International Journal of Research in Engineering and Applied Sciences(IJREAS)
Available online at http://euroasiapub.org/journals.php
Vol. 7 Issue 8, August-2017, pp. 21~38
ISSN (O): 2249-3905, ISSN(P): 2349-6525 | Impact Factor: 7.196

Histomorphology of neuroendocrine system in different multivoltine races of *Bombyx mori* L.

Dr. Yojana S. Muniv[1]
Department of Zoology
Changu Kana Thakur Arts, Commerce and Science College, New Panvel
Maharashtra, India

Prof. Ganesh P. Bhawane[2]
Department of Zoology,
Shivaji University, Kolhapur
Maharashtra, India

Abstract

The histomorphology of neuroendocrine system was studied in different multivoltine races of *Bombyx mori* viz., Kolar gold, Pure mysore and Nistari at adult stage. The retrocerebral complex consisted of neurosecretory cells of the brain and ventral ganglia, corpora cardiaca, corpora allata. In the recent article histomorphology of brain, corpora cardiaca and corpora allata were studied. It was found that, the brain and suboesophageal ganglion were fused together into a mass ringing the oesophagus. In adult of *B. mori* eight paired groups of the neurosecretory cells located symmetrically in the brain were reported in all the races under study. Four types of neurosecretory cells were found viz., A1, A2, B and C. The Corpora cardiac (CC) is paired elongated oval bodies located just behind the brain one on each side of the anterior expanded end of the aorta which terminate just beneath and behind the brain located dorsally over the oesophagus and connected to the brain by nervi corporis cardiaci I II III and IV. The paired corpora allata (CA) are translucent globular bodies situated laterally to the aorta far away from the CC lobes connected to them by nervi corporis allati I.

Keywords: *Bombyx mori*, brain, neurosecretary cells, corpora cardiaca, corpora allata.

Introduction

Neuroendocrine is the secondary system of co-ordination in animal body. It consists of nervous system associated with endocrine glands. Nervous system of insect has many specific cells that can be visualized by the characteristic chromophilic materials present in their cytoplasm. There was extensive literature available on the anatomy and histology of nervous system (Beams and King, 1932; Pflugfelder, 1937; Risler, 1954; Johansson, 1957; Wigglesworth, 1959).

Kopec (1917) opened the field of insect endocrinology by his pioneer experimental work on the moth *Lymantria dispar*. There are four components of insect endocrine system viz., neurosecretory cells of the brain and ventral ganglia, corpora cardiaca, corpora allata. The neurosecretory cells of insects were first described in the pars intercerebralis of the honeybee *Apis mellifera* by Weyer in 1935 (Panov, 1980). The neurosecretory cells of many insect species, including those in the order Lepidoptera, have been stained specifically and mapped (Herman and Gilbert, 1965; Highnam, 1969; Johnson, 1963; Khattar and Maila, 1979; McLeod and Beck, 1963; Mitsuhashi, 1963; Nishimura and Sano, 1981; Panov, 1971; Steel, 1977).

Corpora cardiaca described as paired elongated, milky white structure situated immediately behind the brain, one on each side of the oesophagus covered externally with tissue of sheath (Lyonet, 1762). The corpora allata are paired, almost spherical bodies which lie on each side, ventro-posterior to the corpora cardiaca (Ozluk 1991). Structure of corpora allata firstly reported by Muller (1928) as the visceral ganglia, comparative morphological study of the CA made by Nabert (1913) while their endocrine function reported by Wigglesworth (1934).

From above mentioned literature it becomes cleared that, histomorphology of retro cerebral endocrine complex studied well in various insect species belonging to the different insect orders. From the order Lepidoptera, silkworms both wild and mulberry were actively utilized from the functional point of view and their roles in certain physiological processes were investigated. Although basic structure of retro cerebral endocrine complex of silkworm, *Bombyx mori* is known but the literature on this is fragmentary. Therefore in the present study, the endocrine system of adult in the multivoltine three races in *Bombyx mori* so as to get the wholesome idea about the system.

Material and methods

A) Collection and maintenance of experimental animal:

The experimental animal *Bombyx mori* L. for the present study was reared and maintained under laboratory conditions as per the regimen of Krishnaswami (1978, 1979) in the rearing house of the Department of Zoology, Shivaji University, Kolhapur by providing the fresh leaves of mulberry as food. The adults were used for the further experiments.

B) Tissue preparation for histological studies:

The adult of all three races of *Bombyx mori* were dissected in chilled insect ringer solution under stereoscopic dissecting binocular microscope. The brains were removed from adult to study histomorphology and were immediately fixed either in Stieves or in Bouins fixative**.** Some brains were stored in 70 % alcohol for whole mount. The tissue fixed in Steives fixative after 24 hours washed in 50% alcohol and then transferred into 70% alcohol containing sufficient iodine for half hour. Then they were transferred to 70% alcohol to remove the excess iodine. Tissue fixed in Bouins after 24 hours of fixation transformed to 70% alcohol and 3 changes were given to remove the excess picric acid. Then these tissues were passed through the alcoholic grades and finally through the butanol for complete dehydration. After dehydration the tissues were cleared in the xylene then transferred to the mixture of xylene and wax for cold impregnation and finally embedded in paraffin wax (58^{o}C-60^{o}C). The block prepared was sectioned at 5 to 7μm. The sections and whole mounts were stained by Ewen's Aldehyde fuchin (AF) (Ewen's AF technique) and also by Bargmann's Chrome-alum Haematoxylene Phloxine (CHP) method. After staining observations were made and microphotography were done.

Results

Gross anatomy of neurendocrine system:

i) Cerebral Neuroendocrine system (anatomy) (fig. 1 to 5)

In the adult stage, the brain and suboesophageal ganglion were fused together into a mass ringing the oesophagus. The short but distinct nervus labrofrontalis arises from the anterior surface of the tritocerebral lobes of the brain and connects to the pear shaped frontal ganglion with the help of frontal connective to the brain. The SOG connects the brain to the ventral nervous system. The retro cerebral complex was located behind the brain on either side of the aorta, consisted of paired corpora cardiaca (CC), corpora allata (CA) and associated nerves. The CC elongated paired elongated oval bodies located just behind the brain one on each side of the anterior expanded end of the aorta which terminate just beneath the brain located dorsally over the oesophagus. The CC lobes of the either side connected with each other anteriorly by a bridge and their mesial. The borders were confluent with the outer side of the aortal wall. Each CC lobe was milky white in appearance in freshly dissected adult on the 7th and 8th day of pupal period. They were connected with the brain by four pair of nerves i.e. Nervi Corporis Cardiaci I, II, III and IV (NCCI, NCCII, NCCIII and NCCIV) formed from the axonic pathway of NSC groups of the brain. The axonic pathway of MNC groups i.e. MNSP crosses over the oesophageal foramen forming X shaped chiasma and passes laterally to the foramen and emerges from the posterior face of the brain as nervi corporis cardiaci I (NCCI) which enter into the anterior inner side of CC lobe. The axonic pathway of Lateral Neurosecretory cells (LNC) groups forming LNSP follow the simpler course through the brain and passes parallel outside to the MNSP and emerges from the posterior face of brain as nervi corporis cardiaci II (NCCII) and enter into the CC lobes of the respective side adjacent outside to NCCI. Similarly axons of outer and inner ventral neurosecretory cell group (OVNC and IVNC) forming their pathways which join together in the outer region of the tritocerebral lobe forming neurosecretory pathway VNSP and which emerged from the posterior face of oesophageal foramen as nervi corporis cardiaci III (NCCIII) and enter into the CC lobe of the respective side just adjacent to the NCC II. The axons of anterior and posterior optic lobe neurosecretory cells (AOLNC and POLNC) initially form the distinct pathways. Likewise axons of anterior and posterior neurosecretory cells (ANC and PNC) groups form the distinct pathway. These four pathways of each side join together and emerge from the posterior face of the brain as nervi corporis cardiaci IV (NCCIV) and enter into the CC lobe of the respective side adjacent to the NCC III. The axons of NCCI, II, III and IV forms the axonic core in the lumen of the corpora cardiaca and comes out from the posterior end of each CC lobe as nervi corporis allati I (NCAI) which join the CC lobe to Corpora allata lobe of their own side. Over the CA these NCC I and IV forms the fine branching through which granular NSM probably released into the surrounding haemolymph. In the bridge region where the CC lobes of either side were join the axons of NCC I-IV probably get intermixed after which they probably forms the fine branches which transversed over the aortal wall in a spiral fashion through which also granular NSM probably released into surrounding haemolymph. In whole mount preperations these observations were made. However in the lumen of aorta PF positive granular NSM could not be detected.

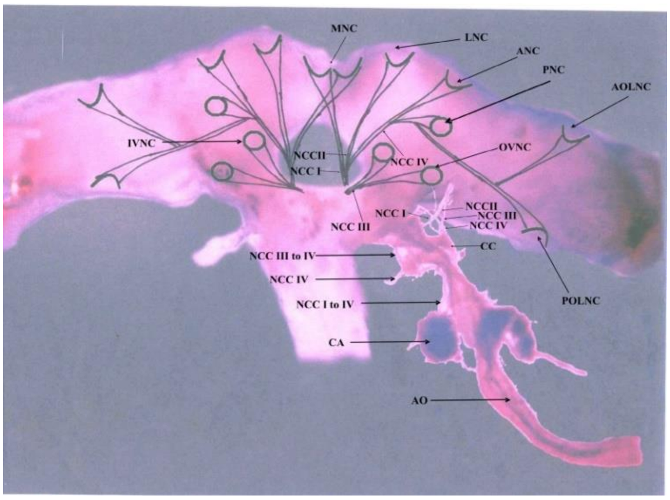

Fig. 1: Diagrammatic representation of brain of adult of *Bombyx mori* L. showing arrangement of neurosecretory cells, CC, CA and Aorta.

The paired corpora allata (CA) are translucent globular bodies situated laterally to the aorta far away from the CC lobes of the respective side connecting to them with the help of NCA I to NCAI V. The CA was firmly attached to the aortal wall by means of connective tissue. Each CA lobe is made from about 25 different oval small lobules and entire organ appears like a bunch of grapes in globular or oval in shape. All these lobules are held together by means of external connective tissue membrane. The lumen of the lobules is filled with the variable amounts of secretion during maturation of ovaries in the pupae of 4 to 8th day old and on the 9th day the size of the glands and lobules and gets considerably reduced. The diagramattic relationship of various endocrine glands shown in Fig.1.

***b)* Neurosecretary cells in brain of adult (histology): (Fig. 6 to 13)**

In the adult of B. mori similar type of the neurosecretory cells as reported in the larval brain were observed. They were located symmetrically in the brain and constitute eight paired groups. They were-

1. Median Neurosecretary cells (MNC)
2. Lateral Neurosecretary cells (LNC)
3. Anterior Neurosecretary cells (ANC)
4. Posterior Neurosecretary cells (PNC)
5. Anterior optic lobe Neurosecretary cells (AOLNC)
6. Posterior optic lobe Neurosecretary cells (POLNC)
7. Inner ventral Neurosecretary cells (IVNC)
8. Outer ventral Neurosecretary cells (OVNC)

1. Median Neurosecretary cells (MNC):

The MNC occupy anterodorsal pars intercerebralis region of the protocerebrum on either side of the brain. Each MNC consist of 4A1, 6A2, 4B and C cells. The diameter of the A1 cells measured 32± 1.06 µm with nucleus diameter 15.7± 2.01 µm in KG, 28.12± 2.9 µm cells diameter with 13.9± 1.19 µm nucleus as diameter in PM and 22.5± 1.02 µm cell diameter with 12.5± 0.09 µm nucleus diameter in Nistari. The A2 cells present in MNC having cell diameter 20.5± 1.2 µm, 19.2± 2.3 µm and 18.9± 1.98 µm with nucleus diameter 10.2± 0.5 µm, 8.9± 1.23 µm and 7.5± 1 µm in KG, PM, and Nistari respectively. The B cell of MNC group having cell diameter 17µm and 7.5µm nucleus diameter in all the races the C cells having diameter 35± 3.54 µm with 15µm nucleus diameter in KG, 30µm of *B. mori* cell diameter with 15µm nucleus diameter in PM while Nistari having 24.5± 3.4 µm cell diameter with 11.25± 1.77 µm nucleus diameter.

2. Lateral Neurosecretary cells (LNC)

The LNC were situated in lateral region of the protocerebrum. The LNC consist of 6A2, 3B and 2C cells. The cell diameter of A2 cells measured 12.9± 1.7 µm in KG, 10.5± 12.9 µm in PM and 9.2± 1.91µm in Nistari with nucleus diameter 6.5± 0.5 µm, 5± 2.0 µm and 4.5± 0.3 µm in KG, PM and Nistari respectively. The B cells diameter measured were 10µm with 5 µm nucleus diameter in *B. mori* races under study. The C cells measured 17.5µm in diameter with 7.5µm nucleus diameter in all the races under study.

3. Anterior neurosecretory cells:

The ANC groups were located aty the anterior region of the protocerbrum. The ANC consist of 4B and 2C cells. The B cells diameter measured 10µm with nucleus diameter 5µm in *B. mori* races. The C cells measured were 17.5μm in cell diameter and 7.5μm in nucleus diameter in the B. mori.

4. Posterior neurosecretory cells:

The PNC groups were occupying at the posterior region of the protocerebrum. It consists of 6C cells and 2B cells. The C cells having diameter 17.5μm with 7.5μm nucleus diameter while B cells having 10 μm cell diameter and 5 μm nucleus diameter in all the races of *B. mori* under study.

5. Anterior optic lobe Neurosecretary cells (AOLNC)

The AOLNC group was located at the anteriorsids of the optic lobe at each hemisphere of brain. It consist of 2A2 and 2B cells. The B cells having diameter 10 μm with 5 μm nucleus diameter. A2 cells having diameter 14.9± 1.2 μm, 13.5± 0.5 μm and 12.6± 0.9 μm with nucleus diameter 7.5± 0.6 μm, 6.9± 1.2 μm and 5.49± 1.02 μm in KG, PM and Nistari respectively.

6. Posterior optic lobe Neurosecretary cells (POLNC)

The POLNC group occupy posterior region of the optic lobe. It consist of 3B and 4C cells. The B cells were observed with cell diameter 10 μm and 5 μm nucleus diameter while C cells measured 17.5 μm in cell diameter and 7.5 μm in nucleus diameter in all the races under study.

7. Inner ventral Neurosecretary cells (IVNC):

In IVNC group 4B cells and 2C cells were observed.

8. Outer ventral Neurosecretary cells (OVNC)

In OVNC group 3 B cells and 2 cells were observed.

iii) Corpora cardiaca: (Fig No. 3 to 6)

The CC elongated paired elongated oval bodies located just behind the brain one on each side of the anterior expanded end of the aorta which terminate just beneath the brain located dorsally over the oesophagus. The CC lobes of the either side connected with each other anteriorly by a bridge and their mesial. The borders were confluent with the outer side of the aortal wall. Each CC lobe was milky white in appearance in freshly dissected adult on the 7th and 8th day of pupal period. They were connected with the brain by four pair of nerves i.e. Nervi Corporis Cardiaci I, II, III and IV (NCCI, NCCII, NCCIII and NCCIV) formed from the axonic pathway of NSC groups of the brain. The axonic pathway of MNC groups i.e. MNSP crosses over the oesophageal foramen forming X shaped chiasma and passes laterally to the foramen and emerges from the posterior face of the brain as nervi corporis cardiaci I (NCCI) which enter into the anterior inner side of CC lobe. The axonic pathway of Lateral Neurosecretory cells (LNC) groups forming LNSP follow the simpler course through the brain and passes parallel outside to the MNSP and emerges from the posterior face of brain as nervi corporis cardiaci II (NCCII) and enter into the CC lobes of the respective side adjacent outside to NCCI. Similarly axons of outer and inner ventral neurosecretory cell group (OVNC and IVNC) forming their pathways which join together in the outer region of the tritocerebral lobe forming neurosecretory pathway VNSP and which emerged from the posterior face of oesophageal foramen as nervi corporis cardiaci III (NCCIII) and enter into the CC lobe of the respective side just adjacent to the NCC II. The axons of anterior and posterior optic lobe neurosecretory cells (AOLNC and POLNC) initially form the distinct pathways. Likewise axons of anterior and posterior neurosecretory cells (ANC and PNC) groups form the distinct pathway. These four pathways of each side join together and emerge from the posterior face of the brain as nervi corporis cardiaci IV (NCCIV) and enter into the CC lobe of the respective side adjacent to the NCC III. The axons of NCCI, II, III and IV forms the axonic core in the lumen of the corpora cardiaca and comes out from the posterior end of each CC lobe as nervi corporis allati I (NCAI) which join the CC lobe to Corpora allata lobe of their own side. Over the CA the NCA I forms the fine branching through which granular NSM probably released into the surrounding haemolymph. In the bridge region where the CC lobes of either side were join the axons of NCC I-IV probably get intermixed after which they probably forms the fine branches which transversed over the aortal wall in a spiral fashion through which also granular NSM probably released into surrounding haemolymph. Corpora cardiaca measured 243.8 ± 8.8 μm in length and 81.3 ± 26 μm in width.

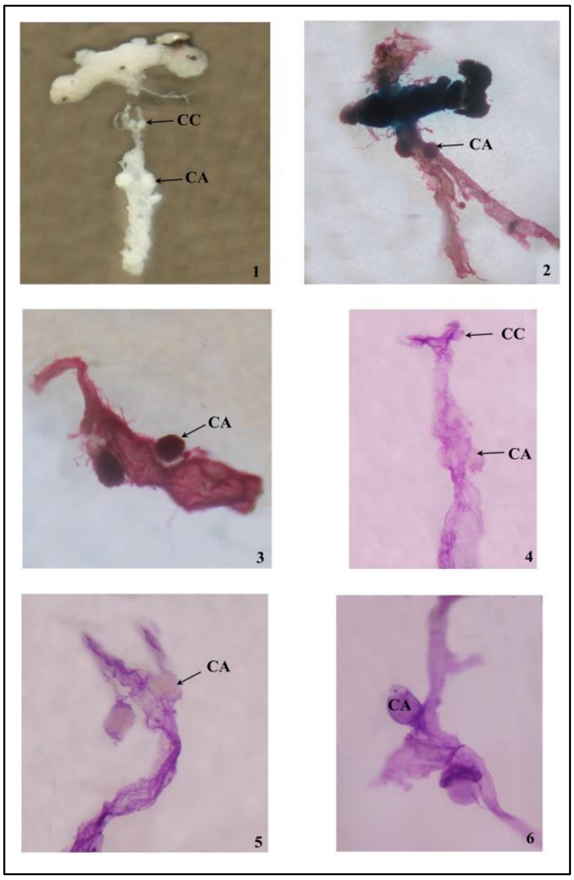

Fig. 2: Whole mount of retro cerebral complex of Pure mysore race stained with PF

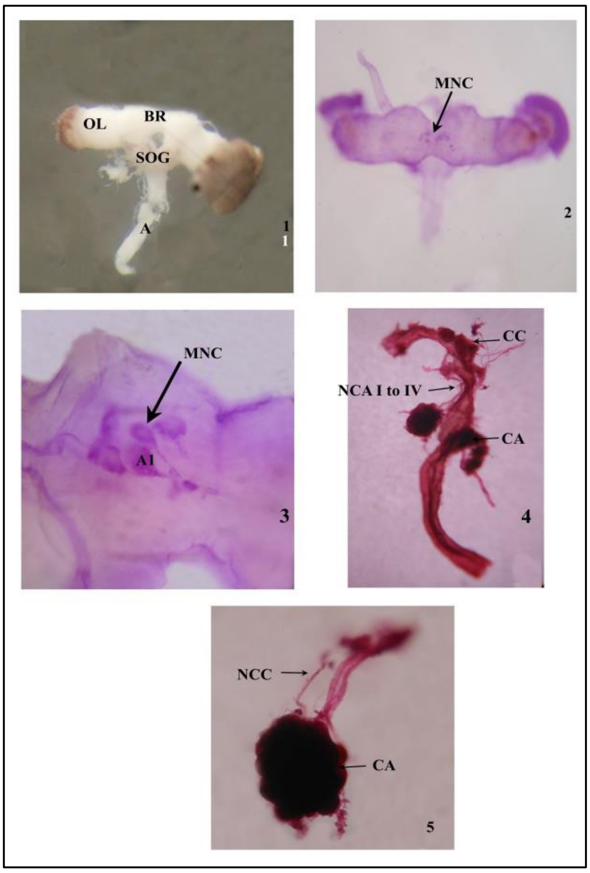

Fig. 3: Whole mount of retrocerebral complex of adult of Kolar gold race unstained(1), brain showing NCS stained with PF(2,3), CA-CC complex(4), CA(5)

CA - Corpora allata; **CC** - Corpora cardiac; **BR** – Brain; **SOG** - Suboesophageal ganglion; **MNC** - Medial Neurosecretory cells; **A1** - A1 cells in MNC; **NCA** - Nervi Corporis Cardiac; **ANSP** - Anterior Neurosecretory Pathway; **LNSP** - Lateral Neurose cretory Pathway; **MNSP** - Medial Neurosecretory Pathway; **PNC** - Posterior Neurosecretary Cells; NCC - Nervi Corporis Cardiaci; **OL** - Optic lobe

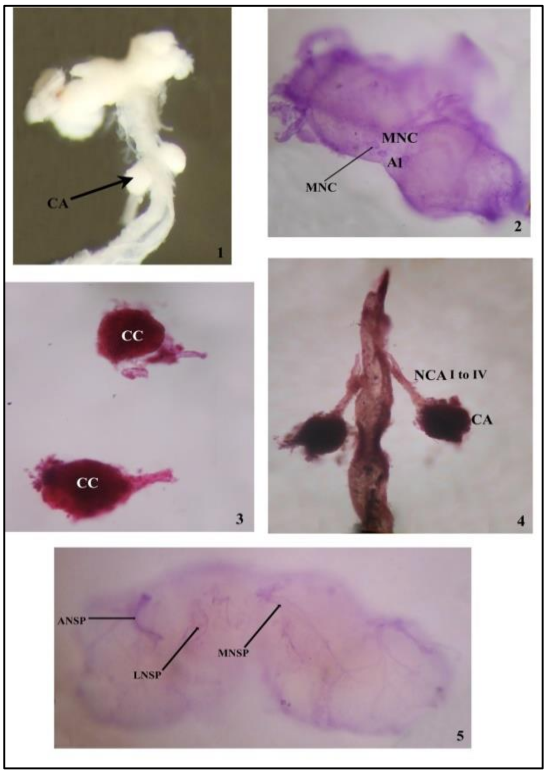

Fig 4: Whole mount of retrocerebral complex unstained(1); stained with PF(2); CC(3)and neurosecretary pathway in brain of adult of Nistari race

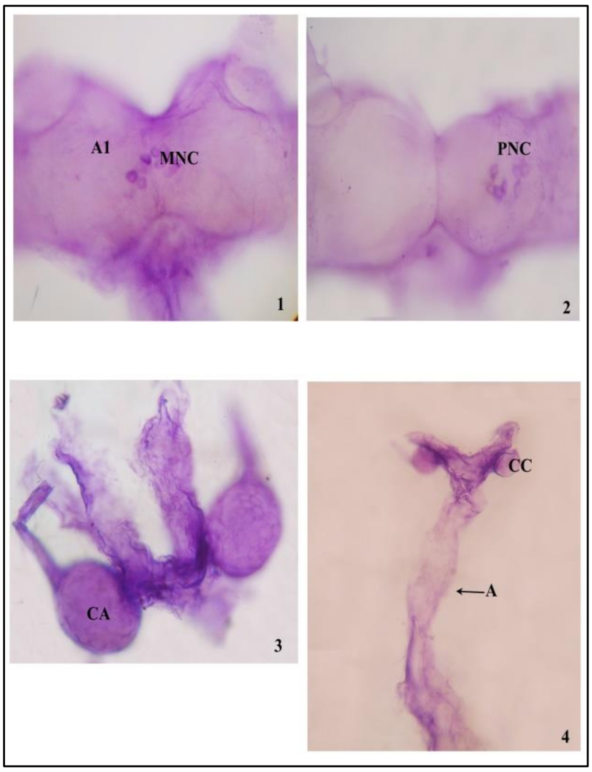

Fig 5: Whole mount of brain showing NCS (1 &2), CA(3) and CA-CC complex of adult of Kolar gold race stained with PF

BR – Brain; **CA** - Corpora allata; **CC** - Corpora cardiac; **CE** - Compound eye; **FG** - Frontal ganglion; **OL** - Optic lobe; **SOG** - Suboesophageal ganglion; **A1** - A1 cells in MNC; **ANC** - Anterior Neurosecretary cells; **MNC** - Medial neurosecretory cells; **LNC** - Lateral Neurosecretary cells; **VNC** - Ventral neurosecretory cells; **IVNC** - Inner ventral Neurosecretary cells; **OLNC** - Optic lobe neurosecretory cells; **PNC** - Posterior Neurosecretary cells; **NCA** - Nervi CorporisAllati; **NCC** - Nervi Corporis Cardiaci

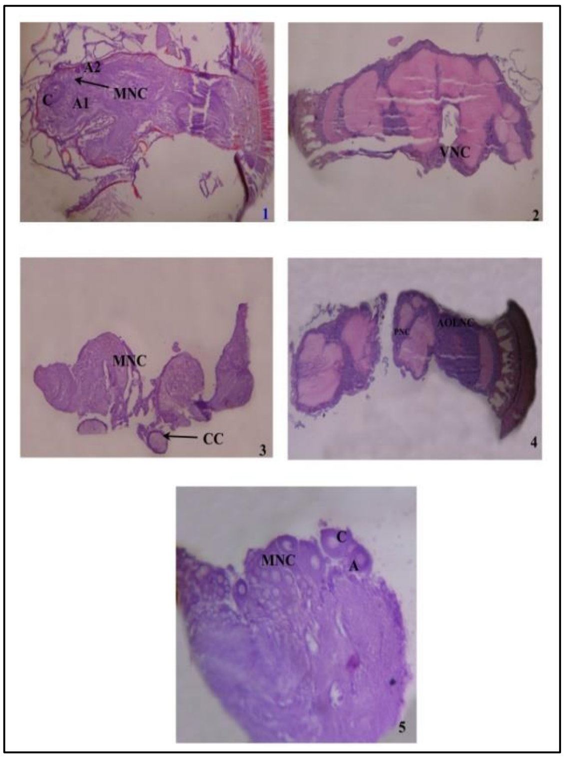

Fig.6: Section passing through adult brain of Pure mysore showing NCS stained with CHP(1,2); PF (3,4)

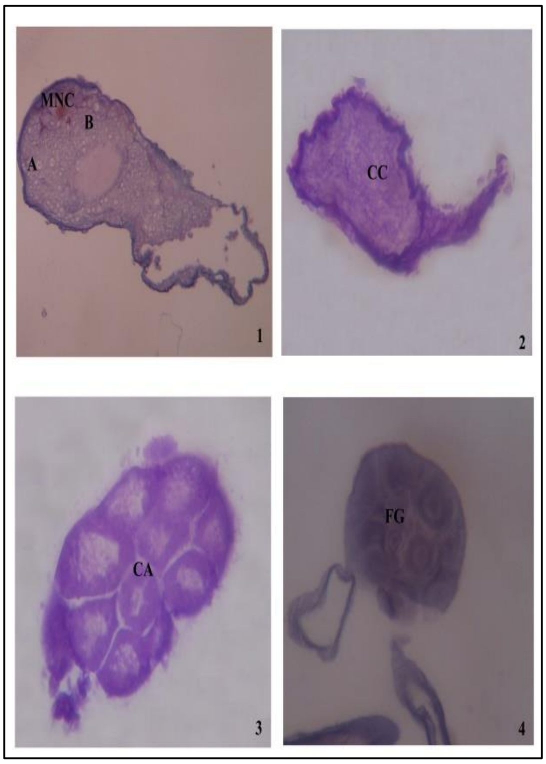

Fig.7: Section passing through adult brain of Pure mysore showing NCS (1); CC (2), CA(3) stained with PF; CA stained wth CHP

International Journal of Research in Engineering and Applied Sciences(IJREAS)
Vol. 7 Issue 8, August-2017
ISSN (O): 2249-3905, ISSN(P): 2349-6525 | Impact Factor: 7.196

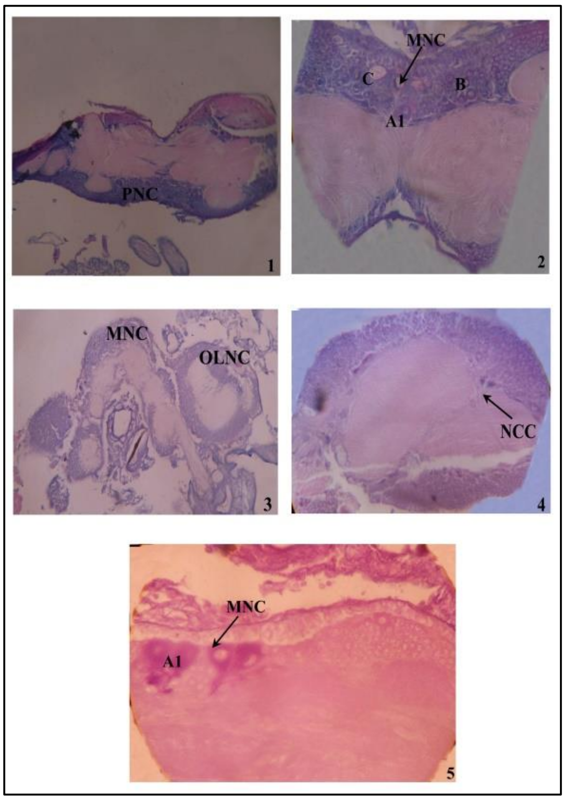

Fig. 8: Section passing through adult brain of Nistari showing NCS stained with CHP

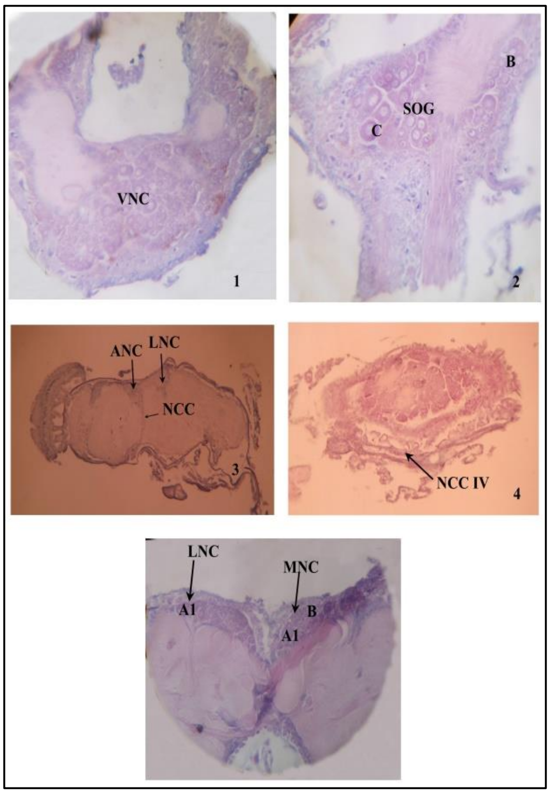

Fig. 9: Section passing through adult brain showing NCS (1,3); Sub oesophageal ganglion(2) of Nistari stained with PF; brain stained with CHP(4)

BR – Brain; **AX** - Axonic nerves; **CA** - Corpora allata; **CC** - Corpora cardiac; **CE** - Compound eye; **FG** - Frontal ganglion; **OL** - Optic lobe; **SOG** - Suboesophageal ganglion; **MNC** - Medial neurosecretory cells; **VNC** – Ventral neurosecretory cells; **LNC** - Lateral Neurosecretary cells; **OLNC** - Optic lobe neurosecretory cells; **OVNC**- Outer ventral Neurosecretary cells; **PNC** - Posterior Neurosecretary cells; **NCA** - Nervi corporis allati; **NCA** - Nervi corporis cardiac; **COC** - Circum oesophageal connective

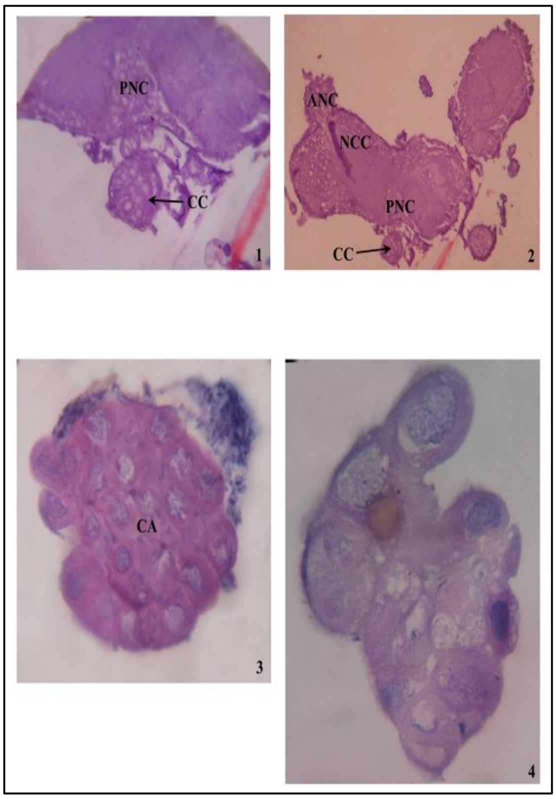

Fig. 10: Section passing through adult brain showing NCS (1,2); CA (3,4) of Nistari stained with PF

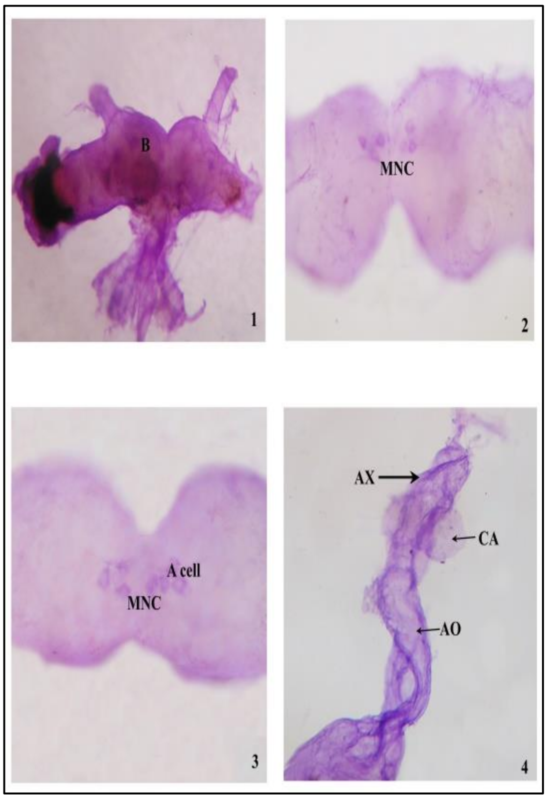

Fig. 11: Whole mount of adult brain (1,2,3); CA(4) of Kolar gold stained with PF showing NCS

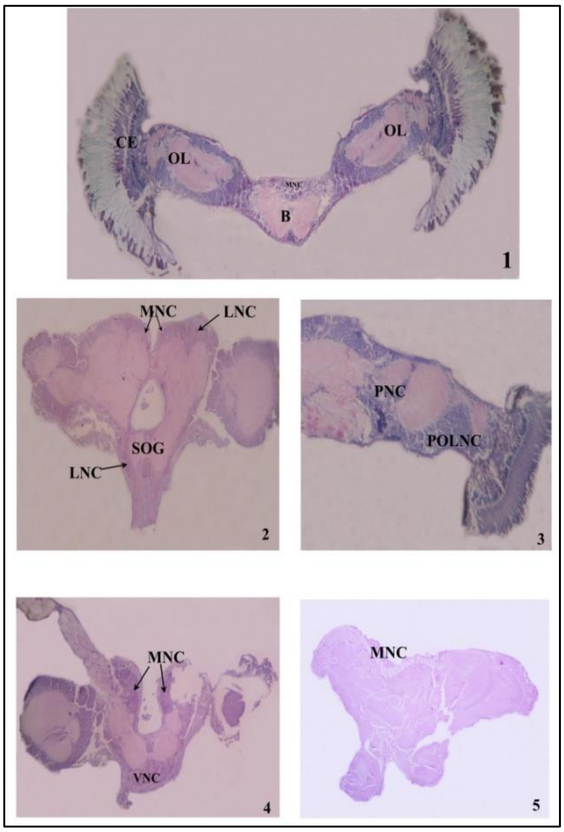

Fig. 12: Section passing through adult brain of Kolar gold race stained with CHP showing NCS

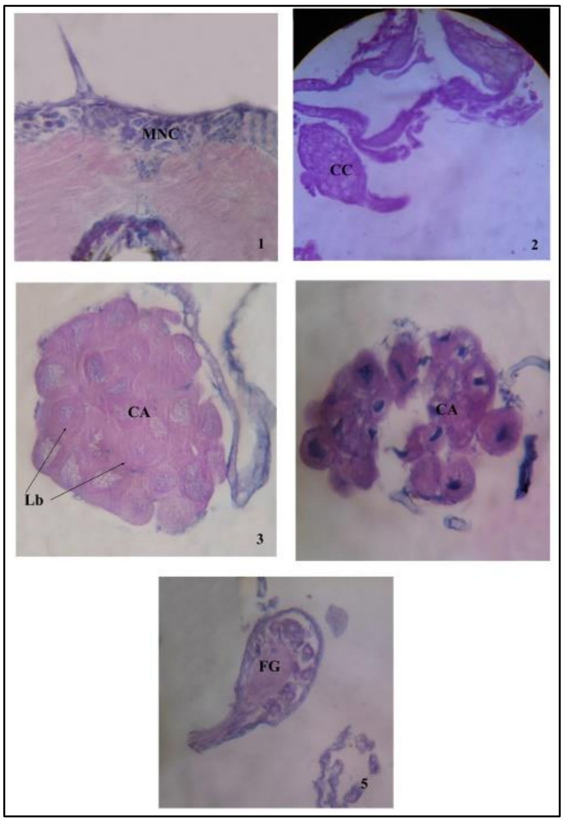

Fig. 13: T. S. of adult brain showing NCS (1); CC (2); CA(3); frontal ganglion (5) of Kolar gold race stained with CHP

BR – Brain; **AX** - Axonic nerves; **CA** - Corpora allata; **CC** - Corpora cardiac; **CE** - Compound eye; **FG** - Frontal ganglion; **OL** - Optic lobe; **SOG** - Suboesophageal ganglion; **MNC** - Medial neurosecretory cells; **VNC** – Ventral neurosecretory cells; **LNC** - Lateral Neurosecretary cells; **OLNC** - Optic lobe neurosecretory cells; **OVNC**- Outer ventral Neurosecretary cells; **PNC** - Posterior Neurosecretary cells; **NCA** - Nervi corporis allati; **NCA** - Nervi corporis cardiac; **COC** - Circum oesophageal connective

iv) Corpora allata: (Fig. 3 to 6)

The paired corpora allata (CA) are translucent globular bodies situated laterally to the aorta far away from the CC lobes of the respective side connecting to them with the help of NCAI. The CA was firmly attached to the aortal wall by means of connective tissue. Each CA lobe is made from about 25 different oval small lobules and entire organ appears like a bunch of grapes in globular or oval in shape. All these lobules are held together by means of external connective tissue membrane. The lumen of the lobules is filled with the variable amounts of secretion during maturation of ovaries in the pupae of 4 to 8th day old and on the 9th day the size of the glands and lobules and gets considerably reduced.

Discussion

Neuroendocrine systems in insect have been described anatomically on the basis of several complimentary investigative approaches. Neurosecretory cell (NSC) somata and neurohaemal organs have been located throughout insect central and peripheral nervous system by the physiological histochemical and ultrastructural detections of neurosecretory material (Rowell 1976; Raabe 1982). An extensive work has been carried on histological structure, mode of action of hormones and functions of cephalic neuroendocrine system of various species of lepidoptera. With regard to staining affinity and other cytomorphological criteria of the neurosecretory cells proposed by earlier workers (Thomsen 1952, 1954 a and b; Nayar 1955; Gabe 1966; Fletcher 1969; Hinks 1971; Prento 1972; Thakare and Tembhare 1975; Panov 1980; Raabe 1982 ; Tembhare and Barsagade 2000).

In the present study, the cephalic neuroendocrine complex of Bombyx mori was studied in detail in adult. The anatomical organization of brain, types of neurosecretory cells within brain and associated structures was studied. The morphological organization of the associated structure in *Bombyx mori* larvae of all the races under study found similar with *Manduca sexta* larvae (Nijhout, 1975; Copenhaver and Truman 1986 a and b). The basic pattern of cephalic neuroendocrine system was reported in insect species of major order e.g Orthoptera by Cazal (1948); hymenoptera by Thomsen (1954a); phasmida by Dupant Raabe (1954) etc. The presence of NSC in the brain was firstly demonstrated by Weyer (1935) in the honey bee, *Apis mellifera*. The corpus allatum as part of the reterocerebral endocrine complex and the endocrine nature of reterocerebral endocrine complex was first time find out by Muller (1928) in the *Blatta orientalis* but the true hormonal nature was established by Wigglesworth (1936) in *Rhodnius prolixus*. This finding was later confirmed by many researchers like Palm (1948), Novak (1951a and b), Nayar (1953, 1955) and Engelmann (1957). The fine structure of corpora allata in alfalfa weevil *Hyperea* studied by Tombes and Smith (2005). The corpus allatum is externally covered by a layer of fat bodies and delicate tracheolar branches.

The structure of corpora cardiaca in orthoptera was investigated by Cazal and Guerrier (1946), in Dytiscidae by Jolly (1945) and in *Locusta migratioria* by Nayar (1954). The neurosecretory material secreted by pars intercerebralis passes to the corpora cardiacd through nervis corporis cardiaci I and get accumulated in corpora cardiaca. This neurosecretory material in *Bombyx mori* (Bounhiol et al., 1953) and Plecoptera (Arvy and Gabe 1954) was carried into corpora allata. The exact role of corpora cardiaca was not known. According to Joly (1945) and Thomsen (1948) it has role in development of egg-chambers while Cameron (1953) reported that, the extract of corpora cardiaca has an influence on the pulsation of heart and peristalsis of intestines in *Periplaneta americana* and *Locusta migratoria.*

In the adult of *Bombyx mori* 8 groups of neurosecretory cells were observed. First four types similar type as present in larvae and additional four groups viz., Anterior optic lobe Neurosecretary cells (AOLNC), Posterior optic lobe Neurosecretary cells (POLNC), Inner ventral Neurosecretary cells (IVNC), Outer ventral Neurosecretary cells (OVNC). The MNC present in anterodorsal pars intercerebralis regionof protocerebrum in all the races under study. The position of MNC groups was variable in many other insects and reported by variously in the mid dorsal and posterodorsal part of the protocerebrum by a number of workers (Awasthi, 1968 and Gillot and Yin, 1972).

The two LNC group were found in the protocerebral hemisphere outside the pars intercerebralis in three races of silkworm in the present study and similar condition was observed in the moth of *Platysemia* (Williams, 1946) in hymenopterous, Ephemeropterous and brachyceros insects (Cazal, 1948), Periplaneta americana (Khan and Fraser, 1962), Mamestra brassicae (Hiruma and Agui, 1982) but the other groups of insects the LNC is found in the region of Pars-intercerebralis (Highnam 1961, Gabe 1966, Gawande 1968, Gupta 1970). However in some insects, the LNC have never been detected (Thomsen, 1954b, Ozeki, 1958, Krishnandan and Ramamurthy, 1971 and Awasthi, 1973b).

In the adult of *Bombyx mori* tritocerebrum lobe of brain the NSC were situated in two groups. One group present along the inner border and the other situated along the outer border known as outer and inner VNC. The ventral neurosecretory cells have been studied by number of insects in Scarabaeoid beetle (Bhawane, 1983), *Dysdercus similis* (Gupta, 1970), *Pterosticus nigrita* (Hoffman, 1970) and *Poecilocera picta* (Saini, 1971). In case of some insects these cells has been absent and reported by Lhoste (1953) and Ozeki (1958) in Dermaptera and Awasthi (1972, 1973a and b) in Heteropterous bug. In the three species of *Bombyx mori* under the study outer and inner group of ventral neurosecretory cells are observed in each tritocerebral hemisphere of the brain. These finding are in agreement with the Sankaye (1981) in the *Buperstid* beetles like *Sternocera laevigata, S. orientalis, S. chrysis Var.Chrysoides* and *Psyloptera fastuosa*. In the present study the brain of moth NSC have been observed in the posterior part (PNC) of protocerebrum. The PNC was also reported in insects like, aphids *Megoursa viciae* (Steel, 1977), *Pectinophora gossupiella* (Raina and Bell, 1978) in *Chilo suppersalis* (Mitsuhashi and Fukaya, 1960), in various lepidopteran insects by (Mitsuhashi, 1963) and in Buprestid beetles (Sankaye, 1981).

The neurosecretory cells have also been observed in the optic lobes of the brain in the many insects such as the fly of *Calliphora erythrocephala* (Thomson, 1952) in *Euraleon nostras* (Arvy, 1956) in the pupae of *Heliothis armigera* (Gawande et al., 1978). The Sankaye (1981) observed the neurosecretory cells along the inner and outer borders of optic lobes in the metallic beetle *Sternocera laevigata*. Therefore two groups of optic lobe neurosecretory cells i.e. AOLNC and POLNC. In the present study the in all the three races of *Bombyx mori* both outer and inner optic lobe neurosecretory cell groups were observed.

There are different types of neurosecretory cells have studied in various insects by different workers, depending upon the size and staining properties of NSC. Ewen (1962), Dogra (1967a, b), Takeda (1972) recognized the two types of NSC viz., A and B, while in the bug *Oncopeltus fasciatus.* Johanson (1957), in the grasshopper *Schistocerca gregaria* (Highnam, 1961), in *Lethocerus indicum* (Bhargava, 1967) and in *Dysdercus similis* Gupta (1970) observed four types viz., A, B, C and D. Johanson (1958) differentiated Nayar's A cells as PF positive and B cells as PF negative. He also described additional C and D types. The C cell type contains flask like PF positive material. Hinks (1971) described in the many lepidopteran species A, B, C and D type of NSC, which is further divided into subtypes such as A into A1, A2, A3 and A4 cells, B into B¬1 and B2 cells, C cells into C1 and C2 depending upon tinctorial properties and the size of the granules of NSM and the location of the NSC. Panov (1978) and Panov and Dovydova (1977) in the neuropterans, orthopterans and mecopterans and Sankaye (1981) in Buprestid beetles differentiated the neurosecretory cells into A and B cell types. Further they subdivided the A cells into A1 and A2 depending upon the staining intensity and the synthetic activity of the cells and also on the basis of tinctorial affinities towards PF and CHP stains and cell size and shape. Only three types of NSC viz., A, B and C cells have been observed in the brain of the adult of silkworm *Bombyx mori.* In the adult brain PF positive A cells are further subdivided into A1 and A2 types depending on the staining intensity and the size of the cells. The a types cells are PF positive but the A1 cells are larger in size with comparatively small nuclei which stain purple with PF stain while, A2 cells are smaller in size and contain less stained NSM.

Corpora cardiaca were paired glandular lobes situated posterior site of the brain and are flanked on either side of the aorta. These appeared white in colour in dissected insects. The gland present on either side is connected with each other by the commisure corporis cardiac (CCC). The corpora cardiaca were simple structure where the nervous and glandular elements were undifferentiated from one another (Cazal, 1947, Arvy and Gabe, 1954, Tembhare and thakare, 1976a, b). In Dictyoptera the glandular part was differentiated from that of nervous.

Lyonett (1762) reported the presence of corpora allata (CA) and regarded them as the nervous structures. The morphological study and the endocrine function of the CA were studied by the Nabert (1913). Wigglesworth (1936) reported the true functional status and called them an integral part of the neurosecretory system of insect. The simplest and most primitive type of CA is a paired lateral structure connected to the paired CC as found in Lepidoptera, Dictyoptera, Coleoptera and Odonata (Novak, 1966, Raabe, 1963). The distribution of various NSC types in the ganglionic chain of three silkworms is similar although minor variations are seen.

Acknowledgement

The authors are thankful to the Head, Department of Zoology, Shivaji University, Kolhapur for providing necessary laboratory facilities and Rajiv Gandhi National Fellowship (UGC,New Delhi) for providing financial support to carry out research work.

References

- Arvy, L. (1956). In Bertil Hanstrom: Zoological Papers (K. G. Wingstrand, ed.) Lund Univ., Lund, Sweden. 47-55.
- Arvy, L. and Gabe, M. (1954). The intercerebralis-cardiacum-allatum system of some plecoptera. Biol. Bull. 106: 1-14.
- Awasthi, V. B (1968). The functional significance at nervi corporis allati I and nervi corporis allati II in *Gryllodes sigillatus. J. Insect Physiol*. 14: 301-304.
- Awasthi, V. B. (1972). Neurosecretory system of the adult flesh-fly *Sarcophaga ruficornis* F. (Diptera: Sarcophagidae). Int. J. Insect Physiol. 21: 1027-1043.
- Awasthi, V. B. (1973a). Neurosecretory system and retrocerebral endocrine glands of *Metochus uniguttatus* (Thumb) (Heteroptera: Belastomatidae). Int. J. Morphol. Embryol. 2: 1-12.
- Awasthi, V. B. (1973 b). Studies in situ on the neurosecretory system and retrocerebral glands of the bug *Spherodema rusticum*. Fabr. (Heteroptera: Balastomatridae). Zool. Beitr. 19: 151-156.
- Beams, H. W. and King, R. L. (1932). Cytoplasmic structures in the ganglion cells of certain orthoptera with special reference to the Golgi bodies, mitochondria, vacuome intracellular trabaculae (trophospongium) and neurofibrillae. J. Morph. 53:59.
- Bhargava, S. (1967). Discription and the distribution of the neurosecretory cells in the brain and ventral ganglia of *Lethocerus indicum. Zool. Anz.* 179: 273-285.
- Bhawane, G. P. (1983). Studies on neuroendocrine of some scarabaeoid beetles. Ph. D. thesis submitted to Marathwada University, Aurangabad.
- Bounhiol, J. J., Gabe, M. and Arvy, L. (1953). Donnes histophysiologiques sur la neurosecretion chez *Bombyx mori* L. et sur ses raports avec les glands endocrines. *Bull. Biol. France-Belgique*. 87: 323-333.
- Cameron, M. L. (1953). Secretion of an orthodiophenol in the corpus cardicum of the insects. *Nature Lond.* 172: 349-350.

- Cazal, P. (1947). Recherches sur les glandes endocrines retrocerebrales des insects. II. Odonates. Arch. zool. exper. gen. 85: 55-82.
- Cazal, P. (1948). Les glands endocrines retrocerebrales des insects (etude morphologique)
- Bull. Biol. (Suppl.). 32: 1-227. Cazal, P. and Gurrier, Y. (1946). Researchers surles glandes endocrines retrocerebroidiennes des insects 1. Etude morphologique chez les orthoptera. Arch. Zool. Exp. Gen. 84 : 303-334.
- Copenhaver, P. F. and Truman, J. W. (1986 a). Identification of cerebral neurosecretory cells that contain eclosion hormone in the moth, *Manduca sexta*. J. Neurosci. 6:1738- 1747.
- Copenhaver, P. F. and Truman, J. W. (1986b). Metamorphosis of the cerebral neuroendocrine system in the moth *Manduca sexta*. J. Comp. Neurol. 249:186-204.
- Dogra, G. S. (1967a). Studies on the neurosecretory system of the *Ranatra elongate* (Fabricius) (Hemiptera: Nepidae) with reference to the distal fate of NCC I and NCC II. J. Morph. 121: 223-240.
- Dogra, G. S. (1967b). Neurosecretory system of Heteroptera (Hemiptera) and role of the aorta as a neurohaemal organ. Nature (London). 215:199-201.
- Dupont-Raabe, M. (1954). Repartition des activities chromatiques dans le ganglion su soerophagian des phasmides mise en evidence d'une regions secretion dans la partie deuto et tritocerebrale. C. R. Acad. Sci. Paris. 238: 950-951.
- Englemann, F. (1957). Die steuerung der overfunktion bei der ovaviparian schabe *Leucophaea maderae* (Fabr.). J. Insect Physiol. 1: 257-278.
- Ewen, A. B. (1962). Histophysiology of the retrocerebral endocrine glands of the alfalfa plant bug, *Adeltphoris leneolatus* (Geoze). J. Morphol 111: 255-269.
- Fletcher, B. S. (1969). The diversity of cell types in the neurosecretory system of the beetle *Balps mucronata*. J. Insect Physiol. 15: 119-134.
- Gabe, M. (1966). Neurosecretion. Pergamon press, oxford.
- Gawande, R. B. (1968). A histological study of neurosecretion in ants. Acta. Entomol. Bohemoslov. 65: 349-363.
- Gawande, R. B., Kale, R. A. and Borle, M. N. (1978). Histological study of neurosecretion in pupa of *Heliothis armigera* (Hubn.) (Lepidoptera: Noctuidae). Entomon. 3(1): 19- 23.
- Gillot, C. and Yin, C. M. (1972). Morphology and histology of endocrine glands of *Zootermophis anqusticollis* Hagen (Isoptera). Can. J. Zool. 50: 1537-1545.
- Gupta, D. P. (1970). Neurosecretory cells in *Dysdercus similis*. J. Zool. Lond. 401-411.
- Herman, W. S. and Gilbert, L. I. (1965). Multiplicity of neurosecretory cell types and groups in the brain of the saturnid moth *Hyalophora cecropia*. Nature. 205: 926-927.
- Highnam, K. C. (1961). The histology of the neurosecretory system of the adult female locust *Schistocerca gregaria*. Quart. J. Sci. 102: 27-38.
- Highnam, K. C. (1969). Neurosecretion in insects. In Gual, C. and Ebling, F. J. G., eds., Progress in Endocrinology, Proc. 3rd Int. Congr. Endocrinology, Mexico. 351-355.
- Hinks, C. F. (1971). Histochemical analysis of the neurosecretory cells in the adult brain of *Triphaena pronuba* (lepidoptera: noctuidae). The Canadian Entomologist. 103(12): 1639-1648.
- Hirama, K. and Agui, N. (1982). Larval-pupal transformation of the prothoracic glands of *Memestra brassicae*. J. Insect Physiol. 28(2): 89-95.
- Hoffmann, H. J. (1970). Neuroendocrine control of diapause and oocyte maturation in the beetle, *Pterostichus nigrita*. J. Insect Physiol. 31: 629-642.

- Johansson, A. S. (1957). The nervous system of milkweed bug *Oncopeltus fasciatus*. Trans Amer. Ent. Soc. 83: 119-1183.
- Johnson, B. (1963). A histological study of neurosecretion in aphids. J. Insect Physiol. 9: 727-739.
- Joly, P. (1945). La fonction ovarienne et son contrôle humoral chez les Dysticides. Arch. Zool. Exp. Gen. 84 : 49-164.
- Khan, T. R. and Fraser, A. (1962). Neurosecretion in the embryos and late stages of the cockroach *Periplaneta americana* (L). Mem. Soc. Endocrinol. 12:349–369.
- Khattar, N. and Maila, Y. K. (1979). Neurosecretory cells in the brain of mature larvae of *Euxoa segetum* Schiff (Lepidoptera: Noctuidae). Zool. Anz. Jena. 203: 114-121.
- Kopec S. (1917). Experiments on metamorphosis of insects. Bull. Int. Acad. Cracovie B: 57- 60.
- Krishnaswami, S. (1978). New Technology of silkworm rearing. CSR and TI Bulletin. 2: 1- 23.
- Krishnaswami, S. (1979). Improved method of rearing youngage silkworm, CSR and TI Bulletin. 3: 1-24.
- Lyonet, P. (1762). Traite anatomique de la chenille qui ronge le bois de soule. La Haye.
- Mcleod, D. G. R. and Beck, S. G. (1963). The anatomy of the neuroendocrine complex of the European corn borer *Ostrinia nubilalis* and its relations to diapause. Ann. Entomol. Soc. Amer. 56: 723-727.
- Mitsuhashi, J. Fukaya, M. (1960). The hormonal control of larval dipause in the rice stem borer, Chilo suppressalis. III. Histological studies on the neurosecretory cells of the brain and post diapauses (in Japanese, English summary). Jpn. J. Appl. Entomol. Zool. 4: 127-134.
- Mitsuhashi, J. Fukaya, M. (1960). The hormonal control of larval dipause in the rice stem borer, Chilo suppressalis. III. Histological studies on the neurosecretory cells of the brain and post diapauses (in Japanese, English summary). Jpn. J. Appl. Entomol. Zool. 4: 127-134.
- Muller, J. (1928). Ube rein eigentuliches dam Nervous sympathicus analoges Nerven system der Eingeweilde bei den insekten. Nova. Acta. Acad. Caesar. Leop. Carol. 14: 71-108.
- Nabert, A. (1913). Die Corpora allata der Insekten. Z. wiss. zool. 104: 181-358.
- Nayar, K. K. (1953). Neurosecretion in *Iphita limbata*. Stal. Curr. Sci. 23: 26-27.
- Nayar, K. K. (1954). The structure of the corpus cardiacum of *Locusta migratoria*. Quart. J. Micr. Sci. 95: 245-250.
- Nayar, K. K. (1955). Studies on the neurosecretory system of *Iphita limbata* Stal. (i) Distribution and structure of the neurosecretory cells of the nerve ring. Biol. Bull. 108: 296-307.
- Nijhout, H. F. (1975). Axonal pathway in the brain-retrocerebral neuroendocrine complex of *Manduca sexta.* Int. J. Insect Morphol. Embyrol. 4: 529-538.
- Nishimura, M. and Sano, Y. (1981). Studies on the neurosecretory cells in the silkworm, *Bombyx mori* (Lepidoptera: Bombycidae). III. Ultrastructural differences of neurosecretory cells in brain of male and female pupae. Appl. Entomol. Zool. 16: 71- 78.
- Novak, V. J. A. (1951a). New aspects of metamorphosis in insects. Nature London. 167: 132.
- Novak, V. J. A. (1951b). The metamorphosis hormones and morphogenesis in *Oncopeltus fasciatus*. Dal. Acta. Soc. Zool. Cal. 15: 1-48.
- Novak, V. J. A. (1966). Insect hormone. Methuen and Co. Ltd., London.
- Ozeki, K. (1958). Effects of corpus allatum hormone on development of male genital organs of ear wig *Anisolubis maritime*. Sci. Pap. Call. Gen. Edu. Tokyo. 8: 69-75.

- Ozluk, A. (1991). The cerebral neurosecretory system in the diversity of the neurosecretory cell types in *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae). Com. Fac. Sci. Univ. An. Series. 9: 33-47.
- Palm, N. B. (1948). Sexual differences in size and structure of the corpora allata in some insects. Klug. Sevens. Vtenskaposakad Handl. Ser. 6: 1-24.
- Panov, A. A. (1978). Neurosecretory cells in the pars intercerebralis of Orthoptera. Zool. Anz. Jena. 201: 49-63.
- Panov, A. A. (1980). Demonstration of neurosecretory cells in insect central nervous system, In Neuroanotomical Techniques. 26-51 (eds., Strausfield, N.J. and Miller, T.A). Springer. Verlag, N.Y.
- Panov, A. A. and Davydova, E. D. (1977). Medial brain neurosecretory cells and the retrocerebral endocrine complex of scorpion flies (Mecoptera: Insecta). J. Gen. Biol. USSR. 38: 79-87.
- Panov, A. A. (1971). A comparison of neurosecretory systems in related species of insects. In Novak, V. and Slama, K., eds., Insect Endocrines. Academa, Praha. 91-95.
- Pflugfelder, O. (1937). Bau, Entwicklung und Funktion der corpora allata und cardiac von *Dixippus morosus*. Br. Z. Wiss. Zool. 249: 477-512.
- Prent, P. (1972). Histochemistry of neurosecretion in the pars intercerebralis corpus cardiacum system of the desert locust, *Schistocerca gregaria* Fabr. C. R. Acad. Sci. Paris. 267: 864-867.
- Raabe, M. (1963). Existence chez divers Insects d'une innervations tritocerebrale des corpora cardiaca. C. R. Acad. Sci. Paris. 257: 1552-1555.
- Raabe, M. (1982). Insect neurohormones, Plenum Press New York.
- Raina, A. K. and Bell, R. A. (1978). Morphology of the neuroendocrine system of the pink bollworm, *Pectinophora gossypiella* during induction, maintenance and termination of diapauses. Ann. Ent. Soc. Am. 71: 375-382.
- Krishnanandam, Y. and Rammurthy, P. S. (1971). A correlative study of the neuroendocrine organs and oocyte maturation in *Pyrilla perpusilla* (Walker). Z. mikrosk. anat. Forsch. Leipzing. 84: 1-29.
- Risler, H. (1954). Die somatische polypoidie in der Entwicklung der Honigbiene (*Apis mellifera* L.) und die wiederherstellung der Diploide bei den Drohnen. Z. Zellforsch. Mikrosk. Anat. 41: 1-78.
- Rowell, H. F. (1976). The cells of the insect neurosecretory sytem: Constancy, Variability and the concept of the unique identifiable neuron. Adv. Insect. Physiol.12: 63-121.
- Saini, R. S. (1971). Neuroendocrine control of oocyte development in *Poecilocera picta*. J. Zool. London. 165: 275-283.
- Sankaye, B. B. (1981). Neuroendocrine studies of some Buprestid beetles. Ph. D. thesis, Marathwada University, Aurangabad.
- Steel, C. G. H. (1977). The neurosecretory system in the aphid Megoura viciae with reference to unusual features associated with long distance transport of neurosecretion. Gen. Comp. Endocrinol. 31: 307-322.
- Takeda, N. (1972). Activation of neurosecretory cells in *Monema flavescens* (Lepidoptera) during diapause break. Gen. Comp. Endocrinol. 18: 417-427.
- Tembhare, D. B. and Barsagade, D. D. (2000). Cdephalic neuroendocrine system in the tropical tasar silkworm, *Anthereae mylitta* (Lepidoptera: Saturniidae). Metamorphic and sericotropic functions. Int. J. Wild Silkmoth and Silk. 5: 21-29.
- Tembhare, D. B. and Thakare, V. K. (1976a). The cephalic neuroendocrine system of the dragonfly, *Orthretum chrysis* (Selys.) (Anisoptera: Libellulidae). Odontologica. 5(4): 355-370.

- Tembhare, D. B. and Thakare, V. K. (1976b). Neuroendocrine control of vitellogenesis in the dragonfly *Orthretum chrysis* (Selys). (Odonata: Libellulidae). Cell Tiss. Res. 177: 269-280.
- Thakare, V. K. and Tembhare, D. B. (1975). Histochemistry of the neurosecretory cells of the pars intercerebralis of the dragon fly, *Orthetrum chrysis* (Selys). Odonatologia. 4: 225-235.
- Thomsen, E. (1948). Effect of removal of neurosecretory brain of adult *Calliphora erythrocephala.* Nature Lond. 161: 439-440.
- Thomsen, E. (1952). Functional significance of the neurosecretory brain cells and the corpus cardiacum in the female blowfly *Calliphora erythrocephala*. Meiz. J. Exp. Biol. 29: 137-172.
- Thomsen, A. (1954a). Observation on the cytology of neurosecretion in various insects (Hymenoptera and Diptera). Pubbl. Staz. Zool. Napoli. 24: 46-48.
- Thomsen, A. (1954b). Neurosecretion in some Hymenoptera. Biol. Skr. 71: 1-14.
- Tombes, A. S. and Smith, D. S. (2005). Ultrastructural studies on the corpora cardiaca-allata complex of the adult alfaalfa weevil, *Hypera postica.* J. Morphol. 132(2): 137-147.
- Weyer, F. (1935). Uber drusenartige Nevervenzellen in Gehirn der Honighiene *Apis mellifera. Zool. Anz.* 112: 137-141.
- Wigglesworth, V. B. (1934). The physiology of ecdysis in Rhodnius prolixus (Hemiptera). II. Factors controlling and metamorphosis. Quart. J. Micr. Sci. 77:191-222.
- Wigglesworth, V. B. (1936). The function of the corpus allatum in the growth and reproduction of *Rhodnius prolixus* (hemiptera). Quat. J. Mic. Sci. 79: 91-121.
- Wigglesworth, V. B. (1959). The histology of the nervous system of insect *Rhodnius prolixus* (Hemiptera). II. The Central Ganglia. Quart. J. Micr. Sci. 100(2): 299-313.
- Williams, C. M. (1946). Physioloy of insect diapauses: the role of the brain in the production and termination of pupal dormancy in the giant silkworm, *Platysamia cercropia.* Biol. Bull., Wood's Hole. 90: 234.