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NEUROANATOMY OF *HAMATOPEDUNCULARIA* SP. (RASTOGI *ET. AL.*, 2005) FROM THE GILLS OF *MYSTUS SEENGHALA* AT MEERUT REGION

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Abstract

Chemical named 5-Bromo indoxyl acetate has been used for first time in India to describe the nervous system of an oviparous monogenean gill parasite, Hamatopeduncularia sp.(Rastogi et. al., 2005).The central nervous system consists of paired cerebral ganglia (cg) from which anterior and posterior neuronal pathways arise. These neuronal pathways are interlinked by cross connectives and commissures. Paired dorsal, ventral and lateral nerve cords emanate from the cerebral ganglia, connected at intervals by transverse connectives. The huge arrangement of dorsal, ventral and lateral nerve cords and their innervations have been examined. The peripheral nervous system (PNS) includes innervations of the alimentary tract, reproductive organs and attachment organs (anterior adhesive areas and haptor). Both the CNS and PNS are bilaterally symmetrical, and better developed ventrally than laterally and dorsally.

Keywords– *Monogeneans, Hamatopeduncularia sp, cholinesterase, dorsal nerve cord, ventral nerve cord and lateral nerve cord.*

Introduction

Monogeneans usually inhabit the skin and gills of their piscine host. According to Chappell (1980), the neurophysiology and neuroanatomy of parasitic animal is an area that has largely been neglected. Nervous system in monogeneans is well-developed and generally resembles the nervous system in Turbellaria.Central nervous system of monogeneans comprises of a bilobed brain, longitudinal cords and connecting transverse commissures forming the so-called orthogon. In the present work 5-Bromo indoxyl acetate has been used for esterases to map the nervous system of *Hamatopeduncularia sp.* (Rastogi *et.al.,* 2005). In the past Parasitologist used acetylthiocholine iodide (AcThI) as substrate to provide a more specific demonstration of Cholinesterase (ChE). The gross spatial arrangement of nerves has been examined.

The CNS is better developed ventrally than dorsally or laterally.It is hoped that such a study will throw some light on the nervous system of monogenea. It may also help researchers to find an anthelmintic drug that may specifically target neural elements of the parasite without substantial side effects on the host.

Materials and Methods

Fishes for present investigation were collected from freshwater bodies of Meerut region. Worms were collected in live condition, and washed thoroughly with distilled water and fixed in cold 4% neutral formaldehyde under cover glass for eight hours and subsequently washed with cold distilled water.

Study of nervous system was made with the help of histochemical localization of esterases, one of the very common neuropharmacological elements, commonly found in the nervous system of all animals including monogeneans as suggested by Halton and Jennings (1964). Microphotographs were taken with the help of Motic image plus software.

Observation and Results

Three species of **Haematopeducnularia*vizH. batrachi, H. orientalis and H. ritaii (Rastogie et.* al. *2005) were obtained from* Mystus seenghala *(Bagiridae).***

Central nervous system (CNS) comprises paired cerebral ganglia (cg) from which anterior and posterior neuronal pathways interlinked by cross connectives and commissures are derived. Peripheral nervous system (PNS) includes innervation of the alimentary tract, reproductive organs, attachment organs (anterior adhesive areas and haptor) and subtegumental muscles. Both CNS and PNS are bilaterally symmetrical, and better developed ventrally than laterally and dorsally.

Cholinergic enzyme histochemical staining

The CNS and PNS were found to be highly reactive for Cholinesterase (ChE), and stained extensively in a dark blue and purple colour.

***Hamatopeduncularia batrachi* (Rastogi *et.al.*, 2005) (fig 1- 7)**

Host: *Mystus seenghala*

No. of hosts found infected: 2

No. of worms collected: 12

Site of infection: Gills

Locality: Meerut

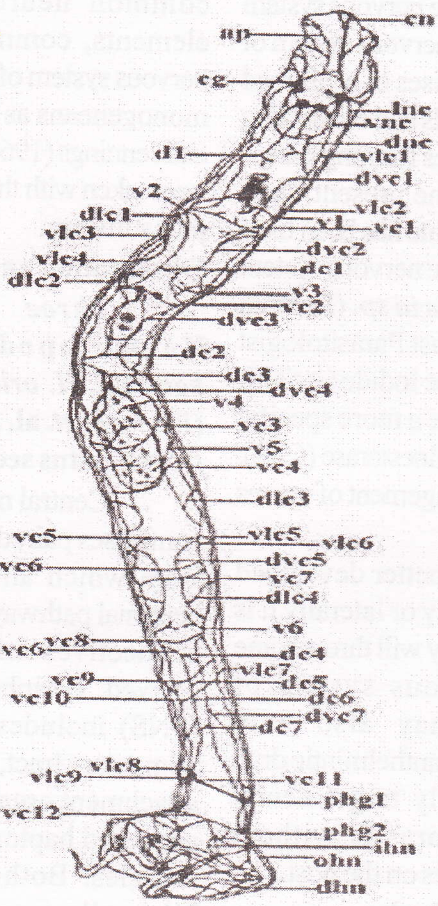

FIG- 1

Fig. 1 Diagram showing the nervous system of *Hamatopeduncularia batrachi* (Rastogi *et. al.*, 2005) as revealed by cholinergic enzyme histochemical staining, Cn (Cerebral nerves); Ap (Anterior projection); Cg (Cerebral ganglia); Vnc (Ventral nerve cord); Lnc (Lateral nerve cord); Dnc (Dorsal nerve cord); V (Ventral neurons); Vc (Ventral transverse connective); Vlc (Ventro lateral connective); Dvc (Dorso ventral connective); Dc (Dorsal transverse connective); Phg (Prehaptoral ganglion); Ohn (Outer haptoral nerve); Ihn (Inner haptoral nerve); Dhn(dorso-lateral haptoral nerve);

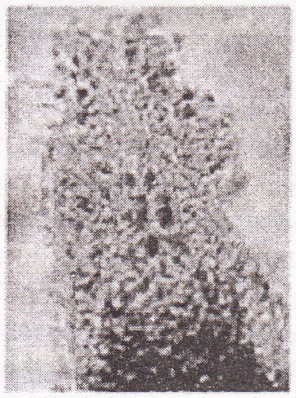

Fig 2 prohaptor region showing cerebral nerve (cn), cerebral ganglia (cg) and anterior projection (ap)

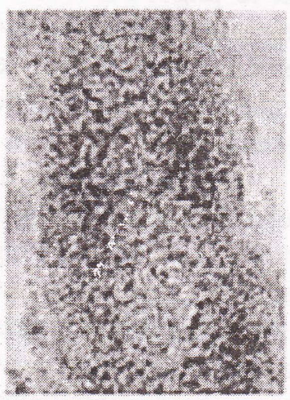

Fig 3 Cirrus region showing ventral neuron (vn) supply to male copoulatory part

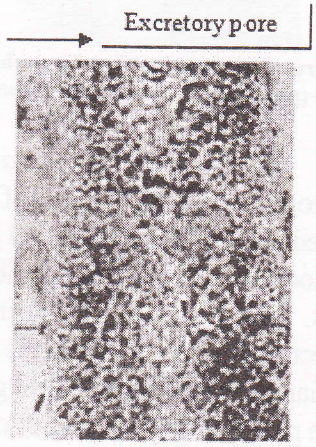

Fig 4 Excretory pore region showing ventral connectives (vc) and dorso ventral connectives (dvc)

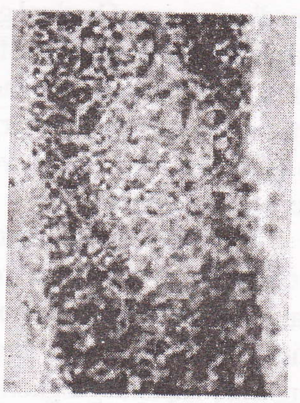

Fig 5 Testis region showing ventral neurons supply to male reproductive organs

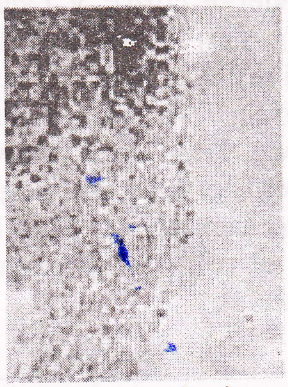

Fig 6 Prehaptoral region showing prehaptoral ganglion (phg) and dorso ventral connective (dvc)

Fig 7 Haptoral region showing haptoral nerves

CNS of *H. batrachi* (Rastogi*et.al.*, 2005) consists of a thick, curved mass of paired cerebral ganglia (cg) located ventrally just anterior to the eyes. Six pairs of thin anterior projections (ap) extend from the anterior median region of the cerebral ganglia. Each projection gives rise to cerebral nerves (cn) that extend anteriorly to enter the head lobes where they innervate the anterior adhesive area. Cerebral ganglia, situated anterior to the pharynx forms a circular path around pharynx and gives out optic nerves (on)and pharyngeal nerves (pn) that innervate eyes and pharynx region respectively.

Two thick and prominent ventral nerve cords (vnc) originate from the cerebral ganglia and run posteriorly, one on either side of the body where each one joins a pre-haptoral ganglion (phg1). Another pair of pre-haptoral ganglia (phg2) is situated one on either side of the body, a short distance posterior to the phg1. Both phg1 and phg2 of either side of the body are connected via two ganglionic connectives. In the region of reproductive glands, two considerably thin branches arise, one from each ventral nerve cord and reconnects to it. In the region of vesicular glands, another two thin branches arise, one from each ventral nerve cord and reconnects to it, adjacent to phg1. Fourteen ventral transverse connectives (vc1-vc14) were also detected, twelve between two ventral nerve cords and two between the phg1 and phg2.

Two considerably thin lateral nerve cords (lnc) arise from the postero-lateral region of the cerebral ganglia and run posteriorly, one adjacent to each lateral margin of the body where they join the phg1. At regular intervals, the ventral nerve cords communicate with the lateral nerve cords by means of three pairs of ventro-lateral connectives (vlc1-vlc3). Two thin dorsal nerve cords (dnc) arise from the postero-dorsal region of the cerebral ganglia and extend posteriorly between the ventral nerve cords where they connect with the anterior phg1. Dorsal nerve cords extend posterior to the Phg2 and enter in the haptoral region to innervate it. The dorsal nerve cords are cross-linked by five dorsal transverse connectives (dc1-dc5). At regular intervals, the dorsal nerve cord communicates, with the ventral nerve cord by means of eight pairs of dorso-ventral connectives (dvc1-dvc8).

Staining for cholinergic elements revealed the presence of seven pairs of large neurons distributed bilaterally down the main body of the worm. All pairs of these cells are located on the ventral side of the body (v1-v7). All of these cells are multipolar. The first two pairs of ventral cell bodies (v1-v2) are positioned at level of cirrus, first pair anterior and second pair posterior to the cirrus, third pair (v3) at the level of reproductive glands, fourth and fifth (v4-v5) between reproductive glands and ovary, sixth (v6) at the level of the testis and seventh (v7) at the level of vesicular glands. Each neuron contains a central nucleus and numerous dense granules. Dendritic processes extend from the ventral cells to connect with the main nerve cords and connectives.

Haptor is extensively innervated by a pair of outer haptoral nerves (ohn) and two pairs of inner haptoral nerves (ihn), which are relatively thick haptoral nerves. The outer of these arise one from each posterior prehaptoral ganglion (phg2) while the inner members are derived from the ventral connective (vc14). The outer and inner haptoral nerves run ventrally in a posterior direction before branching into a plexus of numerous fine nerves in the anterior region of the haptor. There are two dorso-lateral haptoral nerves (dhn) arising one from each posterior prehaptoral ganglion (phg2), and these run posteriorly and eventually branch to supply the marginal hooklets.

***Hamatopeduncularia orientalis* (Rastogi *et. al.*, 2005)** (fig 8-9)

Host: *Mystus seenghala*

No. of hosts found infected: 2

No. of worms collected: 18

Site of infection: Gills

Locality: Meerut

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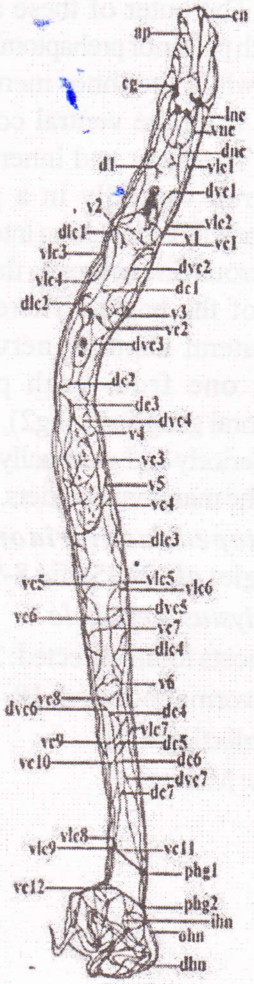


Fig 8 Diagrammatic representation of nervous system of *Hamatopeduncularia orientalis*, Rastogi *et.al.* 2005

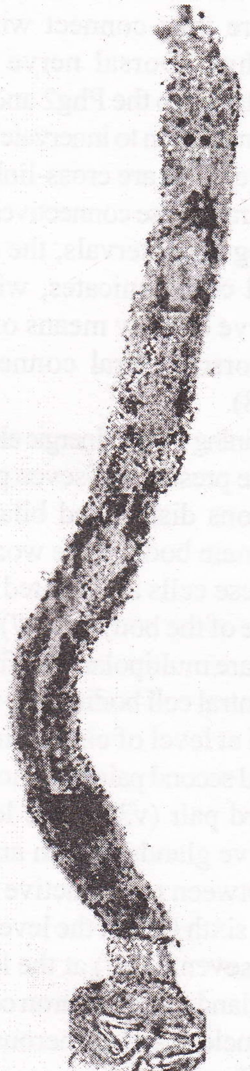


Fig 9 Photomicrograph showing nervous system of *Hamatopeduncularia orientalis*, Rastogi *et.al.* 2005

CNS of *H. orientalis* (Rastogi*et.al*., 2005) consists of a thick, curved mass of paired cerebral ganglia (cg) located ventrally adjacent to the anterior pair of eye spots. Two pairs of thin anterior projections (ap) extend from the anterior median region of the cerebral ganglia. Each projection gives rise to cerebral nerves (cn) that extend anteriorly to enter the head lobes where they innervate the anterior adhesive area. Cerebral ganglia, situated anterior to the pharynx forms a circular path around pharynx and gives out optic nerve (on) and pharyngeal nerves (pn) that innervate eyes and pharynx respectively.

Two thick and prominent ventral nerve cords (vnc) originate from the cerebral ganglia and run posteriorly, one on either side of the body where each one joins a pre-haptoral ganglion (phg1). Another pair of pre-haptoral ganglia (phg2) is situated one on either side of the body, a short distance posterior to phg1. Both phg1 and phg2 on either side of the body are connected via two ganglionic connectives. In the region of gonopore, two considerably thin branches arise, one from each ventral nerve cord and reconnects to it. Twelve ventral transverse connectives (vc1-vc12) were also detected, ten between two ventral nerve cords and two between the phg1 and phg2.

Two considerably thin lateral nerve cords (lnc) arise from the postero-lateral region of the cerebral ganglia and run posteriorly, one adjacent to each lateral margin of the body where they join phg1. At regular intervals, ventral nerve cords communicate with lateral nerve cords by means of eight pairs of ventro-lateral connectives (vlc1-vlc8). Two thin dorsal nerve cords (dnc) arise from the postero-dorsal region of the cerebral ganglia and extend posteriorly between the ventral nerves cords where they connect with the anterior phg1. Dorsal nerve cords extend posterior to the phg2. The dorsal nerve cords are cross-linked by seven dorsal transverse connectives (dc1-dc7). At regular intervals, dorsal nerve cord communicate, with ventral nerve cord by means of seven pairs of dorso-ventral connectives (dvc1-dvc7) and with the lateral nerve cord by means of four pairs of dorsolateral connectives (dlc1-dlc4).

Staining for cholinergic elements revealed presence of seven pairs of large neurons distributed bilaterally down the main body of the worm. Six pairs of these cells are located on the ventral side (v1-v6) and one pair on the dorsal side (d1) of the body. All of these cells are multipolar. First pair of dorsal cell bodies (d1) is positioned posterior to the pharynx. First pair of ventral cell body (v1) is positioned at the level of cirrus, second pair (v2) posterior to the cirrus, third pair (v3) at the level of gonopore, fourth and fifth pair (v4-v5) at the level of the testis and sixth pair (v6) at the level of confluence of intestinal crura. Each neuron contains a central nucleus and numerous dense granules. Dendritic processes extend from the dorsal and ventral cells to connect with the main nerve cords and connectives.

Haptor is extensively innervated by a pair of outer (ohn) and two pairs of inner (ihn), relatively thick haptoral nerves. The outer of these arise one from each posterior prehaptoral ganglion (phg2) while the inner members are derived from the ventral connective (vc12). The outer and inner haptoral nerves run ventrally in a posterior direction before branching into a plexus of numerous fine nerves in the anterior region of the haptor. There are two dorso-lateral haptoral nerves (dhn) arising one from each posterior prehaptoral ganglion (phg2), and these run posteriorly and eventually branch to supply the marginal hooklets.

***Hamatopeduncularia ritaii* (Rastogi *et al.*, 2005)**(Fig 10-15)

Host: *Mystusseenghala*

No. of hosts found infected: 2

No. of worms collected: 21

Site of infection: Gills

Locality: Meerut

Fig 10 Diagrammatic representation of Nervous system of *Hamatopeduncularia ritaii*, in haptoral region Rastogi *et.al.* 2005

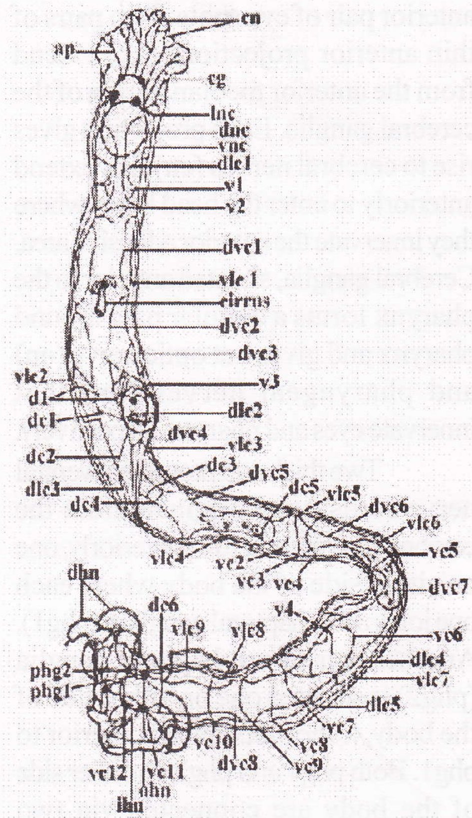
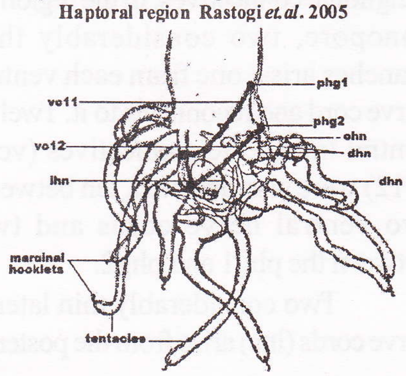


Fig 11 Nerves of *Hamatopeduncularia ritaii* in Haptoral region Rastogi *et.al.* 2005



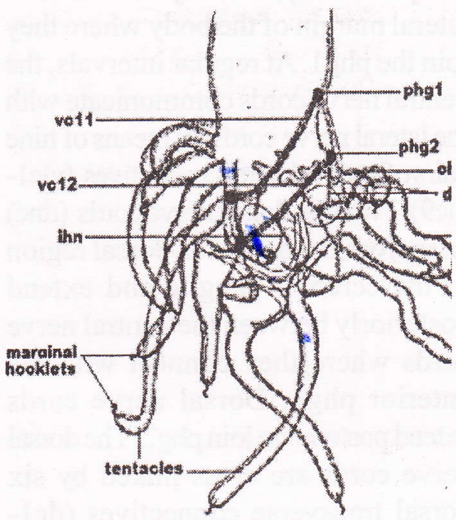


Fig 11 Nerves of *Hamatopeduncularia ritaii* in Haptoral region Rastogi *et.al.* 2005

Fig 12 Photomicrograph showing prohaptoral nerves

Fig 13 Photomicrograph showing postpharyngeal nerves

Fig 14 Photomicrograph showing nerves in testicular region

Fig 15 Photomicrograph showing Haptoral nerves

The CNS of *H. ritaii* (Rastogi *et. al.*, 2005) consists of a thick, curved mass of paired cerebral ganglia (cg) located ventrally just anterior to the eyes. Four pairs of thin anteriorprojections (ap) extend from the anterior-median region of the cerebral ganglia. Each projection gives rise to cerebral nerves (cn) that extend anteriorly to enter the head lobes where they innervate the anterior adhesive area. Cerebral ganglia, situated anterior to the pharynx forms a circular path around pharynx and gives out optic nerve (on) and pharyngeal nerves (pn) that innervate eye spots and pharynx respectively.

Two thick and prominent ventral nerve cords (vnc) originate from the cerebral ganglia and run posteriorly, one on either side of the body where each one joins a pre-haptoral ganglion (phg1). Another pair of pre-haptoral ganglia (phg2) is situated one on either side of the body, a short distance posterior to the phg1. Both phg1 and phg2 of either side of the body are connected via two ganglionic connectives. Twelve ventral transverse connectives (vc1-vc12) were also detected, ten between two ventral nerve cords and two between the phg1 and phg2.

Two considerably thin lateral nerve cords (lnc) arise from the postero-lateral region of the cerebral ganglia and run posteriorly, one adjacent to each lateral margin of the body where they join the phg1. At regular intervals, the ventral nerve cords communicate with the lateral nerve cords by means of nine pairs of ventro-lateral connectives (vlc1-vlc9). Two thin dorsal nerve cords (dnc) arise from the posterior-dorsal region of the cerebral ganglia and extend posteriorly between the ventral nerve cords where they connect with the anterior phg1. Dorsal nerve cords extend posterior to join phg2. The dorsal nerve cords are cross-linked by six dorsal transverse connectives (dc1-dc6). At regular intervals, the dorsal nerve cord communicate, with the ventral nerve cord by means of nine pairs of dorso-ventral connectives (dvc1-dvc9) and with the lateral nerve cord by means of four pairs of dorsolateral connectives (dlc1-dlc4).

Staining for cholinergic elements revealed the presence of four pairs of large neurons distributed bilaterally down the main body of the worm. Three pairs of these cells are located on the ventral side of the body (v1-v3) and one pair on dorsal side of the body. All of these cells are multipolar. The first two pairs of ventral cell bodies (v1-v2) are positioned posterior to the pharynx, third pair (v3) at the level of end of intestinal crura and fourth pair (d1) at level of gonopore. Each neuron contains a central nucleus and numerous dense granules. Dendritic processes extend from the ventral cells to connect with the main nerve cords and connectives.

Haptor is extensively innervated by a pair of outer (ohn) and two pairs of inner (ihn), relatively thick haptoral nerves. The outer of these arise one from each posterior prehaptoral ganglion (phg2) while the inner members are derived from the ventral connective (vc12). The outer and inner haptoral nerves run ventrally in a posterior direction before branching into a plexus of numerous fine nerves in the anterior region of the haptor. There are two dorso-lateral haptoral nerves (dhn) arising one from each posterior prehaptoral ganglion (phg2), and these run posteriorly and eventually branch to supply the marginal hooklets.

Discussion

As the most primitive metazoan phylum, Platyhelminthes occupies a unique position in nervous system evolution. Flatworms occupy a pivotal position in animal evolution when cephalization and an organized nervous system first appear. The flatworm central nervous system (CNS) has an archaic brain located in the head region and paired longitudinal nerve cords, which are cross-linked at regular intervals by transverse commissures. The main longitudinal nerve cords (lateral, ventral and dorsal) are interconnected at intervals by a series ofannular cross-connectives, producing a ladder-like arrangement typical of the platyhelminth nervous system. In turn, the CNS is linked to a simple peripheral nervous system (PNS), consisting of smaller nerve cords and nerve plexuses that supply all the major body structures, in particular the tegumental region, the somatic musculature, the alimentary tract (where present) and reproductive organs.A diverse arrangement of nerve plexuses of varying complexity innervates the subsurface epithelial and muscle layers, and in the parasitic taxa, they are most prominent in the musculature of the attachment organs and egg-forming apparatus, Cable *et. al.* (1996) suggested that the neuropeptide secretions may influence movements of ova &vitelline cells, release of spermatozoa and mehlis gland secretion, peristaltic contraction of the ootype which shape the egg, and regulate the release of egg from the ootype into the uterus. At the level of the haptor, theventral cords provide nerve roots which innervate each clamp.In these respects, the CNS of *Hamatopeduncularia*Yamaguti 1953, resembles in basic structure that of all previously studied flatworms, including monogeneans, digeneans and cestodes (Reuter 1987; Halton and Gustafsson 1996; Reuter *et. al.*, 1998; Reda and Arafa, 2002 and Arafaand Reda,*2002*).

One of the most important developments came through the groundbreaking work of Halton and colleagues who have combined the power of confocal microscopy with immunofluorescence to provide a first detailed map of individual transmitter systems in flatworms (Halton & Gustafsson, 1996; Halton, 2004; Halton& Maule, 2004). This work has greatly improved our understanding of flatworm neuroanatomy. It has also helped to visualize potentially important sites of neurotransmitter activity, which is an important first step towards the elucidation of transmitter function.In its strictest definition, a neurotransmitter is a chemical messenger that is synthesized by a neuron, released at a synaptic junction and is capable of eliciting a response by interaction with specific receptors located on the post-synaptic membrane. In addition, a neurotransmitter must be rapidly inactivated and removed from the synaptic cleft shortly after a response is elicited.

A striking feature of all monogeneans studied hitherto has been the finding of a ring commissure around the mouth (Rohde 1968, Reuter 1987). Rohde (1968) suggested an oral commissure is a characteristic feature, which distinguishes the nervous system of the monogenea from that of digenea.

Acknowledgements

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