Antennal lobe organisation in ant, *Oecophylla smaragdina*: A Golgi study

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Antennal lobes (AL) are the primary olfactory centres of the insect brain, the organisation of which reflects the chemosensory repertoire of the insect. The sensory neurons from the antenna, local neurons (LNs), and projection neurons (PNs) constitute the neuropilar organisation of the AL, which often varies according to the ecology and behaviour of the insects. We explored the organisation of the AL in the ant *Oecophylla smaragdina* through Golgi analysis and other standard histological procedures. Six topographically distinct glomerular clusters were noticed in the AL. AL volume, total number of glomeruli and its spatial organisation were different in the castes of *Oecophylla smaragdina*. We could count 220 glomeruli in the AL of the major worker, whereas only 140 glomeruli were located in AL of minor workers. LNs with their cell body located on the dorsal, dorso-lateral, ventral, ventro-lateral, and medial position of the AL were characterised. Most of the LNs are oligoglomerular. Uniglomerular and multiglomerular PNs were also identified. The results underline a caste specific organisation of the AL in *Oecophylla smaragdina*. The characterised LNs and PNs further reveals the complex neural circuitry involved in the processing of important chemical cues related to the lifestyle of the ant.

Keywords. *Oecophylla smaragdina*; Antennal lobe; Golgi analysis; Local neurons; Projection neurons

1. Introduction

Common designs in structure and function across different animal phyla are testimonies to principles of evolution (Futuyma 2005). Design of the antennal lobe across different animal phyla comprises many conserved features such as spheroidal functional units called glomeruli and input and output pathways that traverse different neuropils (Hildebrand and Shepherd 1997; Strausfeld and Hildebrand 1999). Studies on antennal lobe of *Drosophila*, moths and honeybees have contributed immensely to our knowledge about olfactory-associated memory, principles of olfaction, neural plasticity, neural development and evolutionary aspects of olfaction. Glomeruli are synaptic sites of heterogeneous neurons like local neurons (LNs), projection neurons (PNs) and centrifugal neurons (Anton and Homberg 1999). In the case of insects, olfactory receptor neurons (ORNs) from antennal sensilla converge on glomeruli through sensory tracts found conserved across different phyla (Strausfeld and Hildebrand 1999; Grab et al. 2016). Recent decades have witnessed great interest in ALs of diverse insect orders like Lepidoptera, Hymenoptera and Diptera (Rospars 1983; Rospars and Hildebrand 2000; Galizia et al. 1999b; Smid et al. 2003; Laisseu et al. 1999). The number of glomeruli, their spatial organization and functionally significant variations are noticed in different species like *Aedys aegypti* (Namiki et al. 2014), *Drosophila* (Laisseu et al. 1999); *Camponotus floridanus* (Zube et al. 2008) and *Apis mellifera* (Flanagan and Mercer 1989; Anton and Homberg 1999). Dimorphic antennal lobes of males and females are a common feature observed in Hymenoptera, Lepidoptera, Dictyoptera and Coleoptera (Rospars and Hildebrand 2000; Nishikawa et al. 2008; Kazawa et al. 2009; Zhao et al. 2016; Kollmann et al. 2016). Neuronal populations of antennal lobe and their properties

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are discussed in many studies (Galizia et al. 1999a; Strausfeld 2009; Kristoffersen et al. 2008). Projection neurons (PN) are the sole output tracts that connect AL glomeruli to higher brain centres like the mushroom body and lateral protocerebrum. In the majority of insects, PNs course to higher brain centres through specific tracts classified as antennal-cerebral tracts (ACTs): inner antenna-cerebral tract (iACT), outer antenna-cerebral tract (oACT), medial antenna-cerebral tract (mACT), dorsal antenna-cerebral tract (dACT) and dorso-median antenna-cerebral tract (dmACT) (Hansson and Anton 2000; Zube et al. 2008; Namiki and Kanzaki 2019; Habenstein et al. 2020). Local neurons (LNs) are the most abundant intrinsic neurons of the antennal lobe and they provide horizontal inter-glomerular connections and synapse with PNs and ORNs; their cell bodies are located within the antennal lobe and are involved in synchronization of the PNs and influence spatio-temporal electrical activity of the antennal lobe (Vosshall and Stocker 2007).

Of late, ants have captured the attention of researchers worldwide with their rich behavioural repertoires and experimentally amenable sensory systems which drive those behaviours. They offer a unique biological system in many ways to unravel neural circuits of sensory systems and genes involved and also help us to understand their social behaviours and elaborate communication systems (Gadagkar 2011). Moreover, advents of powerful genetic tools have helped scientists to unravel intricacies of sociality and sensory systems and annotate them with genomes (McKenzie et al. 2016; d’Ettorre 2016; d’Ettorre et al. 2017; Yan et al. 2017).

Understanding functional tracts of antennal lobes is a challenging task in comprehending olfactory perception in ants and is relatively less explored. Research in this line can contribute to our understanding on the intricate neural networks and their functions in ants, which, understandably, possess sophisticated neural networks related to their comparatively complex social life styles. Studies on antennal lobe of ants are few and there is a dearth of knowledge in this field (Zube et al. 2008; Galizia 1999b; Mysore et al. 2009). The present study therefore aims to understand the general organization of antennal lobe in Oecophylla smaragdina in terms of the glomerular number and its spatial organization and the antennal nerve input tracts, as well as to obtain information about the local neurons (LNs) and projection neurons (PNs), which all together add to neuronal constitution of antennal lobe. The study also aims to study the caste specific organization of the antennal lobe of Oecophylla smaragdina which could give us insights into their caste-specific behaviours.

2. Materials and methods

2.1 Cajal’s block impregnation

After removing the cuticle as much as possible from the head of ant immersed, the brain was immersed directly into a 4% silver nitrate solution in the dark for 36 h and washed in several changes of distilled water for about 15 min with constant agitation. The brains were reduced in 1–2% solution of hydroquinone, with 5 ml of neutral formalin. Reduction should last for 24 h. The brains were washed in distilled water and dehydrated in grades of ethanol. Dehydrated samples were cleared in propylene oxide and embedded in Durcupan. After polymerization at 65°C, the blocks were sectioned at 20 µm thickness and observed under an Olympus BX 60 microscope.

2.2 Golgi-rapid technique

Ant heads were severed and fixed in Karnovsky’s fixative for 4 h at room temperature. The fixed specimens were washed with phosphate buffer 3–4 times and the specimen could be stored in buffer in refrigerator. Specimens 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 2 days. The specimens were given a quick rinse in 0.1% silver nitrate until no more red precipitate was formed in the washings.

2.3 Camera lucida drawing

Serial sections were observed using the above-mentioned methods using Olympus BX60 trinocular microscope and analysed for neuronal organization. Three-dimensional reconstruction of the neurons was done by camera lucida drawings. Microphotographs were taken using an Olympus SC35 Camera. Illustration and plates were prepared using HP scanner, and video attachment was used to make measurements. Adobe CS2 software was used for digitally enhancing the photographs.
3. Results

Antennal lobe glomeruli appear in clusters in *Oecophylla smaragdina* (figure 1a-f). Clustered organisation of glomeruli appears to be a common feature observed in many Hymenopterans such as ants and honeybees. Six topographically distinct glomerular clusters were identified in our study and they were named according to the position they occupy in antennal lobe. Clusters located at the dorso-lateral area were categorised as DL clusters. Three distinct clusters were identified from this region and were named DL 1, DL 2, and DL 3, respectively, from the antennal lobe of the major worker (figure 1a-d). DL clusters were absent in antennal lobe of minor workers. Glomeruli located in ventro-lateral area of antennal lobe were categorised into VL clusters. Three distinct clusters VL 1, VL 2, VL 3 were identified from antennal lobe of major workers. A glomerular cluster located in the ventral (V) area was observed from antennal lobe of minor worker and was named V1. Glomeruli in different clusters showed variations in their diameters: diameter of glomeruli ranged from 10 to 50 μm (figures 1, 2, 3). Only a few glomeruli had a size of 50 μm. Total volume of antennal lobe varied significantly between castes. Major workers had an antenna lobe volume of \( \sim 6.96 \times 10^6 \ \mu m^3 \), whereas minor workers had antennal lobe volume of \( \sim 4.3 \times 10^6 \ \mu m^3 \). The total number of glomeruli also differed between major and minor workers. Numbers of glomeruli counted from the serial sections of antennal lobe in major workers were 220, while minors had \( \sim 140 \) glomeruli in the antennal lobe. A few olfactory receptor neuron (ORN) terminals were traced. Antennal nerve innervated glomeruli in multiple tracts (figure 4a). Arbor patterns of a few stained receptor neuron terminals revealed characteristic morphological features in different glomeruli (figure 4a-f). Receptor terminals

Figure 1. Serial sagittal sections of the antennal lobe of the major worker of *O. smaragdina* (Cajal block impregnation). Successive sections were taken at a depth of 25 μm. The white asterisks denote single glomerulus. The dotted line represents the outline of different clusters of the antennal lobe. Clusters on the upper panel are the dorso-lateral (DL): (a) DL 1, (b) DL 2, (c) DL 3. The lower panel represents the ventro-lateral clusters (VL): (d) VL 1, (e) VL 2, (f) VL 3. Scale bar = 50 μm.
Figure 2. Camera lucida drawings of serial sections corresponding to figure 1. The dorso-lateral (DL) and ventro-lateral (VL) clusters of the antennal lobe glomeruli are clear in successive sections. Scale bar – 50 μm.

Figure 3. Camera lucida reconstruction of the glomerular organization of the minor worker of *O. smaragdina*. (a) Ventro-lateral cluster VL1, (b) VL 2, (c) VL 2 and a ventral cluster (V1), (d) VL 3. (e) Glomeruli belonging to VL 2 and VL 3. The arrow indicates the antennal nerve. Scale bar – 50μm.
with thick and thin varicosities were noticed. Receptor neuron terminals innervated whole glomeruli while some had terminals confined to periphery of glomeruli.

Local neurons (LNs) are important cellular constituents of the antennal lobe (figure 6). Different LNs of Oecophylla smaragdina were identified by morphological features in our study. Features like position of their cell body within antennal lobe, location of glomeruli they innervate and characteristic arborisation within glomeruli are striking. LNs with their cell body located on dorsal, dorso-lateral, ventral, ventro-lateral, and medial position of antennal lobe were prominent. LNs with their soma on the medial surface of antennal lobe had a soma size range of 2–5 μm. Three types of medial LNs innervated glomeruli which are topographically distinct. One type innervated a cluster of glomeruli consisting of ~15 glomeruli located close to their cell body (figure 7c-d). Golgi-stained dendritic arbors of these neurons showed a prominent neuronal process entering glomeruli from which numerous branches arise, thereby invading glomeruli densely. We found that dendritic arbors of LN in other glomeruli located away from its cell body are sparse. The second type of LN innervates relatively lesser number of glomeruli which are located in different loci of antennal lobe. The third type of medial LN innervated maximum number of glomeruli within antennal lobe (figure 8a). Degrees of overlap between these LNs are a possibility which could not be deciphered from our study. Two types of LNs with their cell body occupying the dorso-lateral area of antennal lobe were also observed. The first type had a soma diameter of ~ 4μm (figure 6a). This group of LNs innervated about 8 glomeruli. Intra glomerular innervations of these LNs were similar to the features of medial LNs. The second type of dorso-lateral LNs had contrasting features with a soma diameter of ~ 3.8 μm. Relatively thick neuronal processes of this LN invade the glomeruli. Arborisations within the glomeruli were confined to the periphery of the glomeruli (figure 6a). LNs with soma on the ventro-lateral had a
soma diameter of $\sim 4 \mu m$. This type had thick neuronal processes invading the antennal lobe. Within the glomeruli these LNs arborise in a relatively less dense manner because of the very few processes that branch within the glomeruli (figure 6c-d). LNs with soma located on dorsal part of antennal lobe were also observed (figure 9e). They have a soma diameter of $\sim 4.6 \mu m$. Majority of LNs observed were oligo glomerular-innervating only a few glomeruli within the cluster they occupy. LN with arbors innervating whole cluster of glomeruli were also revealed from antennal lobe of Oecophylla smaragdina (figure 8f-g). In addition to these types, many other LNs were identified (figures 6f and 9a-d, f). Cell body of these LNs could not be located, and so they were not included in any of the identified groups observed. We could notice a group of LNs with tufted arbors within glomeruli which were unidentified (figure 9a) and a LN with innervations restricted to $\sim 4$ glomeruli, which had the least number of glomerular innervations. This LN

Figure 5. (a) Sagittal section showing the antennal nerve innervating the glomeruli. White arrows indicate the branches of the antennal nerve. (b) Camera lucida reconstruction of corresponding sections reveals the glomerular innervation at topographically distinct glomeruli. (c) Golgi-impregnated local neuron (LN) shows the arborisation within a glomerular cluster. The arrow indicates the soma of the LN (ventral soma). (d) LN with soma located on the medial surface of the antennal lobe. (e) LN with soma on the dorso-lateral area of the antennal lobe. (f) Camera lucida reconstruction of the corresponding LN. Scale bar – 100 $\mu m$. 

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sends dendritic arbors within the glomeruli with a main neuronal process extending into the glomeruli and only few processes extending from it, thereby the glomerular innervations appear to be very less (figure 6e-f)

Uniglomerular (uPNS) and multiglomerular (mPNs) were identified from the antennal lobe of Oecophylla smaragdina which were grouped in lateral antennocerebral tracts (l-ACT) and medial antenna cerebral tracts (m-ACT). Tracts of PNs were identified with

Figure 6. (a) LN with soma located on the dorso-lateral position of the antennal lobe. White dots indicate the border of the antennal lobe. (b) Camera lucida reconstruction of the corresponding LN. (c) LN with soma located dorso-ventrally. (d) Camera lucida drawing of the corresponding LN. (e) LN showing homogeneous innervation of the glomeruli (sagittal section). Scale bar – 100 μm. (f) Camera lucida reconstruction of the corresponding section.
Figure 7. LNs with soma located on medial surface of the antennal lobe. (c,d) Camera lucida reconstructions of the corresponding LNs. (e) LN innervating a whole cluster within the antennal lobe. (f,g) LNs innervating different cluster of the antennal lobe. Scale bar – 100 μm.
respect to the medial and lateral calyx of the protocerebrum. Two types of multi-glomerular PNs (mPNs) of the l-ACT tracts were identified: Type I multiglomerular projection (mPN) innervates antennal lobe with characteristic filamentous and spiny projections. Dendritic arbors of these PNs in antennal lobe appear to be innervating the whole antennal lobe in a diffuse manner, as revealed in the sagittal sections. Terminal ends of this type of PN in the protocerebrum has blebbled terminals in lateral protocerebrum and extended terminals to the protocerebrum area above the antennal lobe, but the lateral protocerebrum area possess more blebs (figure 12c-d). In unison with the multiglomerular PNs of other insects, the mPNs of *Oecophylla smaragdina* characterised in this study did not have terminals projecting to the calyx of mushroom body. This type of PNs courses to higher brain centres via the l-ACT tracts, which we inferred by position of their tracts positional relation to mushroom body medial calyx. The second type of mPNs innervated relatively small areas within the antennal lobe. However, it lacked projections in protocerebrum area above the antennal lobe. The terminal area of protocerebrum of this PN was further lateral to previous type of PN and the area well correlates with the lateral horn area of protocerebrum observed in other insects. PNs appear to course through the l-ACT tract to higher brain areas. The projection terminals of this PN to lateral protocerebrum had spiny filamentous projection and blebs were absent (figure 12a-b).

Morphological features of uniglomerular projection neurons (uPNs) of *Oecophylla smaragdina* are striking. Four main types of PNs belonging to the m-ACT tracts were revealed by Golgi studies. The first type had a soma located outside antennal lobe with a diameter of \( \sim 2 \mu m \). This uPN had blebbled projections in the lip area of mushroom body calyx and lateral inferior protocerebrum (figure 10a-d). Intra-glomerular innervations of this type of uPN are characteristic. The main neuronal process extended along the outer border of glomeruli and few branches extended from the main process (figure 10c-d). Both showed varicosities in their axonal appearance and innervations of these m-ACT uPNS of the glomeruli were distinct. One group innervated glomeruli located along the lateral border of the antennal lobe, with the axonal tract of these groups emerging from the lateral side of the glomeruli, while the other innervated glomeruli located...
in the medial area of the antennal lobe, the axonal tracts of these m-PNS emerging from the middle of the cluster of the glomeruli they innervated. The fourth group of mPN appear to course to the higher brain centres via the m-ACT. This mPN has projection in the basal ring of the mushroom boy calyx and sends a few collaterals to the protocerebrum (figure 11a-b).

4. Discussion

Each species possess signature features of antennal lobe. Variations under different contexts are most striking among antennal lobes of different insects. In addition, intra-specific variations associated with sexual dimorphism as well as caste-based changes are also
observed (Kuebler et al. 2010; Winnington et al. 1996; Brown et al. 2002). Glomerular units exhibit characteristic spatio-temporal activity patterns to specific odours, indicating odour-specific activity patterns generated within the antennal lobe in response to specific odours.

We explored the organisation of antennal lobe and some of the caste-specific variations in *Oecophylla smaragdina*. Understandably, such differences underlie the behavioural distinctions of each caste and are therefore fundamental to the division of labour prevailing in their societies. Differences in design of antennal lobes of each caste provide crucial information about olfactory-induced behaviours of castes. Knowledge in this regard can be helpful for us in understanding how the diploid genome gets primed during developmental program to function differently in the sensory system of each caste.

Figure 10. (a-b) Uniglomerular projection (uPNs) innervating different glomeruli. Inset shows the blebbled projections of the same PN in the lip area of the medial calyx (sagittal section). (c) Blebbled projections of the same PN in the lip area of the inferior lateral protocerebrum. The arrowhead shows the soma. The tracts are also clear. Scale bar – 100 μm. (d) Camera lucida reconstruction of the corresponding section.
Cajal block impregnation and Golgi staining revealed a characteristic clustered organisation of the antennal lobe of *Oecophylla smaragdina* (figure 1a-f). Clustered organisations of glomeruli within antennal lobe have been reported from prior studies on ants such as *Apheanogaster*, *Camponotus* (Sakura *et al.* 2008; Zube *et al.* 2008) and *Cataglyphis* (Habenstein *et al.* 2020). In *Oecophylla smaragdina*, 5 distinct clusters of glomeruli were observed in the antennal lobe. Glomerular clusters are distinct in their topography within the antennal lobe (figure 1a-f). Clustered arranging of glomeruli has been proven to be functionally significant in many insects, and have provided more insights into the functional compartmentalisation of antennal lobe. For example, in the ant *Camponotus obscuripes*, a cluster of glomeruli occupying the dorsal position of the antennal lobe have been found to be involved in non-sexual pheromonal processing.

4.1 General organisation of the antennal lobe

Cajal block impregnation and Golgi staining revealed a characteristic clustered organisation of the antennal lobe of *Oecophylla smaragdina* (figure 1a-f). Clustered organisations of glomeruli within antennal lobe have been reported from prior studies on ants such as *Apheanogaster*, *Camponotus* (Sakura *et al.* 2008; Zube *et al.* 2008) and *Cataglyphis* (Habenstein *et al.* 2020). In *Oecophylla smaragdina*, 5 distinct clusters of glomeruli were observed in the antennal lobe. Glomerular clusters are distinct in their topography within the antennal lobe (figure 1a-f). Clustered arranging of glomeruli has been proven to be functionally significant in many insects, and have provided more insights into the functional compartmentalisation of antennal lobe. For example, in the ant *Camponotus obscuripes*, a cluster of glomeruli occupying the dorsal position of the antennal lobe have been found to be involved in non-sexual pheromonal processing.
(Yamagata et al. 2006). Each glomerular cluster is distinct with respect to the antennal nerve position. Clustered organisation of glomeruli in hymenopterans especially ants and honeybee seems to be a structural innovation evolved in relation to the higher number of sensory tracts innervating it. Previous studies have

Figure 12. (a) Multiglomerular projection neuron (mPN) belonging to the l-ACT tract. The black arrowhead indicates the antennal lobe innervations and the white arrowhead indicates the lateral protocerebrum innervations of the mPN. (b) Camera lucida drawing of the corresponding mPN. (c) A second type of mPN with characteristic blebbled projections in the lateral protocerebrum. The arrow indicates the axons and blebbled projections in the lateral protocerebrum. (d) Camera lucida drawings of the corresponding mPN.
proven that antennal sensory tracts in ant species like *Camponotus* are extensive and are more prominent compared to other insects (Mysore *et al.* 2009; Nishino *et al.* 2009). Recent studies have highlighted differences in the antennal nerve organisation and its innervations in different castes of the ant *Camponotus* (Nishino *et al.* 2009; Mysore *et al.* 2009). Each olfactory receptor neuron (ORN) bearing specific olfactory receptor converges on specific glomeruli (Vosshall 2000). Sensory tracts innervate distinct clusters of glomeruli which are spatially distinct and probably suggest a functional subdivision of glomeruli. In *Oecophylla smaragdina*, similar to antennal lobe organisation in other ants like *Camponotus*, innervations of the glomerular clusters by antennal sensory tracts were observed. However, a refined and comprehensive understanding of antennal nerve innervations and their relations to the glomerular clusters could not be achieved in our study. Few of the Golgi-stained olfactory sensory terminals within antennal lobe were distinct in their arborisation in the glomeruli and showed morphological properties like varicosities (figure 4a-f). Differences in the arborisation of sensory terminals were observed in some of the stained glomerular loci. We observed sensory receptor terminals innervating the whole of glomeruli as well as ones confined to periphery of the glomeruli. Our results are similar to those reported from honeybee (Mobbs 1982). In Drosophila and honeybee sensilla-specific innervations of glomeruli is delineated well (Couto *et al.* 2005; Fishilevich and Vosshall 2005; Nishino *et al.* 2009). Olfactory terminals coursing through antennal nerve tracts of *Oecophylla smaragdina* distinctly arborise within glomeruli. Since each glomerulus are convergence sites for olfactory sensory neurons bearing specific olfactory receptors (ORs), uniglomerular projection neurons (PNs) and local neurons (LNs), differences in the arborisation suggest a unique structural identity for each glomeruli. Even though glomeruli are considered as the structural and functional units of the antenna, complex neuronal circuitries of diverse neurons within antennal lobe units and their developmental origins are still not understood comprehensively particularly in ants. Hence, investigating these intricacies in ants can enhance our knowledge about the functioning of the olfactory system in a comparative perspective. Studies on diverse insect species therefore can tell us more about innovations incorporated by each species in relation to their life style. Cajal’s block impregnation further reveals asymmetrical glomerular organisation of *Oecophylla smaragdina* in major and minor castes. Glomeruli occupying different clusters showed size variation. Structural asymmetry within glomeruli in each cluster suggests the possibilities of a functionally partitioned antennal lobe in *Oecophylla smaragdina*.

### 4.2 Caste-based differences in the antennal lobe

Caste-based differences observed in the antennal lobe of *Oecophylla smaragdina* were striking. The total numbers of the glomeruli in antennal lobe of major workers (∼240) were significantly different from minors (∼120). In the antennal lobe of major worker, 3 distinct dorso-lateral clusters – DL 1, DL 2, DL 3 – were observed. These clusters were absent in the minor worker. A ventral cluster V 1 was observed from the antennal lobe of the minor worker. Our results corroborate the caste-specific organisation of the antennal lobe glomeruli. Significant changes in volume size of glomeruli and the position within antennal lobe between castes are striking. In minor workers, glomeruli occupying different clusters were conspicuous by the difference in their diameters. Variability in the total volume and number of glomeruli suggests that major workers possess a higher number of ORNs, PNs and LNs. Elaborate neuronal circuitry and the expansion of associated neuropils in major workers are, presumably, bestowed by a differential developmental program. Differential development in majors is presumably an adaptation of the genome to favour division of labour. Reduction in the number and size of the glomeruli in minor castes indicates that antennal lobe glomerular arrangement in each caste is differentially organised. Minor workers possess lesser number of glomeruli in identified clusters with the presence of relatively smaller glomeruli.

Studies on antennal lobe of insects like honeybee and ants like *Camponotus* demonstrate that caste-based differences are reflected in glomeruli. In Attine ants, age-dependent switches in division of labour have been proven to induce changes in the volume of certain glomeruli (Kuebler *et al.* 2010; Winnington *et al.* 1996; Brown *et al.* 2002). A change in volume of glomerular units likely corresponds to differences in elaboration of neuronal processes and is found to be influenced by inputs from olfactory sensory neurons (ORNs). The total number of glomeruli roughly corresponds to the total number of olfactory receptor genes (ORs) Drosophila (Robertson and Wanner 2006). However, recent studies have revealed exceptions to this canonical rule in olfaction (Dobritsa *et al.* 2003; Hallem *et al.* 2004; Couto *et al.* 2005). However, studies on these aspects
of olfaction in Hymenoptera have not been carried out yet. Difference of approximately 80 glomeruli between major and minor worker suggests that major workers may have ~80 olfactory receptors that differ and likely express more OR genes. Since olfactory stimuli are spatially encoded in different glomeruli across the antennal lobe, a relatively higher number of glomerular units in the major workers might provide them with a greater range to process olfactory cues. These prominent differences are probably due to the caste-specific developmental pattern and this developmental phenomenon plays an important role in equipping the majors and minor workers of *Oecophylla smaragdina* to engage in different tasks in their adult life. Studies have conclusively proven that castes in honeybee, bumblebees and ants show differential sensitivity to different sensory cues (Lopez-Riquelme et al. 2006; Spaethe et al. 2007). Threshold-level response to the olfactory cues varies in the castes. Sensitivity of the castes to odour stimuli seems to vary according to the olfactory-driven behavioural tasks they engage in. In *Oecophylla smaragdina*, caste genesis occurs during development. In the adult stage the major and minor workers engage in different tasks without any further switches as they age. Major workers are involved in a wide a range of tasks and therefore need to respond to a greater range of olfactory sensory cues, compared to minor workers concerned with nursing the young ones and are mainly confined to the nest. It is likely that the differential demands of sensory perception, and other requisites of a caste, results in the caste-specific genesis of olfactory system. A differential organisation of the neuropils among the castes as observed in the study is an outcome of this development phenomenon. This probably might have led to an allometric organisation of the antennal lobe in the castes, with a greater volume, greater number of glomeruli and size of the glomeruli in the major workers compared to the minors.

4.2.1 *Projection neurons of Oecophylla smaragdina.*

The main output pathways of antennal lobes are medial antenna-cerebral tract (m-ACT) and the lateral antenna-cerebral tract (l-ACT). These tracts connect the antennal lobe to higher brain centres like lateral protocerebrum and mushroom body calyx. In accordance with these dual innervations patterns of projection neurons tracts, glomeruli are organised into two hemispheres receiving innervations from m-ACT and l-ACT in many hymenopterans. This is a hallmark feature of hymenopteran PN output pathway (Kirschner et al. 2006). Neurons constituting these pathways are distinct in their morphological features and function (Zube et al. 2008). Projection neurons (PNs) belonging to the m-ACT and l-ACT tracts were revealed by Golgi staining. Mainly three types of uniglomerular PNs were discerned from the present study, which are distinct by their innervation pattern in the glomeruli and areas of the protocerebrum to which they project. However, each type of PNs sends dendritic arbors to spatially distinct glomeruli within the antennal lobe. Collaterals of some of PNs project to the lip, basal ring, and dorso-lateral protocerebrum. Multiglomerular PNs belonging to the m-ACT and o-ACT tracts were also revealed by the present study. Features of innervations pattern of different PNs were distinct in the protocerebrum and glomeruli. The presence of atypical PNs innervations patterns suggests the possibility of presence of many non-characterised PNs in insects like *Oecophylla smaragdina*. This might be required in view of a high range of chemosensory demands of the social insect. Classes of PNs involved in conveying these messages to higher centres of the brain are not discerned yet. Morphological features of only a single type of multiglomerular projection were discerned from our study. Features of PNs correspond to the earlier studies conducted on *Periplaneta* which shows similar features of PNs (Malun et al. 1993). Current knowledge about PNs in insects are meagre in terms of its development, anatomy and neurochemistry, except for *Drosophila*, in which studies have started unravelling some interesting aspects about its development and circuitry.

4.2.2 *Local neurons of the antennal lobe:* Cell bodies of LNs are located within antennal lobe and form excitatory and inhibitory synapses with projection neurons (PNs) and olfactory receptor neurons (ORNs). Functional and developmental roles of LNs in majority of insects are still unknown. LNs are crucial for olfactory transformation (Shang et al. 2007). Studies affirm that diverse LNs have a differential origin from distinct neuroblasts located in different loci within the antennal lobe.

We noticed LNs distinct in their arborisation pattern of AL: Oligoglomerular LNs, which are the major group, do not innervate all glomeruli located within the cluster they reside. We also noticed LNs arborising all glomeruli within a cluster. The glomeruli they innervate are topographically distinct. However, LNs do not innervate glomeruli of the contra-lateral antennal lobe. Neurochemical and electrophysiological studies have demonstrated excitatory and inhibitory properties of LNs. They interconnect different glomeruli and form synapses with PNs and ORNs. Despite this, a
comprehensive understanding of the functions of LNs is missing. Our results indicate the possibility of a highly heterogeneous feature of LNs. Morphological features of LNs in *Oecophylla smaragdina* provoke questions about developmental aspects and mechanisms underlying the genesis of these diverse neurons. Social insects like *Oecophylla smaragdina* hugely rely on olfactory cues for most of their life activities. Functions of LNs in transformation and modulation of encoded olfactory stimuli in social insects, which depend on a wide range of olfactory cues, may help us obtain a better understanding the process of olfaction.

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