HISTOMORPHOLOGICAL STUDY OF CEPHALIC NEUROENDOCRINE SYSTEM DURING PUPAL METAMORPHOSIS IN INDIAN WORKER HONEYBEE APIS CERANA INDICA(F.) (HYMENOPTERA: APIDAE)

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ABSTRACT

Honeybee, Apis cerana indica undergoes complete metamorphosis and each stage of development has its own importance. Developmental changes occurs in body structure during metamorphosis are under the control of hormone secreted by neuroendocrine organ. During metamorphosis brain and retrocerebral complex of red eye pupa of Apis cerana indicaworker was studied histologically. Four neurosecretory cell groups with different type of neurosecretory cells were observed in various regions of the red eye pupa brain of worker honeybee. Retrocerebral complex constituting corpora cardiaca and corpora allata loaded with neurosecretory materials. The structure of neurosecretory cells and their number in various region of brain were found unique with different staining properties by using Chrome alum Haematoxylin-Phloxine (CHP) stain.

Keywords: *Apis cerana indica, Brain, Honeybee, Neurosecretory cell, Pupa.*

Introduction

Snodgross (1956) described the anatomical organisation of central and sympathetic nervous system in hymenoptera. Hymenopteran insects complete their development by passing through egg, larva, pupa, and adult developmental stages. During post embryonic development, several neurons in brain formed, degenerated and replaced to perform specific task and memory (Tissot and Stokes 2000;Zars*et. al.,* 2000).

Weyer in 1935 described the cephalic neuroendocrine system in *Apis mellifera*. Scharrer in 1937 noticed the median neurosecretory cells (MNC) groups while, the lateral neurosecretory cells (LNC) groups in insects brain was observed by other workers (Cazal, 1948L'Helias, 1950; L'Hoste, 1952; Gawande, 1968). The ventral(tritocerebralis) neurosecretory cell (VNC) groups noted by Ritcey and Dixon (1969) and the optic neurosecretory cell (ONC) groups by Prasad (1981). Post embryonic brain and mushroom body development were studied by Malun (1998) and Farris *et al.* (1999) by using histological technique.

Apis cerana indica is highly domesticated honeybee in India due to its high pollination efficiency and for apiary byproducts production. Like other hymenopteran insects *Apis cerana indica* complete its development by passing through all four developmental stages. Pupal stage in honeybee is characterised by development of eye and eye colour patterns. Eye colour patterns changes from white to brown to red-black eye during pupa to adult metamorphosis. On that basis honeybeepupa is classified into white eye pupa(early pupa), brown eye pupa (mid pupa) and red- black eye pupa (Late pupa). In the present work detailed study of neurosecretory cells in brain and retrocerebral complex in red eye pupa of worker honeybee *Apis cerana indica*were carried out during pupa to adult metamorphosis.

Materials and methods

Red eye pupae of worker honeybee *Apis cerana indica* were separated from colony and reared in home apiary situated in Warora district Chandrapaur, Maharshtra, India (20º16'53"N79º01'21"E). Brain along with retrocerebral complex of red eye pupa was dissected out from worker bodyin insect saline solution and preserve in bouin's fixative for overnight. In some cases head portion along with first thoracic segment were cut from body of red eye pupa of worker and preserve in bouin's fixative for overnight. Thereafter, tissues were dehydrated in alcohol grades, cleared in xylene and embedded in paraffin wax (58-60^0 C). Serial sections were cut at 4-5 micron thickness and stained with Chrome Alum Haematoxylin-Phloxine (CHP) stain.

Stainingprocedure (Bergman's Chrome alum Haematoxylin Phloxine, Pearse, 1968).

The sections were deparaffinized in xylene and hydrated down to distilled water through series of alcohols in descending order. They were transferred to the mordant Bouin's fluid containing 3% Chrome alum about 12-24 hrs for fixation of NSC. After a thorough wash in running water (1/2 to 1 hour) they were dipped in distilled water, oxidized in acid permanganate ($KMnO_4$-0.15 gm + H_2SO_4-0.1 ml + Distilled water-50 ml).They were dipped in distilled water and decolorized in 2.5% sodium bisulphate solution. The sections were transferred to the staining (CHP) solution (5-10 min) and differentiated in 0.5% acid water, checking intermittently under microscope. They were washed in running water until the sections become blue. The sections were counter stained in 0.5% Phloxine rinsed in 5% Phosphotungstic acid and washed in running water till the sections turned pink. After a quick differentiation in 95% alcohol, they were dehydrated in absolute alcohol, cleared in xylene and mounted in DPX.

Observations

1. Morphological observation

In red eye pupa, bilobed brain was lies above the stomodeum in head capsule, covered by large number of fat bodies and air sacs. All three parts of brain, protocerebrum, deutocerebrum and tritocerebrum and both side of optic lobes were well developed. Large portion of brain was occupied by protocerebrum at dorsal position and was continuous with optic lobes. Both side of deutocerebrum portion of brain were small and contains antennal lobes. Tritocerebral portion was continuing with suboesophageal ganglion leaving entrance for oesophagus. Large, bilateral retrocerebral complex (corpora cardiaca and corpora allata) lies ventrally to the brain. (Fig. 1A & B).

2. Histological observation

Inbrain ofred eye pupa of *Apis cerana indica,*all the three parts of brain, protocerebrum, dueterocerebrum and tritocerebrum and both side of optic lobes were well developed (Fig.2). Large and distinct corpora cardiaca and corpora allata lies ventrally to the brain. Two group of neurosecretory cell were observed in well developed protocerebral lobe of brain as, median neurosecretory cell (MNC) group and lateral neurosecretory cell (LNC) group (Fig.3, 4 and 7) . Deutocerebral portion of brain was small and consist of posterior neurosecretory cell (PNC) group of neurosecretory cells (Fig.5). Tritocerebral parts of brain were well distinct with ventral neurosecretory cell (VNC) group (Fig.6). Four type of neurosecretory cells namely A, B, C1 and C2 were observed in all four groups of NSC. Shape and measurement of NSC are given in table No:1. Median axonal tract (MAT) emerged from MNC was observed in pars intercerebralis region (Fig3). Similar axonal tract were also observed from LNC and PNC (Fig 4, 5). All this pathway joined in tritocerebral region of brain and form nervi corporis cardiaci (NCC).

	A cell	B cell	C1 cell	C2 cell
Staining affinities	Blue Black inclusion	Red inclusion	Bluish inclusion	Bluish inclusion
Shape	Pyriform	Spherical	Ellipsoidal	Ellipsoidal

Table 1: Staining affinities to CHP stain and shape of neurosecretory cell in brain of red eye pupa of worker honeybee. Abbri: A, B, C1 and C2- Neurosecretory cells. CHP-Chrome alum Haematoxylin Phloxine (Bergman's)

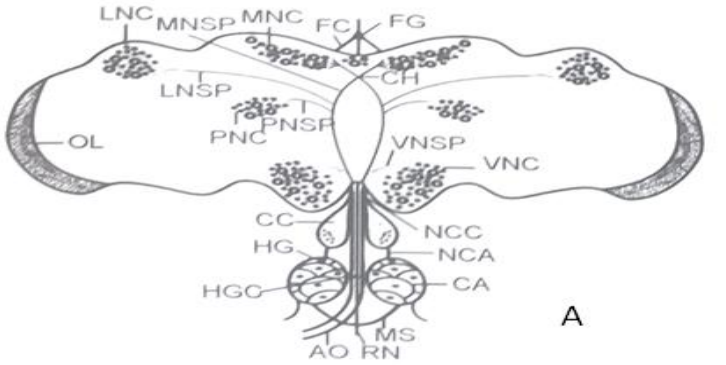
Cell group	MNC				LNC				PNC				VNC			
Type of cell	A	B	C1	C2	A	B	C1	C2	A	B	C1	C2	A	B	C1	C2
Size	12.42 ±0.49	8.21 ±0.29	20.52 ±1.28	13.24 ±0.58	11.06 ±0.02	7.24 ±0.32	16.58 ±0.39	12.09 ±0.24	12.68 ±0.90	12.03 ±0.15	24.07 ±0.80	11.98 ±1.02	10.04 ±0.18	12.03 ±0.18	24.07 ±1.16	12.98 ±1.04

Table 2: Size of neurosecretory cells in median (MNC), lateral (LNC), posterior (PNC) and ventral (VNC) region of brain of red eye pupa in worker honeybee *Apis cerana indica.*

Abbri: A, B, C1 and C2- Neurosecretory cells. CHP-Chrome alum Haematoxylin Phloxine (Bergman's),LNC-lateral neurosecretory cells, MNC-median neurosecretory cells, PNC-posterior neurosecretory cells, VNC-ventral neurosecretory cells.

Retrocerebral complex constitute the corpora cardiaca and corpora allata. The CC was well developed, globular shaped paired milky bodies in the cephalic region lying ventral to the brain. Internally the CC is differentiated into two regions. The anterior (dorsal) glandular region consist of intrinsic neurosecretory cell (INC) while, posterior (ventral) nervous region accommodate axon of cerebral neurosecretory cell containing neurosecretory material (NSM) in variable quantity and some small neurons. The INC stains blue black with CHP stain. INC arranged in linear fashion in pupa. The CC was measured about 64.08±1.98 and 34.20± 0.14 µm in length and width of red eye pupa. Both side of CC were connected to the brain by nervi corporis cardiaci (NCC).

Corpora allata (CA)were oval bodies lying on either side of the esophagus, ventral to the CC. Both the CA connected ventrally by a thick transverse muscle strand (MS). The CC and CA of one side were connected vertically with brain by a thin longitudinal muscle strand. Each CA is internally filled with the spherical epithelial cell generally with intercellular space. The wall of the CA is composed of a thick layer of connective tissue. The CA were connected to the CC by nervi corporis allati (NCA).The size of the CA was measured about 70.12 ± 1.54, µm in diameter in pupa and epithelial cell diameter is 7.12±0.22, µm.(Fig 8-10).



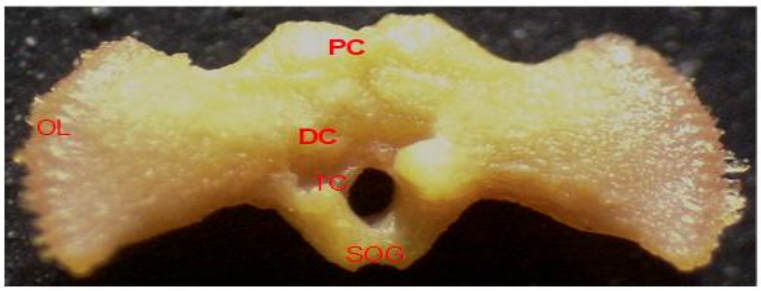


Fig1: A-Topography of neuroendocrine system of red eye pupa of worker honeybee showing neuroendocrine cell and neurosecretory pathway. B- Dissected brain of Red eye pupa of worker honeybee. Abrri.- AO- aorta, CA-corpora allata, CC- corpora cardiaca, CH-chiasmata, DC-deutocerebrum, FC-frontal connective, FG- frontal ganglion,HG- hypocerebral ganglion, hgc- hypocerebral ganglionic conection,LNC- lateral neurosecretory cell,LNSP-lateral neurosecretory pathway, NCA- nervi corporis allati, NCC- nervi corporis cardiaci, MNC- median neurosecretory cell, MNSP- median neurosecretory pathway, OL-otic lobe, PC-protocerebrum, PNC- posterior neurosecretory cell ,PNSP- posterior neurosecretory pathway, RN-recurrent nerve, SOG-suboesophageal ganglion, TC-tritocerebrum, VNC- ventral neurosecretory cell, VNSP- ventral neurosecretory pathway.

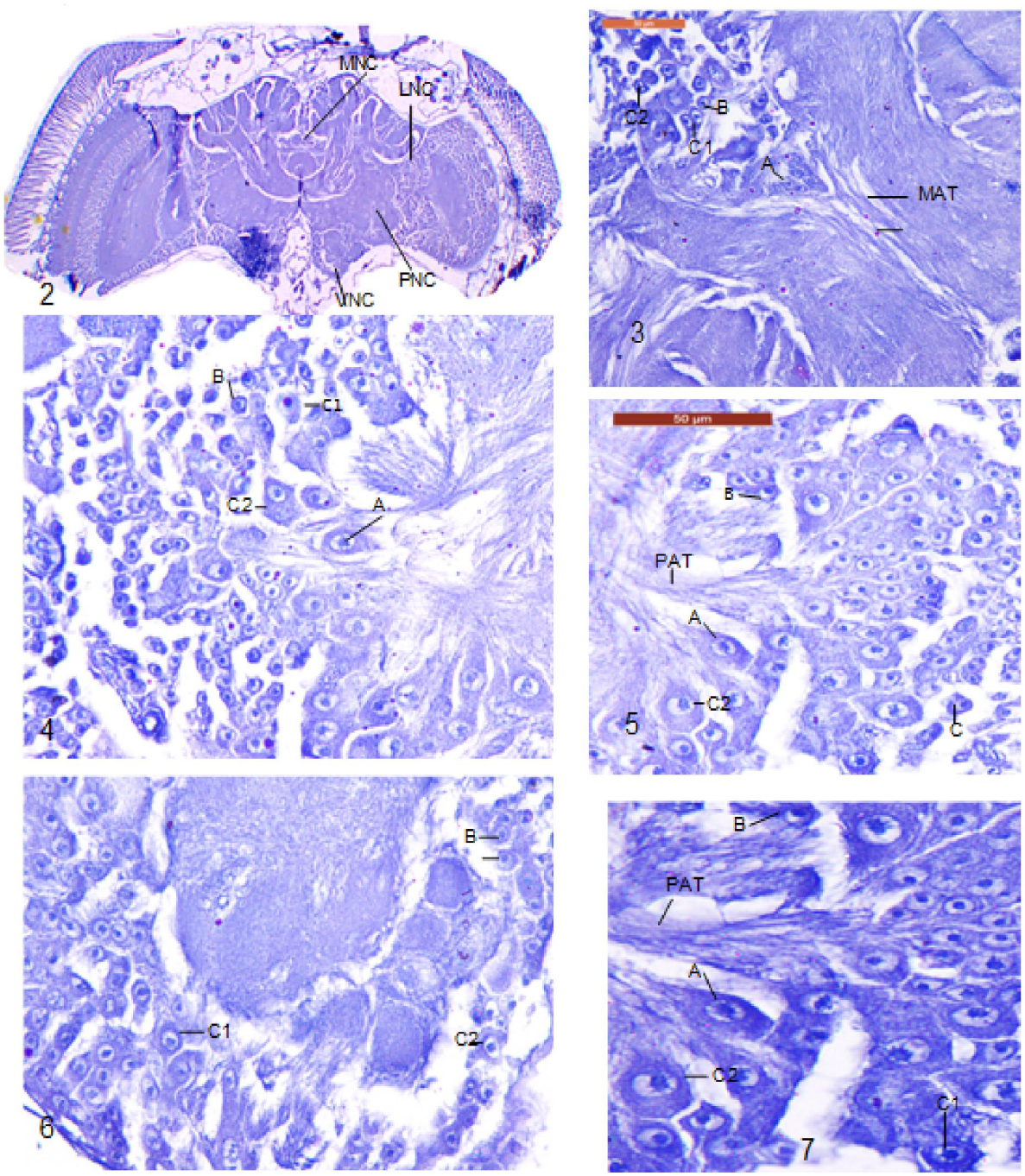


Fig.2. Frontal section of brain of red eye pupa of worker honeybee, *Apis cerana indica* showing position of neurosecretory cells. (CHP-40x)

Fig.3: Section passing through the protocerebrum region of brain to show A,B,C1 and C2 cells in MNC groups. (CHP-400x)

Fig.4: Section passing through the protocerebrum region of brain to show A, B and C1 and C2 cells in LNC groups.(CHP-400x)

Fig.5: Section passing through posterior region of brain to show A, B, C1 and C2 cells types in PNC groups. (CHP-400x)

Fig. 6 : Section passing through tritocerebral region of brain to show B, C1 and C2 cells types in VNC groups. (CHP-400x)

Fig. 7: Magnified view of Fig. 5 to show PNC and PAT in PNC groups.
Abbri:- A- A cell. B- B cell. C1 and C2- C cell. LNC- Lateral neurosecretory cell. MAT-Median axonal tract. MNC- Median neurosecretory cell. PAT-Posterior axonal tract. PNC- Posterior neurosecretory cell. VNC- ventral neurosecretory cell.

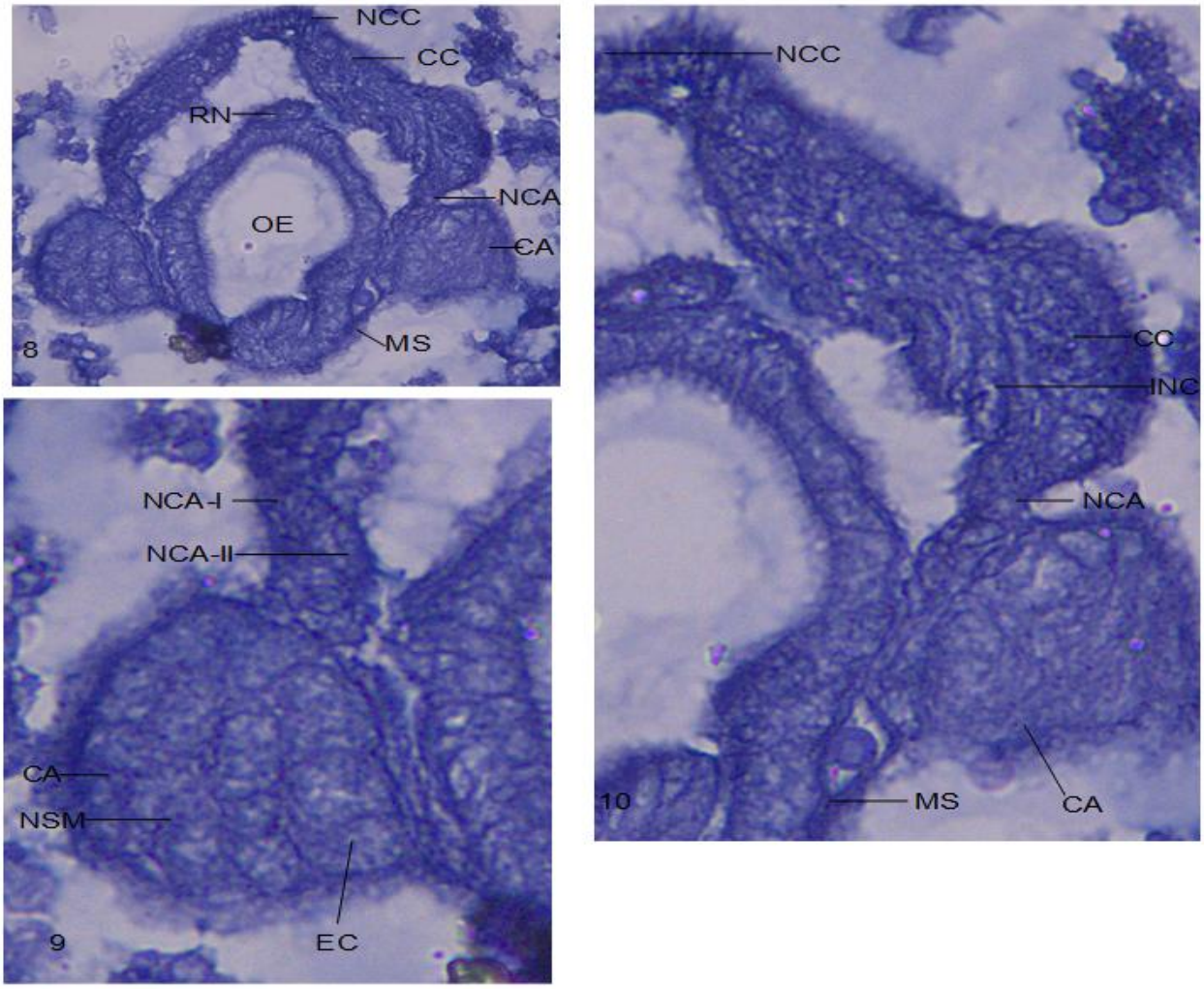


Fig. 8: Section passing through the CC-CA of red eye pupa of worker honeybee.CHP-400X.

Fig 9: Magnified view of fig. 8 to highlight the neurosecretory materials and nerve in CA.
Fig.10: Magnified view of fig. 8 to highlight the CC
Abbri- CA- Corpora allata. CC-Corpora cardiaca.EC-Epithelial cell. INC-Intrinsic neurosecretory cell. NCA- nervi corporis allati. OE- oesophagus. RN-recurrent nerve. NCA- Nervi corporis allati. NCC-nervi corporis cardiaci. NSM-neurosecretory material. MS-muscle strand.

Discussion

In insect only a single pair of MNC groups were described in the pars intercerebralis region of the brain (Weyer, 1935; Schaller, 1937; Laere, 1970; Mishra and Dogra, 1983) while Ritcey and Dixon (1969) reported three group of neurosecretory cell (NSC), medial (MNC), lateral (LNC) groups in protocerebrum and ventral (VNC) group in tritocerebrum in the brain of *Apis mellifera.* Tembhare and Paliwal (1992) 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*. During the present study, total four groups of neurosecretory cell in brain of red eye pupa of worker honeybee *Apis cerana indica*were observed. Single compact median neurosecretory cell (MNC) groupin pars intercerebralis and lateral neurosecretory cell (LNC) group in each hemisphere of protocerebrum was observed. Third group constitute posterior neurosecretory cell (PNC) in deutocerebral part of brain while fourth ventral neurosecretory cell group was observed in tritocerebral part of brain of red eye pupa.

No relation was observed in classification of cerebral neurosecretory cells in various NSC

groups in brain of insects. In Hymenoptera Thomsen (1954a) and Nayer (1955) has classified the cerebral NSC into A and B cell types and suggested that A cell represent active while B cell represent inactive phase during secretory cycle and supported by Gawande (1968) and Gundevia and Ramamurthy (1972). Later on cerebral NSC in the brain of *Apis* where however classified as the large, small and intermediate cells, mostly on the basis of cell size with considering their staining affinities and other characteristic (Ritcey and Dixon 1969). Breed (1983) categorized NSC simply on the basis of position in the brain: medial, lateral I and lateral II. According to Tembhare and Paliwal (1992), the NSC of various groupsclassified into four cells types; A, B, C1, and C2 in the larvae of *Apis dorsata*where A cell were confirmed only in MNC groups in the larvae of *Apis dorsata* worker. Similar observation was found in tropical tasar silkworm, *Anthereamylitta*by Tembhare and Barsagade (2000). In present study, the cerebral neurosecretory cells (CNS) has been classified into A, B, C1 and C2 cell types on the basis of their shape and staining affinities to CHP stainand concluded the presence of A, B, C1 and C2 cells in all groups of neurosecretory cell in brain of worker's red eye pupa of *Apis cerana indica.*

Crossing over of the median neurosecretory pathway (MNSP) commonly occurs in most of the insect including Hymenoptera (Breed, 1983; Mishra and Dogra, 1983; Rybak and Menzal, 2004; Lehman *et al.,* 2006). Breed (1983), noticed the MNSP and lateral neurosecretory pathway (LNSP) arising from the MNC and LNC respectively constituting a single pair of nerve, nervi corporis cardiaci (NCC) and innervate the corpora cardiaca. In the worker's red eye pupa, the axonal tract of MNC after running few distance, cross over to each other. The MNSP joined with LNSP, posterior neurosecretory pathway, PNSP and ventral neurosecretory pathway, VNSP in the tritocerebral part of the brain and emerges out as NCC were observed.

Gawande (1968) and Breed (1983) reported the emergence of the two pair of NCC i.e. NCCI and NCCII from the brains of ants and *Apis mellifera* respectively. Laere (1970) moreover described a single pair of fused NCC-I and NCC-II nerves innervating the CC in the honeybee. In the present study however, only a single pair of nerve NCC was evident in red eye pupa of *Apis cerana indica*.

The intrinsic neurosecretory cells (INC) have been reported in the CC of the Hymenoptera (Cazal, 1948; Thomsen, 1954a; Delerma, 1977). During the present study similar types of intrinsic neurosecretory cell were observed in red eye pupa of *Apis cerana indica.* In *Apis mellifera* (Thomsen, 1954a; Ritcey and Dixon, 1996) observed NCC innervations to the CC and CA in both larvae and adult hymenoptera. In present study it was observed that the NCC innervating the CC and comes out as NCA to innervate the CA in red eye pupa of worker *Apis cerana indica.*In red eye pupa of worker CA with epithelial cell were present on either side of esophagus and fused with it by a circular band of striated muscle and supported the finding of Laere (1970) in *Apis mellifera.*

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