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STUDIES ON NEUROENDOCRINE SYSTEM OF SINGHARA BEETLE, *GALERUCELLA BIRMANICA* (COLEOPTERA : CHRYSOMELIDAE)

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ABSTRACT – The neuroendocrine system of singhara beetle, *Galerucella birmanica* consists of groups of neurosecretory cells in brain, corpora cardiaca, corpora allata, a suboesophageal ganglion, three thoracic and five abdominal ganglia. In each hemisphere of the brain, the medial and lateral groups of neurosecretory cells are located in the mid-dorso-lateral regions of protocerebrum while the ventral and optic groups of neurosecretory cells confined to the tritocerebral and optic lobes, respectively. The axons of medial neurosecretory pathway (MNSP) constitute nervi corporis interni (NCC-I) and similarly, the axons of lateral neurosecretory pathway (LNSP) and ventral neurosecretory pathway (VNSP) collectively constitute another nervi corporis externi (NCC-II). The NCC-I and II emerging out from ventral region of brain and runs for a short distance and terminate into anterior region of corpora cardiaca. The eight groups of the neurosecretory cells- a mid-dorsal, mid-ventral, mid-anterior, mid-posterior, paired antero-lateral and paired postero-lateral groups in the suboesophageal ganglion; six-groups of the neurosecretory cells- the mid-anterior, mid-posterior, paired antero-lateral and paired postero-lateral in the pro-, meso- and metathoracic ganglia and two groups in lateral regions of all abdominal ganglia of ventral nerve cord were found. Neurosecretory cells can be classified in to three types *viz.* 'A', 'B' and 'C' cells on the basics cytomorphological characteristics and staining affinities with paraldehyde fuchsin.

Key words : Neuroendocrine glands, Singhara beetle, *Galerucella birmanica.*

INTRODUCTION

The neuroendocrine systems of insects contribute to the regulation of a wide variety of developmental and behavioral processes, coordinating an animal's responses to both internally and externally derived stimuli. The insect nervous systems have many specific cells that can be visualized by the characteristic materials present in their cytoplasm. Nerve cells with secretory activities shown by specific staining techniques are called "neurosecretory cells". Functionally, they serve as a link between the nervous system and endocrine system. The neurosecretory cells are identified in various parts of central nervous system *viz.* brain, corpora cardiaca, corpora allata, frontal ganglion, suboesophageal ganglion, thoracic and abdominal ganglia. Most such neurosecretory cells (NSCs) make a specific terminal structure in specialized neurohaemal regions in order to release their products into the haemolymph. Many neurohormones identified in insect nervous systems seem to be derived from these NSCs (Ichikawa, 1991). In Insects the neurosecretory cells were found in different groups viz. median,lateral,ventral and optic neurosecretory cells in protocerebrum, deutocerebrum and tritocerebrum parts of brain .Carpora cardiaca are located ventro-posterior of the brain and the hypocerebral ganglion is just ventral to it. In some insects, this endocrine gland is a neuroheamal organ, storing the neurosecretory material coming from the neurosecretory cells of brain. Another endocrine gland connected to the carpora cardiaca is carpora allata. The carpora cardiaca connected to carpora allata by a pair of nerves, nervi corporis allati-I and to suboesophageal ganglion by another pair, nervi carporis allati-II (Ozluk,1991). The carpora allatum has to known functions. In the developing insect, it furnishes a hormone of the prothoracic glands, brings about larval moults. In the adult female, presumably the same carpora allatum hormone stimulates gonadial development, especially yolk deposition in the eggs (Ichikawa and Uwo, 1957). The suboesophageal ganglion is located in the ventro-posterior of the brain and connected to the brain by a pair of connectives. In some insects, these nerves are extremely short and the suboesophageal ganglion is merged into the tritocerebrum by taking oesophagus between brain and itself. The neurosecretery cells are also identified dorsal, ventral and lateral to suboesophageal ganglion. (Fletcher, 1969 and Ozluk, 1991) The insect ventral nerve cord consists of metamerically repeated ganglia subserving the thoracic and abdominal segments. The abdominal ganglia control basic functions such as respiration, circulation, heartbeat, diuresis, hindgut mobility, functions of genetalia and ovipositor and abdominal posture. Some of this control is by efferent innervation of target tissues but hormonal control also is exerted by abdominal neurosecretory cells via release their hormones from neuroheamal organs or other release sites (Nassel, 1996). The neurosecretory cells are classified into various types. One of the criteria is in this classification is the difference in colour of cytoplasm and granules of these cells although the same staining technique is applied (Ozluk,1991). The architecture and histomorphological structure of neuroendocrine system has been extensively studied in various species of beetles (Fletcher, 1969; Hoffmannn, 1970; Schooneveld, 1970; Gundevia and Ramamurty, 1972; Sidhra *et al,* 1983; Panov, 1986 and Pawar, 2008) but our present status of knowledge on the structure and function of the neuroendocrine system in aquatic beetles is still obscure and the present work was, therefore undertaken on the water singhara beetle, *Galerucella birmanica.*

MATERIALS AND METHODS

Large number of adult singhara beetle, *Galerucella birmanica* were collected from local water bodies of Ayodhya, Faizabad and acclimatized the laboratory. The brain and ventral nerve cord consisting of complete chain of suboesophageal, thoracic and abdominal ganglia was dissected in physiological saline solution under stereoscopic binocular microscope and immediately fixed in Boun's solution for 24 hours at the room temperature. Whole mounts were then prepared as described by Dogra and Tandau (1964). Brains containing cells stained with Lucifer Yellow were incubated at 10°C for 2 to 3 hr. Each brain-retrocerebral complex was then freed of extraneous tissue and transferred to a glass slide. After the withdrawal

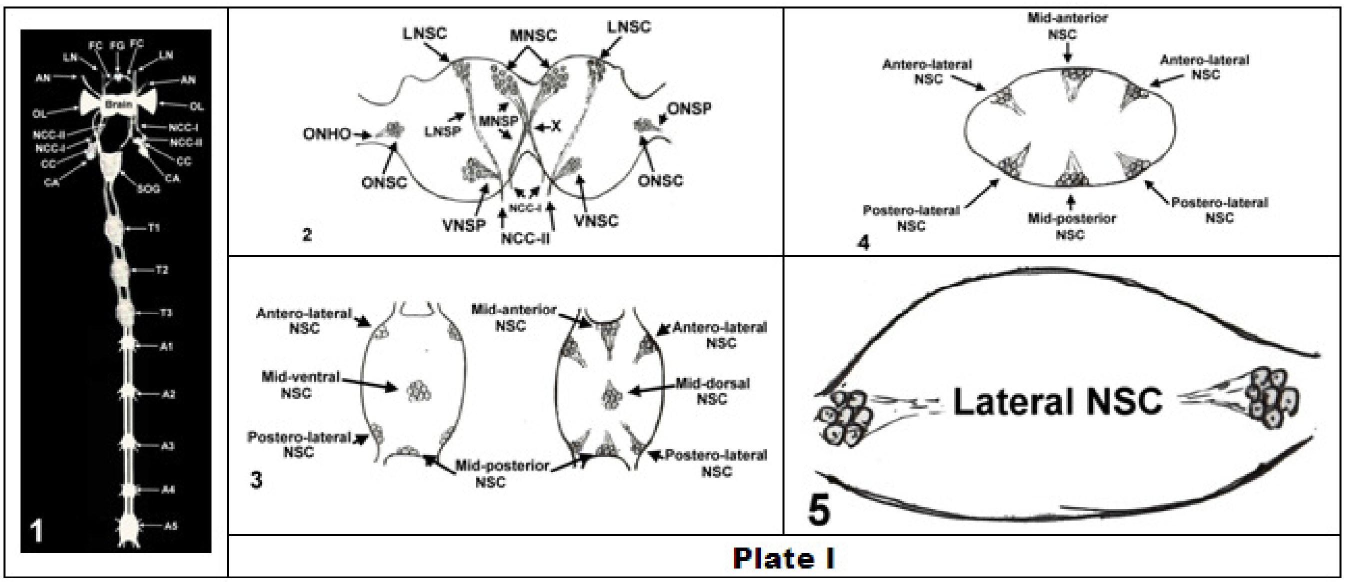

Figure (1) : Cephalic Neuroendocrine System of Adult Singhara beetle, *Galerucella birmanica*. **(2):** Distribution of NSC and neurosecretory pathways in the brain (Camera lucida diagram). **(3 to 5):** Distribution of NSC in subesophageal ganglion, thoracic ganglian and abdominal ganglion, respectively.

Abbr : CA – Corpora allata, CC – Corpora cardiaca, FC – Frontal connective, FG – Frontal ganglion, NCC-I – Nervi corporis cardiaci interni, NCC-II- Nervi corporis cardiaci externi, OL – Optic lobe, SOG – Subsogeal ganglion, T1, T2 and T3 – Pro-, meso- and metathoracic ganglia, A1-A5 – Abdominal ganglia, NSC – neurosecretory cells, MNSC – Median NSC, LNSC – Lateral NSC, VNSC – Ventral NSC, ONSC – Optic NSC, NSP – Neurosecretory pathway, MNSP – Medial NSP, LNSP – Lateral NSP, VNSP – Ventral NSP, ONSP – Optic NSP, ONHO- Optic neurohaemal organ, X- chiasma.

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

Type of NSC	Staining affinity with PF	Cell Size (mm)	Nucleus Size (mm)	Cell Shape	Distribution (Number)
'A' type NSCC hromophilic cells	Dark brown PF Positive	12.40±0.25	8.20±0.10	pyriform	MNSC (10)
'B' type NSC Chromophobic cells	Light brown PF Negative	13.50±0.30	9.32±0.14	spherical	MNSC (18), LNSC (5), VNSC (10), ONSC (12)
'C' type NSC Chromophobic cells	Very lightblue PF Negative	12.00±0.32	8.10±0.15	spherical	MNSC (5), LNSC (2), VNSC (4), (ONSC (6)

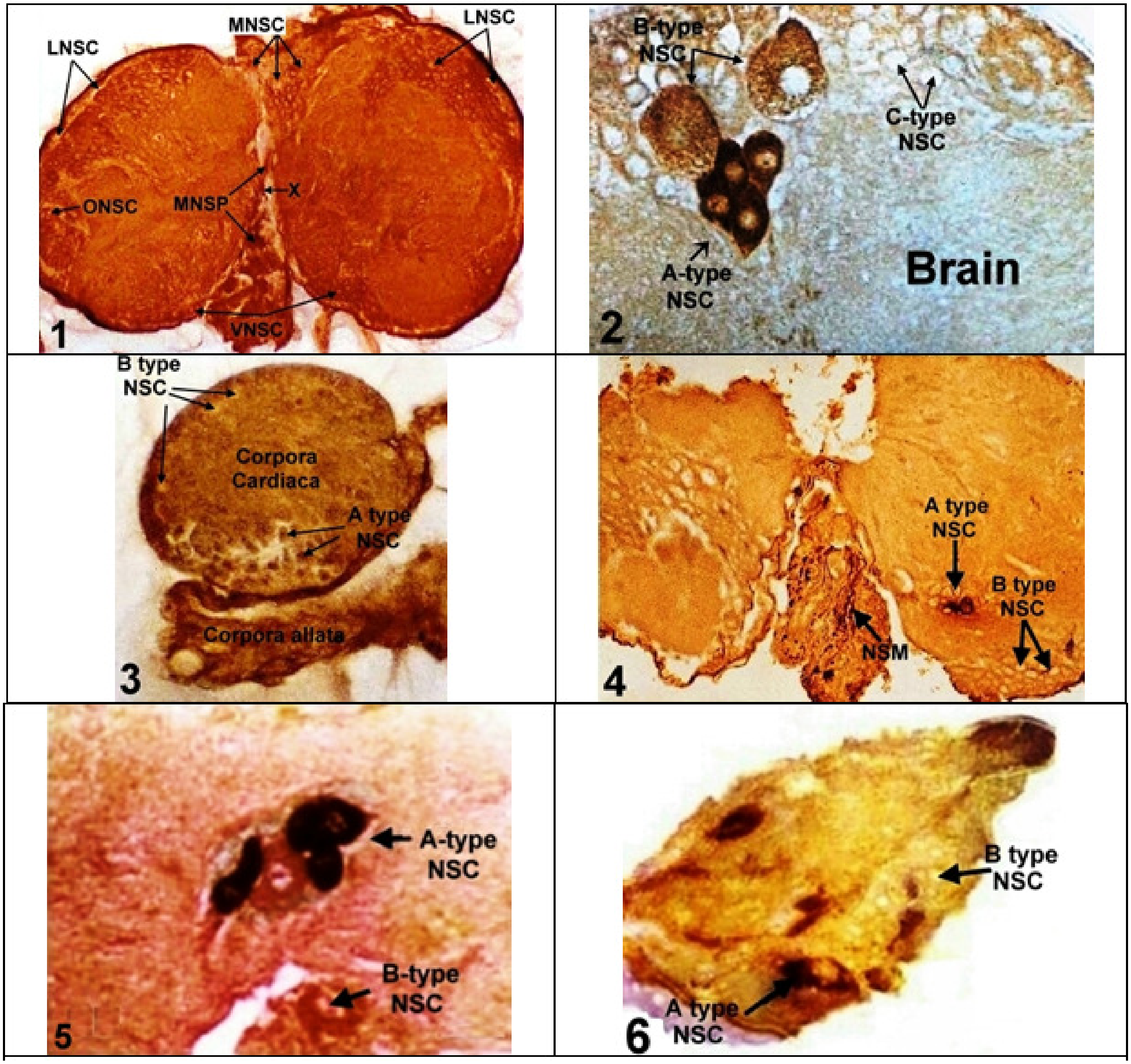

PLATE II

Figures : (1) HLS Brain showing distribution of NSC (×120). **(2)** Sagittal Section of Brain showing 3 types NSC (×480). **(3)** Sagittal section of Corpora cardiaca and Corpora allata showing 2 types of NSC (×240). **(4)** Sagittal section of Suboesophageal ganglion showing 2 types of NSC (×180). **(5)** Sagittal section of thoracic ganglion showing 2 types of NSC (×320). **(6)** Sagittal section of Abdominal ganglion showing 2 types of NSC (×240).

Abbreviations : NSC – Neurosecretory Cells, MNSC – Median Neurosecretory Cells, LNSC – Lateral Neurosecretory Cells, VNSC – Ventral Neurosecretory Cells, ONSC – Optic Neurosecretory Cells, MNSP – Median Neurosecretory Pathway, NSM - Neurosecretory Materials, X – Chiasma.

Table 2 : Cytomorphology, classification and distribution of NSCs in ventral ganglia:

Type of NSC	Staining affinity with PF	Cell Size (mm)	Nucleus Size (mm)	Cell Shape	Distribution
'A' type NSC Chromophilic cells	Dark brown PF Positive	12.40-13.50	8.50-9.10	pyriform	Thoracic, Suboesophageal and Abdominal ganglia
'B' type NSC Chromophobic cells	Light brown PF Negative	13.50-18.50	9.20-11.40	spherical	Thoracic, Suboesophageal and Abdominal ganglia

of all fluid, the tissue was cleared with anhydrous glycerol and gently compressed under the coverslip and photographed immediately using a photomicroscope. Brains containing cells injected with horseradish peroxidase were incubated in the buffered medium for 1 day at 10°C. The tissues were fixed for 6 hr in a mixture of 1% glutaraldehyde and 1% paraformaldehyde in 150 mM buffer solution. After washing in the buffer overnight, the tissues were incubated for 40 min at room temperature in 2 mL of a solution of 0.2% 3,3´-diaminobenzidine and 0.5% Trition X-100 in the same buffer. The tissues were then dehydrated in a graded series of ethanol, cleared with xylene, mounted in DPX, and traced with a camera lucida. The Bouin's fluid-fixed organs were washed thoroughly in the distilled water, dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax at 60-62°C. The 4-5 mm thick sections were cut and affixed to the Mayer's albumenized slides and stained with paraldehyde fuchin (Gomori, 1950).

RESULTS AND DISCUSSION

The neuroendocrine system of singhara beetle, *Galerucella birmanica* consists of groups of neurosecretory cells in brain, corpora cardiaca, corpora allata, suboesophageal ganglion, thoracic and abdominal ganglia. The neurosecretory cells are identified in various parts of the brain and ventral nerve cord.

Cephalic neuroendocrine System: The brain of singhara beetle, *Galerucella birmanica* is a bilobed, white compact nervous structure, lying dorsal to the oesophagus and extended laterally with the well developed optic lobes. It is externally covered with tracheae, air sacs and fat body. The paired corpora cardiaca and the corpora allata form the retrocerebral complex ventrally (Plate I & II).

The cephalic neuroendocrine system of singhara beetle consists of groups of neurosecretory cells in the brain, corpora cardiaca and corpora allata. In each hemisphere of the brain, the medial and lateral groups of neurosecretory cells are located in the mid-darso-lateral regions of protocerebrum while the ventral and optic groups of neurosecretory cells confined to the tritocerebral and optic lobes, respectively. The axons of medial neurosecretory pathway (MNSP) constitute nervi corporis interni (NCC-I) and similarly, the axons of lateral neurosecretory pathway (LNSP) and ventral neurosecretory pathway (VNSP) collectively constitute another nervi corporis externi (NCC-II). The NCC-I and II emerging out from ventral region of brain and runs for a short distance and terminate into anterior region of corpora cardiaca. Similar distribution of neurosecretory cells and nervi corporis cardiaci were also found in many beetles (Fletcher, 1969; Hoffmannn, 1970; Schooneveld, 1970; Gundevia and Ramamurty, 1972; Sidhra *et al.,* 1983; Panov, 1986 and Pawar, 2008).

Distribution, Cytomorphology and Classification of Cephalic Neurosecretory Cells: The types and distribution of neurosecretory cells can be observed after paraldehyde fuchsin staining technique (table 1).

Distribution : In each hemisphere of the brain, four groups of neurosecretory cells are observed distinctly (Plate I, Fig. 2 & Plate II, Fig. 1 and 2), *viz.* the medial neurosecretory cells (MNSC), lateral neurosecretory cells (LNSC), ventral neurosecretory cells (VNSC) and optic neurosecretory cells (ONSC). The medial neurosecretory cells (MNSC) are found in the mid dorsal pars intercerabralis region of the protocerebrum. Both MNSC groups lie close to each on either side of the median furrow and it is often difficult to recorgnize them seprartely. There are 10 'A', 18 'B' and 5 'C' types NSCs in each MNSC group. The lateral neurosecretory cells (LNSC) are found near the mushroom body just beneath the neurolemma in each lateral region of the protocerebrum. Each LNSC group is composed of 5 'B' and 2 'C' types NSCs. The neurosecretory cells (VNSC) are lying lateroventrally in the tritocerebral region of brain close to the optic lobes. Each VNSC group is composed of 10 'B' and 4 'C' cells. The optic neurosecretory cells (ONSC) is located at the root of each optic lobe (figure 3). Each ONSC group is composed of 12 'B' and 6 'C' cells. The axons of the medial, lateral and ventral neurosecretory cells form the medial (MNSP), lateral (LNSP), ventral (VNSP) and optic (ONSP) neurosecretory pathways (NSP) respectively. The MNSP of both hemispheres run obliquely towards the mid-line and they decussate in the middle of the brain so that the MNSP right side come to lie on the left side and vice versa, forming chiasmatic (X) region (figure 3). After crossing over each other, MNSP run postero-ventrally and emerge out of the brain on each side as the fine nerves, the NCC-I. Both the LNSP run independently throughout the brain in antero-posterior direction. Both the VNSP run towards the mid ventral region of the brain and finally combine with the LNSP of their own side and emerge out of the brain as the NCC-II (figure 2). The NCC-I and II emerging out from ventral region of brain and runs for a short distance and terminate into anterior region of corpora cardiaca (figure 1). Distribution of NSCs in four paired groups *viz.* MNSC, LNSC, VNSC and ONSC in *Galerucella birmanica* also found in other beetles *Nebria brevicolis* (Ganagarajah, 1965), *Aulacophora foveicollis* (Saini, 1966), *Blaps mucronata* (Fletcher, 1969), *Pterostichus nigrita* (Hoffmannn, 1970), *Leptinotarsa decemlineata* (Schooneveld, 1970), *Hydrophilus olivaceus* (Gundevia and Ramamurty, 1872), *Mylabris postulate* (Sidhra *et al,* 1983) and in *Cybister tripunctatus* (Pawar, 2008). Presence of neurosecretory cells in the optic lobes of *Galerucella birmanica* has been reported in the present study were also reported in *Periplanela americana* (Beattie, 1971), *Orthetrum chrysis* (Tembhare and Thakare, 1976), *Dineutes indicus* and *Cybister rugulosus* (Barde, 1981) and *Cybister tripunctatus* (Pawar, 2008).

Cytomorphology and Classification: The entire cell body of the NSC is filled with variable quantity of stained neurosecretory material (NSM). In accordance with staining affinity with paraldehyde fuchin and other cytomorphological characteristics, the cephalic neuroendocrine cells can be classified into three types (table 1 and Plate II).

The 'A' type NSC: These are pyriform in shape. Their neurosecretory material (NSM) are granular and stain dark brown with paraldehyde fuchin. They measure about 12.40±0.25 mm in nuclear diameter. These are confined to the MNSC groups only.

The 'B' type NSC: These are spherical in shape and stain light brown in colour with paraldehyde fuchin. They measure about 13.50±0.30mm in cell and 9.32±0.14mm in nuclear diameter. These are found in all groups of NSC.

The 'C' type NSC: These are smaller in size than the 'A' and 'B' type of NSC and stained very light blue in colour with paraldehyde fuchin. They measures about 12.00±0.32mm in all and 8.10±0.15mm in nuclear diameter. The 'C' cells are observed in all groups of NSC in the brain and always occur in less number than that of 'A' and 'B' types of NSCs. Several types of neurosecretory cells have been reported in different species of beetles. Schooneveld (1970) observed seven types of cells in the protocerebrum of *Leptinotarsa decemlineata.* Fletcher (1969) has recorded as many as 13 different types of cells in *Blaps mucronata,* Panov 1989 has observed five types of neurosecretory cells in several beetles and Pawar (2008) has recorded four types of NSCs in *Cybister tripunctatus*. The presence of 'A' type NSC in only in the medial groups in *Galerucella birmanica* seems to be an order-specific feature in coleoptera (Saini, 1966; Fletcher, 1969; Hoffmannn, 1970; Schooneveld, 1970; Gundevia and Ramamurty, 1972; Sidhra *et al,* 1983 and Pawar, 2008).

Corpora cardiaca and Corpora allata: The corpora cardiaca (CC) are white, paired, elongated, partially cylindrical or fusiform bodies lying on the oesophagus dorso-laterally behind the brain. Each CC receives two nerves from the brain, the nervi corporis cardiaci interni (NCC-I) and nervi corporis cardiaci externi (NCC-II) in anterior region. The CC consists of an outer thin transparent membranous peritoneal sheath and inner substance of the CC is filed with axons of neurosecretory cells. 'A' and 'B' types of NSCs are found in corpora cardiaca. The Corpora allata (CA) are paired, oval, gray-white coloured endocrine glands. They are separated from each other and externally covered with the muscles, trachea and fat body. Two types of neurosecretory cells namely chromophilic (AF positive) and Chromophobic (AF negative) cells were also found in *Mylabris pustulata* beetle (Sidhra *et al,*1983). The corpora allata (CA) is covered externally by a thick layer of connective tissue and its central region is filled with epithelial cells.

Distribution, Cytomorphology and Classification of NSCs of Ventral Ganglia: The ventral nerve cord consists of a suboesophageal, three throracic and five abdominal ganglia. The types and distribution of neurosecretory cells found in ganglia of ventral nerve cord can be observed after paraldehyde fuchsin staining technique (table 2).

The neurosecretory system of the ventral ganglia of Singhara beetle, *Galerucella birmanica* consists of three types of neurosecretory cells *viz.* 'A', 'B' and 'C' types. In suboesophageal ganglion and thoracic ganglion has all the three types NSCs where as in abdominal ganglia only two types- 'A' and 'B' NSCs are found. The eight groups of the neurosecretory cells- a mid-dorsal, mid-ventral, mid-anterior, mid-posterior, paired antero-lateral and paired postero-lateral groups in the suboesophageal ganglion; six-groups of the neurosecretory cells- the mid-anterior, mid-posterior, paired antero-lateral and paired postero-lateral in the pro-, meso- and metathoracic ganglia and two groups in lateral regions of all abdominal ganglia of ventral nerve cord were found. Ozluk (1991) reported that neurosecretory cells are found in dorsal,ventral and lateral region of suboesophageal ganglion. The axons of the neurosecretory cells of each region run together and form a distinct neurosecretory pathway extending from the cortical to medullary region and terminating into the respective neurohaemal area or organ. Prasad (1979) observed neurosecretory cells in suboesophageal ganglion, thoracic ganglia and abdominal ganglia of beetles. He also observed large sized neurosecretory cells in last abdominal ganglion. Two types of neurohaemal organs, *viz.* the internal neurohaemal organs (areas) in the suboesophageal and thoracic ganglia and the median dorsal (perisypathetic) neurohaemal organs along with the abdominal ganglia are present. Similar distribution of neurosecretory cells also found in water beetle, *Cybister tripunctatus* (Pawar, 2009). Pawar, 2009 observed four types of neurosecretory cells in venteral ganglia of *Cybister tripunctatus*. Singh *et al,* 2010 observed two types NSCs in thoracic and abdominal ganglia of larva of *Galerucella birmanica*.

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