HISTOMORPHOLOGY OF NEURONAL AND NEUROSECRETORY CELLS IN THE TWO MOLLUSCAN SPECIES B. BENGALENSIS AND S. MACULATA.

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ABSTRACT : Neuronal cells from cerebral ganglia of two molluscan species B. bengalensis and S. maculata were selected, quantitatively and qualitatively differentiated. Study based on the criteria of staining properties in neuronal cells as per size, shape and position of cells in the ganglia and axonal processes. Neurosecretory material were estimated by following histological techniques and finely interpreted comparatively.

Key words: Histomorphology, neuronal cells, molluscan species.

INTRODUCTION:
Central nervous system (CNS) in molluscs is highly suitable for a wide range of neurological studies, due to in part of their relatively simple and consistent neuronal-organization, as well as the unique features such as giant cells. In the early studies scientist have referred cerebral ganglia as the molluscan “brain”. Molluscan nervous system extensively studied by electrophysiologists, comparative neurologists and recently cytologists are interested in structural differentiation with secretion of neurosecretory substances in it (Bonga, 1970). Neuronal network play important role in the initial metamorphosis and further body differentiation and development. Some evidences suggested relation between neurosecretion and reproduction (Apley, 1970; Price, 1976).

Delcomyn (1998), stated that brain process in the information organization, motor output and finely in executive decisions. It also commands important motor behaviors including feeding, mating, defensive, withdrawal and locomotion. Pelluet and Lane, (1961) observed relation between neurosecretion and cell differentiation in ovotestes of two slugs. According to them neurosecreting product also maintain the normal body activities including animal growth, metabolism, and also help for harmonious adaptation with the environment.

Adequate description of the nervous system is lacking in molluscan species and has not published; in fact there is scanty information for any of the other genera (Bullock and Horridge, 1965). Comparative and systematic studies of nervous system of molluscs provides a basis for further work in neuronal anatomy and development, neuroendocrinological processes and the involvement of nervous system in regulation of physiological and behavior aspects of animal. The purpose of this paper is to provide an comparative anatomical and histological description of the neuronal cells and its secretion in two molluscan species snail B. bengalensis and slug S. maculata.

MATERIALS AND METHODS :
The Adult freshwater snail Bellamya bengalensis were collected from Rajaram tank, near Shivaji University, Kolhapur and terrestrial slug Semperula maculata, was collected from Pannala at Arug and Bedug village, Dist. Sangli Maharashtra. These two species were kept for acclimatized under laboratory condition over one week prior to using for experiment. Aquatic snail B. bengalensis and terrestrial slug S. maculata were dissected out to expose nerve complexes from cephalic region. Cerebral ganglia of each species separated form the remaining visceral complexes. Material was fixed in the Bouins fixative for overnight. Dehydrated in alcoholic grade series, cleared in xylene. Paraffin sections at (4-5u) were stained with standard Histological HE and MT staining methods. The neuronal sections from both species were finally considered for comparative neuronal and neurosecretory study.

RESULTS AND DISCUSSIONS:
Morphology of central nervous system (CNS) : The central nervous system of Prosobranch, B. bengalensis and Stylommatophoran S. maculata consist of 11 ganglia i.e. of paired buccal, cerebral, pedal, pleural and parietal ganglia and of single visceral ganglia (Fig: 3, 4). The cerebral ganglia are connected by a very short connective. The more extensive fusion of ganglia seen in more advanced Pulmonate such as Lymnaea (Loose, 1964). The ganglia are surrounded by the peripery, a capsule of connective tissue containing smooth muscle cells, granular cells (Smirina, 1972). Each ganglion of both the species B. bengalensis and S. maculata having natural light pink and white creamy colour respectively. Morphologically, the cerebral ganglion at the periphery contains neurosecretory cells which are taking part in secretion of neurohormones, neromodulators or neurotransmitters in the body.

Cerebral ganglia : Comparatively, the cerebral ganglia of Prosobranch and Stylommatophoran consist of procerebrum, nesocerebrum and metacerebrum. The neurosecretory cells occurs in the cerebral ganglion in B. bengalensis and S. maculata, in these cells cytoplasm stain pink and nucleus blue in standard staining Haematoxyline eosin technique. (Fig: 5, 6 x 400). In B. bengalensis the neurosecretory cells arranged at the periphery i.e. in peripery region. Collectively about 40 NSC were located and found in Dorso-laterally, 64 Dorso-medaly, 58 Ventro-laterally, 70 Ventro- medially. In relation to this NSC of S. maculata were in scattered form including 50 Dorso-laterally, 46 Dorso-medaly, 70 Ventro-laterally, 78 Ventro-medialy to the cerebral ganglia. The NSCs are extended at the center of ganglion up to the commissure. In B. bengalensis morphologically three types of neurosecretory cells were found, Large, Medium and small sized cells. Where as in S. maculata four types, Giant, Large, Medium and small sized cells were observed. The J. Wijdenes et al, (1976) observed five types of cells in some pulmonates D. reticularum and A. horrensis and ten types of cells in Molluscan species H. aspersa related to cerebral ganglionic structure.

Neurosecretory cells (NSCs) : The comparative study of neurosecretory cells is based on the colour of cells taking with histological staining technique, number and their location in the ganglia. Most of cell cytoplasm stained pink and nucleus blue. The result are summarized by table No: 1.

The NSCs are generally oval with well defined nucleus in both species B. bengalensis and S. maculata. When stained with Mallory's triple staining technique showed nucleus stain dark red
and cytoplasm orange-red. (Fig: 9, 10 x 630). The neurosecretory granules in NSCs are found in both species. The neurosecretory cells were unipolar with one axonal process which often divided into collateral in *B. bengalensis* and shortly in *S. maculata*. Gilal cells were found between the neurosecretory cells in both species.

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<th><em>B. bengalensis</em></th>
<th><em>S. maculata</em></th>
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<tbody>
<tr>
<td><strong>Cell types with their location</strong></td>
<td><strong>No. of cells</strong></td>
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<td>Ge-MD-LC</td>
<td>09</td>
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<tr>
<td>Ge-MD-MC</td>
<td>17</td>
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<td>Ge-MD-SC</td>
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<td>Ge-VL-SC</td>
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**Table No. 1:** Neurosecretory cells types in Prosobranch and Stylommatophoran, average numbers in cerebral ganglia. Ge- cerebral, MD- mid- dorsal, LD- lateo- dorsal, VM- ventro- medial, VL- ventro- lateral, L- large, M- medium, S- small. C- Cells, G- Giant.

**Neurohaemal area:** Each neurosecretory cells having axonal process. These axons are seen more prominent in *B. bengalensis* as compare to in *S. maculata*. Only in few cases axons stained with staining technique be traced over some distance In Basommatophore and Prosobranch the neurohaemal area of NSC were located in the peripheries of commissures, connectives and nerves (Bore and Joosse, 1975). The neurohaemal area of Stylommatophora is largely unknown. In a number of species including *H. aspersa*, axoned tracts from the metacerebral cells have been followed into the arteria cerebralis and into the intercerebral nerves which terminate in the connective tissue dorsal to the cerebral ganglia (Kuhlmann, 1963; Nolte, 1965).

Light microscopic investigation of cerebral ganglion in the two molluscan species showed less difference in histological structure. In terms of structural aspects central ganglionic system in *B. bengalensis* assumes much simpler than that of *S. maculata*.

The present study of neurosecretory cells is based on their types, staining properties and location. Based on these criteria it appears that the neurosecretory system of two species investigated shows great similarity, occupying some differences. Most of work up to date on gastropods has been limited to description of special cells in restricted areas of various ganglia in number of molluscan species. As to the cerebral ganglion, it is worth to mentioning that the cerebral neuronal cells were immunonegative for in both active and inactive snails, in fact they are involved in olfactory function. (Chase and Tolloczko 993).

Histologically neurosecretory cell is combined with neurosecretory granules. Neurosecretory cells often contain elementary in which neurosecretory granules were found in molluscan synaptic junction and peripheral nerve fiber (Amosroso, et al, 1964; Baxter and Nisbet 1963; Gerschenfeld 1962). In *H. aspersa* and in numbers of other Stylommatophoran and Prosbranch species it has been studied with classical methods for neurosecretion (Kuhlmann, 1963; cook, 1966). The neurosecretory cells in the cerebral ganglia are playing important role in the metamorphosis and development by secreting neuropeptides and growth hormones. Joosse (1964) noted that the role of neurosecretion in the control of egg laying mechanism of *L. stagnalis* and *Helix aspersa* species showed the neurosecretory cells of the cerebral ganglia have a maximum activity at the time of gamatogenesis (Van mol 1967; Joosse 1964; Smith 1967). Cerebral light green cells in the *L. stagnalis* produces a growth hormone (Geraerts, 1976). In another study vertebrate peptides (ACTH oxytocin, vasoprseen, vasotocin) positively reacting cells have immunocytochemically have been observed in the cerebral ganglia of *L. stagnalis* (Van Nooaden et al, 1980).

Neurosecretory cells in the cerebral ganglia contain definite axonal processes and are also important in the neurosecretion. In *B. bengalensis* it is found long as compare to in *S. maculata*. Hormonogenic function and non synaptic termination of axons have been proposed as essential criteria for considering cells as being “definitely neurosecretory” (Bern, 1962; Bern and Hanadorn, 1965). Neurosecretion has recently been studied to include neurosecretory systems extending to endocrine tissues and controlling the functions “neurosecretomotor innervations” or by secretion of local hormone from neurosecretory neurophilis (Bern and Knowles, 1966).

In addition to this it was found that the fibers mass i.e. neurophilis was present in cerebral ganglia of both the molluscan species *B. bengalensis* and *S. maculata*. Simpson et al. (1964), have found the “neurosecretory neurophil” to be composed of numerous fibers located with elementary granules which appeared to be fine branches of neuritis from cerebral ganglioic neurons. Several neurosecretory endocrine gland complexes have been described in the tentacles in opsthobranchs and Pulmonate molluscan species the work is in process in this direction.
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REFERENCES: