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Organization of the central ganglia in medically important vector snail *Radix acuminata* (Lamarck 1822) (Mollusca: Gastropoda: Pulmonata)

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Abstract
Mollusks have long been used as a model for understanding fundamental processes in neuroscience. The freshwater pulmonate snail *Radix acuminata* (Synonym: *Lymnaea acuminata* Lamarck, 1822) is one of the commonly distributed snails in freshwater bodies of India. This snail serves as an intermediate host for parasitic trematodes and plays a significant role in the transmission of human schistosomiasis and fascioliasis disease. The purpose of this paper is to provide a histological description of the central nervous system, the topography of central ganglia, and types of neurosecretory cells in the central nervous system (CNS) of *R. acuminata*. The CNS consists of paired cerebral, pedal, pleural, parietal ganglia and a visceral ganglion. The ganglia in the form of nerve ring were carefully dissected out and preserved. A routine histological process was followed so as to cut tissues into sections followed by staining. Results obtained showed that all the ganglia are covered by an epineural sheath or perineurium. The central core of each ganglion is called neuropil. The neuropil generally lacks in cell bodies except those of scattered neuroglia and connective tissue cells. The cortex area between the core and perineurium, however, is packed with cell bodies of Neurosecretory cells. The outermost cells are usually the largest, and thus visible topographically in the ganglion. Within the inner layer of connective tissue, the nerve cells are arranged with their cell bodies peripherally and their axons running centrally into the neuropil. Two types of neurosecretory cells, type A and type B cells, are found in almost all ganglia.

Keywords: *Radix acuminata*, CNS, histology, neurosecretory cells

1. Introduction

The central nervous system (CNS) of mollusks is highly useful for understanding the structure and function of the nervous system due to its simple organization. Mollusks have long been used as a model for understanding fundamental processes in neuroscience. For example, work on giant axons of squid led to our first understanding of ionic bases of membrane potentials [1]. In many mollusks, the nervous system is composed of a few and relatively large neurons which played an important role in the study of neuronal mechanisms underlying behavior [2, 3, 4]. The detailed morphology of CNS is available for a number of mollusks [5, 6, 7, 2]. Morphological and histological descriptions of CNS are also available in certain primitive species[8]. Different types of neurosecretory cells (NSCs) have also been identified in many snails [9, 10, 11, 12].

The freshwater snail *R. acuminata* is widely distributed gastropod in Indian inland water bodies [13]. Many freshwater snails including those of genus *Radix* are medically important because they serve as an intermediate vector for helminth parasites which cause helminth-borne diseases of cattle and humans such as fascioliasis and schistosomiasis [14, 15]. A perusal of literature shows that there is no published record on histological details of *R. acuminata*. Hence, the present research was undertaken to elucidate on the gross histological organization of ganglia in the CNS of snail *R. acuminata*.

2. Material and Methods

2.1 Collection and maintenance of experimental animal

Adult snails were collected from cement tanks from local water bodies and maintained in plastic tubs filled with dechlorinated tap water. Snails were fed with chopped mulberry leaves. The water was replaced daily and an excreta, if any, was removed to avoid fouling of water. The water was aerated properly to maintain a sufficient oxygen level. The snail specimen were identified using keys by Ramakrishna and Dey [13].

2.2 Histology

The nerve rings were carefully dissected out without damaging any of the ganglia. Before dissection, animals were narcotized by injecting 1 ml solution of 0.3 M Magnesium Chloride ($MgCl_2$) through the foot into the body cavity. Animals were dissected with the shell removed in Snail ringer solution (*Composition:* KCl 1. 5mM, NaCl 30mM, $MgCl_2$mM, $CaCl_2$ 4mM, Na_2HPO_4 0.25mM and $NaHCO_3$ 18mM). Intact nerve ring along with its component ganglia were fixed in Bouin's fluid for twelve hours, then the ganglia were dehydrated in graded ethanol series, cleared in xylene, and infiltrated with paraffin wax. The ganglia were embedded by orienting them, under a dissecting microscope, in a pool of wax melted in the top of the wax block. These blocks were refrigerated at 4˚C for two hours and then sectioned on a rotary microtome at 7-10 µm thickness. A routine Mayer's hematoxylin and eosin procedure was used for general anatomical observations. Detailed investigations on neurosecretion involved staining by the method of Mallory's triple staining. The majority of central ganglia were stained using Mallory's triple staining. Structures were measured with a calibrated ocular micrometer. The cells containing stained cytoplasmic material (neurosecretory material) were evaluated with regards to types based primarily on the size, shape, and distribution. The laboratory work was carried out in the Department of Zoology, Dr. BAMU, Aurangabad.

3. Results

The CNS of *R. acuminata* consists of a nerve ring constituted by paired cerebral, pedal, pleural, parietal ganglia, and a visceral ganglion. An additional pair of buccal ganglia is also present on buccal mass; however, it is not part of nerve ring and the CNS. Respective commissures connect similar types of ganglia while connectives connect different ganglia. All of the ganglia are roughly spherical with many nerves emerging from them. Histologically, all the ganglia are covered by an epineural sheath or perineurium which is two-layered: the outer thick layer and inner thin layer. The epineural sheath is made up of elastic, fibrous connective tissue as reported in earlier studies [16]. The epineural sheath is penetrated by blood vessels at many places. The central core of each ganglion is called neuropil which lacks in cell bodies except those of scattered neuroglia and connective tissue cells. The cortex area between the core and perineurium, however, is packed with neuronal cell bodies. The outermost cells are usually the largest and thus visible topographically in the whole ganglion. Within the inner layer of connective tissue, the nerve cells are arranged with their cell bodies peripherally and their axons running centrally into the neuropil.

3.1 Neurosecretory cells in the CNS

The NSCs contain fine cytoplasmic particles that stain distinctly which differentiate them from normal neuronal cells. Depending upon the shape, size, and staining properties, neurosecretory cells in the ganglia of *R. acuminata* are classified into two types: NSC type ‘A’ and NSC type ‘B’. The neurosecretory material of both the A and B cell types stain reddish orange with Mallory’s triple stain [Fig 1 & Fig. 2].

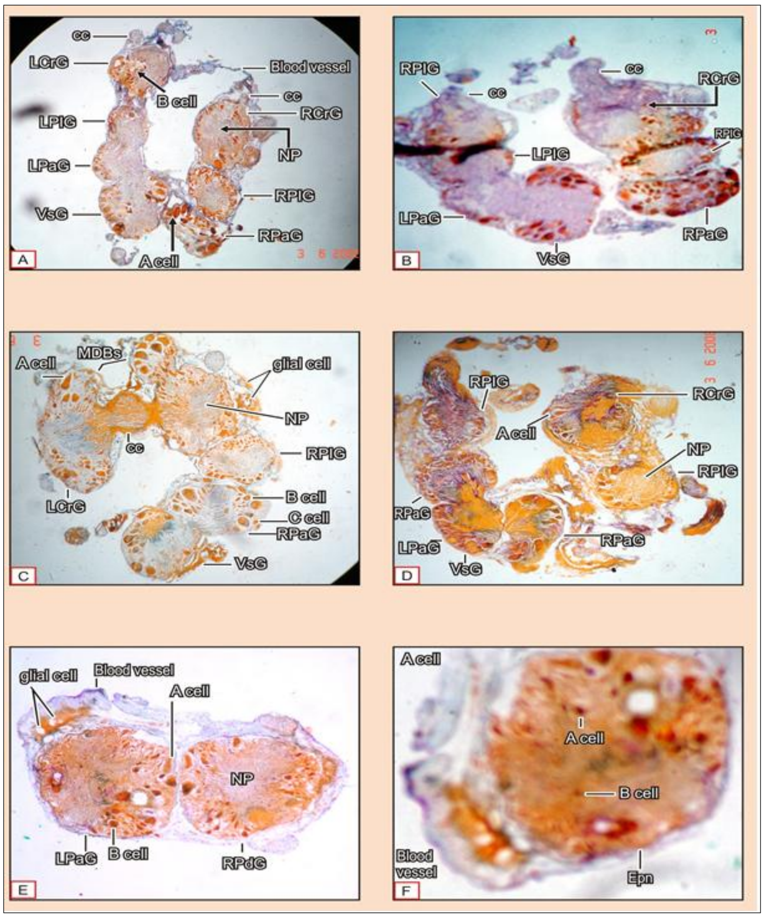

Fig 1: Histomicrographs of different ganglia in *R.acuminata*: *A, B, C and D =V.S. of nerve ring passing through various ganglia in CNS stained with Mallory’s triple stain. D & E = V.S. of pedal ganglion. Abbreviations: Al = Anterior lobe; cc =Cerebral commissure; LBcG =Left Buccal Ganglion; LCrG =Left cerebral ganglion; LPaG=Left parietal ganglion; LPlG = Left Pleural ganglion;MDB =Mediodorsal body, Np =Neuropile; RBcG =Right buccal ganglion; RCrG =Right cerebral ganglion; RPaG =Right parietal ganglion; RPlG=Right pleural ganglion VsG =Visceral ganglion.* Magnification: 100X.

Presence of ‘C’ cell in the CNS have also been reported [17]. Rarely, some NSCs similar in appearance to type C cells could be observed in *R. acuminata,* however, they have not been described here. The type A and type B neurosecretory cells were observed in all the major ganglia of the CNS including cerebral, buccal, pleural, parietal, pedal and visceral ganglia. The extreme periphery of the ganglia is supposed to be the neurohaemal region as it is well vascularized. The number, size, and shape of the NSCs may vary from ganglia to ganglia.

3.1.1 NSC type ‘A’

The type A cells are pyriform in shape and have long axons. These cells are present in all of the ganglia. The length of the cell body ranges from 25-55 µm and are smaller than B cells. The nuclei may be ovoid or pyriform and nuclear diameter is between 6-10 µm. The number of A cells is comparatively lower than those of B cells. They generally lie towards the peripheral regions of the ganglia. They usually have a single nucleolus in the cytoplasm. The cytoplasmic part is filled with deeply stained colloidal neurosecretory material with few vacuolar spaces. The neurosecretory material appears to be within axonic processes suggesting the use of axonic passages for transportation of neurosecretory material**.**

3.1.2 NSC type 'B'

The NSC type B are found in all the ganglia studied. These cells are spherical or somewhat oval, larger than A cells and may be between 50-95 µmm in length. These cells do not have cellular processes and axons. The nuclear diameter is also larger than that of A cells. The nuclear diameter of B cells is 20-25µm while that of A cells is 6-10µm.The nucleus occupies the major portion of the cell body. The number of B cells is more than the number of A cells. B cells are common in the sub-peripheral region of the ganglion. The cytoplasmic portion includes granular neurosecretory material stained red with Mallory's triple stain. The number and size of B cells may vary from ganglia to ganglia.

3.2 Cerebral ganglia

The cerebral ganglia are the largest of all and functionally the most important of all ganglia. The NSCs in the cerebral ganglia can be separated into three different groups depending on their topography: mediodorsal neurosecretory cells (MDCs), caudodorsal neurosecretory cells (CDCs) and, laterodorsal neurosecretory cells (LDCs). These cells are located in a specific area of the cerebral ganglion. Some of these cells are shown to be of endocrine importance as they are involved in the regulation of some important physiological activities performed by the snails.

3.2.1 MDCs

The MDCs are located in the mediodorsal region of cerebral ganglia; they are oval in shape with long axons having a polymorphic nucleus in the center of the cytoplasm. The mediodorsal bodies receive axonal tracts from one of the groups of MDCs. The somatic and nuclear diameters of MDCs measure between 16 to 19µm and 8 to 15µm respectively. Some giant neurons have been observed in MDC groups measuring above 40µm in somatic diameter. The neurosecretory material that they contain is granular.

3.2.2 CDCs

Located dorsally at the caudal region of the cerebral ganglia are groups of caudodorsal neurosecretory cells. CDCs have a somatic diameter between 7 to 11µm while the nuclear diameter measures between 4 to 5µm.

3.2.3 LDCs

LDCs are positioned at the lateral region of each cerebral ganglion. These cells are formed as a result of separation of a group of neurosecretory cells from MDC and CDC groups. The cell diameter of LDCs measures between 12 to 15µm while nuclear diameter measures between 6 to 10µm. The axons of these cells project towards the neuropilar area in the cerebral ganglia [Fig. 2].

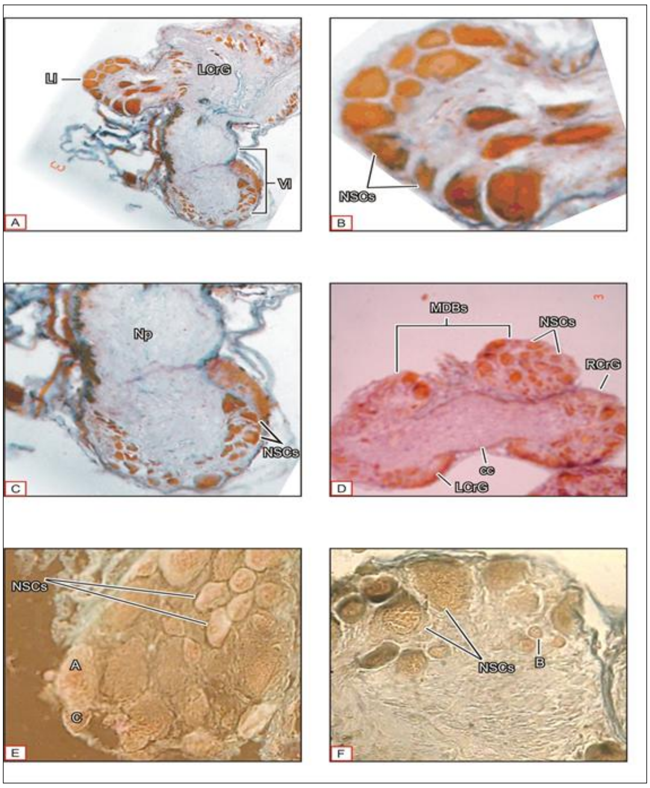

Fig 2: Histomicrographs of cerebral ganglia in *R. acuminata*: *A, B, C and D= V. S. of cerebral ganglia stained with Mallory's triple stain showing its various lobes. E & F= Section of cerebral ganglion stained with Haematoxylin-Eosin stain showing A and B types of NSCs. Abbreviations: A&B = Type A and B NSCs; Al = Anterior lobe; cc =Cerebral commissure; MDB =Mediodorsal body, Np =Neuropile; RCrG =Right cerebral ganglion; LCrG =Left cerebral ganglion; Ll = Lateral lobe; Vl =Ventral lobe Magnification: 100X for A, B, C & D; 400X for E and F.*

The cytoplasm of these cells is characterized by extremely fine granular cytoplasmic neurosecretory material.

3.2.4 Lateral Lobes (LLs)

Each cerebral ganglion is a complex structure with many extensions. The lateral lobe is structural extensions of the cerebral ganglion with functional importance. It arises as a conical or somewhat triangular extension from the anterolateral surface of each cerebral ganglion. It is quite large measuring about 85µm × 48µm. Numerous NSCs are also present in the lateral lobe. Some of the NSCs within lateral lobes are unusually large-sized called giant neurons which measure above 20 µm in somatic diameter and about 15µm in nuclear diameter. Other small-sized NSCs are also observed in lateral lobes. These cells have somatic diameter of about 8µm and a nuclear diameter of about 4µm [Fig.1: A & B].

3.2.5 Anterior lobe (AL)

The AL is a much smaller but important anterior extension of the cerebral ganglion. It arises as a smaller anterior somewhat triangular lobe from laterodorsal surface of each cerebral ganglion [Fig.1: E]. It measures roughly about 50µm × 30µm. The histological details of anterior lobes could not be observed clearly but they are shown to be of great importance in regulation of male sexual behavior in *Lymnaea stagnalis* [18].

3.2.6 Ventral lobe (VL)
VL is largest of the all structural extensions of cerebral ganglia. It arises as a bilobed ventral extension from both of the cerebral ganglia. It measures about 300µm × 100µm. Histologically, the VL is covered by a connective tissue sheath, the perineurium. Ventral lobes contain comparatively less number of NSCs as compared to other regions of the cerebral ganglia. The NSCs of VL are much smaller in size and are located only in the peripheral region of the lobe just inside the perineurium [Fig.1: A & C].

3.2.7 Mediodorsal bodies (MDBs)
Another example of structural extensions of each cerebral ganglion is a pair of MDBs. MDB are attached mediodorsally to the cerebral commissure at the point where cerebral commissure enters into cerebral ganglion of each side [Fig.1: E]. The MDBs are the most important structural extensions of cerebral ganglia because of their role in the regulation of female reproductive activity. Histological analysis showed that they are made up of the single type of secretory cells with very less cytoplasm and the central large-sized nucleus The neurosecretory material accumulates at the junction of cerebral commissure and MDB. Thus, this region may be the release site of neurosecretory product secreted by mediodorsal neurosecretory cells which send axon towards this area. Dorsal bodies of the right side are larger in size than that of the left side. The dorsal bodies are surrounded by a capsule that is continuous with the perineurium of the cerebral ganglion [Fig.1: D & E].

3.3 Pedal ganglia
The dorsal region of the pedal ganglia is occupied by NSC type A. Within the dorsal region of the pedal ganglion, the number of A cells is comparatively than that of B cells. The cell type A measures 18µm to 21µm in somatic diameter and 10.14µm to 13.2µm in nuclear diameter. The rest of the peripheral region of the pedal ganglion is occupied by type B NSCs. The central area of each pedal ganglion constitutes the neuropilar region [Fig.2: E & F]. The morphological and electrophysiological map of neurons and neuronal clusters in pedal ganglia is available for *L. stagnalis* [19].

3.4 Pleural ganglia
Like all other paired ganglia, the right pleural ganglion is a little larger than the left one showing size asymmetry. Type NSC type A in the pleural ganglia send their axons into the pleuroparietal connective. The somatic diameter of type A cells in this ganglia measures 7.8µm to 10.8µm and nuclear diameter measures 3.6µm to 5.4µm. Type B NSCs within pleural ganglia are relatively smaller in size with pyriform shape. Their cell diameter measures about 8.5µm and nuclear diameter is about 5.8µm. In pleural ganglia, the type B cells are relatively abundant than type A cells [Fig.2: A, B, C & D].

3.5 Parietal ganglia
Right parietal is larger than the left parietal ganglion. Comparatively more NSCs are present in the right parietal ganglion. The type A cell can be found on either side of the parietovisceral connective. The cell and nuclear diameter of type A cells measure 18 to 21µm and 10 to 15µm respectively. Type B cells are relatively more in number and measure 9 to 13 *µm* in somatic diameter and 6 to 8µm in nuclear diameter [Fig 2. A, B, C & D].

3.6 Visceral ganglia
It is posterior-most in position and the only unpaired ganglion in the CNS of *R. acuminata*. The number of type A cells is relatively more in the visceral ganglion than those of B cells. Type A cells are quite large in size measuring 21 to 24.6µm in somatic diameter and 15 to 20µm in nuclear size. Type A cells send axons in peripheral portion of the visceral nerves. These cells stain dark orange with Mallory’s triple stain. The type B cells are smaller than A cells. They measure about 10 to 12µm in somatic diameter and 3 to 8µm in nuclear size. Neurosecretory material in the form of plaques is seen in the perineurium of the visceral ganglion [Fig.2: A, B, C & D].

4. Discussion
The comparative studies on histology of cerebral ganglia of gastropods have been reported earlier by many researchers [9, 20, 21, 22]. As in many other freshwater snails, the CNS of *R. acuminata* consists of a nerve ring around esophagus. The CNS in the form of nerve ring comprised of paired cerebral, pedal, pleural, parietal and single visceral ganglia is common feature of many freshwater gastropods [5, 20, 21]. The basic histological details of the ganglia in *R.acuminata* conform to those reported in other freshwater mollusks. The asymmetry between the ganglia of the left side and their right counterparts with respect to the size has been the special feature of the central ganglia of the *R. acuminata*. Such type of size asymmetry is also reported in other snails [23, 20]. The presence of mediodorsal bodies is an important feature of many of the basommatophoran pulmonates and are also present in *R. acuminata* [24, 23, 25, 8, 20]. As in many pulmonates, the lateral lobe or cerebral gland is also present in *R. acuminata* and persist throughout life of the snail.

The presences of prominent inclusions suggestive of secretory activity in the neurons of the gastropods have attracted the attention of a number of investigations [26, 23, 27, 28]. Topographically, three groups of NSCs are found in the cerebral ganglia of *R. acuminata*. The MDCs and LDCs are homologous to those of *G. convexiesculus* [20]. The CDCs are also described in *L. stagnalis*[9]. Similar types of cells have earlier been reported in *Melampus bidentatus* [8] and *R. luteola* [22]. As in *L. stagnalis* and *G. convexiesculus*,

the CDCs of *R. acuminata* sends the axons towards the peripheral part of the cerebral commissure. Joosse [9] speculated that the mediodorsal bodies are endocrine glands controlling ovulation and oviposition. In *R. acuminata*, similar observations were recorded earlier [29] but further investigations are needed to confirm the role of these structures. Both type A and B neurosecretory cells are found in the ganglia of *R. acuminata*. The NSC Type A and type B were also described in the CNS of other freshwater snails [9, 10, 30, 25, 20]. The snail *R. acuminata* shows the neurosecretory material plaques as in *Indoplanorbis exustus* [17]. The neurosecretory material has shown to be phospholipid in nature in members of Planorbidae [30, 20]. The role NSCs in control of reproduction is well established in Mollusks [9, 31, 27, 4]. Advanced studies are essential on further aspects of structural organization of neuroendocrine system in the freshwater snail, *R. acuminata*. The knowledge about histological organization is important in understanding neuroendocrine control of various physiological processes in the life of the mollusks.

5. Conclusion

The CNS of *R. acuminata* consists of the nerve ring comprised of paired cerebral, pedal, pleural, parietal ganglia and single visceral ganglion. All of the ganglia have an epineural sheath called perineurium made up of connective tissue. The central core of each ganglion is the neuropil that lacks in cell bodies except cell bodies of neuroglial and connective tissue cells. The cortex area of each ganglion has a number of cell bodies of Neurosecretory cells. The outer cells in the cortex are larger than the inner ones. The cerebral ganglia are complex in organization and have many lobes such as ventral lobe, lateral lobe, anterior lobe and dorsal bodies. Two types of neurosecretory cells, type A and type B cells, are found in all of the ganglia.

6. Acknowledgment

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7. Conflict of interest

Authors declare that there is no conflict of interest.

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