

Cytological changes in the brain of fourth and fifth instar of worker honey bee *Apis cerana indica* during post-embryonic development

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ABSTRACT

During the post-embryonic development of Indian honey bee, *Apis cerana indica*, brain undergoes enormous change in its anatomical organization. Brain of fourth and fifth instar larva consist of MNC, LNC, PNC and VNC group of neurosecretory cells. Cell types B, C1, C2 are found in LNC, PNC, and VNC while in MNC all cell types A, B, C1, C2 are found. Variation was observed in distribution and size of cell cytoplasm in each hemisphere of brain of fourth and fifth instar larva.

Keywords: brain, Honeybee, Neurosecretory cell, Post-embryonic development,

INTRODUCTION

Indian Honeybee, *Apis cerana indica* can be easily kept in hive and are domesticated for commercial production of honey, other products and as a good pollinator due to its non-aggressive behavior and rarely exhibiting swarming behavior. Under well condition, it built 5-7 vertical and parallel comb in hallow of tree trunk rock crevices under shadow. Single colony per annum on an average may yield 5-6 Kg honey. Most of the research work on Indian honeybee is confined to its distribution, behavior, morphology and social life, comb formation while no significant contribution has been made towards histomorphology of brain, physiology of neurosecretory cell and hormonal activity during post-embryonic development. Snodgrass (1956) described the anatomical organization of central and sympathetic nervous system in hymenoptera. Wayer (1987) observed some neurosecretory cell in the adult brain of worker, drone and queen honeybee, *Apis mellifera* first time. It is now well established from research of certain worker that the central nervous system play an most important role during post-embryonic development, caste differentiation and reproduction in insect (Raabe, 1982; Mishra and Dogra, 1983; Farris *et al.*, 1999; Tembhare and Barsagade, 2000; Norbert and Karl, 2005).

MATERIAL AND METHOD

The fourth and fifth instar larva of *Apis cerana indica* were collected in saline solution during the month of October to April 2006-07 from the well maintained honey bee culture at the department of zoology, RTM Nagpur university campus, Nagpur. The cephalic neuroendocrine organ was dissected out from the larva of *Apis cerana indica* under stereoscopic binocular microscope in insect saline solution. Tissue was fixed in aqueous Bouin's fixative about 16-24hrs duration for histological studies. Thereafter tissue were dehydrated in alcohol grade, cleared in xylene and embedded in paraffin wax (58-60 °C). Serial sections were cut at 4-5-micron thickness and stained with Chrome Alum Haematoxylin-Phloxine (CAHP) and Cameron and Steel's Adehyde Fuchsin-Halmi's mixture (AF).

RESULT AND DISCUSSION

Present study has been undertaken to provide cytology, distribution and classification of cerebral neurosecretory cell and transport of neurosecretory material with their neurosecretory pathway in worker honeybee *Apis cerana indica* during post-embryonic development. Most of the earlier workers describe structure and function of cephalic neuroendocrine system in the honeybee *Apis mellifera* and hormonal regulation of polymorphism (Hannan, 1955; Snodgrass, 1956; Canetti, et al. 1964; Thomsen, 1965; Dogra et al., 1977; Laere, 1970; Breed, 1983; Ritcey and Dixon, 1996a; Farris et al., 1999; Wheeler et al., 2006).

Brain of *Apis cerana indica*, undergoes enormous change in its anatomical organization during the post-embryonic development. Variation was observed in distribution and size of cell cytoplasm in each hemisphere of brain of fourth and fifth instar larvae. All the four types of neurosecretory cells are observed in brain of fourth and fifth instar larva consist of MNC, LNC, PNC and VNC group. In honeybee, some workers described the presence of single pair of MNC groups in the pars cerebralis region of brain (Weyer, 1935; Scaller, 1937; Laere, 1970; Mishra and Dongra, 1983).

In MNC of fourth instar larva A, B, C1, C2, are measuring about 9.06±0.16, 6.78±0.05, 16.58±0.30, and 11.52 ±0.04 µm in diameter respectively. Ritcey and Dixon (1996a) reported three groups of neurosecretory cells, MNC, LNC in protocerebrum and VNC group in tritocerebrum in the brain of *Apis mellifera*. Tembhare and Paliwal (1993) described the six paired groups viz medial, lateral, posterior, deutocerebral, ventral and optic groups of NSC in the brain of drone and queen of *Apis dorsata*. The present study demonstrates the presence of paired groups of MNC, LNC, PNC, in protocerebrum and VNC in tritocerebrum of the 4th and 5th instar larva of *Apis cerana indica*.

The B, C1, and C2 cell types LNC measure about 6.10±0.24, 16.40±0.48, 10.08±0.32 µm in diameter. In PNC B, C1, C2 cell type measuring about 6.78±0.50, 16.40±0.45, and 10.58±0.52 µm in diameter respectively. VNC group cell type B, C1, C2 are measuring about 6.78 ±0.46, 16.58±0.37, 10.58±0.98 µm in diameter respectively. The brain of fifth instar larva is slightly larger than fourth instar larva. A,B,C1,C2 cell types of MNC group measuring about 12.30±0.44, 8.25±0.38, 20.31±0.06, 12.98±0.32 µm in

Table 1: Distribution and size of cell cytoplasm of cerebral neurosecretory cells of brain in 4th and 5th instar of worker honeybee *Apis cerana indica*

NS Cells	4 th instar				5 th instar			
	Cell type				Cell type			
	A	B	C1	C2	A	B	C1	C2
MNC	9.06 ± 0.16	6.78 ± 0.05	16.58 ±0.30	11.52 ±0.04	12.30 ±0.44	8.25 ±0.38	20.31 ±0.06	12.98 ±0.32
LNC		6.10 ±0.24	16.40 ± 0.48	10.08 ± 0.32		6.06 ±0.07	42 ±0.50	12.08 ±0.32
PNC		6.78 ± 0.50	16.40 ±0.45	10.58 ±0.52		9.08 ±0.56	20.05 ±0.62	12.05 ±0.64
VNC		6.78 ±0.46	16.58 ±0.37	10.58 ±0.98		9.08 ±0.58	16.40 ±0.28	12.04 ±0.28

Abbr : A,B,C1,C2- Neurosecretory cell types. LNC- Lateral neurosecretory cell, MNC- Mediam neurosecretory cell, PNC-Posterior neurosecretory cell, VNC-Ventral neurosecretory cell.

diameter respectively. In LNC B, C1, C2 cell measuring about 6.06 ± 0.07 , 16.42 ± 0.50 , 12.08 ± 0.32 μm in diameter respectively. In PNC B, C1, C2 cell type measuring about respectively 9.08 ± 0.56 , 20.05 ± 0.62 , and 12.05 ± 0.64 μm in diameter. VNC group of neurosecretory cell consist of B, C1 C2 cell type measuring about 9.08 ± 0.58 , 16.40 ± 0.28 , 12.04 ± 0.28 μm in diameter respectively (Table 1).

In hymenoptera Thomsen (1954a) and Nayar (1955) have classified the cerebral neurosecretory cell into A and B type and suggested that A cell represent the active while B cell represent inactive during secretory cycle. The cerebral NSC in the brain of *Apis* where however classified as the large, small and intermediate cells mostly on the basis of their staining affinities and other characteristic (Ritcey and Dixon, 1996a). Breed (1983) categorized NSC simply on the basis of position in the brain: medial, lateral I and lateral II. In the present study, vertebral neurosecretory cells has been classified into A,B,C1,C2 on the basis of their staining affinity to the various selective stains and some variation in their cytomorphological feature. Median neurosecretory pathway joined with lateral neurosecretory pathway, posterior neurosecretory pathway and ventral neurosecretory pathway in the tritocerebral part of brain and emerge out as nervi corpori cardiaci.

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