
Maxillary palp glomeruli and ipsilateral projections in the antennal lobe of *Drosophila melanogaster*

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The antennal lobe was examined by Golgi-silver impregnation to differentiate the glomeruli depending on the source and types of inputs. Thirty-five of the 43 'identified' olfactory glomeruli were Golgi-silver impregnated in the present study. Seven glomeruli compared to three, reported previously, were found to be targets of maxillary palp chemosensory neurons. These include glomeruli VA3, VC2, VM5, VA7m/VA7l of the ventral antennal lobe and DC2, DC3, DM5 of the dorsal antennal lobe. The number of glomeruli receiving the maxillary palp sensory projections tallies with the number of *Drosophila* olfactory receptors (seven) reported to be expressed exclusively in the maxillary palp. Twenty-eight Golgi-impregnated glomeruli were found to receive input from the antennal nerve. The ratio of glomeruli serving the maxillary palp to those serving the antenna (~ 1 : 5) matches with the ratio of *Drosophila* olfactory receptors expressed in these two olfactory organs respectively. In addition to glomerulus V, glomeruli VP1-3, VL1, VL2a/2p and VC3m/3l were found to receive ipsilateral projections. Thus, additional ipsilateral glomeruli have been identified.

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1. Introduction

The olfactory system of *Drosophila* has been in the forefront as a model to understand the process of olfaction through anatomy (Stocker 2001), molecular genetics (Keller and Vosshall 2003) and behaviour (Devaud 2003). The olfactory organs, namely the antennae and the maxillary palps of *Drosophila* are discrete structures and bear characteristic olfactory sensilla along with mechanosensory trichoid sensilla. Apart from olfactory hairs, the antenna, in its sacculus, has presumptive hygro- and thermoreceptive sensilla (Shanbhag *et al* 1995). Thus, the antennal lobe (AL) receives input from the funiculus of the antenna, the maxillary palp, and the sacculus. In *Drosophila*, the surfaces of funiculus of antenna and maxillary palp bear different types of sensory hairs. The funiculus has about 200 sensilla basiconica (BS), 150 sensilla trichoidea (TS) and 60 sensilla coeloconica (CS) while the surfaces

of the maxillary palp have 60 BS (Shanbhag *et al* 1999, 2000). While the axons from the olfactory sensilla of the antenna and the sacculus course in the antennal nerve, axons from sensilla on the maxillary palp, occur in the labial nerve. The axons of these sensory neurons terminate in distinct neuropils of the AL called glomeruli.

The glomerular organization of *Drosophila* AL was studied by Stocker *et al* (1983) who identified 19 glomeruli. Stocker *et al* (1990) made further studies on the sensory projections to the glomeruli and reported the presence of 35 glomeruli in the antennal lobe. More recently Laissue *et al* (1999) provided a three-dimensional map of AL using confocal microscopy and reported the presence of 43 glomeruli in the AL with an addition of eight glomeruli compared to previous reports. About 1200 antennal afferent neurons from each side have bilateral projections that extends into the contralateral AL. Five of the glomeruli of each AL are reported to receive

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Abbreviations used: AL, Antennal lobe, DOR, *Drosophila* olfactory receptor.

ipsilateral input, with the fibres terminating in the ipsilateral AL only. Thus the antennal lobe of *Drosophila* has a glomerular organization which is apportioned into antennal, maxillary, saccular and ipsilateral glomeruli.

Previous studies reported that sensory projection from the maxillary palp innervate glomeruli VM1, VM2 and VA2 of the AL (Singh and Nayak 1985). Stocker *et al* (1990) stated that five glomeruli of the AL are targets of maxillary palp sensory neurons but specified only glomeruli VA4, VA3 and DM2 to be the recipients of these projections. There are 57 known *Drosophila* olfactory receptor (DORs) genes (Vosshall *et al* 2000). Olfactory neurons in *Drosophila* are likely to express only a single DOR gene (Vosshall *et al* 2000). The sensory projections of neurons expressing a given olfactory receptor gene, converge at topographically fixed glomeruli in the antennal lobe (Gao *et al* 2000). Seven DOR genes are found solely in the maxillary palp, where they appear randomly interspersed but have non-overlapping expression (Vosshall *et al* 2000). The glomerular organization is invariant in terms of number and position. Previous studies have attempted to provide an explanation for the arrangement of the glomeruli in terms of functional and/or spatial arrangements of olfactory sensilla. The glomeruli involved in partitioning of the AL into antennal, maxillary, saccular and ipsilateral projections have not been clearly identified. An analysis of Golgi-silver impregnated olfactory projections of *Drosophila* was undertaken to identify the glomeruli receiving maxillary projections and the glomeruli receiving ipsilateral projections. Such an analysis also permits us to deduce the glomeruli receiving the antennal projections. Recent description of the glomerular organization by Laissue *et al* (1999), a definitive study of the sensilla on the funiculus by Shanbhag *et al* (1999, 2000), description of the DOR genes of *Drosophila* and their expression in the olfactory organs (Vosshall *et al* 2000), assisted the analyses of our observations.

2. Materials and methods

A certified culture of *Drosophila* Oregon-K wild type flies from *Drosophila* Stock Centre at University of Mysore was obtained and maintained in the laboratory, and used for our experiments. Fibres of the antennal nerve were Golgi-silver impregnated by Golgi-rapid and Golgi-Colonier methods. Selectivity of staining could be enhanced by cutting only antennae or maxillary palps, and retaining the other parts of the head intact.

2.1 Golgi-rapid technique

The flies aged 3 or 4 days were cooled, dissected and fixed in Karnovsky's fixative 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. Subsequently, heads were incubated in a mixture containing 0.5 volume of 4% osmium tetroxide (Sigma Aldrich, O-5500) and 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 incubated in 0.75% silver nitrate solution in the dark for 48 h. The heads were 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 µ.

2.2 Golgi-Colonier 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 above two steps were repeated once more. The specimen was washed with distilled water, dehydrated and embedded in soft araldite and sectioned at 30 µ thickness.

3. Results and discussion

The observations are based on about one hundred Golgi-impregnated antennal lobes. The anterior part of the AL has relatively higher aggregation of glomeruli than the posterior. The categorization of the projections as maxillary and antennal projections, in the present study relies on exclusive impregnations of maxillary fibres or antennal nerve fibres in a preparation and ambiguous terminals were not considered. Preparations with only ipsilateral projections were also obtained. No fibre was seen to project to the antennal lobes from the pharyngeal or accessory pharyngeal nerves. The nomenclature of the glomeruli in the present study is based on confocal reconstruction of AL by Laissue *et al* (1999).

3.1 Maxillary projections

The maxillary chemosensory neurons deviate antero-dorsally and separate from the mechanosensory fibres with which they share the labial nerve. They enter the AL and a total of seven glomeruli were found to be occupied by maxillary projections (figure 1a–g). They were found to occupy glomeruli VA3, VM5, VC2 and VA71/VA7m of the ventral antennal lobe, DC3, DC2 and DM5 of the dorso-medial antennal lobe. These glomeruli are represented in

a preparation shown in figure 1a–c. They form a cluster being located in the vicinity of each other. These glomeruli are small and approximately uniform in size ranging from 8 to 15 μm in diameter. The identification of glomeruli occupied by maxillary projections has also been done based on several preparations wherein sensory neurons of some of the maxillary palp glomeruli were impregnated (figure 1d–g). A semi-schematic diagram of the maxillary palp glomeruli is provided in figure 3c. The observations in the present study reveal sensory fibre projections in DM5, VA3, and VC2 glomeruli that are class I glomeruli as per classification of Laissue *et al* (1999). Glomeruli DC2, DC3 observed in the present study are the class II glomeruli and the class III glomeruli

to which fibres been seen projecting are VA7m, VA7l, VM5. One of the ‘new’ glomerulus (DM5) identified by Laissue *et al* (1999), was found to be innervated by maxillary palp projections.

Glomeruli VP1-3, VL1, VL2a/2p and VC3m/3l receive only ipsilateral projections (figure 2a–b). The contralateral sides are devoid of any projections in the illustrations shown. The glomeruli receiving sensory projections, revealed by Golgi impregnation in the present study are listed in table 1. Innervation of a glomerulus by sensory fibre provides a direct evidence of glomerulus. Glomerulus-like aggregations of fibres are some times seen in the vicinity of the antennal lobe, but lying outside the antennal lobe, having no association with the antennal nerve

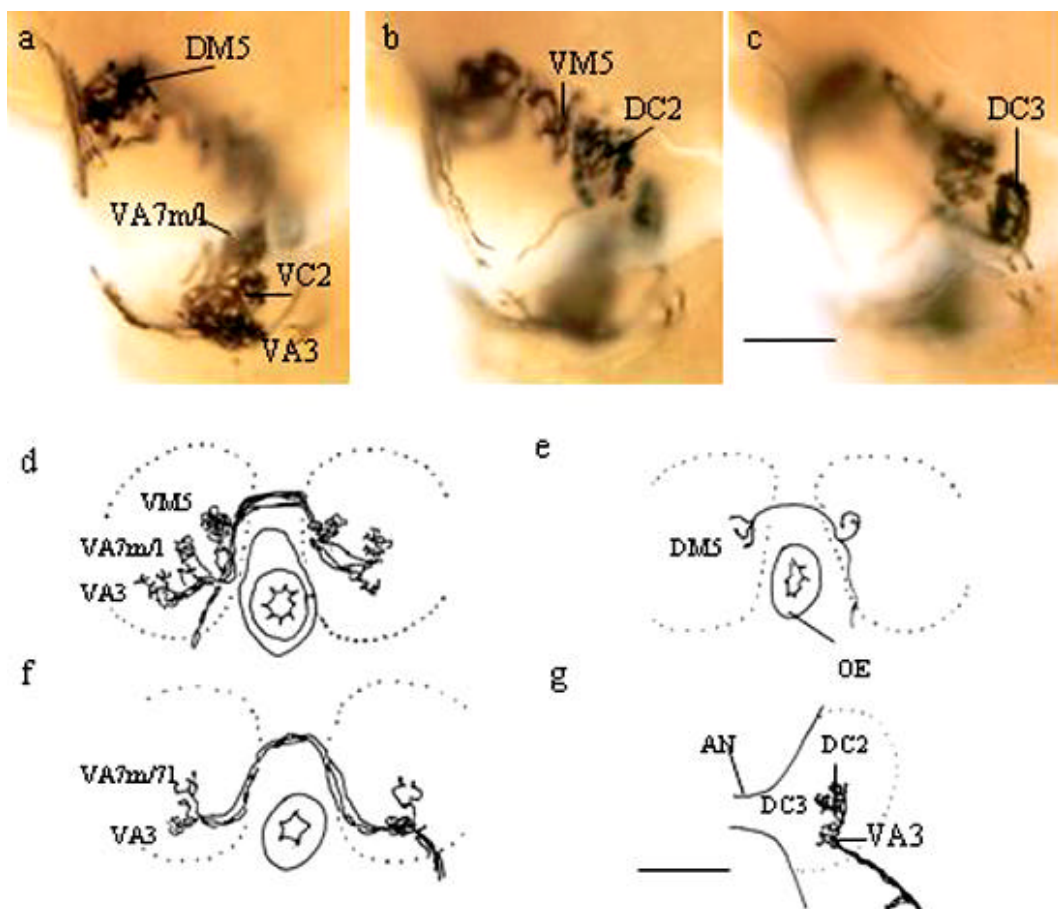


Figure 1. Golgi-silver impregnated maxillary nerve projections in the antennal lobe. Photomicrographs of a preparation showing the glomeruli of the maxillary projections photographed at successively deeper focal planes (a–c). (a) The most anterior plane showing glomeruli DM5, VA7m, VC2 and VA3. (b) Shows glomeruli VM5 and DC2. (c) The most posterior focal plane showing glomerulus DC3. (d–e) Camera lucida reconstructions of maxillary palp projections from frontal serial sections of the head of *Drosophila*. Dotted lines show the outline of the antennal lobe. The projections to glomeruli VM5, VA7m, and VC2 are seen in (d), to glomerulus DM5 is seen in (e). Three neurons, two of which project to glomerulus VA3 and one projecting to VA7m/1 are seen in (f). A sagittal section (g) show projections to glomeruli VA3, DC3 and DC2. Dorsal on top. Anterior to the left for (g) only. AN, Antennal nerve; OE, Oesophagus. Scale: (a–c) 20 μm , (d–f) 30 μm .

(figure 2b). It is therefore necessary to establish the identity of an olfactory glomerulus by verifying the innervation. The schematic illustration provided in figure 3, summarises the projections of the maxillary palp projections. The present study has revealed 7 glomeruli to be targets of maxillary projections as compared to three reported by Singh and Nayak (1985) and Stocker *et al* (1990) (figure 3a–c). VC3l and VL2a are the additional targets of fibres that are exclusively ipsilateral (figure 4a–c), in addition to the ones represented by Stocker *et al* (1983, 1990). All the glomeruli impregnated in the present study are listed in table 1.

3.2 Functional identity of glomeruli

The identity of glomeruli by confocal analysis (Laissue *et al* 1999) is established by using nc82 neuron-specific antibody. Golgi-impregnations show that the sensory projections from antennal/labial nerve innervate these glomeruli. The Golgi technique that impregnates neurons was found to stain 35 of the 43 known glomeruli in the present study (listed in table 1). A powerful technique utilizing expression of DOR genes in the antenna, visualized by *in situ* hybridization using antisense digoxigenin riboprobes (Vosshall *et al* 2000) has been used to localize receptors in the olfactory organs. The projections from sensory neurons expressing a particular DOR converge on to one or sometimes two glomeruli (Clyne *et al* 1999; Gao and Chess 1999). Thus sensory neurons that are believed to be functionally similar converge upon a glomerulus. Though glomeruli in the antennal lobe reflect the olfactory receptor expression in the antenna, olfactory neurons respond to more than one olfactory stimulus. In *Drosophila* the maxillary sensory neurons respond either by being inhibited or excited to more than one stimulus (de Bruyne *et al* 1999). Likewise multiple glomeruli in honeybee antennal lobe have been shown to respond to a

single odour by optical imaging of calcium pioneered by Galizia *et al* (1999). Novel techniques have been employed recently to conduct functional studies on olfaction in *Drosophila* (de Bruyne *et al* 1999; Ng *et al* 2002; Fiala *et al* 2002; Wilson *et al* 2004). Ng *et al* (2002) have employed synaptophluorin – a pH sensitive variant of green fluorescent protein that produces pH sensitive fluorescence changes during synaptic activity. They found that odours activate a distinct group of glomeruli. The activity of projection neurons was studied by Fiala *et al* (2002) using cameleon – a genetically encoded calcium sensor, to monitor odour evoked activity changes. These studies provide a functional correlation of the stereotypy of projections neurons to odour stimuli. Wang *et al* (2003) have expressed a Ca²⁺ sensitive fluorescent protein to demonstrate that the glomerular response is dependent on the odourant receptor expressed by a receptor neuron. These studies suggest a strong correlation between sensory neuron and projection neuron activity. Recently, Wilson *et al* (2004) employed a more direct approach by adopting whole-cell clamping and subsequent dye injection to find that second order projection neurons displayed broader tuning and a more complex response than the sensory neurons, indicating lateral interactions through local interneurons. The glomerulus in the AL is thus a site where transformation of olfactory input takes place, influenced not only by sensory neuron input, but also by the interaction of sensory neurons, projection neurons and the local interneurons.

3.3 Maxillary projections

As compared to a complex mosaic of sensilla on the funicular surface (Shanbhag *et al* 1999), the maxillary palp is relatively simple. About 60 sensilla basiconica are located on maxillary palp (Singh and Nayak 1985; Shanbhag *et al* 2000). All of them are innervated by two receptor

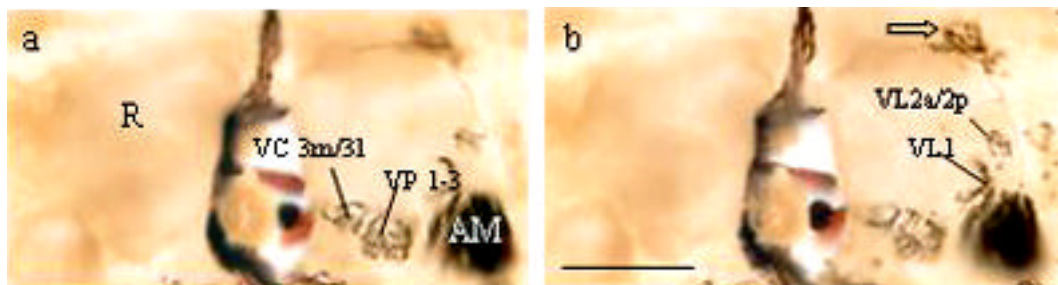


Figure 2. (a, b) A frontal section through the antennal lobe of *Drosophila*, at two focal planes showing glomeruli that are innervated by ipsilateral fibres. Glomeruli VC3m/3l and VP 1–3 are shown in (a), glomeruli VL2a/2p, and VL1 are seen in (b). The right antennal lobe (R) is devoid of any projections. Aggregation of terminals that appear like glomeruli are some times seen outside the AL (arrow). AM, Antennal mechanosensory tract. Scale: 50 μ m.

neurons (Singh and Nayak 1985). Studies by de Bruyne *et al* (1999) provide an electrophysiological analysis of the maxillary palp sensilla and demonstrate that one of the two neurons of each sensillum is inhibited by the stimulus while the other is excited. Thus there are six functional types of sensilla. The maxillary projections were shown to project to three glomeruli VM1, VM2 and VA2 by Singh and Nayak (1985) (figure 3a) and to glomeruli DM2, VA3 and VA4 by Stocker *et al* (1990) (figure 3b). The number of glomeruli observed in present study, which

is seven (figures 1 and 3), compares well with the number of olfactory receptors expressed in maxillary palp (Vosshall *et al* 2000). These olfactory receptors expressed in the maxilla are exclusive to maxilla (Vosshall *et al* 2000). One of interesting facts is segregation of maxillary input from antennal input. It is quiet likely that the odours sensed by maxilla are different for the ones sensed by antenna. In male moths, pheromone-receptive neurons project to an exclusive macroglomerular complex, which is independent of antennal projections (Christensen and Hildebrand 1987). The maxillary information flow in *Drosophila* may also be independent in nature. As the maxillary input is segregated from antennal input, it would be interesting to observe whether projection neurons are also exclusive for glomeruli of maxilla. We do not yet know whether the maxillary palp glomeruli respond to inputs from antennae or not, which is possible due to local interneurons. The number of DOR expressed is 57 and seven of them are expressed in maxilla while 32 are expressed in funiculus, which is a ratio of about 1 : 5. A similar ratio is found in the glomeruli of antennal lobe

Table 1. Bilateral and ipsilateral antennal glomeruli and maxillary palp glomeruli of *Drosophila*, found to be innervated by Golgi-silver impregnation.

Antennal glomeruli	
Glomerulus	Bilateral
D	Bilateral
DA1	Bilateral
DA2	Bilateral
DA3	Bilateral – S
DA4	Bilateral
DC1	Bilateral
DL1	Bilateral – S
DL2V	Bilateral – S
DL5	Bilateral – S
DM2	Bilateral
DM6	Bilateral
DP1m	Bilateral
DP1l	Bilateral
VA1d	Bilateral
VA1m	Bilateral
VA1l	Bilateral
VA2	Bilateral
VC1	Bilateral
VM1	Bilateral – S
VM2	Bilateral – S
VM3	Bilateral – S
VM4	Bilateral
	Ipsilateral
V	Ipsilateral – S
VC3m	Ipsilateral
VC3l	Ipsilateral
VL1	Ipsilateral
VL2a/2p	Ipsilateral
VP1-3	Ipsilateral
Maxillary palp glomeruli	
Glomerulus	Bilateral
DC2	Bilateral
DC3	Bilateral
DM5	Bilateral
VA3	Bilateral
VA7m/7l	Bilateral
VC2	Bilateral
VM5	Bilateral

S, Glomeruli of sacculus.

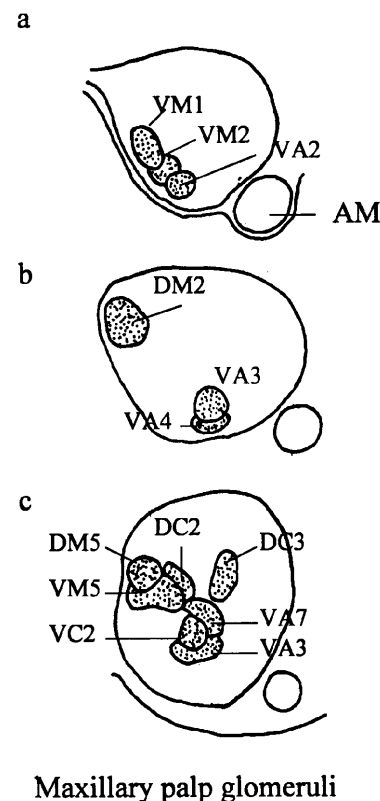
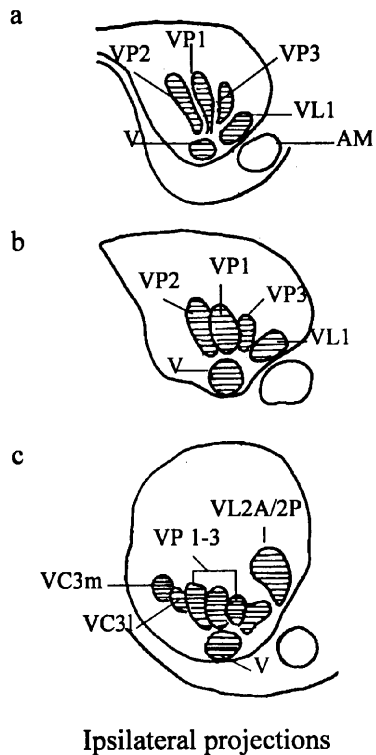


Figure 3. Semi-schematic representation of frontal view of glomeruli in the antennal lobe, innervated by chemosensory projections from the maxillary palp as described by Singh and Nayak (1985) (a), by Stocker *et al* (1990) (b) and observed in the present study (c).



Ipsilateral projections

Figure 4. Olfactory glomeruli innervated by ipsilateral fibres as described by Stocker *et al* 1983 (a), Stocker *et al* (1990) (b) and present study (c). See text for description.

assuming that the rest of the glomeruli are antennal glomeruli. The antennal lobe also has an identified group of glomeruli that serve the input from sacculus. They are V, VA2, VM1–3, DA3, DM1 and DL1–3 (Shanbhag *et al* 1995). The maxillary projections do not target any of these glomeruli.

3.4 Ipsilateral projections

The significance of ipsilateral fibres is still not very clear. About 1/6th of sensory neurons from antenna are believed to have ipsilateral projections as revealed by deafferentation studies by Stocker *et al* (1990). Stocker *et al* (1983) suggested that ipsilateral fibres help in osmotropotaxis, demonstrated in *Drosophila* by Borst and Heisenberg (1982). Stocker *et al* (1983) suggested that ipsilateral fibres enter from arista, which provides sensory input for stretch. However such input would have been a mechanosensory input and expected to course in the antennal mechanosensory nerve. Foelix *et al* (1989) suggests that arista sensilla to be thermosensitive. The presumptive thermosensitive sensilla of sacculus (Shanbhag *et al* 1995) however projects to bilateral glomeruli

as revealed by cobalt back filling. A smaller number of arista sensory fibres (Foelix *et al* 1989) might be one of the reasons for an ill-defined glomerulus (example: V). Depending on the input, the AL can be classified as antennal glomeruli, maxillary glomeruli, glomeruli of the sacculus, and ipsilateral glomeruli. There are some overlaps between these groups. Future studies can focus on the processing of information related to these partitions.

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