Golgi analysis of tangential neurons in the lobula plate of *Drosophila melanogaster*

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The lobula plate (LP), which is the third order optic neuropil of flies, houses wide-field neurons which are exquisitely sensitive to motion. Among Diptera, motion-sensitive neurons of larger flies have been studied at the anatomical and physiological levels. However, the neurons of *Drosophila* lobula plate are relatively less explored. As *Drosophila* permits a genetic analysis of neural functions, we have analysed the organization of lobula plate of *Drosophila melanogaster*.

Neurons belonging to eight anatomical classes have been observed in the present study. Three neurons of the horizontal system (HS) have been visualized. The HS north (HSN) neuron, occupying the dorsal lobula plate is stunted in its geometry compared to that of larger flies. Associated with the HS neurons, thinner horizontal elements known as h-cells have also been visualized in the present study. Five of the six known neurons of the vertical system (VS) have been visualized. Three additional neurons in the proximal LP comparable in anatomy to VS system have been stained. We have termed them as additional VS (AVS)-like neurons. Three thinner tangential cells that are comparable to VS neurons, which are elements of twin vertical system (tvs); and two cells with wide dendritic fields comparable to CH neurons of Diptera have been also observed. Neurons comparable to VS cells but with 'tufted' dendrites have been stained. The HSN and VS1-VS2 neurons are dorsally stunted. This is possibly due to the shape of the compound eye of *Drosophila* which is reduced in the fronto-dorsal region as compared to larger flies.

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1. Introduction

Insect visual systems provide amenable models for exploring the principles of design and function of vision in general. Special abilities like flight, foraging and navigation in insects demand a highly efficient visual system. Admiring the abilities of insect visual systems and opto-motor responses, Egelhaaf and Kern (2002) state: 'despite their small brain, insects easily outperform current man-made autonomous vehicles in many respects'. Apart from their ability to sense colours, polarized light and recognition of patterns, compound eyes of insects can detect motion that guides appropriate opto-motor responses. Each ommatidium of the compound eye contains eight

Keywords. *Drosophila*; lobula plate; motion perception; vision

Abbreviations used: AVS, Additional vertical system; HS, horizontal system; HSE, horizontal system equatorial; HSN, horizontal system north; HSS, horizontal system south; MARCM, mosaic analysis with a repressible cell marker; *omb*^{H31}, optomotor-blind^{H31}; VS, vertical system.

photosensitive retinula cells (R1-R8). The retinula cells R1-R6 synapse with precision on to lamina interneurons (Nicol and Meinertzhagen 1992), while R7-R8 retinula cells synapse in the second optic neuropil – the medulla (see figure 1). The medulla is connected with the lobula complex consisting of the anterior lobula and the posterior lobula plate. The distal lobula plate (region adjacent to the medulla) receives input from frontal visual field, while proximal lobula plate receives input from the lateral or caudal visual field (Eckert and Bishop 1978). Cells with dendritic profiles, parallel to the axis of the visual information flow, are termed as the columnar elements. The perpendicular ones are the tangential fibres.

1.1 *Motion perception*

Motion perception is one of the remarkable abilities of the fly visual system. Flies can perceive and follow moving objects. Movements of the fly, or its head, cause displacement of the visual field and this is termed as visual flow field. By studying the behaviour (Srinivasan *et al* 1999), motion perception has been analysed in flies. The underlying neural substrates have been studied by anatomical and electrophysiological techniques (Hausen 1984; Egelhaaf and Borst 1993). Apart from eliciting appropriate optomotor responses (yaw, pitch and role), perception of motion has been demonstrated to be instrumental in course-correction by computing the visual input to each of the compound eyes. Distance perception in insects is achieved by peering. When a locust moves its head, the amplitude and angle of movement of the image on the retina are computed to gauge the distance of objects. While landing, appropriate motor programmes elicit extension of legs in preparation to landing depending on the rate of expansion of an image on the retina and odometry in flies is made possible by rate of flow of the field during flight. Thus motion perception plays a significant role in many of the visually mediated behaviours of insects. The lobula plate region in the fly's brain has been shown to contain neurons, with large dendritic fields (Pierantoni 1973, 1976; Strausfeld 1976; Dvorak *et al* 1975; Hausen 1976, 1981, 1984; Hengstenberg *et al* 1982; Krapp *et al* 1998), that are sensitive to movement in the visual field. Through extensive electrophysiological and anatomical studies, 21 classes of lobula plate neurons have been identified and 10 classes of such neurons are well-characterized (Hausen 1981, 1984). This classification is based largely on arborization patterns of dendritic fields in the lobula plate. A detailed account of the anatomical types of lobula plate neurons in Diptera (*Musca*, *Phaenicia* and *Calliphora*) is provided by Hausen (1984). The lobula plate motion-sensitive neurons are presynaptic to the descending giant neuron that constitute a major component of the descending pathway through the neck connectives (Strausfeld and Bassemir 1985). More recently these descending neurons have also been shown to be presynaptic onto the motor neurons regulating the musculature of the halteres in Diptera (Chan *et al* 1998). The descending giants neurons thus regulate flight by computing visual input.

1.2 *Optomotor responses and lobula plate neurons in* Drosophila melanogaster

Compared to the wealth of information available on the lobula plate of other Diptera (*Musca*, *Calliphora* and *Phenicia*), the neurons of the *Drosophila* lobula plate are not well explored. Despite an exciting possibility of a genetic dissection of motion perception and application of molecular biological tools, relatively few motion-sensitive mutations or structural mutations of the lobula plate have been identified and characterized (Heisenberg *et al* 1978; Bulthoff and Buchner 1985; Bausenwein *et al* 1986). A brief description of the lobula plate horizontal cells and vertical cells based on semi-thin sections was provided by Heisenberg *et al* (1978) while studying optomotor-blind^{H31} (*omb*^{H31}) mutation. They found that the mutant *omb*^{H31} has reduced lobula plate neurons. The turning reaction in flight and during walking is strongly reduced in *omb*^{H31} flies. Fischbach and Dittrich (1989) have described the dendritic organization of horizontal system (HS) neurons briefly. A brief account of some of the lobula plate neurons of *Drosophila* has been presented by us elsewhere (Rajashekhar and Shamprasad 2001). Recently, Scott *et al* (2002), applying mosaic analysis with a repressible cell marker (MARCM) technique, described the HS and the vertical system (VS) neurons. A common cell-lineage for the VS cells comprising six neurons and a common lineage for HS comprising three neurons has been proposed (Scott *et al* 2002). Buchner (1984) used 2-deoxy glucose technique to analyse the regions of the lobula plate associated with reference to motion perception. Similar analyses were later carried out on visual mutants *omb*^{H31} and *lobula plate-less*^{N684} (*lop*^{N684}) (Bulthoff and Buchner 1985). To aid further studies on the lobula plate of *Drosophila* through molecular genetic techniques that have been developed recently, a study on the tangential neurons of the lobula plate of *Drosophila* was carried out. Dendritic arbors of neurons representing the major classes of motion- sensitive neurons have been visualized and described, based predominantly on Golgi-silver impregnation. Surveying the anatomical types of lobula plate neurons eight anatomical categories of cells have been observed.

2. Materials and methods

A certified culture of *Drosophila melanogaster* Oregon-K wild type flies was obtained from *Drosophila* Stock Centre at University of Mysore and maintained in the laboratory. Neurons of the lobula plate were Golgi-silver impregnated by Golgi-rapid and Golgi-Collonier methods. Fibre tracts and gross neural arrangement were studied by Bodian-Protargol silver staining. Horseradish peroxidase technique was also applied for visulaizing some of the neurons. However, the illustrations provided are Impregnations of Golgi-silver techniques, only due to their clarity in revealing the dendritic arbors.

2.1 *Golgi-rapid technique*

The flies aged 3 or 4 days were cooled, dissected and fixed in Karnovsky's fixative (Karnovsky 1965) in phosphate buffer for 4 h at room temperature. The fixed specimens were washed with phosphate buffer thrice and specimens were stored in buffer at 4°C. Subsequenly, heads were incubated in a mixture containing 0·5 volume of 4% osmium tetroxide (Sigma Aldrich, O-5500) and 20, 30 or 50 volumes of 2·5% potassium dichromate in the dark for two days. The heads were given a quick rinse in 0·1% silver nitrate (Merck, DA1 DR51009) until no more red precipitate was formed in the washings. Heads were then incubated in 0·75% silver nitrate solution in the dark for 48 h and washed with distilled water, dehydrated and embedded in soft araldite (Fluka, 44610). Saggital and frontal sections were cut on a sliding microtome, at a thickness of 30 μm.

2.2 *Golgi-Collonier technique*

The dissected flies were incubated in a mixture containing one volume of stock 25% glutaraldehyde and 4 volumes of 2·5% potassium dichromate for 2–3 days. The specimens were washed in 0·1% silver nitrate and subsequently incubated in 0·75% silver nitrate solution for 12–18 h in the dark. The process of chromation and silver impregnation was repeated once. The specimens were washed with distilled water, dehydrated and embedded in soft araldite and sectioned at 30 μm thickness.

2.3 *Bodian-Protargol silver staining*

Flies were anaesthetized by cooling on ice, the heads were dissected and a slit was made at the occiput. They were fixed in alcohol : acetic acid : formaline for 4 h at room temperature. At the end of fixation, heads were washed in 70% ethanol and dehydrated. Specimens were embedded in paraplast plus (Sigma) and sectioned at 10 μm thickness. Deparaffinized sections were hydrated and incubated in 2% aqueous solution of silver proteinate in the presence of copper turnings in the dark at 37°C for 48 h. Afterwards slides were rinsed with distilled water and treated with the reducer solution (1% hydroquinone and 5% sodium sulphite in 100 ml solution) for 10–15 min. Slides were rinsed well with distilled water and treated again with silver proteinate solution in contact with copper metal for 24–48 h. The sections were rinsed and treated for 10–15 min with the reducer solution and washed with distilled water.

Sections were then treated with 1% solution of gold chloride in 1% citric acid and acetic acid in bright light at 25°C for 10–15 min. Sections were quickly and briefly rinsed in distilled water. Differentiation was done by dipping the section in 2% oxalic acid, until sections appeared bluish-red. Slides were rinsed very quickly with distilled water and fixed in 5% sodium thiosulphate solution for 15 min. They were washed in distilled water, dehydrated and mounted in Entellan. Serial sections from above mentioned techniques were observed using Olympus BX60 trinocular microscope and analysed for the neuronal organization. Drawings were made using a camera lucida attachment (U-DA) for three-dimensional reconstruction of neurons. Microphotographs were taken using Olympus SC35 camera.

3. Results

The prominent optic lobes in the optic pathway are lamina, medulla and lobula complex that includes the lobula and lobula plate. The present description concentrates on the third optic neuropil, the lobula plate. The lobula and lobula plate are arranged with their axis perpendicular to the retina. Lamina and medulla have uniform thickness from frontal to caudal region. The same holds good for the lobula also. However the lobula plate tapers from distal end to the proximal end (figure 1), measuring 45 μm at its distal end and 22 μm at its proximal end in a section passing through the dorso-ventral mid-point of the lobula plate. In a frontal section the *Drosophila* lobula plate is nearly oval measuring 80 μm in width from proximal to the distal part and 115 μm from dorsal to the ventral (figure 2a). The Golgi-impregnated neurons in the present study of lobula plate were broadly classified following the classifications of lobula plate neurons in *Calliphora* (Hausen 1981). The descriptions of neurons given below are based on dendritic arrangements in the lobula plate, which are of functional significance (Fischbach and Dittrich 1989). Therefore cell bodies are not depicted in camera lucida reconstructions.

3.1 *Horizontal system*

Similar to the horizontal system (HS) of other Diptera the lobula plate of *Drosophila* has three neurons (figure 2a, b, c, d). The dorsal neuron, which by convention is the horizontal system north (HSN) neuron, has its neurites and dendritic arbors entering the lobula plate near the dorsal margin of lobula plate neuropil. It extends till the anterior margin compared to the axis of the main neurites (figure 2b, c, f). The arbors branch centrifugally and ventrally. There are very few dendritic arbors in the region dorsal to main neurites (figure 2b, f). Minor variations do occur in the arborization. However, the arbors cover about a third of the lobula plate neuropil in a frontal aspect. The horizontal equatorial (HSE) cell is the middle one and shows two major branches and has dendritic disposition in such a way that the arbors are distributed on either sides of the axons (figure 2d, f). The horizontal system south (HSS) neuron covers half the lobula plate neuropil area in the ventral part (figure 2d, f). The three neurons (HSN, HSE and HSS) thus have overlapping visual fields (figure 2d, f). Their dendritic terminals however are more concentrated in the distal lobula plate than the proximal lobula plate.

3.2 *Vertical system*

The vertical system (VS) neurons are so named by virtue of their predominantly dorso-ventral branching pattern, confined to a narrow region of the lobula plate. Five Golgi-impregnated neurons comparable to description of VS neuron in Diptera (Hausen 1981) and *Drosophila* (Scott *et al* 2002) were observed in the present study (figure 3a, b, g, h). Five of them are clearly seen in the Bodian-Protargol stained prepartion (figure 2a). The vertical system neurons occupy the posterior layers 3 (Lop3) and 4 (Lop4) of the lobula plate (figure 2b). The most distal VS neuron is the VS1 that has its arbors predominantly ventral to the main dendrites with the main dendrite exhibiting a sickle-shaped profile (figure 3a, b, g, h). The geometry shows one or two dendrites on the dorsal side and large number of lateral branches in the medial and ventral side. The branches narrow down in width and the field of dendrites tapers posteriorly. VS2 neuron (figure 3a, b, g) is the second in the stack of VS and has dendrites comparable to VS1 but with a prominent dorsal dendritic branch. The dendritic arbors of VS3 (figure 3a, b, g) and VS4 (figure 3a, b, g) resemble the branching pattern shown by typical VS neurons of larger Diptera by virtue of having dorsally and ventrally branching major dendrites. This gives the VS3, VS4 and VS5 neurons a 'T'-shape typical of VS neurons in Diptera. Simultaneous Golgi-impregnation of two or three VS neurons in the same preparation was useful in ascertaining their identity. Comparison was also made with results of Scott *et al* (2002).

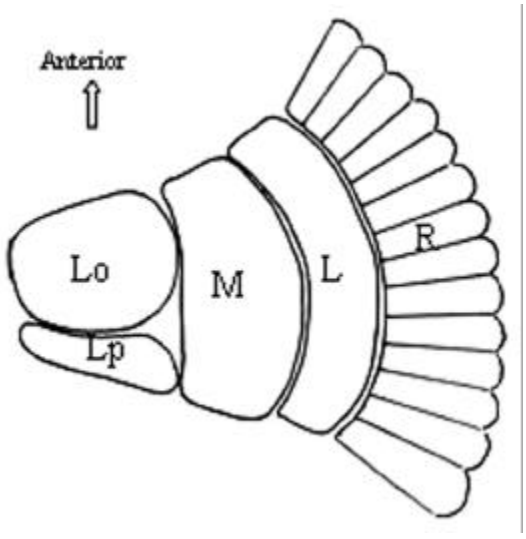

Figure 1. Semi-schematic representation of the optic neuropils of *D. melanogaster* as seen in a horizontal section. The cellular cortex surrounding the neuropil is not shown. R, Retina, consisting of ommatidia; L, lamina; M, medulla; Lo, lobula; Lp, lobula plate.

3.3 *Additional VS neurons*

In addition to the six described above that conformed to VS, three neurons that have VS anatomical traits were observed in the proximal quarter of the lobula plate. Two of them (AVS1 and AVS2) have a main dorso-lateral dendrite, giving out branches (figures 3c and 5a). AVS3 lacks this dorso-lateral branch. All three neurons have clear dorso-ventral dendritic patterns.

3.4 *h-cells*

These categories of cells have been reported to co-exist along with the HS cells (Dvorak *et al* 1975). Some of our preparations have Golgi-silver-impregnated neurons that show clear presence of h-cells in association with HS cell. They are named in the description below as hs-north, hs-equatorial and hs-south (figures 4a and 6a). Figure 6a shows all the three neurons of HS and their hs partners. From our analysis they appear to occur in layer II (Fischbach and Dittrich 1989) of the lobula plate whereas HS system is placed in zone I comparable to the HS the

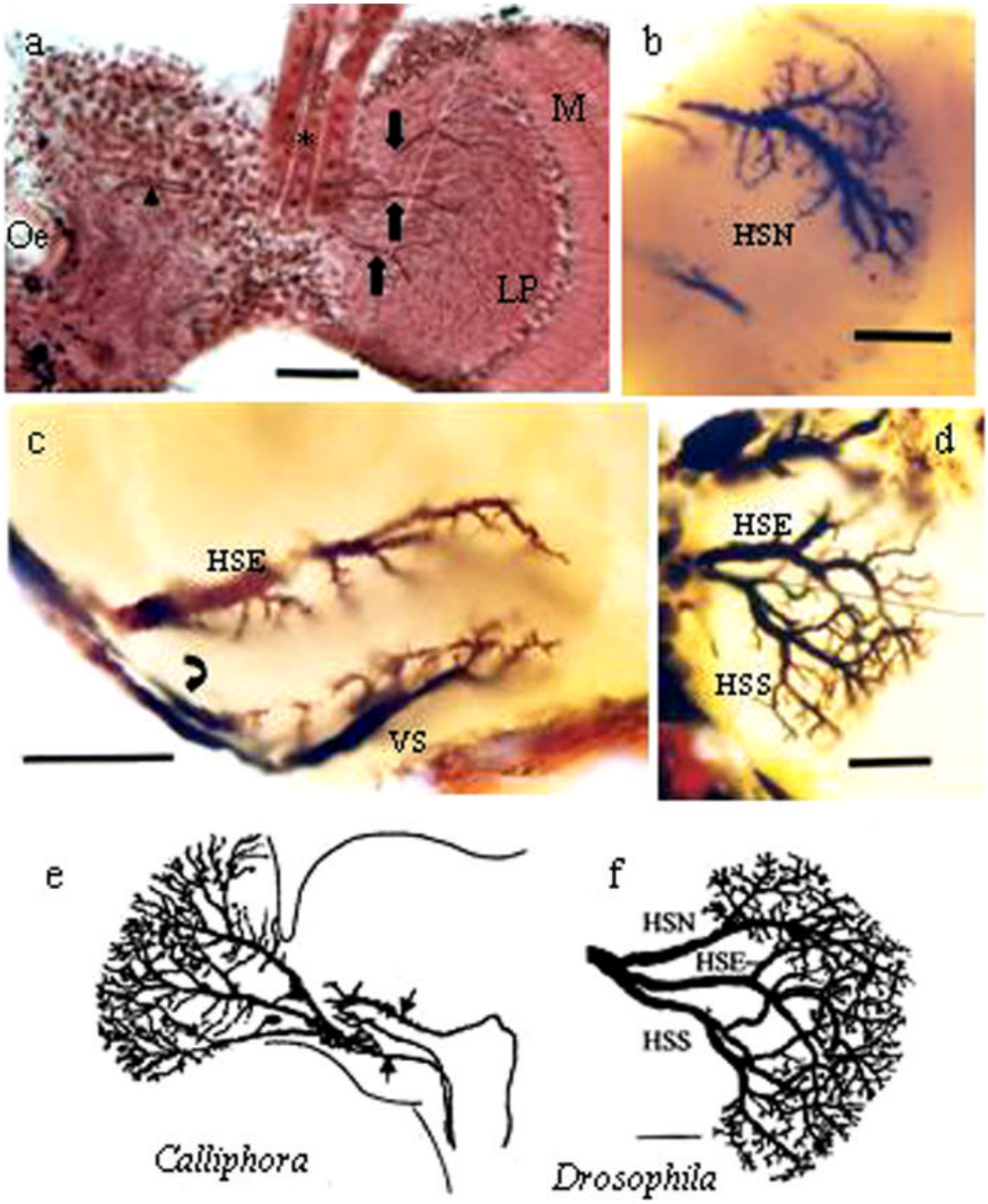

Figure 2. The horizontal system in the lobula plate of *D. melanogaster*. (**a**) Bodian-Protargol stained frontal section through the lobula plate region showing the three neurons (arrows) that constitute the horizontal system. The axons (arrow head) of these neurons project to the lateral protocerebrum adjacent to the oesophagus (Oe). LP, Lobula plate; M, medulla; *M1 muscle of the proboscis. Dorsal on top. (**b**) Dendritic arbors of a Golgi-silver impregnated HSN neuron in the dorsal lobula plate. Dorsal on top and lateral to the right. (**c**) A horizontal section of the head of *Drosophila* showing the lobula plate region. Golgi-impregnated HSE neuron that occupies layer I of the lobula plate and the dendritic arbors of two vertical system neurons (VS) in layer IV region are seen in the posterior. Profiles of two dendrites are clearly seen at the place indicated by a curved arrow. Anterior on top and lateral to the right. (**d**) Dendrites of the HSE and HSS neurons seen in frontal section of the lobula plate. (**e**) The full complement of HS neurons of lobula plate of *Calliphora* (redrawn from Starusfeld 1976) *in situ* on the left as compared to the complement of HS neurons in the lobula plate of *Drosophila*. (**f**) Note the reduced dorsal component of HSN in *Drosophila* that has stunted arbors. Scale (**a**) 15 μm; (**b**) (**d**) and (**f**) 20 μm; (**c**) 10 μm.

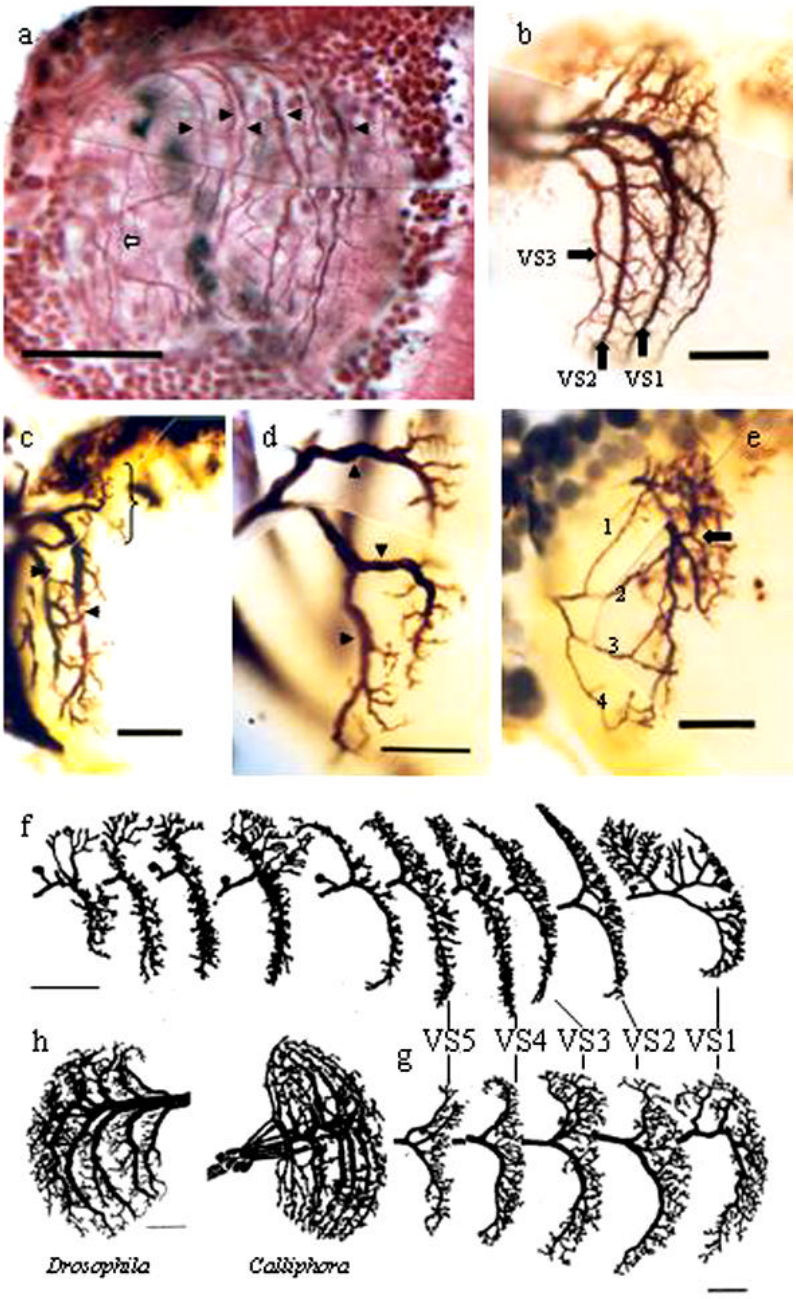

Figure 3. Dendritic arbors of Bodian-Protargol stained and Golgi-silver impregnated neurons in frontal sections through the lobula plate region of *Drosophila*. (**a**) Montage of VS neurons. Five of them are clearly visible in the microphotograph (arrow heads). Profiles of thin vertical system-like dendrites are seen in the proximal region of the lobula plate (white arrow). (**b**) Dendrite of three neurons of the vertical system, VS1, VS2 and VS3 indicated by arrows. (**c**) VS-like neurons, which we have termed as additional VS neurons (see text and figure 5) are seen in the proximal lobula plate. They are indicated by arrow-heads. The bracket shows distinct dorsal component of arbors of these neurons. (**d**) An atypical tangential element with tufted arbors arising from three branches (arrow heads) of dendrites in the lobula plate. (**e**) Dendrites of a neuron with four branches (numbered 1, 2, 3 and 4), terminating in the distal lobula plate. Dendrites of this neuron are found in close proximity of a thin vertical neuron (arrow; also see figure 5). The dendrites of neurons shown in **c**, **d** and **e** have predominantly dorso-ventral arbors. (**f**) Individual VS neurons of *Calliphora* (redrawn after Krapp *et al* 1998) and of *Drosophila*. (**g**) The VS neurons of *Calliphora* are typically 'T'-shaped with their main dendrites, placed in the medial region branching dorsally as well as ventrally. The VS neurons VS1 and VS2 are sickle-shaped while VS3-5 resemble the VS neurons of *Calliphora*, being nearly 'T'-shaped. The equivalents of VS1-VS5 are marked. (**h**) Full complement of VS neurons of *Calliphora* (redrawn from Strausfeld 1976) and of *Drosophila*, *in situ*. Scale: 15 μm for **a–d**; 30 μm for **e**, **g** and **h**; 60 μm for **f**.

hs also preferentially arborise in the distal lobula plate. HS and hs have similar arborization except that axons are thinner in diameter.

3.5 *Twin vertical system*

Bishop and Bishop (1981) described vertical system (VS) neurons and their synaptic relationship in *Phaenicia sericata*. They used Lucifer-yellow and revealed the presence of thinner tangential cells that are comparable in geometry to VS neurons but are thinner in size and named them as thin vs. They co-occur with VS neurons. In the present study similar neurons have been observed (figures 3a and 5d). These neurons also have a predominantly dorso-ventral course of dendrites in relation to the main dendrites and are sickle shaped. The arbors of two such neurons have shown in figure 5d. It is likely that more such neurons are present in lobula plate as can be seen in Bodian-Protargol silver stained preparations (figure 3a).

3.6 *Wide field neurons*

Cells that arborise with a wide dendritic field occupying most of the lobula plate were also observed. One of them has thin dendritic branches and branches profusely in the proximal lobula plate (figure 4b). This is comparable to the CH neurons of Diptera. The other one has four main dendritic branches, which are placed almost equidistant to one another (figures 3e and 5b). These branches are devoid of terminal arbors except in the most proximal region.

Compared to the VS neurons that have large number of branches, cells with tufted dendrites have been observed. Such a neuron illustrated in figures 3d and 5c has three major branches with the terminals arborizing in dorsal, medial and ventral lobula plate confined to a narrow dorso-ventral disposition. Cells with dendritic geometry similar in some respect to the HS were found in the medulla and ventral lobula plate (figure 6b, c). They are comparable to M cells identified by Heisenberg *et al* (1978).

4. Discussion

Due to an antero-posterior optic chiasma, the frontal visual field is projected to posterior medulla. A retinotopic projection of the medulla on to the lobula plate causes the distal lobula plate to receive visual information from frontal visual fields. Distal lobula plate has a larger neuropil compared to the proximal region (figure 1). This suggests the significance of frontal visual fields in insect vision. In the fronto-equatorial part of the compound eye the angular distance between adjacent ommatidia are the smallest, indicating a higher visual equity (Beermsa *et al* 1975).

Antero-posteriorly the lobula plate in *Drosophila* is divided into four equal-sized layers (Fischbach and Dittrich 1989) and the tangential neurons of the lobula plate occupy specific layers. The organization of HS appears to be a conserved feature among Diptera. Diptera studied so far (*Musca*, *Calliphora* and *Phaenicia*) have three horizontal elements (HSN, HSE and HSS) (Hausen 1984). In the present study horizontal elements of *Droso-*

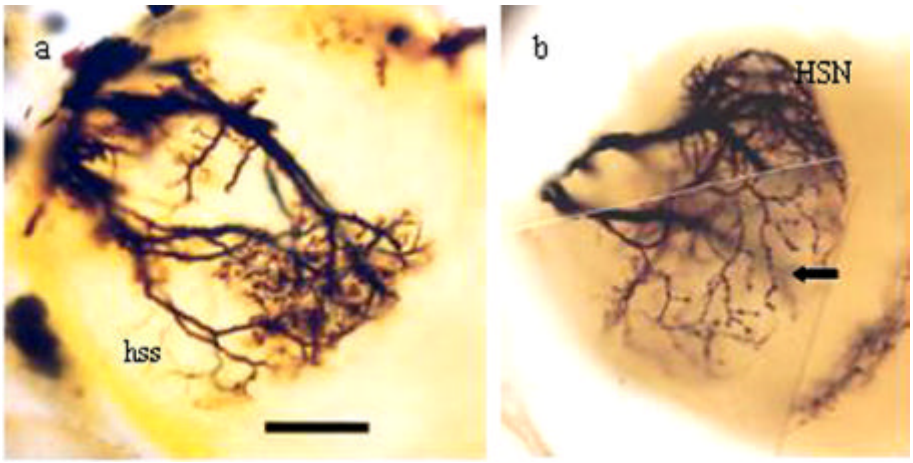

Figure 4. Golgi-silver-impregnated profiles in the lobula plate. (**a**) A 'h-cell' (hss) found in close proximity of HSS in the ventral lobula plate. (**b**) Dendrites of a wide field neuron (arrow) found ventral to a HSN neuron shown in a photo-montage of the same section. Dorsal on top and lateral to the right. Scale: 20 μm for both **a** and **b**.

phila (figure 2a–d), which were earlier reported by Fischbach and Dittrich (1989) using Golgi studies and recently by Scott *et al* (2002) using MARCM technique have been observed. The horizontal system in *Drosophila* was identified by Heisenberg *et al* (1978) using semi-thin serial sections stained by toluidine blue. These neurons occupy the two anterior layers of the lobula plate known in *Drosophila* (Fischbach and Dittrich 1989). The horizontal system in Diptera, has been shown by electrophysiological technique and simultaneous dye filling to be sensitive to ipsilateral progressive movement and horizontal equatorial cells in addition responds to contralateral regressive movements (Hausen 1981, 1984). Laser ablation of HS neuron by Geiger and Nassel (1981) also implicate horizontal cells to be involved in detecting back to front motion. The HS in all the Dipterans studied so far including *Drosophila* (present study) terminate in lateral protocerebrum (figure 1a). While electrophysiological analysis in *Drosophila* is still lacking, the function of HS has been hypothesized by observing the optomotor torque of *Drosophila* (Blondeau and Heisenberg 1982). The mutant *optomotor blind* (*omb*^{H31}) has its

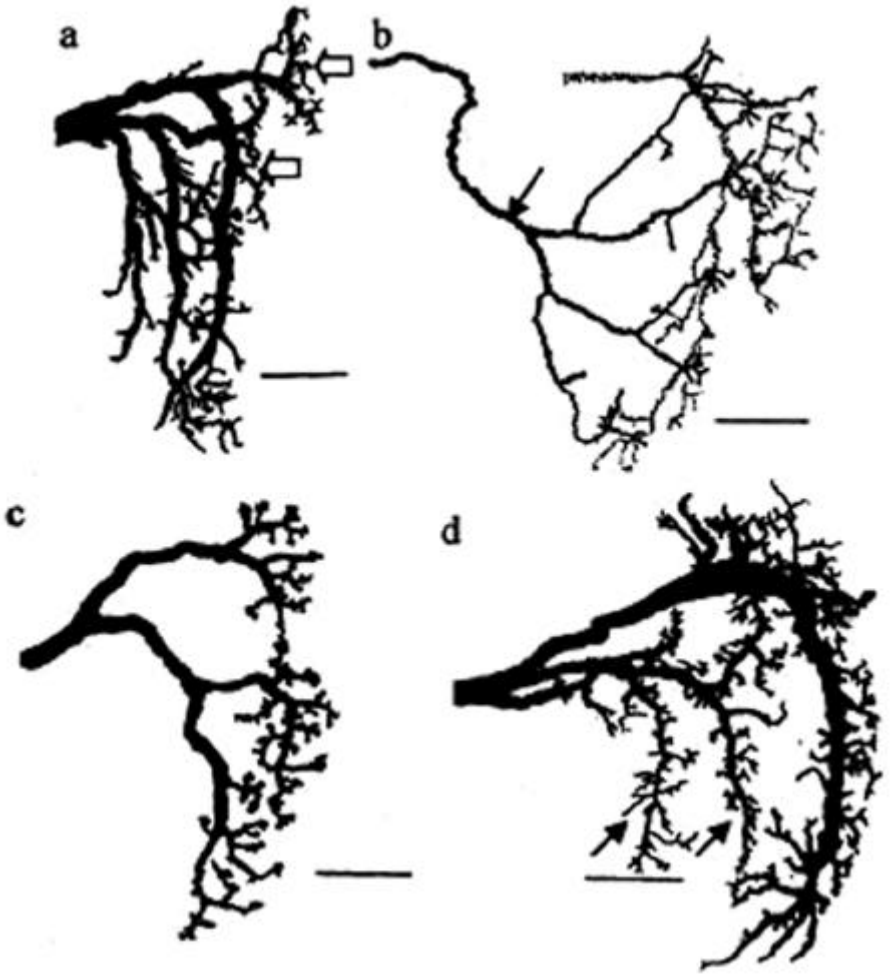

Figure 5. Camera lucida reconstructions of dendritic arbors that predominantly arborise dorso-ventrally, in the lobula plate. (**a**) Three neurons of the additional vertical system in the proximal lobula plate (also see figure 3). Open arrows indicate dorsal arbors that project relatively more anteriorly to the vertical profiles of the neurons. (**b**) A tangential element that has a major branch (arrow) traversing the lobula plate producing four branches terminating in the distal lobula plate in close vicinity to a twin vertical system neuron (stippled). (**c**) A tangential element with three tufts of arbors (also see figure 3d). Two neurons of the twin vertical system (arrow) shown in comparison to the dendritic profiles of VS1 neurons from the same Golgi-silver impregnated preparation. Some dendrites of VS1 neuron are not shown. Scale: **a–d** 20 μm.

horizontal and vertical system missing and has impaired yaw, pitch and roll (Blondeau and Heisenberg 1982). These observations suggested that horizontal cells in *Drosophila* are involved in optomotor turning reaction (Heisenberg *et al* 1978; Bausenwein *et al* 1986). The region occupied by the horizontal neurons (layer 1 and 2) were shown by Buchner *et al* (1984) to display higher metabolic activity by 2-deoxyglucose autoradiography to back to front motion in the wild type as well as in *omb*^{H31} flies (Bulthoff and Buchner 1985), suggesting their involvement in respective type of motion perception.

The neurons of the VS were studied in flies by Eckert and Bishop (1978), Hengstenberg (1982), Hengstenberg *et al* (1982) and Eckert (1982). The VS neurons observed in the present study (figure 3a, b, g) share all the features of VS neurons of *Calliphora* (Hengstenberg 1982; Hengstenberg *et al* 1982). The VS neurons were shown to respond to directionally sensitive vertical movement (Eckert and Bishop 1978) and were believed to constitute a system for detecting vertical motion in the visual field. Krapp *et al* (1998) observed the responses of VS neurons in *Calliphora* and demonstrated that individual VS neurons are tuned to sense optic flow. Thus the array of VS neurons in flies are specialized to aid in perceiving optic flow caused by self-motion in flies. The VS neurons observed in *Drosophila* during the present study and recently by Scott *et al* (2002) may have similar function as revealed by their geometry. The first neuron in the VS1-system (figure 3a, b, g), when compared to its equivalents in *Calliphora* (Hengstenberg *et al* 1982) and *Phaenicia* (Eckert 1982) is strikingly different. It lacks the elaborate dorsal component compared to the VS1 in *Calliphora*. Due to horizontal branching, the visual field of VS1 of *Calliphora* is involved in both horizontal and vertical motion perception. Eckert (1982) therefore called these neurons as VH neurons. The reduced number (six) found in *Drosophila* is possibly due to antero-posterior reduction of the compound eye as compared to other larger flies.

4.1 *The dorsal lobula plate*

Though conserved in terms of number and their major dendritic patterns, the neurons in the dorsal part of the proximal lobula plate of *Drosophila*, show noteworthy differences compared to other flies. The neurons of HS and VS occupying this region are the HSN (figure 2b) and VS1-VS3 (figure 3a, b, g) in *Drosophila* and VS1-4 in case of larger flies (Strausfeld 1976). The dorsal dendritic arbor of HSN is considerably stunted compared to dorsal components of HSN of larger flies (figure 2b, e, f). Likewise the dorsal components of VS1 and VS2 neurons in *Drosophila* are reduced (figure 3b, g) as compared to dorsal branches of VS1 and VS2 neurons of larger flies (figure 3f, h). The main dendritic trunks of VS neurons in *Drosophila* are placed in the dorsal part of the lobula

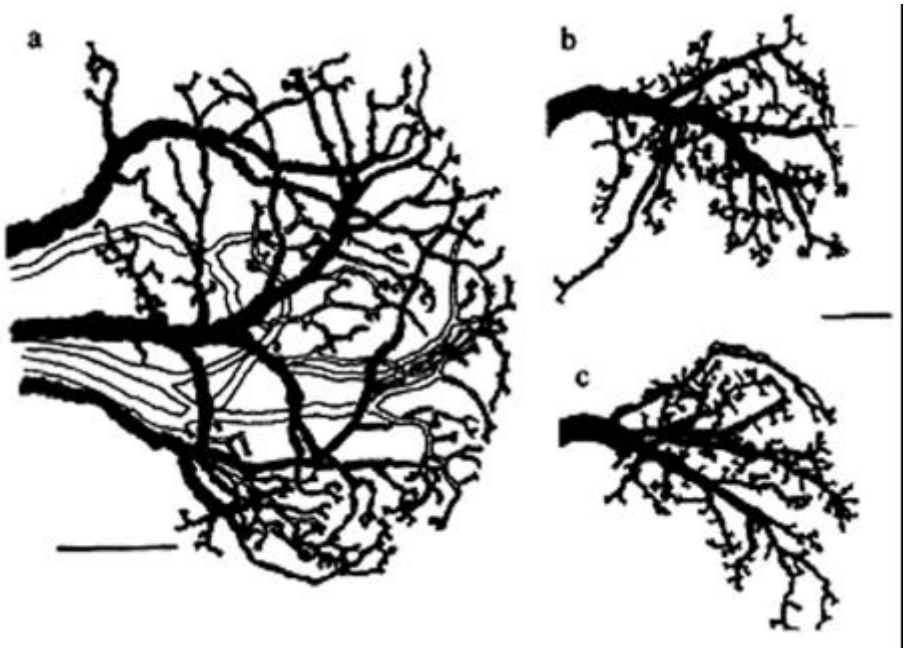

Figure 6. Camera lucida reconstructions of Golgi-silver impregnated neurons. (**a**) h-cells (shown in outline) in relation the HS neurons (black) in a preparation. (**b**), (**c**) Reconstructions of two M cells. Scale: **a–c** 20 μm.

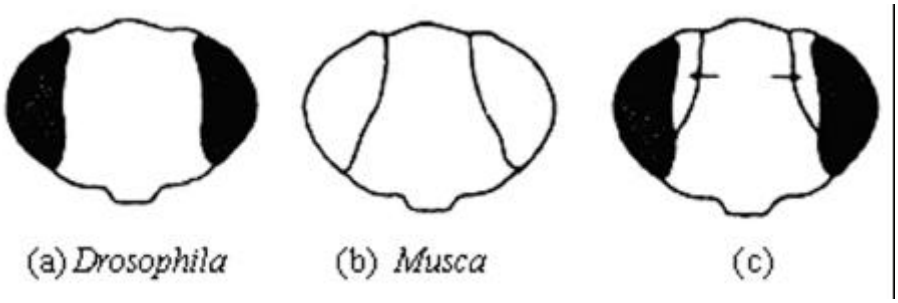

Figure 7. The head of *D. melanogaster* has smaller compound eyes, particularly in the fronto-dorsal region. This illustration is prepared by magnifying the frontal view of head of *D. melanogaster* to match the head of *Musca* in size and overlapping the two images. If the head of *Drosophila* were to be as large as that of *Musca*, the compound eye of *Musca* would exceed that of *Drosophila* by an area indicated by arrows in (**c**). Thus, the eyes of *Drosophila* are smaller, particularly in the fronto-dorsal region. Visual input to this region projects to the dorsal part of the proximal lobula plate, which as shown in the present study, has reduced arbors HS and VS neurons.

plate compared to the medial T-shaped vertical cell trajectories of their counterparts in *Calliphora* and *Phaenicia* (Eckert and Bishop 1978; Bishop and Bishop 1981; Hengstenberg 1982; Hengstenberg *et al* 1982). The VS1-2 cells in *Drosophila* resemble a sickle-shaped dendritic pattern (figure 3b, g). These variations in distal lobula plate can be explained by the differences observed in morphology of compound eye of *Musca* and *Calliphora*. A comparison of the head of *Drosophila* and the region occupied by its compound eyes and that of *Musca* are shown in figure 7. The antero-dorsal part of the compound eye of *Musca* is larger than that of the compound eye of *Drosophila*. *Musca* has about 20% more area and therefore an enhanced visual field in the antero-dorsal part. This region in flies is also of higher acuity as shown by Beersma *et al* (1975). The dorso-distal lobula plate is the region serving the antero-dorsal visual field of Diptera due to its internal and outer chiasma. This explains the anatomical variations found in HSN and of the vertical system (VS1-VS2) of *Drosophila*. This feature of compound eye may also influence the dendritic arborizations of other lobula plate neurons. It is likely that the reduced size of the compound eye in *Drosophila* as compared to that of *Musca* also reduces the overlapping field of vision, which is believed to be about 16% (Bausenwein *et al* 1986). A reduction in the overlapping field of the compound eyes of *Drosophila* may also cause corresponding changes in neural circuitry in the optic neuropils.

Scott *et al* (2002) demonstrated that the horizontal system consisting of three neurons develop from one neuroblast. The vertical system has been shown to contain six VS neurons that have common lineage. The number of VS cells suggested by Heisenberg *et al* (1978) based on semi-thin serial section and silver staining was 5–7. Five of the six known VS neurons demonstrated by MARCM technique (Scott *et al* 2002) have been successfully stained in the present study (figure 3g). The blowfly has eleven VS neurons (figure 3f, Eckert and Bishop 1978; Krapp *et al* 1998). The reduced number of VS may be due to smaller size of compound eye of *Drosophila* that has ~ 800 ommatidia compared to ~ 2800 ommatidia in *Musca* (Chapman 1998; Strausfeld 1976). The presence of vertical-like cells, termed as additional vertical system cells (AVS) (figures 3c and 5a) in the present study, is an interesting feature. They occupy the most proximal part of the lobula plate. Scott *et al* (2002) are of the opinion that additional cells sharing the structural and functional features of VS1-6 are unlikely without sharing the lineage and gene expression with this group. However, our observations do present such cells whose lineage is as yet unknown. By virtue of their structure they appear to share functional characteristics of VS neurons. All VS neurons have an interesting feature of a dorsal bistratified nature occupying at least two layers of lobula plate (Scott *et al* 2002). This feature is also shown by additional vertical system neurons except the AVS3. Bistratified arbors have been suggested to be required for vertical motion detection (Scott *et al* 2002).

Homologous neurons of related species can be compared for anatomical purposes and limited functional deductions. The knowledge about anatomical types of neurons in lobula plate of *Drosophila* is limited. Therefore comparison is based on the observations made on other flies (Hausen 1984). Cells that are similar to the HS neurons are seen in the lobula plate (figure 4a) but they have thinner dendrites compared to HS neurons. Such cells were described by Dvorak *et al* (1975). Similar cells have not been reported after that. However Hausen (1984) suggested the possibility of these being centrifugal horizontal (CH) cells (Hausen 1976; Hengstenberg and Hengstenberg

1980). Dendrites of h-cells described in the present study (figure 4a) coexist with HS dendrites. The CH cells of larger Diptera respond to contralateral and ipsilateral progressive movements.

Our observations have revealed certain cell types in lobula plate of *Drosophila*, which are not homologous in anatomical terms to any of the previously described Dipteran lobula plate neurons. For example, the tufted cell (figures 3d and 5c) possibly has functional characteristics of VS neurons by comparison. However, functions of wide field neurons such as the one shown in figure 4b cannot be predicted. The number and types of tangential neurons in the lobula plate is small compared to neurons of other optic neuropils. Three thinner tangential cells that are comparable in geometry to VS neurons, referred to as twin vertical system (tvs) cells by Bishop and Bishop (1981), were observed (figure 5d) in the present study. Such cells have not been described before. Heisenberg *et al* (1978) had observed in semi-thin-sections of *Drosophila*, cells that are termed as M cells. Comparable cells have been visualized (figure 6b, c) and they have anatomical features similar to HS. Golgi technique stains about 1% of neural cells, randomly in a preparation. The tangential neurons of lobula plate are reputed to be refractory to Golgi-impregnation. Significant types of neurons have been stained. Recent developments in anatomical techniques, particularly applicable to *Drosophila* will most certainly fill the existing gaps.

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