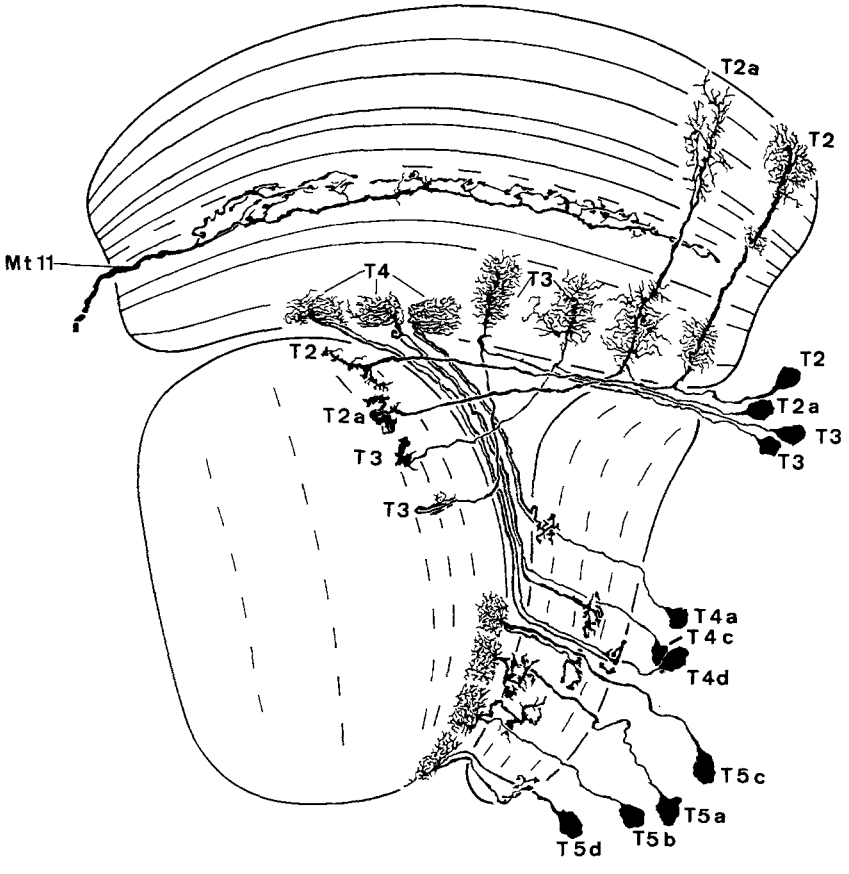


Fig. 14. Composite of camera lucida drawings showing *T2*, *T3*, *T4*, and *T5* cells. A medulla tangential element (*Mt11*) invading the medulla via Cuccatti's bundle and arborizing in layer M6 is shown as well. *T2* and *T3* connect the proximal medulla (layer M9) with the lobula (layers Lo2 and Lo3). *T2* cells furthermore project into the distal medulla. The variant most often impregnated by the Golgi method is stratified and arborizes in layer M5 and in layers 1 and 2. The main fiber of the *T2a* variant bifurcates in layer M5 and branches through layers M1–5. This form also shows a less pronounced restriction to layer M9 in the proximal medulla and seems to project into layer Lo3 instead of Lo2. The cell bodies of the *T* cells lie clustered together with those of *C2* and *C3* (see Fig. 3A) between the posterior rim of the medulla cortex and the lobula plate cell body layer. Several (probably 4) types of *T4* and *T5* cells occur in each visual column. They differ in the depth of their terminal projections into the lobula plate (see Table 1). *T4a* and *T5a* terminate in Lop1 where the large dendrites of the horizontal cells can be found. *T4b* (not shown) and *T5b* terminate in layer Lop2, *T4c* and *T5c* in Lop3. *T4d* and *T5d* arborize in layer Lop4 at the level of the giant dendrites of the VS cells

via the inner optic chiasm up to the inner face of the medulla. There it bifurcates, giving rise to the linking fiber connecting a bushy specialization in the innermost medulla layer with terminal arborizations in the lobula plate. On its way to the lobula plate the linking fiber closely follows its cell body fiber. Comparison of the arborization levels in the lobula plate of 80 Golgi-stained *T4* neurons in wild-type flies points to the existence of two to four different variants of *T4* neurons (see Table 1). At least one type of *T4* neuron in other Diptera synapses upon *VS* cells (Strausfeld and Bassemir 1983; Strausfeld et al. 1984). Strausfeld (1984) argues that *T4* neurons play a key role in the computation of directional sensitivity. Buchner et al. (1984) identified four different layers of the lobula plate that have preferences for different directions of movement. The most anterior layer of the lobula plate (corresponding in thickness to layer Lop1) is sensitive to movement from front to back. The next layer (corresponding to Lop2) is sensitive to movement from back to front. The next deeper layer (Lop3) is sensitive to upward movement, while the most posterior layer (Lop4) detects downward movement. If *T4* really plays a key role in the detection of the direction of movement, one should not be surprised to find several types of *T4* cells (Fig. 14; Table 1). Ultrathin serial sections of Golgi-impregnated *T4* cells are required to determine how many different connectivity types exist in each column. It should be noted that the terminals of the *T5* neurons (see section 3.3.9.), which are very similar to *T4*, arborize at four distinct levels in the lobula plate (see Table 1). *T4* and *T5* terminals at four levels of the lobula plate are seen in the micrograph of Fig. 29B.

3.3.7. Neurons connecting the proximal medulla exclusively with the lobula (T2 and T3 cells). The cell bodies of *T2* and *T3* cells are clustered posteriorly in the space left by the medulla and lobula plate neuropiles (Fig. 14). Their fibers travel along the inner face of the medulla to their respective columns where they bifurcate in a T-like fashion, sending one branch distally into the medulla and the other into the isotopic column of the lobula.

The dendrites of *T3* neurons arborize within single columns of the proximal medulla, bypassing the innermost layer in which the arborizations of *T4* predominate. They send their axons into the lobula neuropile where they terminate in the third layer.

T2 neurons differ from *T3* neurons in two respects. While their arborizations in the proximal medulla are very similar to those of *T3* neurons, *T2* cells possess additional dendrites in the distal medulla. Type *T2* arborizes in layers M1, M2, and M5. Type *T2a* bifurcates in layer M5 and branches throughout layers M4-M1. The axons of *T2a* cells terminate in the lobula at the same level as *T3* axons; their terminals have, however, a larger lateral extent (Fig. 14). The terminals of *T2* are located in the second layer of the lobula (Fig. 14). Both *T2* types have often been seen in male and in female *Drosophila* in neighboring columns. *T2* and *T3* neurons have been described in several other dipteran species (Strausfeld 1970, 1976).

3.3.8. Neurons connecting the proximal medulla with the lobula and the lobula plate (Y cells). The cell bodies of *Y* cells are situated in the rind of the lobula plate. They typically have dense arborizations within several columns of the lobula plate neuropile. Characteristically, their dendrites within the lobula plate are not much stratified. The main fiber of the *Y* cells bifurcates in the inner chiasm, sending one or several branches into the lobula and the other one upstream into the proximal medulla (Figs. 9, 15). In our wild-type preparations of *Drosophila*, no *Y* cell has ever been seen to enter the distal medulla. This is similar to the situation in the butterfly *Pieris brassicae* (Strausfeld and Blest 1970). However, the *Y5* cell of *Musca* (Strausfeld 1976) seems to be an exception.

3.3.9. Neurons connecting the lobula plate and lobula (T5, T1p and T1 cells). One of the most easily identifiable and therefore rather conserved cell types in the dipteran optic lobe is *T5* (Strausfeld 1970, 1976). *T5* cells have many structural and possibly also functional features in common with *T4* neurons (see Fig. 14, and section 3.3.4. for functional aspects). They differ, however, in connecting the most superficial layer of the lobula (Lo1) with the lobula plate. Quantitative evaluation of the position of the terminal arborizations of 120 *T5* neurons (Table 1) suggests that there are four different kinds of *T5* neurons (Fig. 14). These correspond to the four layers of the lobula plate with preferences for different directions of motion (Buchner et al. 1984; see section 3.3.6.). In the micrograph of Fig. 29B all four levels of *T* cell terminals in the lobula plate are visible. We believe that these variants coexist in each visual column. This could explain the high frequency of Golgi impregnation of *T4* and *T5* cells.

The cell bodies of *T5* neurons stain in the rind of the lobula plate. The shape of this neuron type with stout lobula plate terminals and fine lobula dendrites suggests that the main information flow mediated by *T5* is from the lobula to the lobula plate (see Discussion). The opposite seems to be true for translobula-plate neurons (*Tlp* neurons; Figs. 4, 5), but without electron microscopy the polarity in neither type of neuron, *T5* or *T1p*, is clear. The existence of *Tlp* neurons in other Diptera is not well established. As opposed to *Y* cells the arborizations of *Drosophila Tlp* cells in the lobula plate are stratified. The cells seem to connect at least several neighboring columns of the lobula plate with isotopic columns in the lobula. The retinotopic fields of the *Tlp5* neuron within the lobula and the lobula plate are rather large (Fig. 5). It is nevertheless listed here as a columnar neuron since its main fiber is clearly oriented parallel to the main axis of the columns. Postembryonic growth in this neuron class has presumably therefore followed the rules for columnar neurons.

A translobular neuron (*Tl1* neuron) projecting into the lobula plate has been found twice in the columns subserving the most frontal visual field (Fig. 16). It is uncertain whether this neuronal type occurs throughout the lobula complex. Another type, *Tl2*, is also depicted in Fig. 16. The neuron's shape is unusual, since its axon bifurcates in the middle of the lobula neuropile into two equal processes, which project via the inner optic chiasm into the lobula plate where both branch at all levels. The arborizations of *Tl2* spare only the deepest and most superficial layers of the lobula. The neuronal shape of *Tl2* within the lobula complex is similar to that of the *Lccn1* neuron (see Fig. 16 and section 3.4.3.).

3.3.10. Intrinsic neurons of the lobula or lobula plate. In contrast to the medulla, the lobula and lobula plate of *Drosophila* do not seem to contain many intrinsic neurons that

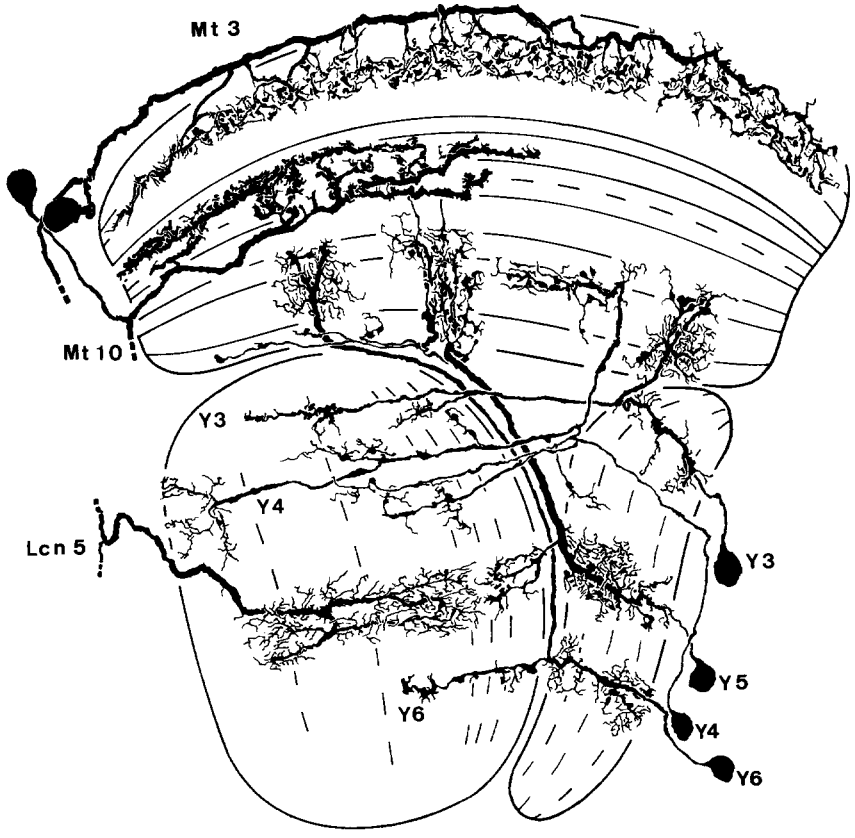


Fig. 15. Composite of camera lucida drawings showing various types of *Y* cells and medulla tangentials. *Y* cells connect the lobula plate with retinotopic regions of the proximal medulla and lobula. Their cell bodies are located behind the lobula plate neuropile as those of T4 and T5 neurons (see Fig. 14). Note that the *Y* cells do not extend into the distal medulla neuropile. *Mt10* is an example of a tangential neuron that subserves only a part (in this case the posterior part) of the visual field, quite in contrast to *Mt3*, which extends superficially throughout the distal medulla

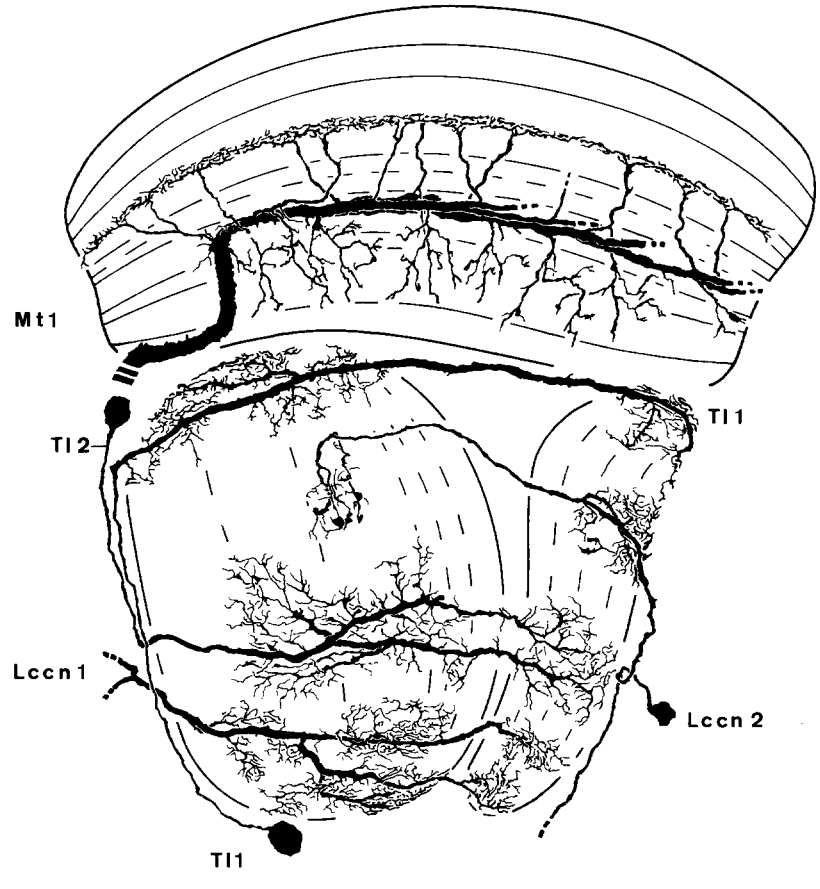


Fig. 16. Composite of camera lucida drawings showing various types of neurons in the optic lobe. *Tl1* and *Tl2* are translobula neurons projecting into the lobula plate. *Lccn1* and *Lccn2* are lobula-complex columnar neurons, which connect the lobula with the lobula plate via the inner optic chiasm. *Lccn* axons project via different routes into the central brain. The dendrites of the wide-field *Mt1* tangential element are bistratified. The arborizations in M4 arise from ascending fibers branching from the main dendrites in the serpentine layer. Descending fibers give rise to the arborizations in M8 and M9

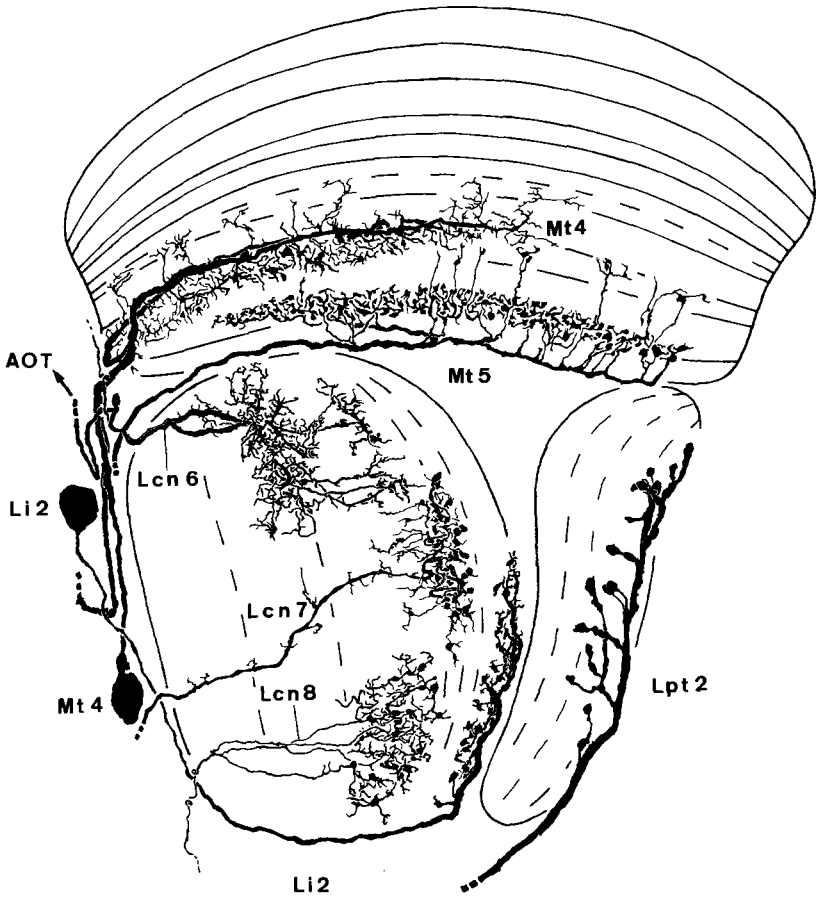


Fig. 17. Composite of camera lucida drawings showing different types of lobula columnar neurons (*Lcn6–8*), medulla tangential elements (*Mt4, Mt5*), a lobula plate tangential (*Lpt2*), and a lobula intrinsic neuron (*Li2*). The central brain projection of *Mt4* is shown in Fig. 21C, that of *Lcn6* in Fig. 23B

stain. This agrees with what is known from other Diptera. For the lobula, only two true candidates *Li1* (Fig. 6) and *Li2* (Fig. 17) have been detected in *Drosophila*. *Lpi*, depicted in Fig. 7, is an amacrine of the lobula plate residing entirely within that neuropile. *Tm23* and *Tm24* (Fig. 11) also arborize exclusively in the lobula and may function as lobula intrinsic neurons. Nevertheless, it seems reasonable to classify these neurons as *Tm* neurons because their cell body is located in the distal medulla cortex and their axons project through the medulla neuropile. As already stated above these neurons are reminiscent of the lamina monopolar neuron *L5*, which sometimes lacks any obvious branches in the lamina while arborizing in the medulla (see Fig. 24).

3.4. Columnar neurons connecting the optic lobe with the central brain

The columnar neurons discussed so far are intrinsic to the optic lobe. Based upon the position of their cell bodies it seems likely that they are formed either by the outer optic anlage (e.g., lamina monopolar cells, *Tm* and *TmY* cells) or by the inner optic anlage (e.g., *T4, T5* and *Y* cells). The neurons now to be discussed may be of different origin. The cell bodies of some lie in the cell body rind of the central brain and are, therefore, not formed by the optic anlagen.

Examples of columnar neurons of the lobula complex projecting to the central brain are seen in Figs. 5, 6, 11, 15–17. No neuron has a receptive field as narrow as a single visual column. Although the retinotopic input connections into the lobula complex have as fine a grain as in the lamina, the output connections are much coarser. Some projection areas of these neurons within the central brain are shown in Figs. 23, 28. The central brain receives input from multiple sets of isomorphic neurons projecting into many different areas. Our account of these optic foci is rather incomplete. For *Musca* a much more comprehensive account exists (Strausfeld 1976).

3.4.1. Lobula columnar neurons (LCN). A common feature of columnar neurons connecting the lobula with the central brain (Figs. 5, 6, 11, 15–17) is that they never arborize in the most anterior layer where the *T5* dendrites reside (Fig. 14). Different types branch at different levels of the lobula neuropile. Axons of isomorphic neurons are bundled beneath the lobula neuropile and project into the central brain. One of the large tracts is the anterior optic tract (AOT) which is used by several such isomorphic sets (Fischbach and Lyly-Hünerberg 1983). Central projections of *LCN* neurons are shown in Figs. 23B, 28D–F.

3.4.2. Heterolateral columnar neurons connecting the lobulas (HLCN). In Diptera, different kinds of heterolateral lobula columnar neurons connect special parts of both lobulas via the great commissure. Strausfeld (1979) found such elements in the binocular visual field of male *Calliphora* by cobalt injections, and recently Strausfeld and Wunderer (1985) described marginal heterolateral connections between the lobulas. In *Drosophila*, heterolateral neurons were seen first in the mutant *sine oculis* (Fischbach 1983a). Unfortunately, only fragments of these neurons have been stained in wild-type preparations. These, however, prove

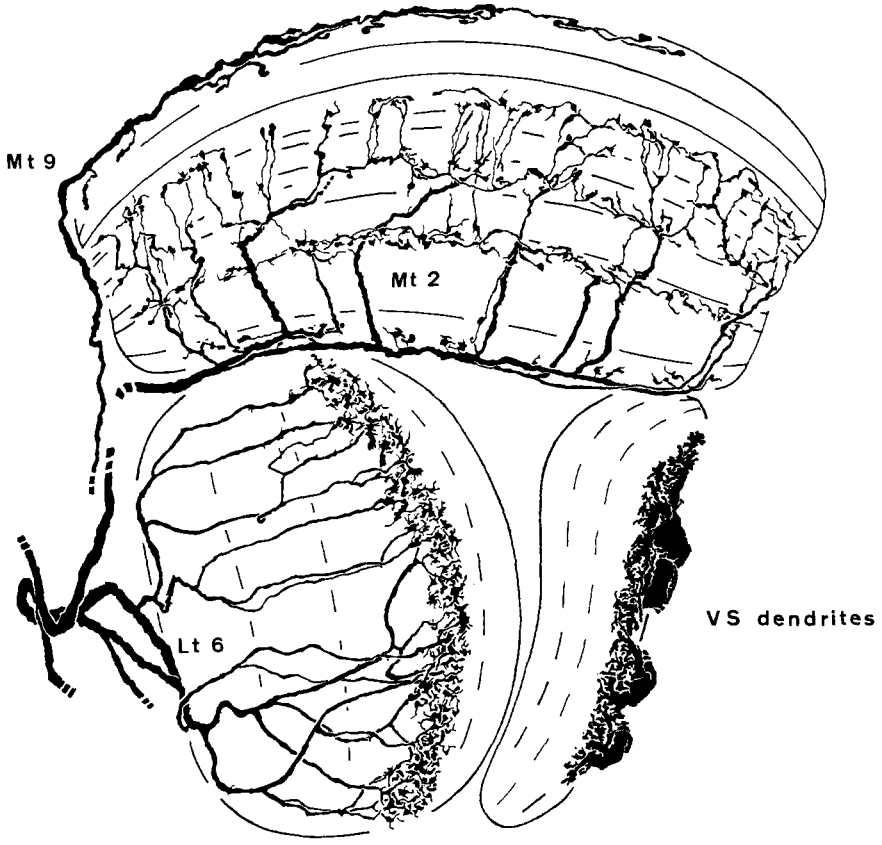


Fig. 18. Composite of camera lucida drawings showing various types of tangential elements in the optic lobe. The strip-field element *Mt9* forms a network on top of the medulla neuropile, only sparing the most posterior part of it. *Mt2* on the other hand has specializations throughout M3–M6, M7 and M10. The distal arborizations are formed by many linking fibers ascending from the main axon collaterals, which arborize in M10. With regard to this feature, *Lt6* shows a comparable structure. The linking fibers arising at the base of Lo6 give rise, however, to a narrow network of arborizations in only one layer (Lo3). The central brain projections of the lobula tangential *Lt6* are shown in Fig. 22B. In layer Lop4 of the lobula plate, dendrites of *VS* cells are shown. These and other vertically extending neurons cannot be represented completely in a horizontal view of the optic lobe

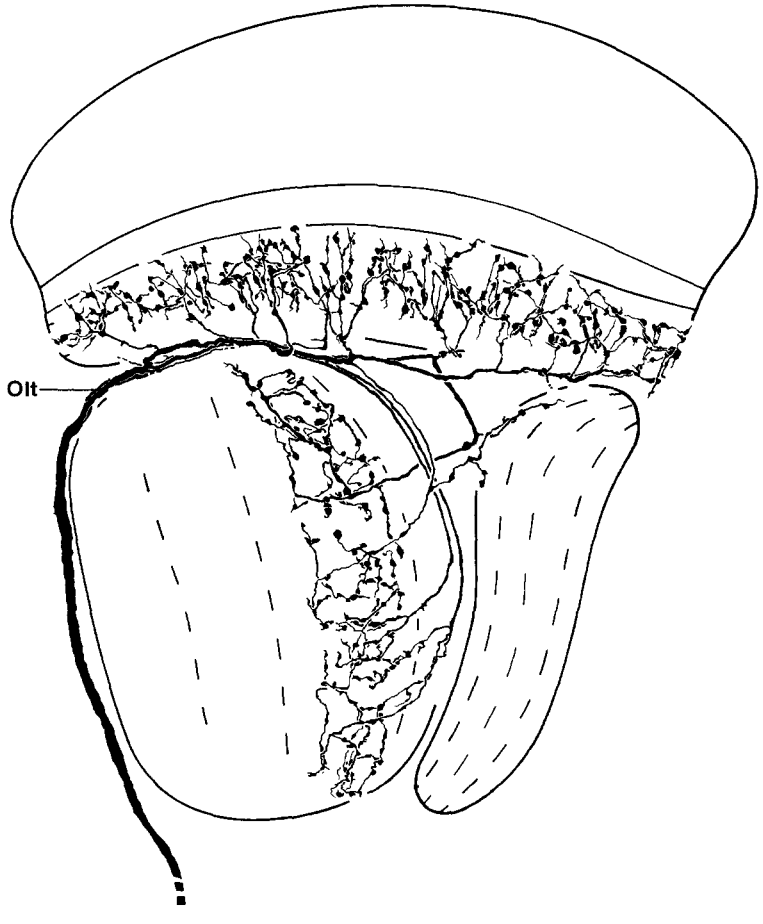


Fig. 19. Camera lucida drawing showing a tangential element of the optic lobe (*Olt*) that arborizes in the proximal medulla, the lobula, and much less extensively in the lobula plate. Medulla and lobula arborizations are linked by several fibers running in the inner optic chiasm

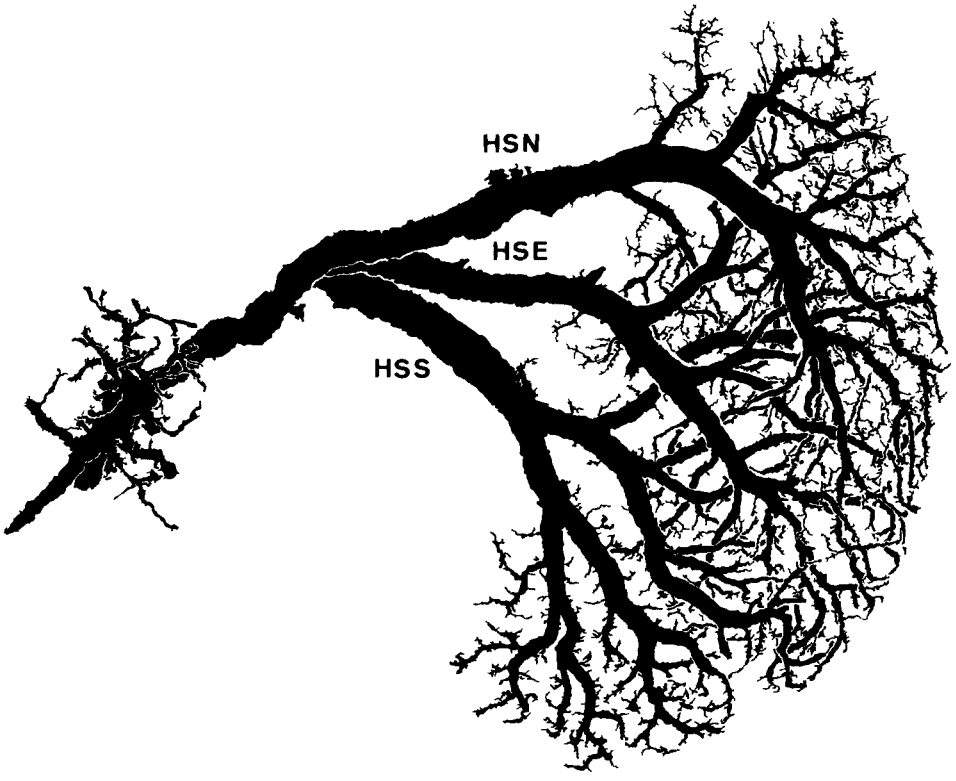


Fig. 20. Camera lucida drawing showing the dorsal (*HSN*), equatorial (*HSE*) and ventral (*HSS*) giant horizontal cells of the lobula plate in a vertically sectioned preparation, which was kindly lent to us for evaluation by N.J. Strausfeld. The cell bodies were not impregnated. They are known, however, to lie in the proximal rind of the lobula plate

that such neurons exist in wild-type *Drosophila* as well. Silver-impregnated sections suggest that many axons from the lobula enter the great commissure (e.g., Fig. 2 of Fischbach and Heisenberg 1981).

3.4.3. Columnar neurons with branches in lobula and lobula plate (lobula-complex columnar neurons, LCCN). Two different types of columnar neurons connecting lobula plate and lobula with the central brain have been stained (*Lccn1* and *2*). They differ in the position of their cell bodies, and their axons use different routes towards the central brain (Fig. 16).

3.5. Tangential neurons

Tangential neurons connect layers of the optic lobe with the central brain or with the contralateral optic lobe. Typically their dendrites and axonal terminals extend throughout the entire retinotopic map or throughout large parts of it. The developmental origin of tangential neurons is heterogeneous and seems to be different from that of optic lobe intrinsic columnar neurons. Most medulla tangentials are generated early by the first proliferation of the outer optic anlage to produce imaginal neurons (Meinertzhagen 1973; Hofbauer 1979).

That the distinction between tangential and columnar neurons is not merely a superficial one depending solely on shape is stressed by the specific defects of structural brain mutants. The optic lobe rudiment of the double mutant *sol so* (*small optic lobes* combined with *sine oculis*) completely lacks optic lobe intrinsic columnar cell types (Fischbach and Technau 1984). It is made up of tangential neurons, and medulla, lobula, and lobula plate tangentials can still be distinguished. In flies of the mutant *disco* (*disconnected*) the optic lobe rudiment still contains tangential neurons (Fischbach and Heisenberg 1984) although it has never been innervated by imaginal or larval photoreceptor axons due to the failure of normal target recognition by Bolwig's nerve (Steller et al. 1987). Optic lobe intrinsic columnar neurons die in mutant pupae (Steller et al. 1987). Therefore, it seems that the maintenance of tangential neurons depends much less upon innervation of the optic lobe by photoreceptor axons than does that of columnar neurons (see also Fischbach 1983a; Nässel and Sivasubramanian 1983; Nässel et al. 1987).

3.5.1. Lamina tangential neurons. To avoid confusion we would like to remind the reader that according to our definition of columnar neurons (section 3.1.) we do not consider the lamina wide-field neurons (section 3.3.2.) to be true tangential neurons. There is, however, one true tangential neuron innervating the lamina cortex (*Lat* in Figs. 3B, 24G). This *Drosophila* neuron, only fragments of which are seen in our Golgi impregnations, is presumed to be serotonergic since it binds the corresponding antibodies (E. Buchner, personal communication). A similar serotonergic neuron (*Tan3*) has been impregnated in *Calliphora* and other insects (Nässel et al. 1983, 1985). In *Calliphora* this neuron has recently been redescribed to be bilateral and has been renamed *LB05HT* (Nässel et al. 1987). The cell bodies of the two *LB05HT* neurons lie in the posterior central brain, one on each side.

In Fig. 24H a giant cell body can be seen inside the outer optic chiasm just beyond the lamina neuropile. We believe this soma to be one of the optic lobe pioneer cells (*OLPs*, Tix et al. 1989), early differentiating neurons of the larval optic lobe. These neurons are known to persist into the adult stage and their axons project anteriorly across the first optic chiasm. It has been suggested that these neurons may play an essential role for the proper internal orga-

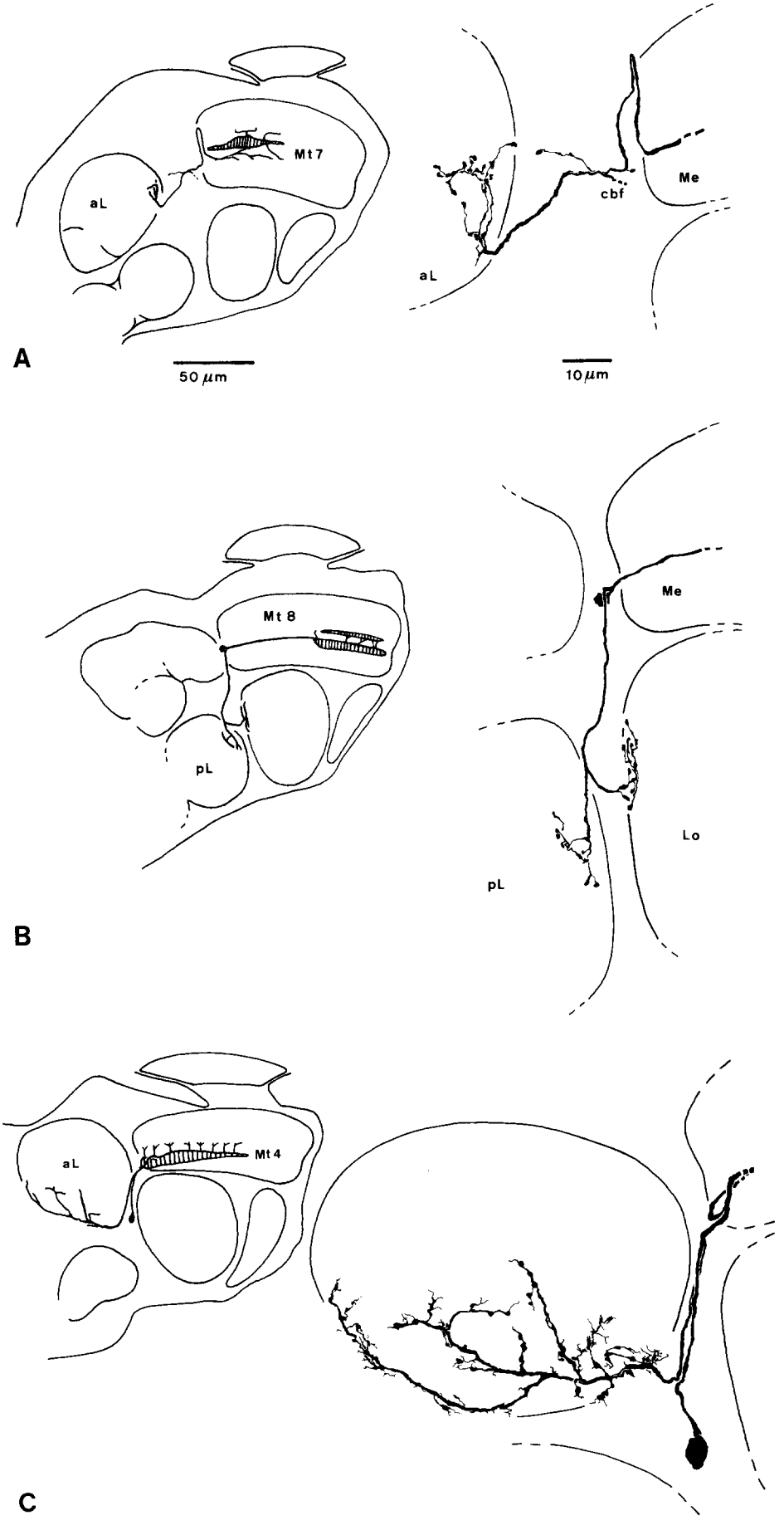


Fig. 21 A–C. Examples of medulla tangentials with terminal arborizations in the lateral protocerebrum. Depicted are the terminals of *Mt7* (**A**), *Mt8* (**B**), and *Mt4* (**C**). On the left a low-power survey view is given, while on the right camera lucida drawings of the terminals are shown at higher magnification. The medulla arborizations are shown in Figs. 7, 6, 17 respectively. The *scale bars* in **A** apply to Figs. 21–23

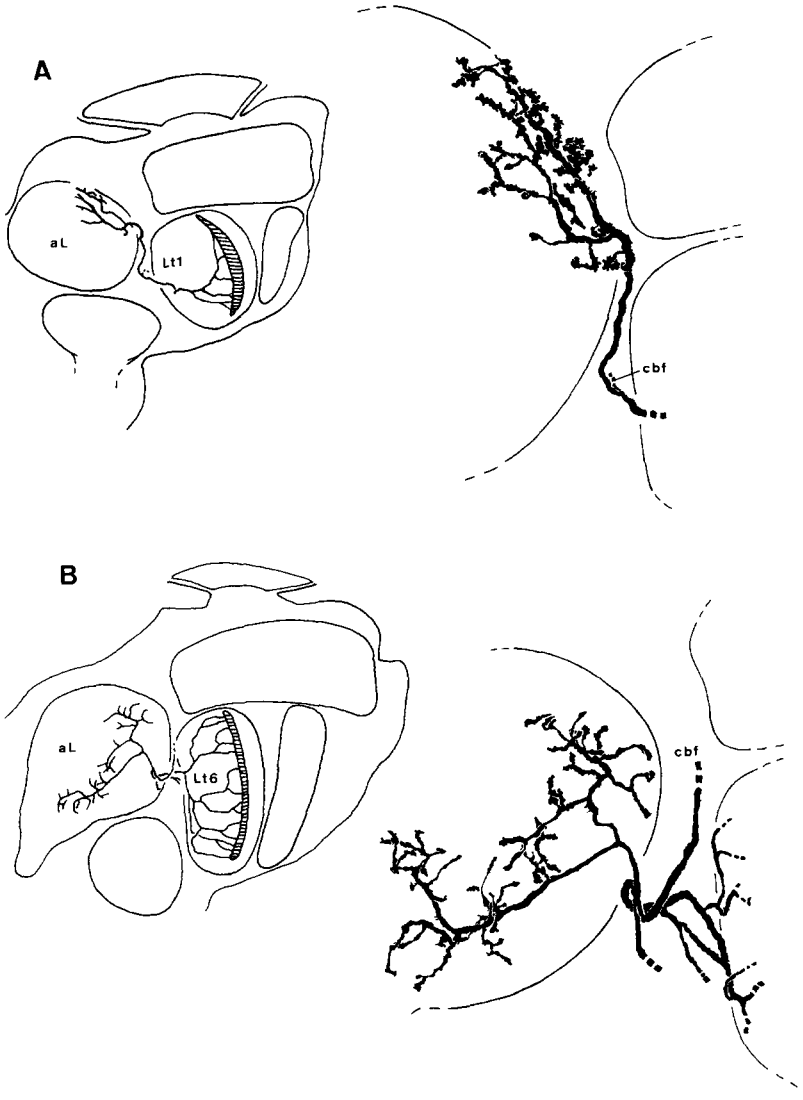


Fig. 22A, B. Examples of lobula tangentials with terminal arborizations in the lateral protocerebrum. Depicted are the terminals of *Lt1* (**A**) and *Lt6* (**B**). On the left a low-power survey view is given, while on the right camera lucida drawings of the terminals are shown at higher magnification. Compare with Figs. 5, 28A (*Lt1*) and Fig. 18 (*Lt6*), respectively

nization of the optic lobe (Fischbach and Technau 1987; Tix et al. 1989). Such an organizing (pioneering) function of the *OLPs* is corroborated by the fact that in the *irre C* mutant misrouting of their axons precedes the formation of an irregular first optic chiasm (Boschert et al. 1989). Fig. 24G and H are from the same fly, and the axon of the *Lat* neuron projects along that of the giant neuron across the first optic chiasm towards the posterior optic tract. During development the larval *OLPs* may well guide the centrifugal growth of *Lat*, which starts during late third instar (Ohlsson and Nässel 1987; Nässel et al. 1987).

3.5.2. Medulla tangential elements. Our list of medulla tangential neurons is incomplete and many have only been depicted fragmentarily. One reason for this is that in many cases it was extremely difficult to produce camera lucida drawings from stained tangentials because such preparations often contained also groups of impregnated columnar neurons. Another difficulty was to follow bilateral neurons to the contralateral optic lobe when there was extensive staining of the central brain. Furthermore, many tangential neurons are unique and their staining frequency is accordingly fairly low. We believe, however, that the elements (*Mt1–15* and *Olt*) shown in Figs. 14–19, 21, 27–28 demonstrate the principles of the organization of medulla tangential neurons.

The cell bodies of most medulla tangential neurons are situated anteriorly to the medulla neuropile. According to Meinertzhagen (1973) and Hofbauer (1979) these cells are derived from the outer optic anlage. They differentiate earlier than the columnar neurons because they are produced first. This also explains their anterior position. From the work of Ohlsson and Nässel (1987) with *Calliphora*, we have to assume that cell bodies of medulla-intrinsic, tangentially orientated, serotonergic giant amacrine cells are close by and of similar origin. Element *Mt14* (Fig. 27E) seems to be a representative of these neurons. It is listed here because we believe that developmentally it is more related to tangential neurons than to the small amacrines described in section 3.3.3.

Not all cell bodies of medulla tangential neurons are positioned anterior to the medulla neuropile. On example is *Mt4*, the cell body of which lies in front of the lobula neuropile (Figs. 17, 21C). Similar to *Mt7, Mt8,* and *Mt10* (Figs. 6, 7, 15) this tangential element covers only part of the visual field, quite in contrast to more typical representa-

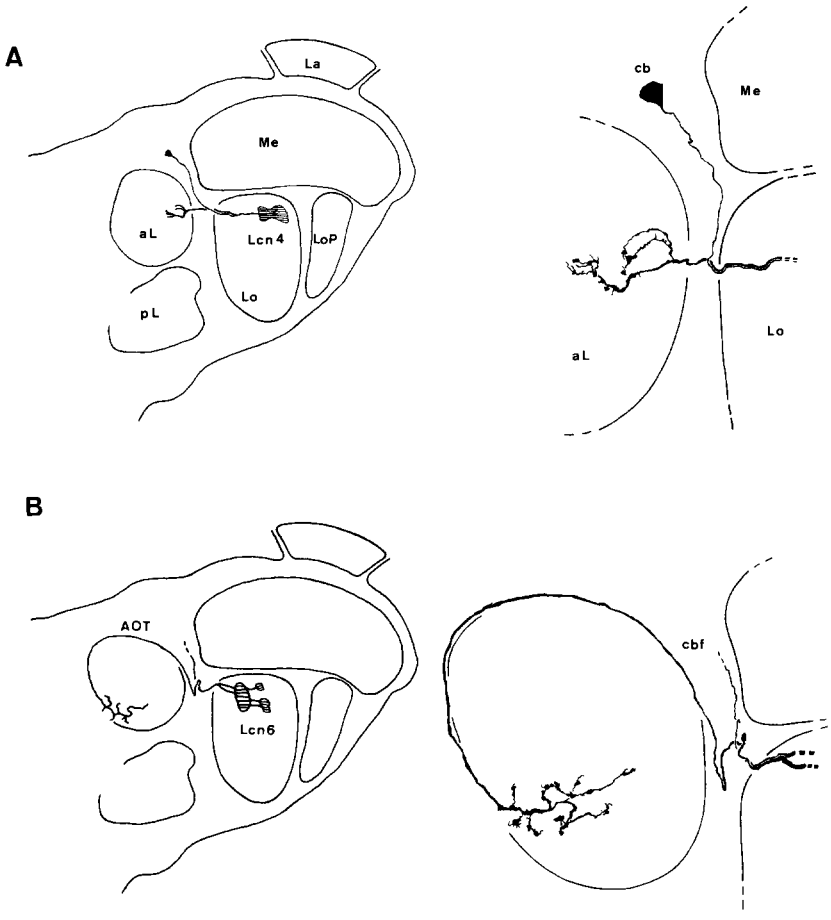


Fig. 23A, B. Example of *Lcn* neurons terminating in different optic foci, shown in low-power plan (left) and high-power camera lucida (right). In **A** Lcn4 is shown (see Fig. 11) and in **B** *Lcn6* (see Fig. 17). The latter projects through the anterior optic tract (*AOT*) in subbundle S4 (see Fig. 28D). Note that the axons of one kind of *Lcn* all project via the same fiber tract into the same optic focus. Different optic foci obtain information via different sets of retinotopically organized fiber systems

tives of tangential elements like *Mt1* (Fig. 16), *Mt2* (Fig. 18), *Mt3* (Fig. 15), *Olt* (Figs. 19, 27B), and *Mt13–15* (Fig. 27).

The *Olt* (optic lobe tangential element; Figs. 19, 27B) is an interesting case. It seems to constitute the telodendritic arborizations of a tangential neuron that enters the proximal medulla neuropile and the outer third of the lobula (sparing the *T5* layer, however). Medulla and lobula arborizations are connected by several linking fibers through the inner optic chiasm. These linking fibers roughly follow the rules for isotopic columnar connections.

Similar to columnar neurons, tangential neurons are characterized by their stratifications. Their axons leave the medulla at the level of the serpentine layer and project to either the central brain or the contralateral medulla via Cuccatti's bundle. Tangential axons may enter the medulla at the level of the serpentine layer (e.g. *Mt15*) or at its proximal face (e.g., *Mt1, Mt5, Mt2*, and *Olt* in Figs. 16–19). In other cases tangential axons project along the anterior edge of the distal medulla neuropile (e.g., *Mt9* in Fig. 18). This diversity reflects the different roots of Cuccatti's bundle visible in silver-stained preparations (Fig. 1F). The distal root also seems to contain the axons of the lamina tangential (Fig. 3B) and of the *OLP* neurons (Fig. 24H).

3.5.3. Lobula plate tangentials. The lobula plate is especially well known because of the giant tangential neurons, grouped into horizontal (*HS*) and vertical (*VS*) systems (Pierantoni 1976; Hausen 1976; Heisenberg et al. 1978). The *HS* neurons of the lobula plate are part of the optomotor pathways. This has been shown electrophysiologically in *Calliphoridae* (review: Hausen 1981). In support of this evidence, the optomotor responses of the *Drosophila* mutants *optomotor blind*^{H31} (*omb*) and *lobula plate-less* (*lop*) are defective (Heisenberg and Wolf 1984). The *omb* mutant lacks the *HS* neurons while in *lop* these neurons are depleted of their input neurons *T4* and *T5* (Fischbach 1983b; Fischbach et al. 1989).

The *HS* system consists of three neurons (Figs. 20, 29) which together span the innermost layer of the lobula plate (layer 1 of the lobula plate in Figs. 3–19). *HSN* is the dorsal horizontal neuron, *HSE* the equatorial, and *HSS* the ventral horizontal neuron. They project into the ipsilateral posterior slope of the central brain.

The *VS* neurons of *Drosophila* have been described by Heisenberg et al. (1978). These neurons have vertically oriented dendrites in the most posterior layer of the plate covering lateral parts of the retinotopic map. A smaller dendrite branches to the most anterior layer of the lobula plate covering a dorso-frontal retinotopic area. This distribution has been discussed as being well suited for the participation of *VS* cells in roll control (Blondeau and Heisenberg 1982). The *VS* neurons enter the lobula plate dorsally and their main dendrite turns ventrally. Therefore they cannot be illustrated as a whole in a horizontally oriented diagram of the optic lobe. Fig. 18 shows the camera lucida projections of the main *VS* dendrites in a 35 µm horizontal section.

Only fragments of two other tangential elements of the lobula plate (*Lpt1* in Fig. 6 and *Lpt2* in Fig. 17) are depicted

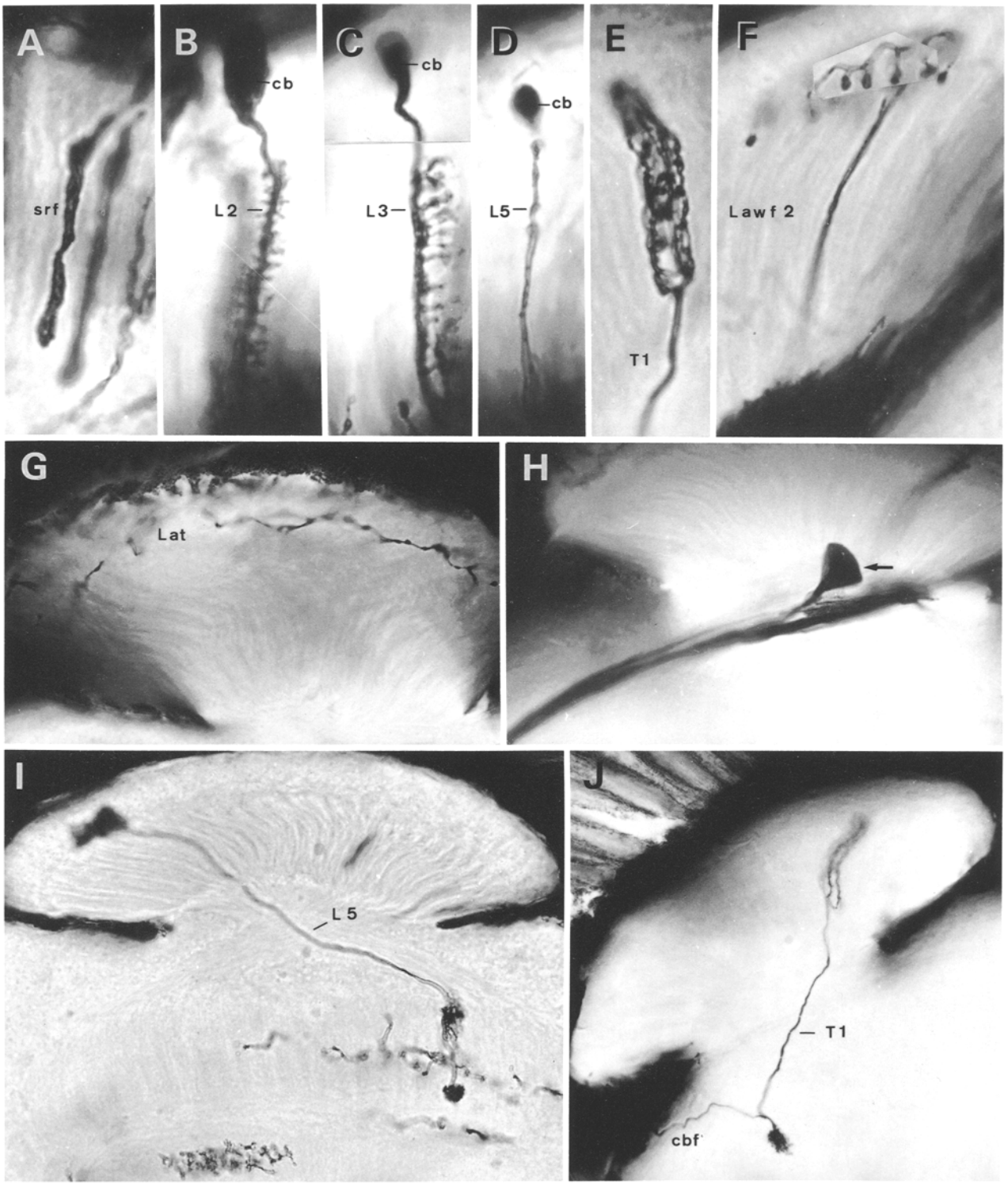


Fig. 24A–J. Examples of Golgi-impregnated columnar neurons in the lamine (**A–F, I, J**), compare with Fig. 3. In **G** branches of the lamina tangential neuron (*Lat*, probably homologue to *LBO5HT*; Nässel et al. 1987) extend between the cell bodies of the lamina monopolar neurons (compare (Fig. 3B). In **H** the huge cell body of a giant optic lobe tangential element in the first optic chiasm probably belongs to the optic lobe pioneer neurons (OLPs; Tix et al. 1989); *srf* short retinula fibers, *cb* cell body, *cbf* cell body fiber. **A–F** ×2100; **G–J** ×800

in this account. In large flies, however, e.g., in *Calliphora*, more than 20 different kinds of tangential neurons in the lobula plate exist (Hausen 1981). Therefore, our list of tangential neurons in the lobula plate of *Drosophila* has to be extended.

3.5.4. Lobula tangentials. Finally, the lobula also contains many different kinds of tangential neurons that possess stratifications at specific levels only. As judged by light mi-

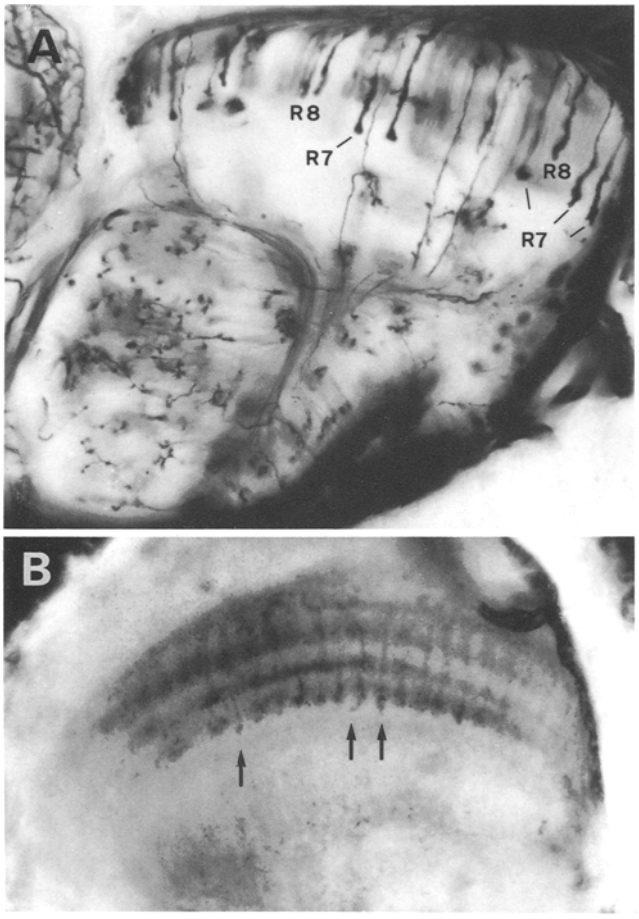


Fig. 25A, B. Projection of long retinula fibers *R7* and *R8* into the medulla. **A** Golgi-Colonnier preparation showing the different depths of *R7* and *R8* terminals. The Golgi rapid preparation in **B** (courtesy of U. Hanesch) shows the entire array of *R7* and *R8* terminals. *Arrows* point to especially long *R7* axons. ×640

croscopic criteria (see Discussion) most of them seem to have their dendritic arborizations inside the lobula, while their axons project to different regions of the central brain. Typical examples of those are *Lt7* (Fig. 4), *Lt1* (Figs. 5, 22A, 28A), *Lt6* (Figs. 18, 22B), and *Lt2* and *Lt8* (Fig. 28B). The latter two neurons look very similar but occupy different layers of the lobula. Fewer lobula tangential elements seem to be centrifugal (e.g., *Lt3*, Fig. 6; *Lt4*, Fig. 7, *Lt10*, Fig. 28C).

The *Olt* neuron (Figs. 19, 27B) may be regarded as a special case of a lobula tangential element. It invades the lobula via the second optic chiasm. In visual mutants depleted of many columnar cell types the input-deprived lobula is frequently compensatorily innervated by medulla tangential neurons via the second chiams (e.g., in *sine oculis*, Fischbach 1983a).

Another unusual case is the *Mt8* neuron (Figs. 6, 28B). It sends an axonal collateral to the lobula where it terminates in a varicose arborization, which occupies roughly the same retinotopic region as its dendritic specializations in the medulla.

Although several types of lobula tangential elements are depicted, we have to concede again that the documentation of such elements in the present paper is far from complete. Nearly nothing is known about their physiological properties. In a recent study using activity staining of *Drosophila* neurons in response to visual stimuli, Bausenwein (1988) found that the lobula is functionally organized into different layers similar to the other visual neuropiles. So far 3 different layers have been labeled by the H^3-deoxyglucose method. In the 2 deepest of these layers (one in Lo4 according to our judgement, the other in layer Lo6), the label does not seem to reside in columnar elements (Bausenwein 1988). In contrast to what is seen in the lobula plate (Buchner et al. 1984), labeling of lobula layers seems to be more sensitive to contrast frequency than to direction of movement (Bausenwein 1988).

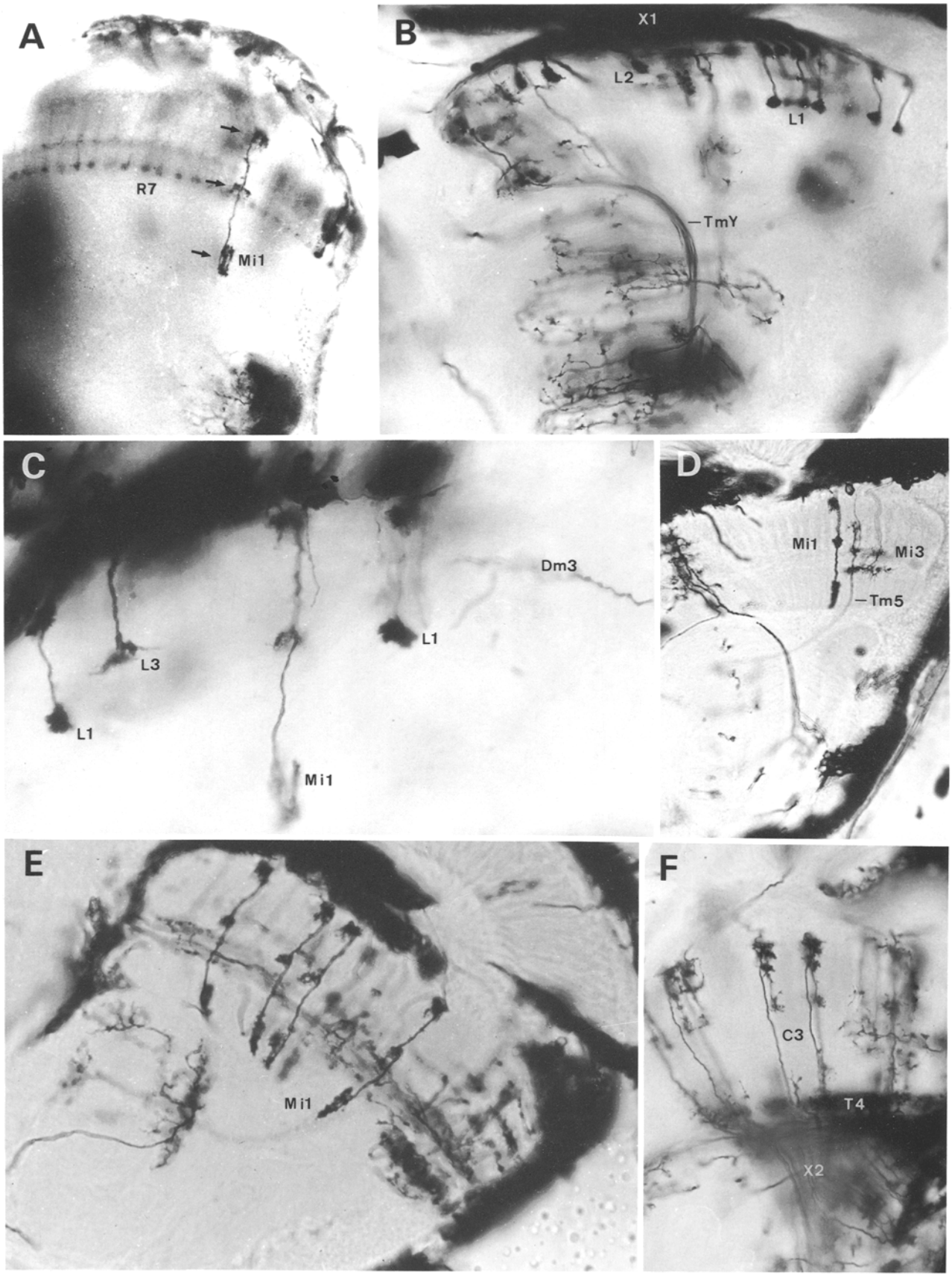
Discussion

1. How complete is the list of neurons?

The chemical mechanism of Golgi impregnation is still unknown. Neurons seem to be stochastically impregnated, i.e., each Golgi preparation shows a unique pattern of stained neurons (Strausfeld 1980b). The relative frequency at which representatives of neuronal types turn up is a function of the number of cells per class and possibly also of the position of their cell bodies because accessibility to the staining solutions is important. This is clearly demonstrated by the effects of cutting the eyes, the antenna, or the neck prior to fixation. The position of stained neurons can be partially directed by these procedures, e.g., an acceptable frequency of impregnation of lamina neurons requires a cut in the eye. Therefore, the glass splinter method we used (see Materials and methods), which leads to random cuts in the head cuticle, may have been essential for being able to impregnate that many different neuronal cell types.

Inspite of the high number of impregnated neurons we cannot be sure that we have seen all types. This is especially true for those neurons occurring only once or a few times in the optic lobes. In fact we stated already in the result section that the accounts of medulla and lobula complex tangential elements are incomplete. Further work on those is needed. With regard to columnar neurons we are more confident. We may have missed only few. We identified in *Drosophila* the whole complement of lamina-medulla connections known to exist in other Diptera (Strausfeld and Campos-Ortega 1972; Strausfeld and Nässel 1981). The number of identified columnar elements of the medulla is comparable to that found in *Musca* (Strausfeld 1976).

Fig. 26A–F. Examples of columnar neurons in the medulla illustrating how the relationship of layers in the optic lobe can be established with the help of certain marker neurons. For example, the small-field, tristratified *Mi1* neuron is impregnated in **A** and **C–E**. Its 3 specializations (*arrows* in **A**) mark 3 medulla layers (actually 1, 5, and 9–10, see Fig. 3A). In **A** the relationship to the *R7* terminals (in layer M6) is seen. In **C** it is apparent that the 2 distal arborizations of *Mi1* fall level with the 2 *L1* specializations in layer M1 and M5, while *L3* terminates clearly above layer M5 (actually in layer M3). In **D** *Mi1* can be used to measure the depth of *Tm5* and *Mi3* arborizations; **E** supports our conclusion from looking at hundreds of *Mi1* neurons that there is no obvious gradient in the shape of these neurons throughout the medulla. In fact, we believe this to be true for most other columnar cell types as well (e.g., arrays of *L1* in **B**, and of *C3* and *T4* in **F**). *X1* first optic chiasm, *X2* second optic chiasm. **A, B** ×600; **C** ×1270; **D** ×422; **E, F** ×690


A
R7
Mi1
B
X1
L2
L1
TmY
C
Dm3
L1
L3
L1
Mi1
D
Mi1
Mi3
Tm5
E
Mi1
F
C3
T4
X2

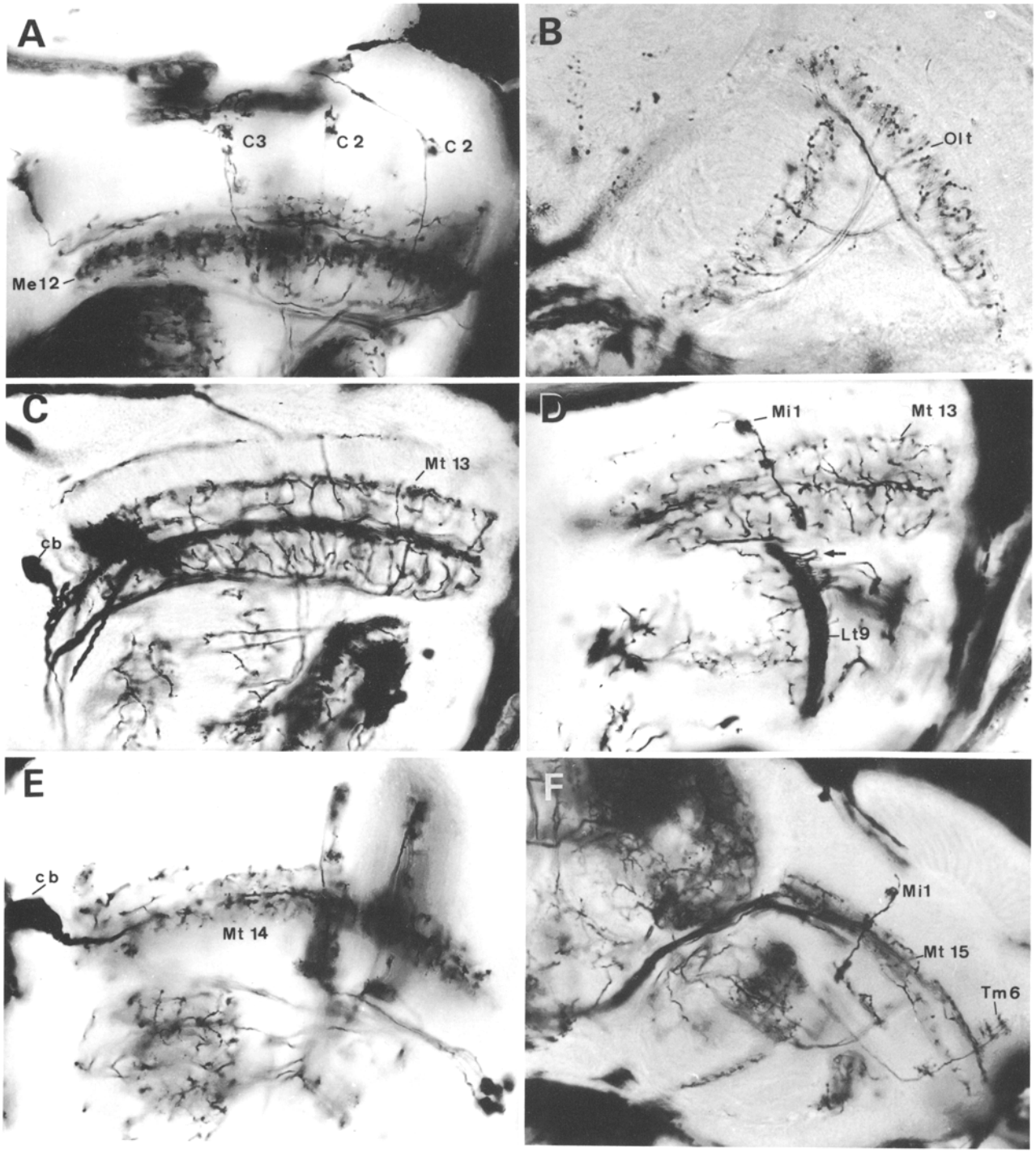


Fig. 27 A–F. Examples of medulla tangential elements. In **A** a tangential element (*Mt12*) arborizing in the proximal medulla is shown. In **B** the telodendritic arborizations of the optic lobe tangential neuron *Olt* are shown (see Fig. 19 for camera lucida drawing). **C** and **D** show arborizations of element *Mt13* in different horizontal sections of the same optic lobe. In **D** it is also seen that the lobula tangential *Lt9* forms a narrow strip of arborizations in the T5 layer. The dendrites of this neuron are frequently observed to form conspicuous loops extending into the inner optic chiasm (*arrow*). Element *Mt14* in **E** is remarkable as it does not possess an axon. It may therefore also be regarded as a giant medulla amacrine cell. Finally, in **F** a telodendritic element (*Mt15*) entering the medulla at the level of the serpentine layer is shown. Here again, *Mi1* marks the different medulla layers. Parts of *Tm6* are seen as well. **A, B** × 550; **C, D** × 440; **E** × 650, **F** × 380

2. Do we overestimate the number of columnar cell types due to structural variability?

It is quite apparent that no two neurons in the optic lobe are identical. The justification then to group cells into classes is that variation is discontinuous. Objective criteria for the classification of cells are the position of cell bodies, the extent and layers of arborizations, and the kind of specializations (fine branching, blebbed or bulky appearance). Variability of individual cell shapes may be accounted for

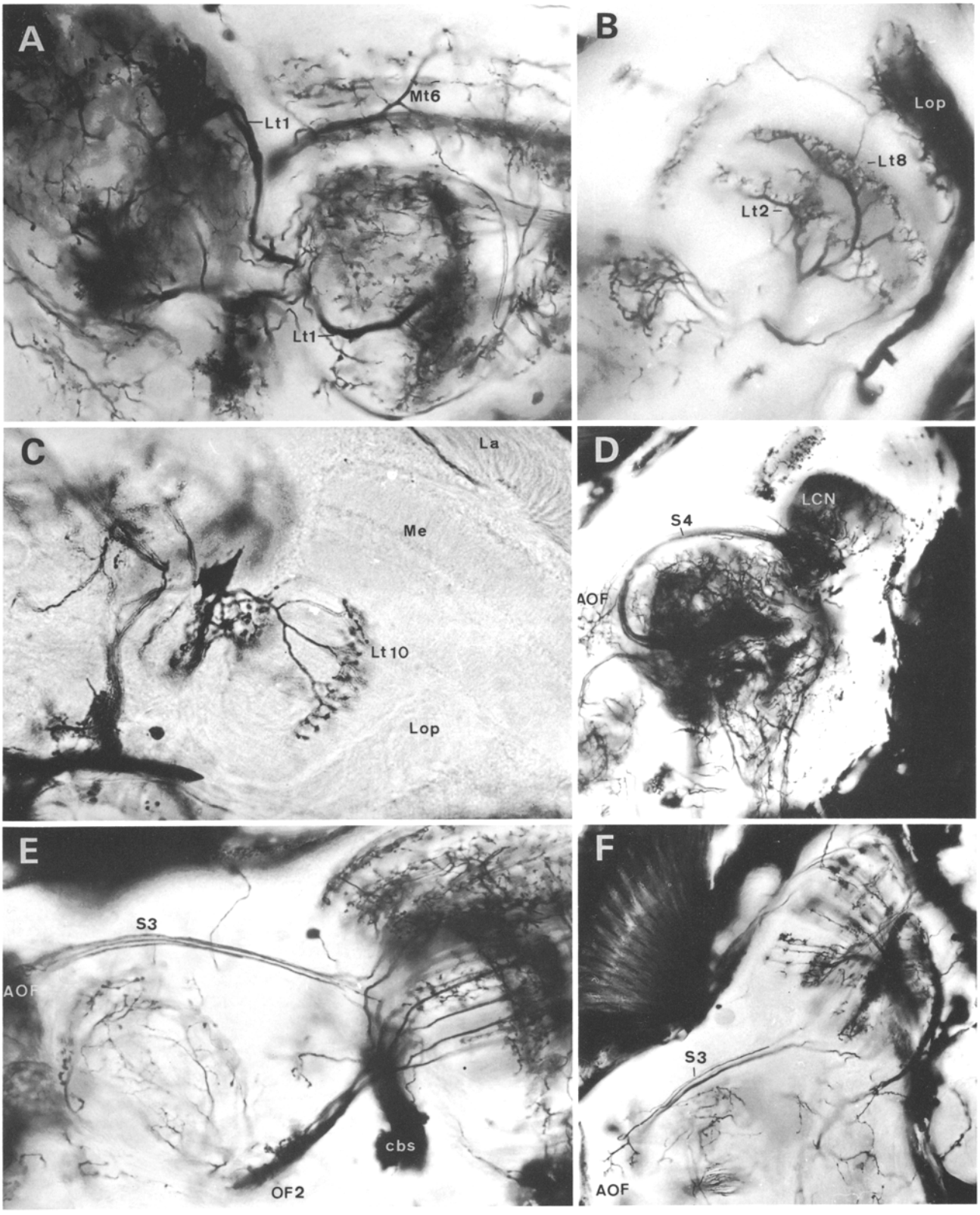


Fig. 28 A–F. Examples of lobula-complex neurons and their projections into optic foci of the central brain. *Lt1* in **A** should be compared with the camera lucida drawing of this cell type in Figs. 5 and 22 B. *Lt2* and *Lt8* in **B** are lobula tangentials of comparable shape, which arborize at different layers. The linking fibers of *Lt10* in **C** arise at the base of Lo6 and give rise to terminal arborizations in layers Lo2/3. D–F show fibers in different sub-bundles of the anterior optic tract (S3 and S4; Fischbach and Lyly-Hünerberg 1983; see Fig. 23 B for single neuron projection in S4). *AOF* anterior optic focus; *cbs* cluster of *LCN* cell bodies, the axons of which project into optic focus no. 2 (*OF2*). **A, B** ×660; **C** ×550; **D, F** ×310; **E** ×440

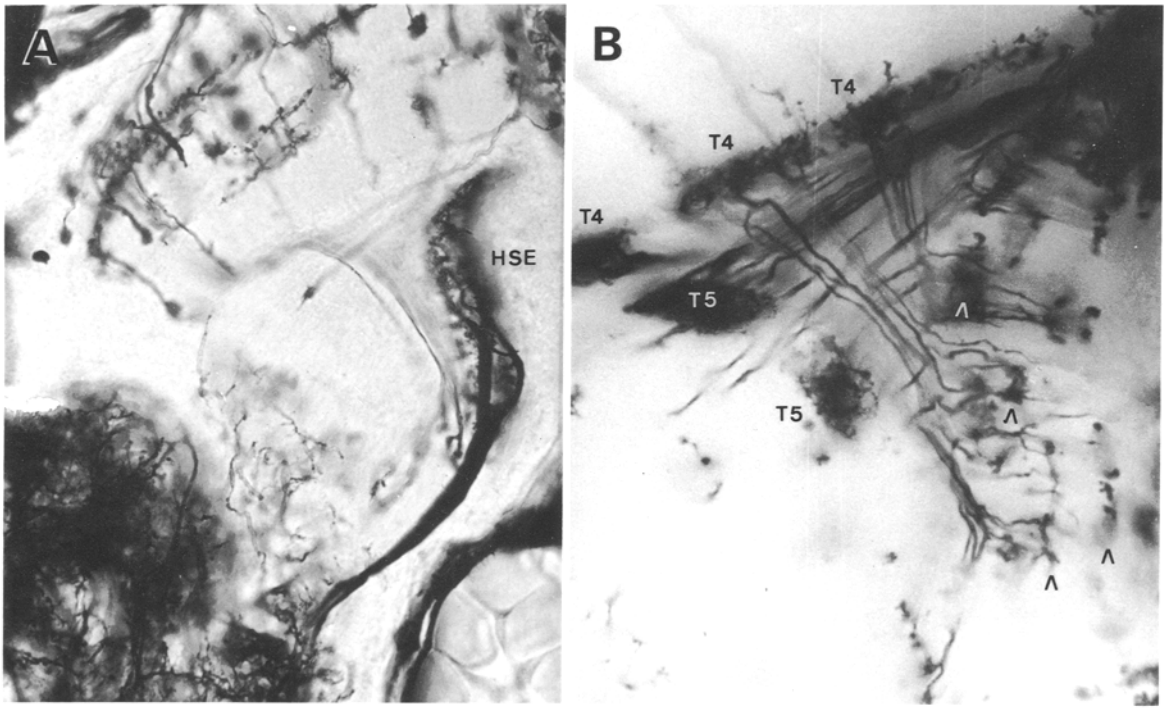


Fig. 29A, B. **A** Horizontal neuron in the lobula plate (*HSE*); **B** *T4* and *T5* neurons arborizing within what seem to be at least 4 different layers in the lobula plate (see *arrowheads*). **A** ×620; **B** ×1300

by developmental noise (Macagno et al. 1973) and position effects. Furthermore, it is now well established that insect brains display structural plasticity in response to environmental factors (e.g., Technau 1984; Kral and Meinertzhagen 1989). Random exploratory processes are known to participate in the growth of neurons (e.g., Goodman et al. 1982; Stürmer 1988) and should be reflected in fine branching. Comparison of *Tm4* and *Tm4a* (Fig. 8), as well as of *Tm5(Y)* neurons (Figs. 9–11, 13), give an impression of neuronal variability that might result from the nature of growth cone exploration.

In some cases partial Golgi impregnation might conceivably have contributed to the variability of the Golgi shapes documented in this paper, e.g., one might argue that *Tm3Y* (Fig. 13) might represent the normal form of *Tm3* (Fig. 8). It is ultimately hard to disprove the validity of the objection, but we do not believe that it is valid as this type of variability was cell-type specific. In the case of *TmY* cells we never found a missing lobula plate branch although in several types it also is rather tiny (e.g., in *TmY2, TmY8, TmY10*, Fig. 13). Partial impregnation of axons was occasionally observed. Its frequency seemed to increase with axonal size.

If there are strong structural gradients in the optic lobe of *Drosophila* along the antero-posterior or dorso-ventral axes, it may be possible to misclassify members of the same neuronal set that are impregnated in different parts of the lobe as being members of different neuronal sets. Staining of neuronal assemblies with cobalt in the lobula of large Diptera, for example, has revealed structural gradients and suggested that the number of neuronal classes in the lobula was formerly overestimated (Strausfeld and Hausen 1977; Strausfeld and Nässel 1981). The picture, however, may be different in the medulla of *Drosophila*. Most columnar cell types described in the present study are taken from this neuropile, which displays a gradual increase of its volume in the antero-posterior direction. In all closely inspected cases this gradation is, however, not reflected by an obvious change of neuronal shape within a given isomorphic class. The following cell types may illustrate this to the reader: long visual fibers (Figs. 25, 26A). *Mi1* neurons (Fig. 26), and *T4* (Figs. 26F, 29B). Therefore, we believe that most cell types classified in this study as different are not interpretational errors due to structural gradients.

We cannot, however, exclude that in some neuronal assemblies a stronger variability is expressed, e.g., we mentioned the possibility that *Tm8* and *Tm22* (Fig. 9) or *Tm1, Tm2*, and *Tm9* (Fig. 8) might not represent different cell types. Therefore, in *Drosophila*, methods like cobalt injection into the central brain or antibody binding studies, which expose entire neuronal cell assemblies, are now urgently needed to complement the present results (e.g., Buchner et al. 1988).

3. Are there specialized neuropile regions subserving certain parts of the visual field?

It has become apparent in recent years that the compound eye of insects may contain functionally specialized regions and that the structural separation of functional subsystems begins at the receptor level. A well-known example is the male-specific chasing behavior of *Musca* and *Calliphora* (Land and Collett 1974), which correlates with specific subtypes of receptors in the frontal eye region (Franceschini et al. 1981) and with specializations at the level of the neuropile (Strausfeld 1980a; Hardie 1983).

Wada (1974) first described specialized receptors in the marginal zone of the compound eye of *Calliphora*. Hardie (1984) has shown that in *Musca domestica* and *Calliphora erythrocephala* the medulla terminals of marginal *R7* and *R8* receptors (specialized for the detection of the E-vector of polarized light) differ from the majority of receptor terminals (see also Fig. 2a, d of Nässel et al. 1988). Furthermore, Strausfeld and Wunderer (1985) describe in *Calliphora* specific interneurons in the corresponding region of that neuropile after cobalt injection into the retina. *Drosophila* also possesses specialized marginal *R7* and *R8* receptors with large rhabdomere diameters (unpublished EM observations). The medulla terminals of these have not been Golgi impregnated.

We think it is likely that in *Drosophila* as in larger flies the neuropile underlying specialized regions of the compound eye shows special features. It is only in the frontal visual field that certain behaviors of *Drosophila* can be elicited, e.g., the landing response (Fischbach and Bausenwein 1988). Due to its stochastic nature the Golgi method is certainly not a good tool to identify confidently such singularities. It is, nevertheless, noteworthy that several cell types, which have been impregnated repeatedly, have been seen only in neuropile areas subserving the frontal visual field. Examples are *Tm28* and *Mt8* in Fig. 6 and *Tm26* in Fig. 11.

4. Functional versus morphological polarity of neurons in the optic lobe

Insect neurons differ from the classical picture of a vertebrate neuron in that the information flow from dendrites to axon does not converge upon the cell body. The cell bodies of insect neurons are typically far removed from the neuropile and are connected to those of their parts that process synaptic information by a thin cell body fiber only. Nevertheless, most neurons in the optic lobe of *Drosophila* examined by light microscopy display morphological polarity. If we assume – as we believe to be reasonable – that the main information flow is from the compound eye to the central brain, the morphological polarity of the majority of neurons in the optic lobe can be used to correlate structural specializations with dendritic or axonal function. Although this argument is generally not liked by electron microscopists who point to the abundance of feedback synapses, it is most clearly demonstrated by the terminals of the receptor axons in the lamina and medulla (see Figs. 3, 24) and by the morphological polarity of most *Tm* and *TmY* neurons. Fine branching seems to be a feature of (mainly) postsynaptic dendrites while a varicose and baggy appearance seems to be a characteristic feature of (mainly) presynaptic axonal terminals perhaps reflecting the presence of intracellular organelles such as mitochondria. This is also well demonstrated by olfactory receptors in the antennal lobe and by the endings of relay neurons in the calyx of the mushroom bodies and the lateral protocerebrum (Borst and Fischbach 1987).

Once such features are detected they can be used to predict the functional polarity of a minority of the neurons in the optic lobe as being centrifugal. Examples are *C2* and *C3* cells (Fig. 3A) and the lobula tangential *Lt3* depicted in Fig. 6. The features can even be applied to make guesses about the polarity of central brain neurons (Hanesch et al. 1989). It should, however, be kept in mind that insect neurons are usually pre- and postsynaptic at the same fine branches (e.g., Armett-Kibel et al. 1977; Ribi 1981; Strausfeld and Bassimir 1983). Therefore, predictions of polarity based upon the light microscopic overview need to be validated by physiological experiments.

5. Conserved and variable neurons in the optic lobe of arthropods and the problem of homology

The overall organization of the optic lobe underlying compound eyes in arthropods is surprisingly similar (Hanström 1928; Strausfeld and Nässel 1981). This supports the idea that not only the compound eye of arthropods (Paulus 1979), but the underlying neural structures as well are of monophyletic origin. Accordingly some insect terminology (lamina, medulla, lobula, serpentine layer, etc.) has been applied in crustaceans (Strausfeld and Nässel 1981). Many neurons in the arthropod optic lobe are likely to be homologues, i.e., derived from a common ancestor. It is, therefore, not surprising that we have been able to name homologous counterparts of a considerable number of neurons in the optic lobe of *Drosophila* and *Musca* or other dipteran species (see Results section and below). On the other hand, the documented differences of even rather conserved homologous neurons in those fly species (Fig. 3C) may explain why so far this was not possible for all neurons. The tracing of the evolutionary history of neurons cannot be done directly, and the establishment of homology between neurons of recent species has to be done by use of one or several of the following criteria:

(1) Criterion of homotopy. This criterion applies if the neurons under consideration occupy the same relative position in the different organisms. The retinula cells *R1–6*, *R7* and *R8* of *Musca* and *Drosophila* are good examples. Their homology can easily be established by considering their positions in the ommatidium. The value of the above criterion becomes clearer when one tries to draw homologies between all of the lamina monopolar neurons of *Drosophila* and *Musca*. The shape of *L4* neurons is fairly different in both species (see Fig. 3C). Nevertheless, homology of the neurons can be assumed because the 4 other lamina monopolar neurons per cartridge (*L1, L2, L3, L5*) are easily identified in both species, so that the remaining monopolar neuron of the cartridge has to be *L4*. The criterion of homotopy (together with that of specific quality, see below) can also be used to establish, e.g., homology between *C2, C3, T1, T2, T3, T4,* and *T5* neurons of different flies.

(2) Criterion of continuity. If neurons diverge in function and shape from each other, the demonstration of intermediate stages can prove homology. In general, it will not be possible to infer neuronal structures from fossils, but continuity can be shown by comparison of recent species with the inclusion of the developmental domain. For example, it is fessable that certain neurons (of the same or of different species) reveal their homology at an early stage of differentiation only. Unfortunately, our attempts to apply the Golgi procedure to the nervous system of *Drosophila* larvae and pupae have failed so far. Other methods of labeling of neuronal populations in larvae and pupae, however, seem to be promising (e.g., Nässel et al. 1987). Shaw and Meinertzhagen (1986) have studied the evolutionary changes at well-

defined synaptic connections made by homologous neurons of the lamina cartridge in representative Diptera from a monophyletic series of 14 families. They showed that within the phylogenetic series, the ensemble postsynaptic to photoreceptor axons changes from the primitive dyad to a tetradic configuration in more recent Muscomorpha. Two new postsynaptic elements from an amacrine cell are added to the postsynaptic elements of *L1* and *L2*. Meinertzhagen and Shaw (1986) argue that within a phylum, evolution occurs mainly by rearranging synaptic connections between preexisting homologous sets of neurons, whereas the generation of new neuron classes is most likely to have been important at the divergence of the major phyla.

In the present paper some evidence has been presented that *Tm* and *TmY* neurons of Diptera may be regarded as homologous. We have shown that intermediate forms do exist and believe that both classes of cell types are derived from a common ancestor (see section 3.3.5.). This supports the notion that new neuronal classes can emerge by neuron duplication (Goodman 1977) and subsequent modification of the duplicate.

(3) Criterion of specific quality (homomorphy). The neurons under consideration may be homologous if they resemble each other with regard to general shape, connectivity, and, most important, gene expression. Using Golgi analysis we can make no statements about connectivity and gene expression, and the shape criterion alone does not seem to be very compelling. However, in case of, e.g., the *Tm1, C2, C3, T1, T2, T3, T4,* and *T5* neurons of *Musca* and *Drosophila* the similarities in shape and the homotopy of cell bodies and dendritic as well as axonal arborizations leave no doubt about their mutual relatedness. Some T neurons seem to be especially well-conserved structures (see Fig. 3D). *T1* neurons are recognizable not only in *Drosophila* and *Musca,* but even in *Limulus* and decapodes (Hanström 1928; Strausfeld and Nässel 1981). One might even suspect that the *T2* neurons of crayfish (Strausfeld and Nässel 1981) are homologous to the dipteran's *T4* cells, although the crayfish lacks the division of the lobula complex into lobula and lobula plate (as do most insects).

If the shape criterion is the only criterion that can be applied, homology between neurons with altered shapes in different species cannot be established. This is especially the case for many medulla columnar neurons (with the exceptions mentioned throughout the text). In the medulla, interspecific variability of neuronal shape seems to be much more pronounced than in the lamina. Although it is clear that, for example, the *Drosophila Tm* and *TmY* neurons are homologous to the *Tm* and *TmY* cells in other Diptera (or even in crustaceans; Strausfeld and Nässel 1981), stating homology between individual types seems to be mere speculation in many cases. Before listing homologous types of neurons in a comprehensive table we prefer to wait for more information about individual cell types, e.g., for information about antibody binding, connectivity, and transmitters used.

What is the reason for the relative increase of interspecific variability of neurons in the medulla as compared to the lamina? It is tempting to speculate that the lamina is involved in more basic computational functions, early steps of visual processing that are essentially the same in all visual systems, while species-specific mechanisms may be localized more centrally.

6. Multiple retinotopic maps in the optic lobe and the segregation of functional pathways

The number of different kinds of columnar neurons in the optic lobe, especially in the medulla, is high. The functional significance of stratification is the key to an understanding of this abundance. Neuronal stratification makes direct synaptic contacts between many columnar cell types less likely while at the same time it enables numerous synaptic connections between others. Specifically, it is quite clear that the different lamina monopolar cells communicate to different sets of *Tm* and *TmY* (see section 3.3.4.).

Therefore, the large number of columnar neurons reflects the segregation of many parallel functional pathways, which are all retinotopically organized. There are clearly many types of columnar neurons in each column, each with a similar field of view, so that this segregation into parallel functional pathways is overlaid on and has to be distinguished from the existence of parallel pathways imposed upon the optic neuropiles by the repetitive organization of the retina. While the parallel neurons of the same kind in different columns specify spatial information, the parallel neurons of different type inside the same column specify different 'meanings' of visual information. Activity labeling experiments (Buchner et al. 1984; Bausenwein 1988) suggest that the specification of 'meaning' increases centripetally.

The peripheral segregation of functional pathways is one of the main postulates derived from our anatomical description of the visual system. It is supported by physiological evidence from larger flies and by specific behavioral defects of structural mutants of the optic lobe in *Drosophila* as will be discussed below.

7. Is the structural organization of the optic lobe adapted for independent genetic modifiability of functions?

Brains are the result of evolutionary change. This should be reflected in their structure, which is not expected to be optimized for the execution of present functions alone (e.g., Dumont and Robertson 1986). Only such brain organizations survived that could readily be modified in the course of evolution. If different functional pathways share common building blocks (e.g., receptor cells, movement detectors), independent evolution of functions by genetic modification of these common elements is not possible. Therefore, a peripheral separation of neuronal pathways for diverse visual functions might have been of selective advantage. As a consequence, visual functions can be altered at several levels rather specifically by mutations. If we assume that the neuronal cell types of different functional pathways are specified by different (although overlapping) sets of genes, the optic lobe provides an abundance of target sites for visual function-specific gene actions. This has been experimentally confirmed by the isolation of *Drosophila* mutants with defects in certain behavioral subroutines only (Benzer 1971; Fischbach and Heisenberg 1981, 1984; Hall 1982).

Recent advances in vision research in insects have emphasized the existence of parallel visual pathways (for reviews see Wehner 1983; Heisenberg and Wolf 1984; Fischbach and Heisenberg 1984; Hardie 1986). In *Drosophila,* movement detection relies solely on receptors *R1–6* (Heisenberg and Buchner 1977), while in fast phototaxis adult *Drosophila* use *R7* and *R8* receptors at high light intensities only (Hu and Stark 1977). In many cases different functions rely on input derived from different sets of photoreceptors

(Wada 1974; Wehner 1983; Strausfeld and Wunderer 1985). In extreme cases this may even result in divided compound eyes and optic lobes, e.g., in males of the mayfly *Baetis rhodani* (Nässel 1988a). In *Calliphora* and *Musca* examples for a nearly complete regional separation of visual functions include the sexual dimorphism in chasing behavior (Land and Collett 1974). A dorso-frontal region of the eye of male *Musca domestica* has specialized *R7* receptors (Franceschini et al. 1981), which terminate in the lamina and synapse preferentially upon *L3* neurons (Hardie 1983). At the isotopic position of the lobula, male-specific interneurons are found (Hausen and Strausfeld 1980). Thus, the sexually dimorphic behavior of *Musca* nicely exemplifies not only that specific behaviors use modified neuronal pathways, but also that these pathways are under genetic control. It is in *Drosophila* that the gentic control of different functional pathways can best be analyzed.

We hope that some of the data presented in this paper will serve as a wild-type reference for detailed structural characterizations of optic lobe mutants, and that the comparison thereby enabled will help to elucidate structure-function relationships in the visual system of the fly as well as to unravel genetic and epigenetic algorithms (Stent 1981) combined in its development.

Acknowledgements. The experimental work presented here was initiated and nearly completed in the laboratory of Martin Heisenberg. Many thanks to him for his strong support and his interest in our work. We are indebted to Mrs. Claudia Götz for excellent technical assistence, and to Bernhard Bausenwein, Jośe Campos-Ortega, Martin Heisenberg, and Gerhard Technau for useful comments on the manuscript. Special thanks to Ian Meinertzhagen and an anonymous referee whose suggestions helped a lot to improve the text. Thanks also to Mrs. Ulli Hanesch who provided some of her Golgi-rapid preparations for evaluation. The work was supported by DFG grants to K.-F.F.

References

Aceves-Piña EO, Booker R, Duerr JS, Livingstone MS, Quinn WG, Smith RF, Sziber PP, Tempel BL, Tully TP (1983) Learning and memory in *Drosophila* studied with mutants. Cold Spring Harbor Symp Quant biol 48:831–840

Armett-Kibel C, Meinertzhagen IA, Dowling JE (1977) Cellular and synaptic organization in the lamina of the dragon-fly *Sympetrum rubicundulum*. Proc R Soc Lond [Biol] 196:385–413

Bausenwein B (1988) Neuronale Aktivitätsmarkierung während visueller Flugsteuerung von *Drosophila melanogaster*. Dissertation. Julius-Maximilians-Universität Würzburg, FRG

Benzer S (1971) From the gene to behavior. J Am Med Assoc 218:1015–1022

Blest AD (1961) Some modifications of Holme's silver method for insect central nervous system. Q J Micr Sci 102:413–417

Blondeau J, Heisenberg M (1982) The three-dimensional optomotor torque system of *Drosophila melanogaster*. Studies on wild-type and the mutant *optomotor blind*^{H31}. J Comp Physiol 145:321–329

Borst A, Fischbach K-F (1987) Golgi- and degeneration studies of the antennal lobes of *Drosophila melanogaster*. J Neurogenetics 4:115–117

Boschek CR (1971) On the fine structure of the peripheral retina and the lamina of the fly *Musca domestica*. Z Zellforsch 110:336–349

Boschert U, Ramos R GP, Tix S, Technau GM, Fischbach K-F (1989) Genetic and developmental analysis of *irreC*, a genetic function required for optic chiasm formation in *Drosophila*. J Neurogenetics (submitted)

Braitenberg V (1967) Patterns of projections in the visual system of the fly. I. Retina lamina projections. Exp Brain Res 3:271–298

Braitenberg V, Debbage P (1974) A regular net of reciprocal synapses in the visual system of the fly, *Musca domestica*. J Comp Physiol 90:25–31

Braitenberg V, Strausfeld NJ (1970) The compound eye of the fly (*Musca domestica*): Connections between the cartridges of the lamina ganglionaris. Z vergl Physiol 70:95–104

Buchner E, Buchner S, Bülthoff I (1984) Deoxyglucose mapping of nervous activity induced in Drosophila brain by visual movement. J Comp Physiol [A] 155:471–483

Buchner E, Buchner S, Crawford G, Mason WT, Salvaterra PM, Sattelle DB (1986) Choline acetyltransferase-like immunoreactivity in the brain of *Drosophila melanogaster*. Cell Tissue Res 246:57–62

Buchner E, Bader R, Buchner S, Cox J, Emson PC, Flory E, Heizmann CW, Hemm S, Hofbauer A, Oertel WH (1988) Cell-specific immunoprobes for the brain of normal and mutant *Drosophila melanogaster*. Cell Tissue Res 253:357–370

Bülthoff I, Buchner E (1985) Deoxyglucose mapping of nervous activity induced in *Drosophila* brain by visual movement. II. *optomotor blind*^{H31} and *lobula plate-less*^{N684}, visual mutants. J Comp Physiol [A] 156:25–34

Burkhardt W, Braitenberg V (1976) Some peculiar synaptic complexes in the first visual ganglion of the fly *Musca domestica*. Cell Tissue Res 173:287–308

Cajal SR, Sánchez D (1915) Contribucion al conocimiento de los centros nerviosos de los insectos. Parte I, retina y centros opticos. Trab Lab Invest Biol Univ Madr 13:1–168

Campos-Ortega JA, Strausfeld NJ (1972a) Columns and layers in the second synaptic region of the fly's visual system. The case for two superimposed neuronal architectures. In: Wehner R (ed) Information processing in the visual system of arthropods. Springer, Berlin Heidelberg New York

Campos-Ortega JA, Strausfeld NJ (1972b) The columnar organization of the second synaptic region of the visual system of *Musca domestica* L. I. receptor terminals in the medulla. Z Zellforsch 124:561–585

Campos-Ortega JA, Strausfeld NJ (1973) Synaptic connections of intrinsic cells and basket arborizations in the external plexiform layer of the fly's eye. Brain Res 59:119–136

Colonnier M (1964) The tangential organization of the visual cortex. J Anat 98:327–344

Datum K-H, Weiler R, Zettler F (1986) Immunocytochemical demonstration of γ-amino butyric acid and glutamic acid decarboxylase in *R7* photoreceptors and *C2* centrifugal fibers in the blowfly visual system. J Comp Physiol 159:241–249

Dumont JPC, Robertson RM (1986) Neuronal circuits: An evolutionary perspective. Science 233:849–853

Ewer J, Rosbash M, Hall JC (1988) An inducible promoter fused to the *period* gene in *Drosophila* conditionally rescues adult *per*-mutant arrhythmicity. Nature 333:82–84

Fischbach K-F (1983a) Neural cell types surviving congenital sensory deprivation in the optic lobes of *Drosophila melanogaster*. Dev Biol 95:1–18

Fischbach K-F (1983b) Neurogenetik am Beispiel des visuellen Systems von *Drosophila melanogaster*. Habilitation Thesis. University of Würzburg

Fischbach K-F, Bausenwein B (1988) Habituation and sensitization of the landing response of *Drosophila melanogaster*: II. Receptive field size of habituating units. In: Hertting G, Spatz H Ch (eds) Modulation of synaptic transmission and plasticity in nervous systems. NATO ASI Series, Springer, Berlin Heidelberg New York, pp 369–385

Fischbach K-F, Götz CR (1981) Das Experiment. Ein Blick ins Fliegengehirn: Golgi gefärbte Nervenzellen bei *Drosophila*. BiuZ 11:183–187

Fischbach K-F, Heisenberg M (1981) Structural brain mutant of *Drosophila melanogaster* with reduced cell number in the medulla cortex and with normal optomotor yaw response. Proc Natl Acad Sci USA 78:1105–1109

Fischbach K-F, Heisenberg M (1984) Neurogenetics and behaviour in insects. J Exp Biol 112:65–93
Fischbach K-F, Lyly-Hünerberg I (1983) Genetic dissection of the anterior optic tract. Cell Tissue Res 231:551–563
Fischbach K-F, Technau G (1984) Cell degeneration in the developing optic lobes of the *sine oculis* and *small optic lobes* mutants of *Drosophila melanogaster*. Dev Biol 104:219–239
Fischbach K-F, Technau G (1987) Mutant analysis of optic lobe development in *Drosophila*. In: Elsner N, Creutzfeld O (ed) New frontiers in brain research. Georg Thieme Verlag, Stuttgart New York
Fischbach KF, Barleben F, Boschert U, Dittrich APM, Gschwander B, Houbé B, Jäger R, Kaltenbach E, Ramos RGP, Schlosser G (1989) Developmental studies on the optic lobe of *Drosophila melanogaster* using structural brain mutants. In: Singh RN, Strausfeld NJ (eds) Neurobiology of sensory systems. Plenum Press
Franceschini N, Hardie R, Ribi W, Kirschfeld K (1981) Sexual dimorphism in a photoreceptor. Nature 291:241–244
Garen SH, Kankel DR (1983) Golgi and genetic mosaic analyses of visual system mutants in *Drosophila melanogaster*. Dev Biol 96:445–466
Goodman CS (1977) Neuron duplications and deletions in locust clones and clutches. Science 197:1384–1386
Goodman CS, Raper JA, Ho RK, Chang S (1982) Pathfinding of neuronal growth cones in grasshopper embryos. In: Subtelny S, Green PB (eds) Developmental order: its origin and regulation. Alan R Liss, New York, pp 275–316
Hall JC (1982) Genetics of the nervous system in *Drosophila*. Q Rev Biophys 15:223–479
Hall JC (1984) Genetic analysis of behavior in insects. In: Kerkut GA, Gilbert LI (eds) Comprehensive Insect Physiology Biochemistry and Pharmacology 9:287–373
Hanesch U (1986) Der Zentralkomplex von *Drosophila melanogaster*. PHD Thesis, University of Würzburg
Hanesch U, Fischbach KF, Heisenberg M (1989) Neuronal architecture of the central complex in *Drosophila melanogaster*. Cell Tissue Res 257:343–366
Hanström B (1928) Vergleichende Anatomie des Nervensystems der wirbellosen Tiere. Springer, Berlin Heidelberg New York
Hardie RC (1983) Projection and connectivity of sex-specific photoreceptors in the compound eye of the male housefly (*Musca domestica*). Cell Tissue Res 233:1–21
Hardie RC (1984) Properties of photoreceptors R7 and R8 in dorsal marginal ommatidia in the compound eyes of *Musca* and *Calliphora*. J Comp Physiol 154:157–165
Hardie RC (1986) The photoreceptor array of the dipteran retina. TINS 9:419–423
Hardie RC (1987) Is histamine a neurotransmitter in insect photoreceptors? J Comp Physiol 161:201–213
Harris WS, Stark WS, Walker JA (1976) Genetic dissection of the photoreceptor system in the compound eye of *Drosophila melanogaster*. J Physiol (Lond) 256:415–439
Hausen K (1976) Functional characterization and anatomical identification of motion sensitive neurons in the lobula plate of the blowfly *Calliphora erythrocephala*. Z Naturforsch 31 C:629–633
Hausen K (1981) Monocular and binocular compution of motion in the lobula plate of the fly. Verh Dtsch Zool Ges 49–70
Hausen K, Strausfeld NJ (1980) Sexually dimorphic interneuron arrangements in the fly visual system. Proc R Soc Lond [Biol] 208:57–71
Hauser-Holschuh H (1975) Vergleichend quantitative Untersuchungen an den Sehganglien der Fliegen *Musca domestica* and *Drosophila melanogaster*. Dissertation Tübingen
Heisenberg M (1980) Mutants of brain structure and function: What is the significance of the mushbroom bodies for behavior? In: Siddiqi O, Babu P, Hall LM, Hall JC (eds) Development and Neurobiology of *Drosophila*. Plenum Press, New York, pp 373–390
Heisenberg M, Buchner E (1977) The rôle of retinula cell types in visual behavior of *Drosophila melanogaster*. J Comp Physiol 117:127–162
Heisenberg M, Wolf R (1984) Vision in *Drosophila*. Genetics of microbehavior. Springer, Berlin Heidelberg New York
Heisenberg M, Wonneberger R, Wolf R (1978) Optomotor blind^{H31} – A *Drosophila* mutant of the lobula plate giant neurons. J Comp Physiol [A] 124:287–296
Hofbauer A (1979) Die Entwicklung der optischen Ganglien bei *Drosophila melanogaster*. Dissertation, Universität Freiburg
Hu KG, Stark WS (1977) Specific receptor input into spectral preference in *Drosophila*. J comp Physiol 121:241–252
Jacob KG, Willmund R, Folkers E, Fischbach KF, Spatz H Ch (1977) T-maze phototaxis of *Drosophila melanogaster* and several mutants in the visual system. J Comp Physiol 116:209–225
Kirschfeld K (1967) Die Projektion der optischen Umwelt auf das Raster der Rhabdomere im Komplexauge von *Musca*. Exp Brain Res 3:248–270
Kirschfeld K (1973) Das neurale Superpositionsauge. Fortschr Zool 21:229–257
Konopka R, Wells S, Lee T (1983) Mosaic analysis of a *Drosophila* clock mutant. Mol Gen Genetics 190:284–288
Kral K, Meinertzhagen IA (1989) Anatomical plasticity of synapses in the lamina of the optic lobe of the fly. Philos Trans R Soc Lond [Biol] 323:155–183
Land MF, Collett TS (1974) Chasing behavior of houseflies (*Fannia canniculuris*). J Comp Physiol 89:331–357
Macagno ER, Lopresti V, Levinthal C (1973) Structure and development of neuronal connections in isogenic organisms. Variation and similarities in the optic system of *Daphnia*. Proc Natl Acad Sci USA 70:57–61
Meinertzhagen IA (1973) Development of the compound eye and optic lobe of insects. In: Young D (ed) Developmental neurobiology of arthropods. Cambridge University Press
Meinertzhagen IA (1989) Fly photoreceptor synapses: their development, evolution and plasticity. J Neurobiol 20:276–294
Meinertzhagen IA, O'Neil S (1988) The lamina cartridge in the optic lobe of *Drosophila*. Soc Neurosci [Abstr] 14:376
Nässel DR (1988a) Serotonin and serotonin-immunoreactive neurons in the nervous system of insects. Prog Neurobiol 30:1–85
Nässel DR (1988b) Immunocytochemistry of putative neuroactive substances in the insect brain. In: Salanki J, Rósza KS (eds) Neurobiology of invertebrates: Transmitters, modulators and receptors. Akadémiai Kiadó, Budapest
Nässel DR, Sivasubramanian P (1983) Neural differentiation in fly CNS transplants cultured in vivo. J Exp Zool 225:301–310
Nässel DR, Hagberg M, Seyan HS (1983) A new, possibly serotonergic neuron in the lamina of the blowfly optic lobe: an immunocytochemical and Golgi-EM study. Brain Res 280:361–367
Nässel DR, Meyer EP, Klemm N (1985) Mapping and ultrastructure of serotonin-immunoreactive neurons in the optic lobes of three insect species. J Comp Neurol 232:190–204
Nässel DR, Ohlsson L, Sivasubramanian P (1987) Differentiation of serotonin-immunoreactive neurons in fly optic lobes developing in situ or cultured in vivo without eye discs. J Comp Neurol 255:327–340
Nässel Dr, Holmquist MH, Hardie RC, Håkanson R, Sundler F (1988) Histamine-like immunoreactivity in photoreceptors of the compound eyes and ocelli of the flies (*Calliphora erythrocephala* and *Musca domestica*). Cell Tissue Res 253:639–646
Nicol D, Meinertzhagen IA (1982) An analysis of the number and composition of the synaptic populations formed by photoreceptors of the fly. J Comp Neurol 207:29–44
Ohlsson LG, Nässel DR (1987) Postembryonic development of serotonin-immunoreactive neurons in the central nervous system of the blowfly, *Calliphora erythrocephala*. I. The optic lobes. Cell Tissue Res 249:669–679
Paulus HF (1979) Eye structure and the monophyly of the Arthropoda. In: Gupta AP (ed) Arthropod phylogeny. Van Nostrand Reinhold Comp, New York, pp 299–383

Pierantoni R (1976) A look into the cockpit of the fly. The architecture of the lobular plate. Cell Tissue Res 171:101–122

Power ME (1943) The effect of reduction in numbers of ommatidia upon the brain of *Drosophila melanogaster*. J Exp Zool 94:33–71

Ribi WA (1981) The first optic ganglion of the bee. IV. Synaptic fine structure and connectivity patterns of receptor cell axons and first order interneurones. Cell Tissues Res 215:443–464

Shaw SR (1981) Anatomy and physiology of identified non-spiking cells in the photoreceptor-lamina complex of the compound eye of insects especially Diptera. In: Roberts A, Bush BMH (eds) Neurons without Impulses. Cambridge University Press

Shaw SR (1984) Early visual processing in insects. J Exp Biol 112:225–251

Shaw SR, Meinertzhagen IA (1986) Evolutionary progression at synaptic connections made by identified homologous neurons. Proc Natl Acad Sci USA 83:7961–7965

Steller H, Fischbach K-F, Rubin G (1987) Disconnected: A locus required for neuronal pathway formation. Cell 50:1139–1153

Stent GS (1981) Strength and weakness of the genetic approach to the development of the nervous system. Annu Rev Neurosci 4:163–194

Stocker RF, Singh RN, Schorderet M, Siddiqi O (1983) Projection patterns of different types of antennal sensilla in the antennal glomeruli of *Drosophila melanogaster*. Cell Tissue Res 232:237–248

Strausfeld NJ (1970) Golgi studies on insects. Part II. The optic lobes of Diptera. Philos Trans R Soc Lond 258:135–223

Strausfeld NJ (1971) The organization of the insect visual system (light microscopy). II. The projection of fibres across the first optic chiasm. Z Zellforsch 121:442–454

Strausfeld NJ (1976) Atlas of an insect brain. Springer, Berlin Heidelberg New York

Strausfeld NJ (1979) The representation of a receptor map within retinotopic neuropil of the fly. Verh Dtsch Zool Ges 72:167–179

Strausfeld NJ (1980a) Male and female visual neurons in dipterous insects. Nature 283:381–383

Strausfeld NJ (1980b) The Golgi method. Its application to the insect nervous system and the phenomenon of stochastic impregnation. In: Strausfeld NJ, Miller TA (eds) Neuroanatomical techniques. Insect nervous system. Springer, Berlin Heidelberg New York

Strausfeld NJ (1984) Functional neuroanatomy of the blowfly's visual system. In: Ali MA (ed) Photoreception and vision in invertebrates. Plenum Publishing Corporation.

Strausfeld NJ, Bassimir UK (1983) Cobalt-coupled neurons of a giant fibre system in Diptera. J Neurocytol 12:971–991

Strausfeld NJ, Blest AD (1970) Golgi study on insects. Part I. The optic lobes of Lepidoptera. Philos Trans R Soc Lond [Biol] 258:81–134

Strausfeld NJ, Campos-Ortega JA (1972) Some interrelationships between the first and second synaptic regions of the fly's (Musca domestica) visual system. In: Wehner R (ed) Information processing in the visual system of arthropods. Springer, Berlin Heidelberg New York

Strausfeld NJ, Campos-Ortega JA (1973a) The L4 monopolar neurone: A substrate for lateral interaction in the visual system of the fly *Musca domestica*. Brain 59:97–117

Strausfeld NJ, Campos-Ortega JA (1973b) L3, the 3rd 2nd order neuron of the 1st visual ganglion in the "neural superposition" eye of *Musca domestica*. Z Zellforsch 139:397–403

Strausfeld NJ, Campos-Ortega JA (1977) Vision in insects. Pathways possibly underlying neural adaptation and lateral inhibition. Science 195:894–897

Strausfeld NJ, Hausen K (1977) The resolution of neuronal assemblies after cobalt injection into neuropil. Proc R Soc Lond 199B:463–476

Strausfeld NJ, Nässel DR (1981) Neuroarchitecture of brain regions that subserve the compound eyes of crustacia and insects. In: Handbook of sensory physiology Vol. VII/6B. Springer, Berlin Heidelberg New York

Strausfeld NJ, Wunderer H (1985) Optic lobe projections of marginal ommatidia in *Calliphora erythrocephala* specialized for the detection of polarized light. Cell Tissue Res 242:163–178

Strausfeld NJ, Bassemir UK, Singh RN, Bacon JP (1984) Organizational principles of outputs from dipteran brains. J Insect Physiol 30:73–93

Stürmer CAO (1988) The trajectories of regenerating retinal axons in the goldfish. II. Exploratory branches and growth cones on axons at early regeneration stages. J Comp Neurol 267:69–91

Tanouye MA, Wyman R (1980) Motor outputs of giant nerve fibres in *Drosophila*. J. Neurophysiol 44:405–421

Technau GM (1984) Fiber number in the mushroom bodies of adult *Drosophila melanogaster* depends on age, sex and experience. J Neurogenetics 1:113–126

Technau GM, Heisenberg M (1982) Neural reorganization during metamorphosis of the corpora pedunculata in *Drosophila melanogaster*. Nature 295:405–407

Tix S, Minden JS, Technau GM (1989) Pre-existing neuronal pathways in the developing optic lobes of *Drosophila*. Development 105:739–746

Trujillo-Cenóz O (1965) Some aspects of the structural organization of the intermediate retina of Dipterans. J Ultrastruct Res 13:1–33

Tully T (1988) On the road to a better understanding of learning and memory in *Drosophila melanogaster*. In: G Hertting G, Spatz H Ch (eds) Modulation of synaptic transmission and plasticity in nervous system. NATO ASI Series, Springer, Berlin Heidelberg New York

Wada S (1974) Spezielle randzonale Ommatidien von *Calliphora erythrocephala* Meig. (Diptera, Calliphoridae): Architektur der zentralen Rhabdomeren-Kolumne und Topographie im Komplexauge. Int J Insect Morphol Embryol 3:397–424

Wehner R (1983) Celestial and terrestrial navigations: Human strategies – insect strategies. In: Huber F, Markl H (eds) Neuroethology and behavioural physiology. Springer, Berlin Heidelberg New York, pp 366–381

Accepted July 10, 1989