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STUDIES ON THE ANTENNAL LOBES OF THE STINGLESS BEE *TETRAGONULA IRIDIPENNIS* (SMITH)

Joseph Tom and Martin J Babu*

Department of Zoology, St. Berchman's College, Changanassery 686101, Kerala
*Email: martinbabu25@gmail.com (corresponding author)

ABSTRACT

***Tetragonula iridipennis* (stingless bees) are common foragers and pollinators on a wide range of plants. Though the ecosystem services of these insects are acknowledged, sensory perception- especially olfactory and visual perceptions of these insects are less understood. This study explores the primary olfactory centre of the brain with an aim to understand the primary organization of the antennal lobe of *T. iridipennis* and also to get an overview of the glomerular arrangement through hematoxylin and eosin staining method. This can provide insights about olfactory sensory perception, as 70 glomeruli were found in total from both left and right antennal lobes. The glomeruli were distributed along the periphery of the antennal lobe. Total number of glomeruli indirectly reveals that *T. iridipennis* has relatively less olfactory receptors.**

Key words: *Tetragonula iridipennis,* antennal lobe (AL), glomeruli, distribution, histochemical, olfactory, hematoxylin, eosin,

Antennal lobe (AL) of insect brains process olfactory and other chemo sensory inputs from the antenna and in some from the maxillary palps. (Strausfeld and Hildebrand 1999). AL is constituted of heterogeneous population of local inter neurons (LNs), projection neurons (PNs) that relay information to high olfactory centers in the protocerebrum, and axon terminals of antennal olfactory receptor neurons (ORNs) (Anton and Homberg, 1999). The olfactory glomeruli of AL of both vertebrates and invertebrates are conspicuous and well-delineated anatomically (Hildebrand and Shepherd, 1997). About 43 glomerulii are reported from the AL of *Drosophila*; *Aedes aegypti*; 32; locusts and social wasps ; ~ 1000 (Hansson and Anton 2000). The interaction between the olfactory receptor neurons, local neurons and projection neurons process the sensory inputs from the sensory neurons into a spatiotemporal code before it is sent to higher brain centers (Laurent 2002; Stopfer and Laurent 1999).

AL glomerulii process general odours, pheromones and other plant emitted odours (Rössler et al. 1998; Berg et al. 2002; Shields and Hildebrand 2001; Hillier et al. 2006). Synapses between receptor neuron terminals and central neuron processes are located within these glomeruli. Individual glomeruli can be identified by their position within the AL (Flanagan and Mercer, 1989; Galizia et al., 1999). Considerable variations within the AL are also noticed (Schachtner et al., 2005).Total glomeruli, their loci within the AL, loci of soma groups, the diversity in the type of the neuron populations that constitutes the AL organization etc are variations noticed across species. Stingless bees are eusocial insects of the tropical and subtropical regions and are major pollinators for a large number of plants in these geographical realms.

Tetragonula iridipennis is the common stingless bees found in India (Smith, 1854). Our knowledge is scarce about olfactory sensory perception and other related aspects in insects like *T. iridipennis*. In the present study, the glomerular organization of the antennal lobe is explored through basic histochemical procedures. Architecture of the antennal lobe reflects the olfactory repertoire of the insect; glomerular size, pattern of distribution, the total number of antennal lobe glomeruli are all good indicators of an insect's olfactory sensory acuity. As the number of the glomeruli increase so does the olfactory sensitivity of an insect. The results of the studies can be helpful in understanding the sensory sophistications of a social insect with a range of contrasting behavioral repertoires.

MATERIALS AND METHODS

Tetragonula iridipennis worker females were collected from the nests built on the crevices of building premises in and around Changanasserry. Bees were collected with an aspirator, cold anaesthetized by keeping in the refrigerator for one day and sacrificed. After removing the mouth parts and cuticle the heads were then fixed in 4% paraformaldehyde for 24 hr. To minimize tissue distortion from diffusion currents specimens were dehydrated in a graded ethanol series from water through 10%, 20%, 50%, 90%, 100% ethanol. After dehydration clearing of the samples was done using xylene. Tissue was then embedded in paraffin and micro sections of 5µm were taken using a rotary microtome. Serial sections tissue samples of the dissected-out brains were immersed in the filtered Harris hematoxylin for 1 min and rinsed with tap water. Tap water was exchanged till the water was clear. Sections were immersed in eosin stain for 1-2 min and rinsed with tap water. Tap water was exchanged till the water was clear. Sections were dehydrated in ascending alcohol solutions (50%, 70%, 80%, 95% x 2, and 100% x2), subsequently they were cleared in Xylene (2X). H and E stains nuclei and other basophilic structures as blue and cytoplasm and acidophilic structures as light to dark red.

RESULTS AND DISCUSSION

The AL glomeruli of *T. iridipennis* are arranged in the outer border of the AL, (Figure 1,2a,2b). The average diameter of the AL is ~260 µm. Variations in the diameter of the glomeruli is not significant. (Figure 3,4). The average diameter of the glomeruli is 30 µm (Table 1). Most of the glomeruli appears monomorphic, except for few in the medial loci of the AL. The monomorphic nature and the uniform distribution of the glomeruli along the outer order of the AL are contrasting features of *T. iridipennis.* We could identify nearly ~35 glomeruli each from the left and right ALs. (Table 2). The number of glomeruli is a signature feature of AL in insects and it is species specific. Often the number of glomeruli an insect possess is a reliable indicator of the olfactory sensitivity of the insect as the total number of glomeruli roughly corresponds with the number of olfactory receptors (ORs). AL of *T. iridipennis* appears less complex with comparatively less number of glomeruli. The average size of the glomerulus is 30 µm. The size of the glomerulus seems to be constituted by the synapses at glomerular loci. Comparing the size of the AL glomeruli of *T. iridipennis with* other insects suggest olfactory inputs and olfactory processing in the AL of *T. iridipennis* to a lesser degree.

The antennal lobe of *Tetragonula* though similar in its morphological features to other hymenopterans shows subtle yet significant variations. The number of glomeruli an insect possess is a reliable indicator of an insect's chemosensory acuity. ~70 glomeruli were identified from both the antennal lobes and unique pattern of glomerular distribution were revealed in the present studies. AL glomeruli of *Tetragonula* are distributed on the periphery. This pattern and the homogeneity in the size of the glomeruli is contrasting feature of AL of *Tetragonula*. This contrasts with the AL glomeruli seen in other insects, where glomeruli

FIg. 1

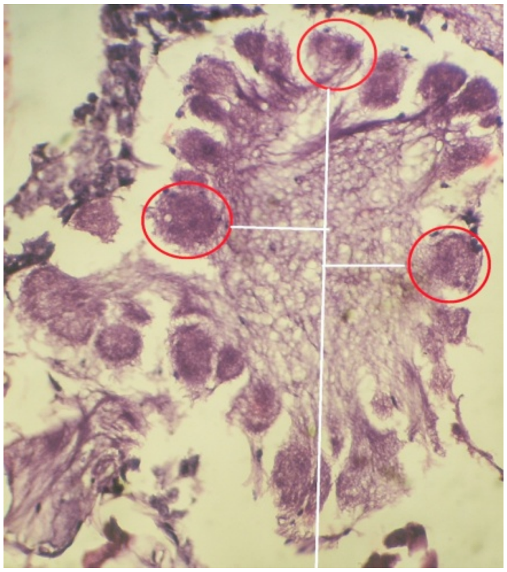
Fig. 2. Left antennal lobe

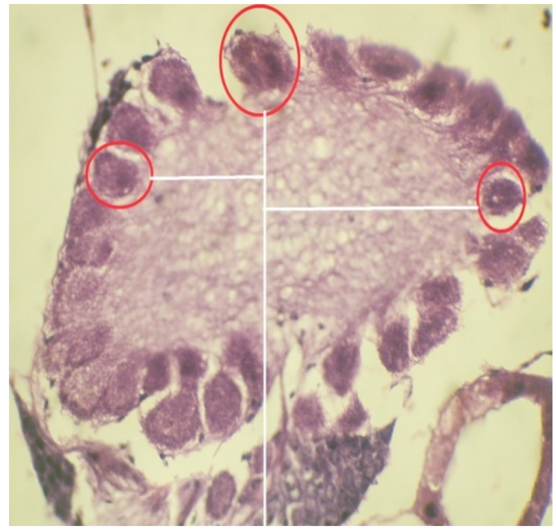
Fig. 3. Right antennal lobe

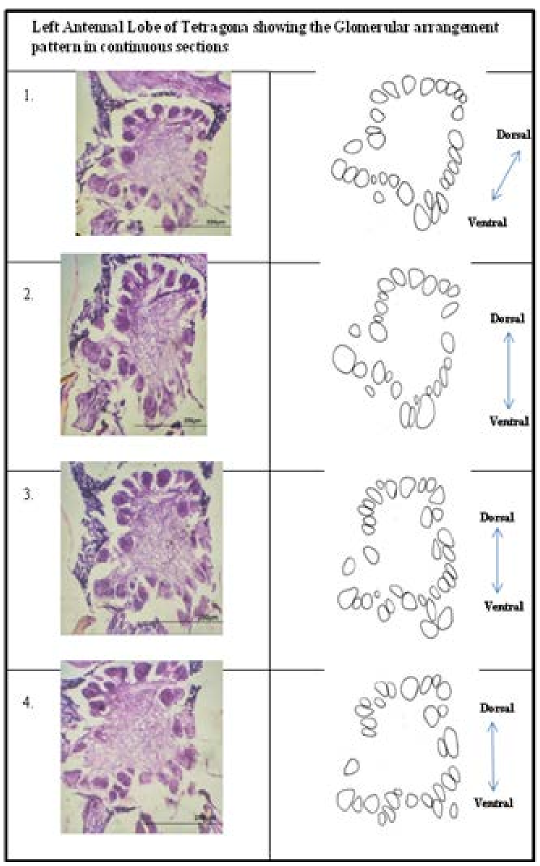

Fig. 4

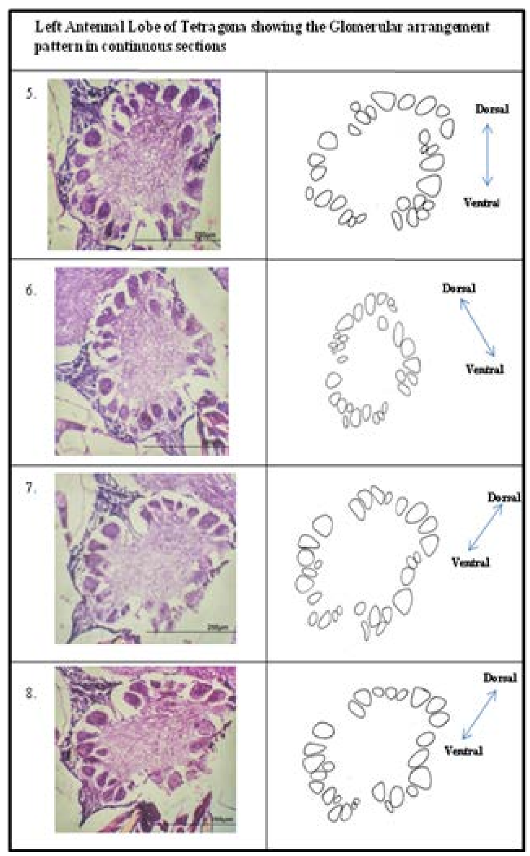

Fig. 5

are more or less distributed more or less throughout the antennal lobe volume. Significance of this pattern of the glomerular organization needs to be further probed. Total number of glomeruli is taken as an index of the insect's olfactory sensitivity. With a total number of ~70 glomeruli, *Tetragonula* probably relies less on olfactory sensory inputs. It is likely that *Tetragonula* depends more on visual sensory inputs in addition to the mechanosensory receptors.

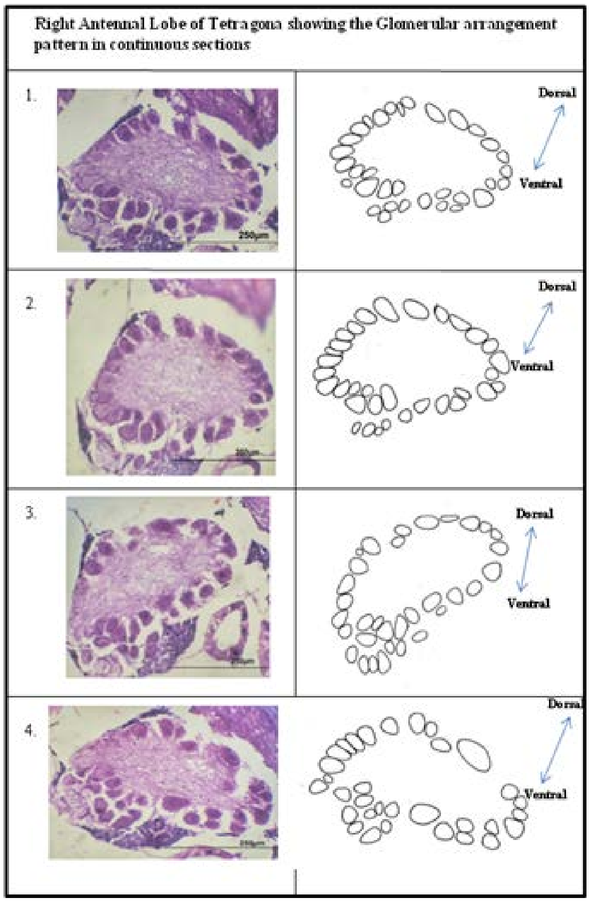

Fig. 6

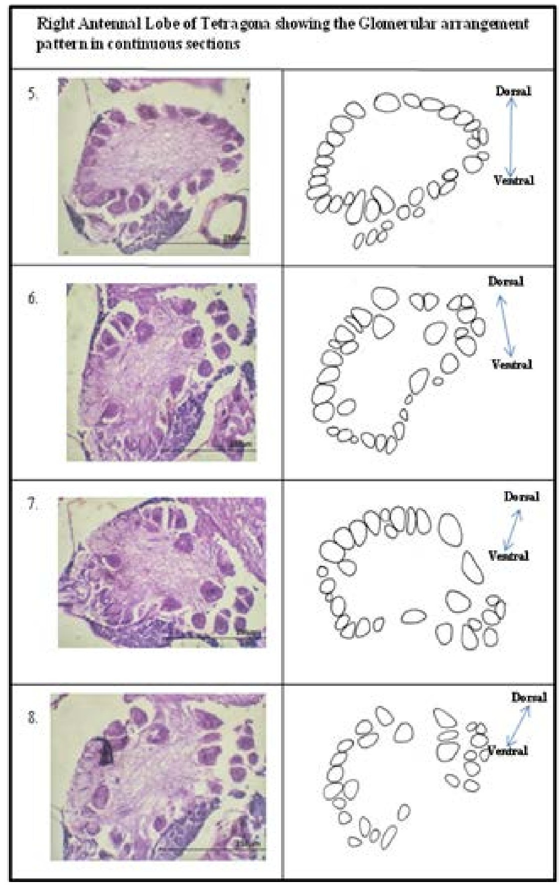

Fig. 7

Table 1. Morphometry of antennal lobe and glomeruli

No.	~Diameter of Left Antennal Lobe (µm)	~Average Diameter of Glom-eruli Of Left A.L (µm)	~Diameter of Right Antennal Lobe (µm)	~Average Diameter of Glom-eruli Of Right A.L (µm)
1	252	30	236	28
2	248	32	240	28
3	260	30	268	30
4	236	30	280	25
5	236	32	280	27

Table 2

No.	~No. of Glomeru-li in left Antennal lobe	~No. of Glom-eruli in right Antennal lobe
1	30	33
2	27	33
3	34	33
4	30	32
5	29	35
6	29	33
7	28	29
8	27	27

In social insects a total number of ~70 glomeruli is a relatively less number in comparison to other social insects like ants which have glomeruli in the range of 300-400.Therefore it is easy to presume that *Tetragonula* depend on other sensory modalities also. Sensory perception aspects of *Tetragonula* needs to be ascertained. Social insects need a superior communication system to maintain its social fabric. Therefore, the question of the fundamental properties of other sensory system is needed to be analyzed with reference to the ecology of the *T. iridipennis.*

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