

# HISTOMORPHOLOGICAL STUDIES ON THE NEUROSECRETORY CELLS IN THE BRAIN OF THE BEETLE *Cybister tripunctatus* (OL) (COLEOPTERA: DYTISCIDAE)

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## KEY WORDS

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## ABSTRACT

The cephalic neuroendocrine system is well developed in the adult water beetle, *Cybister tripunctatus*, consisting of the neurosecretory cells in the brain, corpora cardiaca and corpora allata. The brain is well distinct, bilobed structure situated on mid-dorsally on the pharynx in the head of adult beetle. The neurosecretory cells are distributed symmetrically and distinguished into five-paired groups viz. medial, lateral, ventral, posterior and optic in each hemisphere of the brain.

## INTRODUCTION

The neurosecretory cell of the brain and other ganglion of the central nervous system are extensively studied in large number of the insect in order to explore their structure and function (Novok, 1975). To a great extent the cytomorphological details, neurosecretory cells types and distribution as paired medial, lateral and ventral neurosecretory cells in the brain of *Cybister limatus* (Prasad, 1979). Beside this Barde (1981) has already reported the presence of optic neurosecretory cells in optic lobes in *Cybister regulosus*.

With the help of various specific staining techniques, the neurosecretory cells (NSC) are distinctly recognized in various region of the brain. These techniques not only provide topographical picture of the neurosecretory cell but evaluate both, the qualitative and quantitative characteristic of the neurosecretory material so that different cell types and stages of activity of the neurosecretory system during physiological state can easily be recognized.

In Coleopteran, most of the literature is available on phytophagous rather than carnivorous insects and there is perhaps, no substantial work in the cephalic neuroendocrine system of various respects in *Dytiscid* beetles. During the present study the histomorphology, classification and distribution of the neurosecretory cells (NSC) in the brain of the water beetle, *Cybister tripunctatus* have been elucidated for the first time.

## MATERIALS AND METHODS

The beetles were mainly collected from the perennial ponds

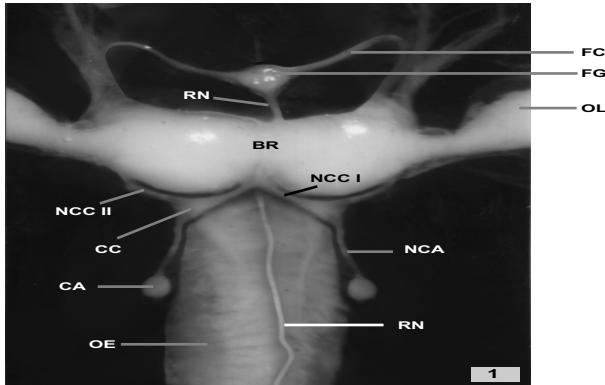
and rice fields located at Pauni, District Bhandara and acclimatized in the laboratory. The brain of adult beetle was dissected out, gently fixed in Bouin's fluid for 18-24h, dehydrated in 30% to absolute alcohol, cleared in xylen and embedded in paraffin wax at 58-60°C. About 4-6 $\mu$  thick sections were cut and mounted on the slides and stained with Bergmann's chrome-alum-haematoxyline phloxine (CHP), Delphin's Alcian Blue phloxine (ABP), Gurr's Azan and Ewen's aldehyde fuchsin (AF) staining techniques.

## RESULTS

The brain is a bilobed structure situated on the dorsal surface of the pharynx in the middle of the head region. It is anteriorly connected to the frontal ganglion with the paired frontal connectives, and distally with the paired corpora cardiaca (CC) with the nervi corporis cardiac interni (NCC I) and nervi corporis cardiaci externi (NCC II). Each CC is connected to the corpora allata (CA) with a nerve, nervi corporis allati (NCA), (Fig. 1). With the help of various specific staining techniques, the neurosecretory cells (NSC) are distinctly recognized in various region of the brain. The NSC consists of large glandular bodies filled with variable quantity of neurosecretory material (NSM). The NSM is also observed in the axon of the NSC and they terminate into the corpora cardiaca (CC).

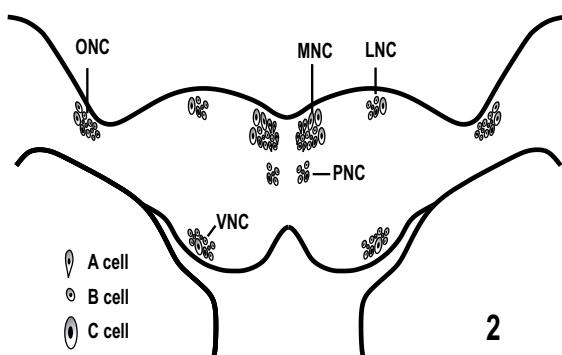
### Cytomorphology and classification of the NSC (Fig. 2)

On the basis of cytomorphological characteristics, such as, shape, size, cytoplasmic contain and cyclical activity of the nuclei and staining affinities of the NSM in the cell bodies and axons of the NSC (Table 1), they are classified into various



**Figure 1: *In situ* structure of cephalic neuroendocrine system in the beetle**

BR: Brain; CA: Corpora allata; CC: Corpora cardiaci; CE: Compound eye; FC: Frontal connective; FG: Frontal ganglion; NCA: Nervi corporis allati.;NCC-I: Nervi corporis cardiaci interni; NCC-II:Nervi corporis cardiaci externi; OE: Oesophagus; OL: Optic lobe; RN: Recurrent nerve



**Figure 2: Diagrammatic representation of distribution of neurosecretory cells in the brain**

LNC: Lateral neurosecretory cells; MNC: Medial neurosecretory cells; ONC: Optic neurosecretory cells; PNC: Posterior neurosecretory cells; VNC: Ventral neurosecretory cells

types as follows:

#### A cells

They can easily be identified from the other NSC due to their specific staining affinities. They stain blue black with CHP, purple with AF, blue with ABP and red with Azan. They are mostly pyriform in shape. They measured  $18.45 \pm 2.05\mu\text{m}$  and their nuclei about  $10.25 \pm 2.05\mu\text{m}$  diameter. The A cells bear large number of granules stained deeply with CHP and Aldehyde Fuchsin. The granules are scattered throughout the cytoplasm.

#### B cells

The B cells stain red with CHP, ABP, green with AF and blue with aniline blue of Azan stain techniques. The NSM stain well in the cell bodies with phloxin of CHP and appears as fine granules, while in AF, the NSM as well as the cytoplasm stain uniformly as green. The B cells are spherical in shape. The B cell can be classified into two subtypes on the basis of their size as follows.

**The B<sub>1</sub> cells:** They measures about  $14.35 \pm 2.05\mu\text{m}$  and nuclei  $10.25 \pm 2.05\mu\text{m}$  in diameter.

**The B<sub>2</sub> cells:** They measures about  $18.45 \pm 2.05\mu\text{m}$  and nuclei  $9.57 \pm 1.367\mu\text{m}$  in diameter.

#### C cells

The C cells are larger than A cells and are ambhibious in staining affinity. The NSM stains blue in CHP, light purple in AF, blue in ABP and red in azan staining techniques. The cytoplasm stains with the counter stains. They are spherical or oval in shape. The C cells can be classified into two sub types as follows:

**The C<sub>1</sub> cells:** They measures  $22.55 \pm 2.05\mu\text{m}$  and nuclei measure about  $9.56 \pm 1.36\mu\text{m}$  in diameter.

**The C<sub>2</sub> cells:** They measures  $28.70 \pm 2.36\mu\text{m}$  in cell and  $14.35 \pm 2.05\mu\text{m}$  nuclear diameter.

#### The distribution of the NSC

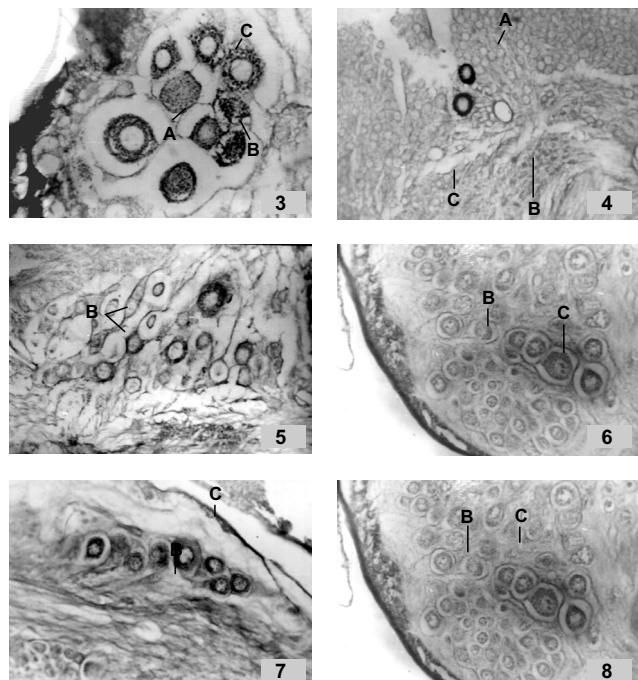
The NSC are located in five regions in the brain and form five paired distinct groups as follows:

#### The medial neurosecretory cells (Figs. 3 and 4)

Two groups of the neurosecretory cells, the medial neurosecretory cells (MNC) lie close to each other in the anterodorsal region of the pars intercerebralis medialis in the protocerebrum on either side of the median furrow. Both the MNC groups are so closely situated that, it is often difficult to recognize them separately. Each group is composed of 8 A cells, 20 B<sub>1</sub> cells and 7 C<sub>1</sub> cells. The A and B<sub>1</sub> cells are intermingled with each other while the C<sub>1</sub> cells are situated peripherally at anterior and posterior region.

#### The lateral neurosecretory cells (Fig. 5)

A small group of lateral neurosecretory cells (LNC) is situated in the pars intercerebralis lateralis region of the protocerebrum



**Figure 3 to 8: Section passing through the brain showing (3) medial A, B and C neurosecretory cells (AF, X80) (4) medial, A, B and C neurosecretory cells, magnified (AF, X160) (5) lateral B neurosecretory cells. (AF, X128); (6) ventral B and C neurosecretory cells (AF, X128) (7) posterior B and C neurosecretory cells (AF, X128) (8) optic B and C neurosecretory cells (AF, X128)**

**Table 1: Cytomorphology, classification and distribution of neurosecretory cells in the brain**

Cell type	Staining	Affinity	CHP	AF	ABP	Azan	Size ( $\mu\text{m}$ )	Diameter	Shape	Cell	Nuclei	Distribution Group	Number
A cell	Blue-Black	Purple	Dark blue	Dark red	Dark blue	Dark red	18.45 $\pm$ 2.05	10.25 $\pm$ 2.05	Pyriform	Spherical	Spherical	MNC	8
B <sub>1</sub> cell	Dark red	Purple NSG in greenish	Dark red	Dark red	Dark blue	Dark blue	14.35 $\pm$ 2.05	10.25 $\pm$ 2.05	Spherical	Spherical	Spherical	MNC	20
B <sub>2</sub> cell		cytoplasm					18.45 $\pm$ 2.05	9.57 $\pm$ 1.36	Spherical	Spherical	Spherical	LNC	5
C <sub>1</sub> cell	Blue-black NSG in reddish cytoplasm	Purple NSG in brownish cytoplasm	Blue NSG in reddish cytoplasm	NSG in brownish cytoplasm	Red granules in bluish cytoplasm	Red granules in bluish cytoplasm	22.55 $\pm$ 2.05	9.56 $\pm$ 1.36	Spherical	Spherical	Spherical	MNC	2
C <sub>2</sub> cell							28.70 $\pm$ 2.36	14.36 $\pm$ 2.05	Oval	Spherical	Spherical	PNC	7
												LNC	3
												VNC	3
												ONC	3

CHP - Chromalum Haematoxiline Phloxin; AF – Aldehyde Fuchsin; ABP – Alcian blue; MNC, LNC, PNC, VNC, ONC – Medial, Lateral, Posterior, Ventral, Optic neurosecretory cells respectively.; NSG – Neurosecretory granules.

in each hemisphere of the brain. They are located in the anterior region in between the MNC group and corpora pedunculata in each half of the protocerebrum. Each LNC group contains 5 B<sub>1</sub> cells and 3 C<sub>2</sub> cells. The B<sub>1</sub> cells form the single cluster and the C<sub>2</sub> cells lie on the periphery of the LNC group. The A cells are absent.

#### The ventral neurosecretory cells (Fig. 6)

The NSC, constitute a distinct group of ventral neurosecretory cells (VNC) in the lateral part of each tritocerebral lobe. Each VNC group is composed of 7 B<sub>2</sub> cells and 3 C<sub>2</sub> cells. These cells are scattered amongst large number ordinary and some giant neurons. The A cells are lacking.

#### The posterior neurosecretory cells (Fig. 7)

The paired groups of posterior neurosecretory cells (PNC) are observed in the midposterior region of the protocerebrum. Each PNC group consists of 14 B<sub>1</sub> cells and 2 C<sub>1</sub> cells. The A cells are lacking.

#### The optic neurosecretory cells (Fig. 8)

A distinct group of optic neurosecretory cells (ONC) is observed at the root of each optic lobe. Each ONC group is composed of 7 B<sub>2</sub> cells and 3 C<sub>1</sub> cells. The A cells are lacking.

## DISCUSSION

During the present study the histomorphology, classification and distribution of the neurosecretory cells (NSC) in the brain of the water beetle, *Cybister tripunctatus* have been elucidated for the first time.

The localization of paired medial, lateral and ventral NSC (MNC, LNC, VNC) in the brain of *Cybister tripunctatus* resembles with that in other beetles (Fletcher, 1961; Lalitha et al., 1979; Gundevia and Ramamurti, 1972a; Prasad, 1979; Rajendran and Ramalingam, 1979b; Sidhra et al., 1983; Gundevia, 1983; Panov and Melnikova, 1986; Panov, 1989). Besides MNC, LNC and VNC, some workers reported presence of optic neurosecretory cells (ONC) in the optic lobes, Noirot (1957) in *Calotermis flavicollis*, Thomsen (1965) in *Calliphora erythrocephala*, Baettie (1971) in *Periplaneta americana*, Khan (1976) in *Blatta orientalis*, Tembhare and Thakre (1976a) in *Orthetrum chryris*, Barde (1981) in *Dineutes indicus* and *Cybister regulosus*, Prasad (1981) in *Poekilocerus pictus*, *Periplaneta americana*, *Belostoma indicum*, *Polistes hebraeus*

and *Sandrocottus de jeani*, Andrew (1988) in *Tramea virginia* and Tembhare and Paliwal (1990) in *Apis dorsata*. The presence study also reveals the presence of well-defined paired groups of neurosecretory cells in optic lobes of *Cybister tripunctatus*.

The number of NSC constituting various groups appears to be the species-specific phenomenon. The presence of a small number of comparatively large size NSC in the pars intercerebralis (PI) of *C. tripunctatus* corroborates to the findings of Fletcher (1961), Siew (1965a), Gundevia and Ramamurti (1972a), Govardha et al. (1978), in Coleoptera and also in other insects (Deoras and Bhaskaran, 1966-1967a; Hsiao and Fraekel, 1966; Dogra, 1967a; Raina, 1974; Kurad and Thakare, 1980). On the other hand some workers have reported presence of NSC of small size occurring in large number (Highnam, 1961; Dogra, 1967d; Awasthi, 1969; Dogra and Even, 1970; Tembhare, 1973).

On the basis of staining affinity and other cytological characteristics, earlier workers (Thomsen, 1952; 1954b; Nayar, 1955; Highnam, 1961; Delphin, 1965; Hinks, 1971a; Thakare and Tembhare, 1975; Panov, 1979, 1980) classified the NSC of various groups into the cell types like, A, B, C and even into subtypes like, A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub> etc. The A cells seem to occur in PI of almost all the insects (Raabe, 1982). In Coleoptera maximum number of cell types was described by Fletcher (1961) who ascribed 13 types of cells in the beetle, *Blaps mucronata* and 7 types by Schooneveld (1970) in *Leptinotarsa decemlineata*. In *C. tripunctatus*, moreover, only three types of the NSC viz. A, B and C can be recognized similar to that in *Galeruca tunaceti* (Siew, 1965a) and *Mylabris pustulata* (Sidhra et al., 1983). The B and C cells in various groups in the brain of *C. tripunctatus* are further classified into two subtypes B<sub>1</sub> and B<sub>2</sub> and C<sub>1</sub> and C<sub>2</sub> respectively on the basis of their size variations. The neurosecretory cells of various groups in the brain of *C. tripunctatus* frequently show change in cell and nuclear size and quantity of cytoplasmic inclusion in the perikarya and axons suggesting cyclical activity of synthesis and release of neurosecretory material in accordance with the physiological, reproductive or developmental requirement of the insects. (Gawande, 1969; Khan, 1969; Singh and Arif; 1981; Muszynska-pytel, 1987; Lee et al., 1991; Peric-Mataruga V et al., 2008).

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