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The nervous system of the pike-tapeworm *Triaenophorus nodulosus* (Cestoda: Pseudophyllidea)—ultrastructure and immunocytochemical mapping of aminergic and peptidergic elements

Natalia M. Biserova,[1] Margaretha K. S. Gustafsson,[2,3] Maria Reuter,[2] and Nadezhda B. Terenina[3]

[1]Institute of Inland Water Biology, Russian Academy of Science, 152742 Borok, Yaroslav District, Russia
[2]Department of Biology, Abo Akademi University, FIN-25200 Abo, Finland
[3]Institute of Parasitology, Russian Academy of Science, 117071 Moscow, Russia

Abstract. The nervous system of the adult pike-tapeworm *Triaenophorus nodulosus* was studied to identify nerve cells and fibers immunoreactive to serotonin (5-HT) and RFamide (RF) on whole-mount preparations and frozen sections. Neurons immunoreactive to 5-HT were seen solely in the central nervous system, while those immunoreactive to RF occurred in the peripheral nervous system as well as in the central nervous system. In the scolex, both types of nerve fibers were found. While the gonads were not innervated by either fiber type, the reproductive tract showed RF-immunoreactive nerves. On the ultrastructural level, five types of neurons and three types of release sites and a neuromuscular junction could be distinguished. Levels of 5-HT, measured spectrofluorimetrically, were found to be lower in the tapeworm than in the tissues where it resides in its host, indicating a possibility that the parasite absorbs this bioamine from its environment.

Additional key words: 5-HT, RFamide, neuron types, neurotransmitter release sites, spectrofluorimetry

During the last two decades, knowledge of the neuroanatomy of flatworms has grown (for review, see Reuter & Gustafsson 1995). For the parasitic flatworms, however, ultrastructure of neuronal elements is still rather poorly known, and information on the nature and number of neuronal mediators in flatworms in general is sparse. To fill part of this gap, we have studied the nervous system (NS) of the pike-tapeworm *Triaenophorus nodulosus* (PALLAS 1781) with immunocytochemical, ultrastructural, and spectrofluorimetric methods. *T. nodulosus* parasitizes the intestine of pike as an adult and parasitizes other economically important fishes, such as burbot, as a plerocercoid, the last larval stage. The first parasitic larval stage, the procercoid, develops in fresh-water crustaceans.

Apart from the studies of Michajlow (1932, 1933, 1934) little information is available on the histology, especially the neuroanatomy, of the Triaenophoridae. Gustafsson (1973), investigating cellular composition in the neck region of plerocercoids of *T. nodulosus*, noted that the cells surrounding the main nerve cords constitute as much as 19% of the total cell population. In light microscopical studies of the general neuroanatomy of *T. nodulosus,* Kotikova & Kuperman (1977) monitored acetylcholinesterase activity during ontogenesis and documented formation of nerve plexuses and cholinergic nervous elements in the scolex and an increase in the number of longitudinal nerve cords at each successive stage of development; Biserova et al. (1991) used the glyoxylic-acid method to study the aminergic NS of adults and showed evidence of serotonin in ganglia of the brain, connecting commissure, and main nerve cords. Ultrastructure of sensory structures in the scolex was investigated by Biserova et al. (1991); and ultrastructure of the integument, frontal glands, and excretory system was examined by Kuperman (1973, 1988) and Kuperman & Davydov (1982a,b). We have extended the study of adults of *T. nodulosus* by comparing patterns of peptidergic and aminergic nerve elements, distinguishing types of nerve cells and neurotransmitter-release sites, and measuring serotonin (5-HT) concentrations.

The nervous system of flatworms can be subdivided into central and peripheral elements (see Reuter &

[a] To whom correspondence should be addressed.
Phone: 358 21 2654603; FAX: 358 21 2654748;
E-mail: magustaf@ra.abo.fi

Gustafsson 1995 and Reuter et al., in press, for discussion of terminology). The central nervous system of *T. nodulosus* consists of the bilobed brain and the two main nerve cords (MC's). The peripheral nervous system comprises all the other longitudinal cords (the minor cords), ring commissures in the cortical parenchyma, and nervous structures associated with the genital organs.

Methods

Immunocytochemistry

Adult specimens of *Triaenophorus nodulosus* were obtained from the intestine of pike *(Esox lucius)* caught in brackish water in the Finnish SW archipelago. Some worms were fixed directly in Stefanini's fixative (2% paraformaldehyde and 15% picric acid in 0.1 M phosphate buffer [PBS] pH 7.6) for 20 h at 10° C. Others were "skinned" according to the method of Gustafsson (1991) by transfer to distilled water for 3 h at room temperature, then fixed for 20 h in Stefanini's fixative. Skinning removes the tegument, which is impermeable to the antibodies applied to whole mounts. After fixation, the worms were transferred to 10% sucrose solution in PBS for 2 to 3 days at 4°

Skinned worms were stained as whole mounts (Gustafsson 1991) with the indirect immuno-fluorescence method (Coons et al. 1955). Rabbit anti-RFamide (146 III) diluted 1:500 and rabbit anti-serotonin (Incstar, USA) diluted 1:500 were used. Incubation time in primary antibodies was 5-7 days. Rhodamine isothiocyanate (TRITC) was used as secondary antibody, incubation time 20 h. The worms were mounted flat in 50% glycerin in PBS and stored at -20° Controls included omission of primary antiserum, substitution of primary antiserum with non-immune rabbit serum, and liquid phase pre-adsorption of primary antiserum with 5-HT and RFamide in excess.

Unskinned worms were embedded in Tissue and cut at 20 (xm on a Bright cryostat. Sections were collected on gelatin-coated slides and stained and mounted in the same fashion as the whole mounts but with an overnight incubation in primary antibodies and a 1-h incubation in secondary antibodies, which were

Figs. **1-14.** Left side (Figs. 1-7): 5-HT-immunoreactive; right side (Figs. 8-14): RF-immunoreactive. Adults of *Triaenophorus nodulosus.* Scale bar, 50 (xm in all photomicrographs.

Fig. 1. Scolex, "en face" view of whole-mount preparation showing 5-HT-IR fibers in the bilobed brain (arrowhead) and the two MC's originating at the brain (long arrows). Note 5-HT-IR cell bodies along the MC (small arrow).

Fig. 2. Neck region, whole-mount preparation showing the pattern of 5-HT-IR nerve cells along the MC's (arrows).

Fig. 3. Scolex, frontal section showing the pattern of 5-HT-IR nerve cells and fibers in the brain. Note large multipolar cell body in the brain commissure (arrowhead) and small cell bodies in the periphery of the brain ganglion (arrows). Thin 5-HT-IR processes (small arrows) fill the neuropile and extend toward the surface of the scolex.

Fig. 4. MC (bent arrow) composed of many 5-HT-IR fibers running longitudinally. Note many bipolar 5-HT-IR cell bodies (arrows) along the cortical border of MC and many thin processes (arrow pair) extending to the cortical parenchyma and tegument (right side).

Fig. 5. Scolex, section showing varicose 5-HT-IR processes (arrow pair) extending from the brain ganglion (bent arrow) to the hook region (arrowhead).

Fig. 6. Neck region, transverse section showing 5-HT immunoreactivity in the two MC's (arrows) and the ring commissure (arrow pairs) in the cortical parenchyma. Medullary parenchyma (M).

Fig. 7. Section showing 5-HT immunoreactivity in the MC (long arrow) and genital duct (short arrow). Note 5-HT-IR terminals (arrow pair) close to basal lamina of surface tegument.

Fig. 8. Neck region, whole-mount preparation showing the pattern of RF-IR nerve fibers and cells. Note the two MC's (arrows) and the cell bodies in the ring commissures (small arrows).

Fig. 9. Higher magnification of whole-mount preparation with RF-IR cell bodies (arrow) in ring commissures. Note the branching and varicose nerve fibers (arrow pair).

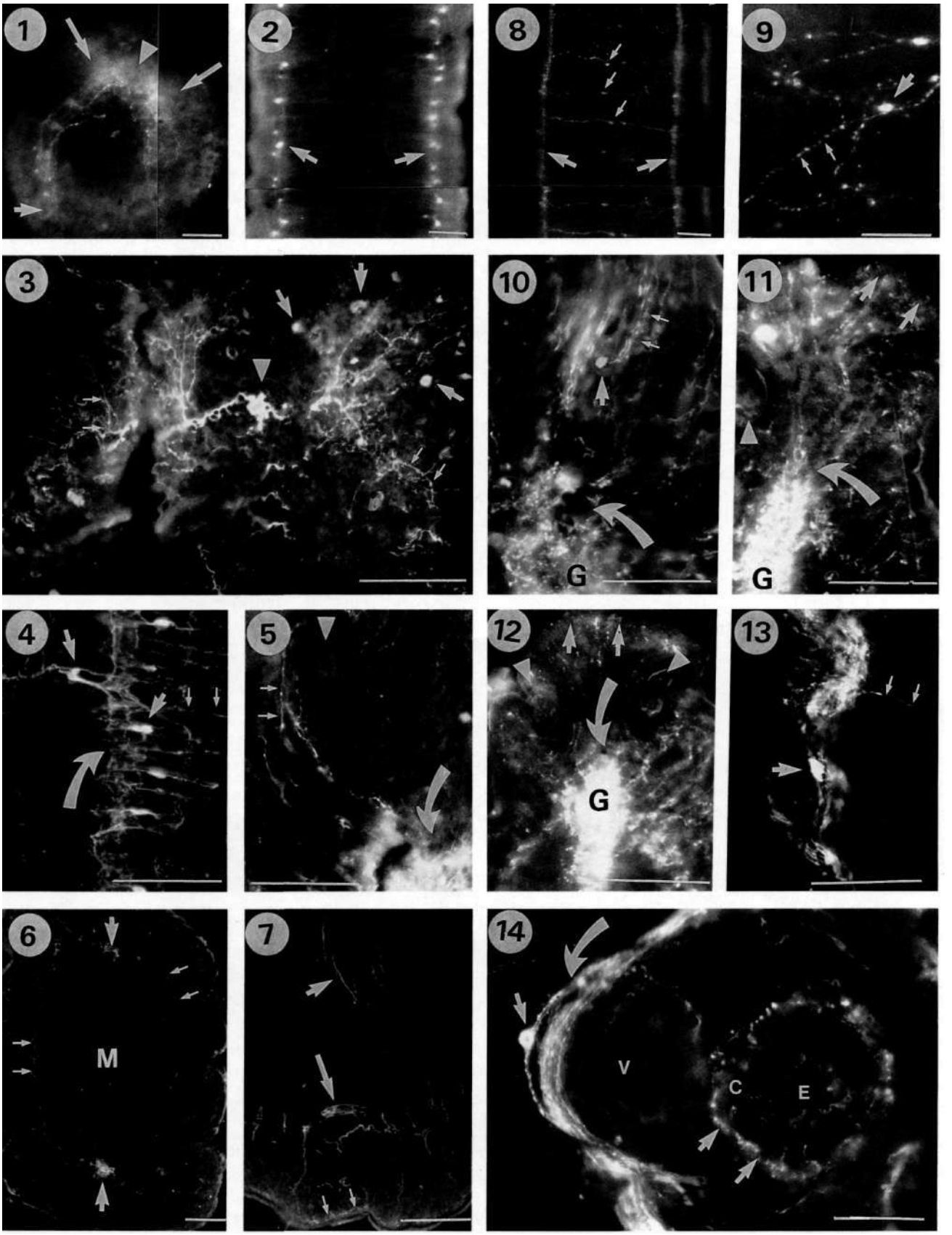
Fig. 10. Scolex, section with RF-IR fibers in brain ganglion (G). Note large cell body (bent arrow) at the surface of brain ganglion and small cell body (short arrow) in nerve extending toward hook region (arrow pair) and surface of scolex.

Fig. 11. Section of neuropile filled with RF-IR fibers (bent arrow). Varicose fibers run from brain ganglion (G) to frontal plate ending in terminals below basal lamina (short arrows). Hook (arrowhead).

Fig. 12. Section of neuropile filled with RF-IR fibers (bent arrow). Note RF-IR fibers close to hooks (arrow heads) and RF-IR terminals beneath basal lamina of tegument (short arrows).

Fig. 13. MC, section showing many RF-IR fibers and one cell body (arrow). Note thin RF-IR fiber (arrow pair) extending toward cortical parenchyma.

Fig. 14. Section of genital tract with large bipolar RF-IR cell body (single arrow) in MC (bent arrow) filled with RF-IR fibers. Note RF-IR fibers (arrow pair) in wall of cirrus sac (C) and ejaculatory duct (E). Vagina (V).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labeled with TRITC or fluorescein isothiocyanate (FITC). Controls like those for whole mounts were also executed.

The immunostained material was examined in a Leitz Orthoplan microscope fitted with filter-blocks 12 and N2; photomicrographs were produced with an Olympus model PM 10 ADS automatic photomicrographic system.

The controls for all immunostained material were negative.

Electron microscopy

Adults of *T. nodulosus* obtained from the intestine of pike from the Rybinsk reservoir (Yaroslav District, Russia) were collected in glucose-supplemented (1 g/1) standard Hank's solution and then fixed in 2% glutaraldehyde in 0.1 M sodium-cacodylate buffer (pH 7.2) at room temperature for 2 h. Post fixation was performed in 2% osmium tetroxide in the same buffer for 2 h, dehydration was through ethanol or acetone, and embedment was in Araldite (37° and 60° C). Ultrathin sections were examined with a JEM 100C transmission electron microscope ( ).

Spectrofluorimetry

Fresh and frozen (—20 to —70° C) adults of *T. nodulosus* obtained in the Rybinsk reservoir were extracted with butanol. Concentrations of 5-HT in the extracts were measured by (a) the spectrofluorometric method of Maickel et al. (1968) based on the reaction of 5-HT with o-phthaldialdehyde; (b) the method of Udenfriend (1962), measuring excitation (295 nm) and emission (540 nm) wavelengths after induction of 5-HT fluorescence with strong acid (HC1 3N); and (c) the method of Snyder et al. (1965), measuring excitation (385 nm) and emission (495 nm) wavelengths after treatment with ninhydrin in a hot near-neutral solution. Fluorescence was measured in a Hitachi MPF-4 spectrofluorimeter using 1-cm quartz cuvettes. Serotonin creatinic sulfate (5-hydroxytryptamine, Reanal) was used as the standard.

Concentration of 5-HT was also measured in the intestinal tissue of the pikes from which the worms were obtained.

Results

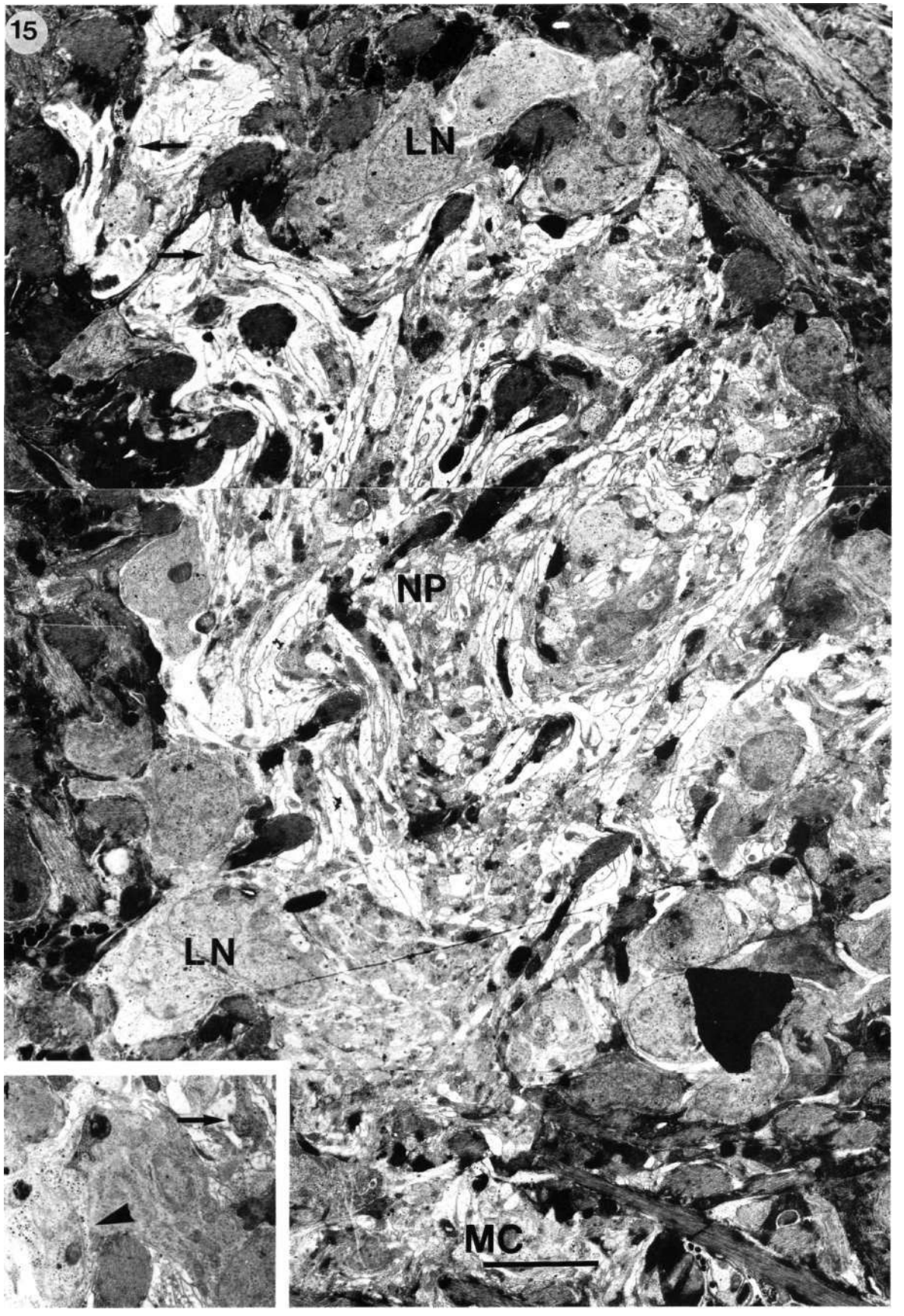
Immunocytochemistry

5-HT-immunoreactivity (Figs. 1-7). The NS of whole mounts stained strongly with anti-5-HT The brain, composed of two lateral ganglia connected by a commissure (Fig. 1), and the two main cords (Fig. 2), running from the brain to the posterior end of the body, were clearly highlighted by the stain. In the neck and proglottids, immunoreactive (5-HT-IR) cell bodies were clearly confined to the MC's (Fig. 2), and their processes extended across the worm from one MC to the other, forming transverse ring commissures in the cortical parenchyma (the commissures appear weak in Fig. 2 because they lie out of the plane of focus). No 5-HT-IR cell bodies were observed in the peripheral nervous system—i.e., along the minor longitudinal cords or in the ring commissures.

Staining with anti-5-HT was similar in frozen sections (Figs. 3-7). A large multipolar 5-HT-IR cell body was revealed in the brain commissure and smaller 5-HT-IR cell bodies in the periphery of the brain ganglia (Fig. 3). The ganglia were filled with 5-HT-IR nerve fibers. A dense population of 5-HT-IR cell bodies lay along the MC's (Fig. 4). Most of these cells were bipolar with extensions reaching to the basal lamina of the tegument. From the brain ganglion, 5-HT-IR nerves were evident running close to the hooks and parietal plate in the scolex (Fig. 5). In the neck (Fig. 6) both MC's and the ring commissure showed immunoreactivity, and in mature proglottids (Fig. 7) 5-HT-IR nerves could be seen extending from cell bodies along the MC's toward the vitelline glands and genital atrium. No 5-HT immunoreactivity was observed around the ovary, uterus, or testes.

RF-immunoreactivity (Figs. 8-14). Staining with anti-RFamide produced a different picture. While the general morphology of the NS, with the bilobed brain and the pair of MC's, was revealed by this stain, as with anti-5-HT, an additional four pairs of minor longitudinal cords were evident: dorsal and ventral pairs, and dorso-lateral and ventro-lateral pairs close to the MC's. Transverse ring commissures in the cortical parenchyma connected the main as well as the minor nerve cords. The ring commissures were highest in

Fig. 15. Overview of brain lobe ultrastructure in adult worm, *Triaenophorus nodulosus.* Anterior part to left, posterior part to right. Neuropile (NP) is filled with processes containing vesicles of various sizes and densities as well as large lucent processes. In the periphery of the brain are groups of light neurons (LN). Origin of a main nerve cord (MC) is seen in the lower right corner. Profiles of processes with large dense granules in upper left corner (arrows). Muscle fibers lie at the periphery of the brain. Inset: Neurosecretory cell (arrowhead) with large dense granules (arrow). Scale bar, 10 u.m. .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number in the neck region, becoming sparser in the mature proglottids. Distribution of the RF-IR cell bodies differed clearly from that of the 5-HT-IR cell bodies (cf. Figs. 8 and 2). Most RF-IR cell bodies were in the peripheral nervous system; only scattered RF-IR cell bodies lay along the MC. In the neck region a consistent pattern was observed, with one or two RF-IR cell bodies situated in the middle of the transverse ring commissure and sending processes to both MC's (Fig. 8). The RF-IR nerve fibers filling the MC and forming the ring commissures were varicose and formed branches (Fig. 9).

Staining of frozen sections by anti-RF was intense. Brain ganglia were filled with RF-IR nerve fibers extending toward the top of the scolex and ending in terminals beneath the tegument (Figs. 10, 11). Numerous varicose RF-IR fibers filled the brain ganglia and extended toward the parietal plate and hooks (Fig. 12). RF-IR cell bodies along the MC's were sparse (Fig. 13). In the mature proglottids, such cells were large and bipolar. Reactive nerve fibers were associated with the genital aperture, cirrus sac, and cirrus (Fig. 14).

Electron microscopy

Serial longitudinal and transverse sections of the scolex and anterior part of the strobila showed the bilobed brain filled with aggregations of neuronal processes (Fig. 15) and the MC's, measuring about 10-20 |xm in diameter. From the two ganglia, numerous thin processes filled with electron-dense granules (120-140 nm in diameter) extended anteriorly, penetrating the musculature of the hooks and reaching the parietal plate. Neuronal cell bodies lay in groups at the periphery of the ganglia, commissure, and MC's (Fig. 15). Occasionally neurons were seen in the central part of the ganglia, between the neuronal processes. The total number of cells in the bilobed brain is approximately 80, with 11 cells in the commissure and about 35 cells in each ganglion. Five types of neurons were distinguished (Figs. 15-20): "light" neurons, multipolar and unipolar neurons, neurosecretory cells, and interneurons.

"Light" neurons (Figs. 15, 16) were the dominating type of neuron. They occurred both in the brain and along the MC's. The cells had large, round, euchromatic nuclei, round nucleoli, and electron-lucent cytoplasm. The nucleo-cytoplasmic ratio was approximately 1:1. Deep invaginations of the plasma membrane were observed. These neurons were characterized by round or sometimes irregular granules (75 X 100 nm) of various densities. Groups of free ribosomes filled the cytoplasm while mitochondria and e mic cisternae were sparse.

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Neuropile (Figs. 15, 20-24). Processes of si types formed the neuropile of the brain commi ganglia, and MC's. Large electron-lucent proc containing microtubules, elements of SER, and mitochondria were seen (Figs. 15, 20, 22), as w smaller processes filled with vesicles of different and densities. Processes filled with large dense ules of the neurosecretory type, large lucent ves and neurotubules were occasionally observed (15, 24). All processes tightly adjoined each othe

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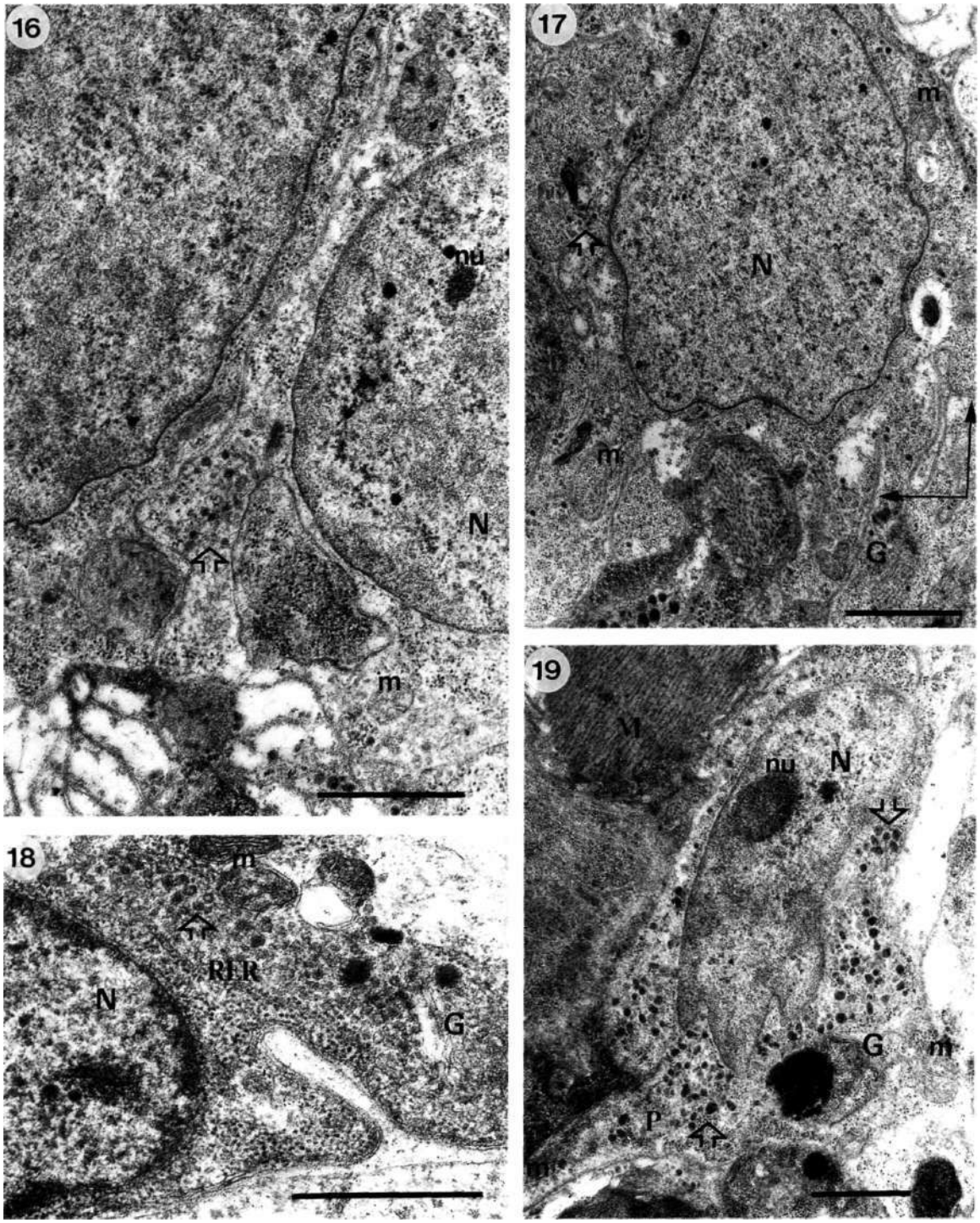
Unipolar neurons (Fig. 18) occurred in the periphery of the ganglia and along the MC's in the neck region. They were usually unipolar. The large nucleus was surrounded by a thin layer of cytoplasm of medium electron density. Mitochondria, smooth endoplasmic reticulum (SER), RER, Golgi complexes, microtubules, and small, round vesicles or vesicles of moderate density occurred in clusters where the single process originated from the cell body.

Neurosecretory cells (Figs. 15 inset, 19). Only two cells of this kind were observed in the scolex of each specimen examined. One cell body lay close to the commissure, with a process extending into the MC. The nucleus was oval and contained a large nucleolus. The nuclear membrane formed protrusions close to the starting point of the main process. The organelles were ordered in their distribution: round electron-dense granules with a diameter of 90-120 nm and SER and RER were located mostly close to the origin of the main process; at the opposite pole were mostly free ribosomes and microtubules. A prominent Golgi complex was observed near the nucleus.

Interneurons (Fig. 20). In the central part of the ganglion and in the MC's were small neurons with an oval nucleus and a thin layer of cytoplasm. In the ganglia, the perikarya of such interneurons were tightly covered by nerve endings forming synaptic contacts.

Neuropile (Figs. 15, 20-24). Processes of several types formed the neuropile of the brain commissure, ganglia, and MC's. Large electron-lucent processes containing microtubules, elements of SER, and a few mitochondria were seen (Figs. 15, 20, 22), as well as smaller processes filled with vesicles of different sizes and densities. Processes filled with large dense granules of the neurosecretory type, large lucent vesicles, and neurotubules were occasionally observed (Figs. 15, 24). All processes tightly adjoined each other.

"En passant" synapses (Figs. 20, 22). Presynaptic terminals containing dense-cored vesicles (80 nm in



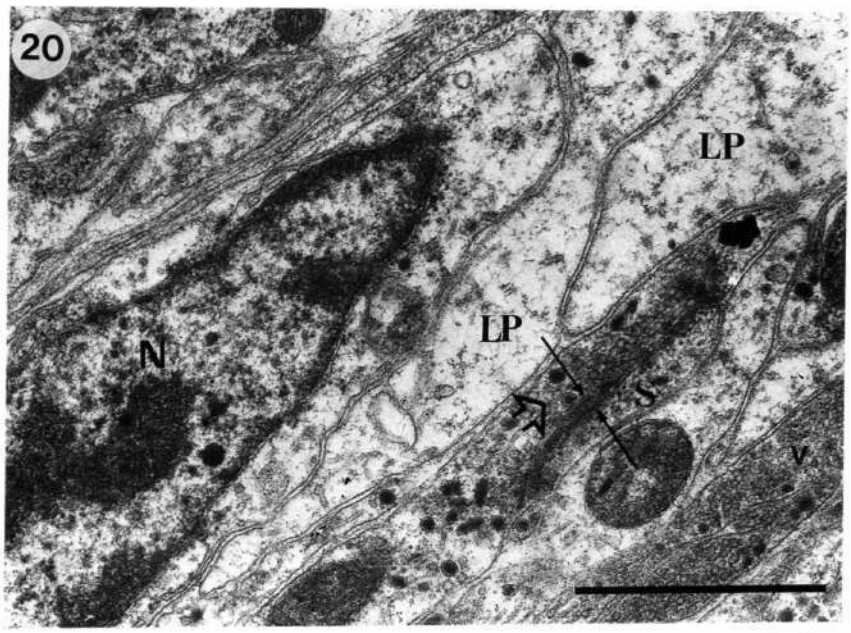
Figs. 16-19. Ultrastructure of neuronal cell bodies in adults of *Triaenophorus nodulosus.* Scale bars, 1 jxm. .

Fig. 16. Light neurons with euchromatic nuclei (N), small amount of electron-lucent cytoplasm and group of granules of different densities (empty arrow). Nucleolus (nu), mitochondrion (m), large lucent processes (LP).

Fig. 17. Large multipolar neuron with round nucleus (N), deep invaginations of plasma membrane (long arrows), and small granules (empty arrow). Golgi complex (G), mitochondrion (m).

Fig. 18. Unipolar neuron in MC with dense-cored vesicles (empty arrow), RER, and Golgi complex (G). Nucleus (N), mitochondrion (m).

Fig. 19. Neurosecretory cell in brain ganglion with large dense granules (empty arrows). Nucleus (N), nucleolus (nu), Golgi (G), mitochondrion (m), origin of neuronal process (P), muscle fiber (M).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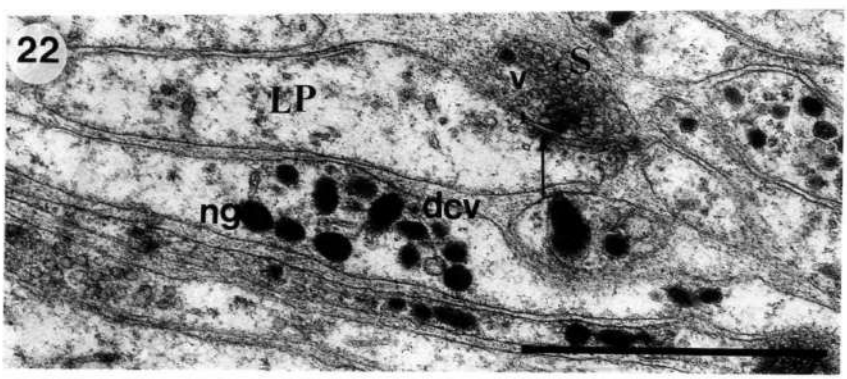
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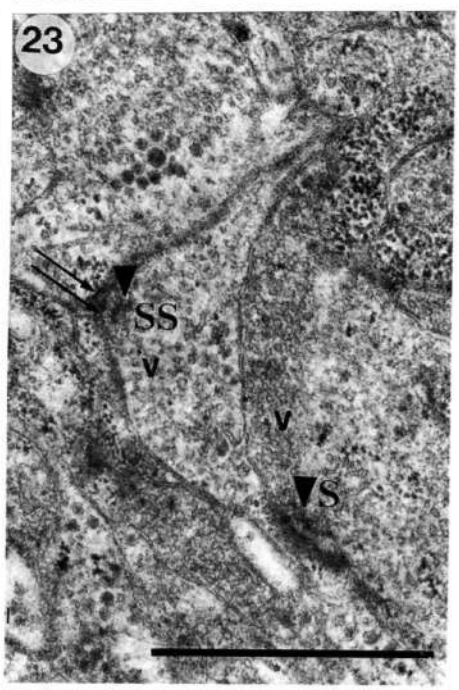
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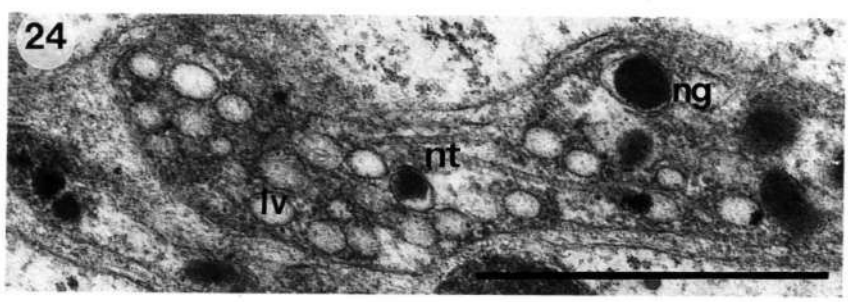
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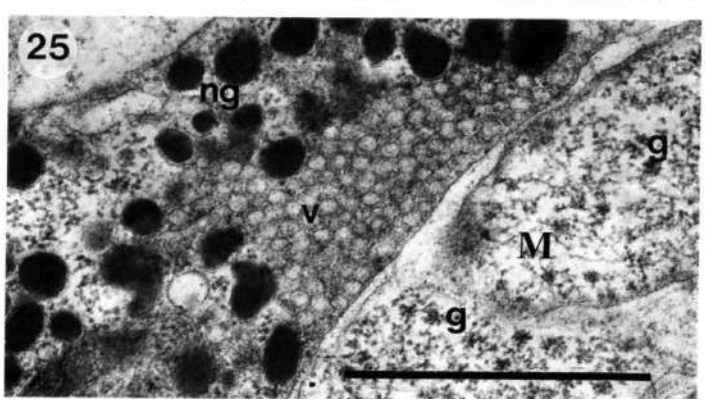
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diameter) and small clear vesicles were found in the neuropile of the brain and in the MC's. Membranes at the site of contact were thickened, and the synaptic cleft was distinct. These "en passant" synapses usually appeared between neural processes.

Single and shared synapses (Figs. 21, 23). Presynaptic terminals containing small, round, clear vesicles were frequently found. In these, a synaptic cleft separated the pre- and postsynaptic terminals. The postsynaptic membrane was thickened in most synapses, but some synapses lacked postsynaptic densities. In the presynaptic terminal, a paramembranous density was observed close to the thickened presynaptic membrane. This kind of synapse occurred in two variants: either the presynaptic terminal ended on one postsynaptic element (single synapses; Figs. 21, 23), or the presynaptic membrane was shared by two postsynaptic elements (shared synapses; Fig. 23).

Paracrine release and neuromuscular junctions (Fig. 25). Processes with neurosecretory granules (100-140 nm) were found passing close to the MC's and the excretory ducts. They penetrated between large muscular fibers and formed characteristic contacts with muscle cells, particularly at glycogen-containing parts of the muscles. These nerves contained large (90-145 nm) electron-dense granules and clusters of clear vesicles near the membrane. No thickenings of the parallel membranes were evident. Neuromuscular junctions were found in the scolex, where the large muscle fibers of the scolex musculature send fine processes into the neuropile of the ganglion lobes (Figs. 15, 25). Other neuromuscular junctions were found close to the MC's.

Spectrofluorimetry

All three spectrofluorimetric methods used revealed 5-HT (Table 1). The amount of 5-HT in adult worms ranged from 0.297 to 1.411 u.g/g wet wt. The intestinal wall of the host also showed 5-HT and at significantly higher levels, ranging in concentration from 0.560 to 2.854 (xg/g wet wt (Table 2).

Discussion

Immunocytochemistry

Our immunocytochemical staining for aminergic and peptidergic elements shows the same general organization to the nervous system of adult pike-tapeworms, *Triaenophorus nodulosus,* that Kotikova & Kuperman (1977) found by mapping acetylcholinesterase activity (Fig. 26). We can conclude that *T. nodulosus* has at least three types of neuronal-signal substances in its NS, the bioamine serotonin, the peptidergic neuronal substance RF-amide, and cholinergic elements (see introduction for references). In addition to the parts of the nervous system revealed by our immunocytochemical staining, median commissures connecting the MC's through the medullary parenchyma show cholinesterase activity (Kotikova & Kuperman 1977). We found no median commissures immunoreactive to 5-HT or RF-amide. Similarly, Gustafsson et al. (1994) found no median commissures reactive to either 5-HT, RFamide, or neuropeptide F in another pseudophyllidean tapeworm, *Diphyllobothrium dendriticum.* Further investigations are needed to clarify the nature of these commissures. Generally, neuronal-signal substances occur in separate sets of neurons in fiatworms (for references, see Reuter & Gustafsson 1995).

The general form of the NS of cestodes is evident from immunostaining of whole mounts (Gustafsson 1991; Gustafsson & Eriksson 1992; Gustafsson et al. 1993), and staining of frozen sections reveals even more details. The scolex, which is especially difficult

Figs. 20-25. infrastructure of neuronal processes and release sites in adults of *Triaenophorus nodulosus.* Scale bars, 1 .

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Fig. 20. Interneuron in brain ganglion. Note "en passant" synapse (S) in neuropile with dense-cored vesicles (empty arrow) in presynaptic terminal and thickened synaptic membranes (double arrows). Terminals filled with small clear vesicles (v), large lucent processes (LP), nucleus (N).

Fig. 21. Single synapses (S) with presynaptic terminal filled with small clear vesicles (v), synaptic cleft, pre- and postsynaptic densities (arrow pair), paramembranous density (arrowhead).

Fig. 22. Large lucent processes (LP) in central part of MC with neurotubules; large dense granules (ng) and dense-cored vesicles (dcv). Note "en passant" synapse (S) with presynaptic terminal filled with small clear vesicles (v) and postsynaptic density (arrow).

Fig. 23. Single (S) and shared synapses (SS) with presynaptic terminals filled with small clear vesicles (v). Pre- and postsynaptic densities (arrow pair) and paramembranous densities (arrowheads).

Fig. 24. Process with neurotubules (nt), large dense granules (ng), and large lucent vesicles (lv).

Fig. 25. Neuromuscular junction. The terminal faces the non-contractile part of a muscle cell (M) and is filled with large dense granules (ng) and small clear vesicles (v). Glycogen (g).

Table 1. Concentration of 5-HT in adults of *Triaenophorus nodulosus.*

5-HT content (u,g/g wet weight) determined widi		
Butanol/o-phthaldialdehyde (Maickel et al. 1968)	Butanol-HCl (Udenfriend 1962)	Butanol-Ninhydrin (Snyder et al. 1965)
0.489-0.495	0.297-0.778	0.481-1.411
(3)	(7)	(8)

Numbers in parentheses indicate number of experiments (worms).

to stain in whole mounts (Gustafsson 1991; Gustafsson et al. 1994, 1995a), is best studied in section; it can be seen in *T. nodulosus* to have both 5-HT-IR and RF-IR fibers in the brain ganglia and commissure, with processes reaching close to the hooks and the parietal plate, often terminating at the basal lamina of the tegument of the scolex. This conforms with data on the intense peptidergic and aminergic innervation in holdfast organs in other parasitic flatworms (for review, see Gustafsson 1992).

The 5-HT-IR and RF-IR cells along the MC's of *T. nodulosus* correspond in size and shape, as well as distribution, to the nerve cells Gustafsson (1973) described surrounding the MC's. The 5-HT-IR and the RF-IR material is localized to separate sets of neurons, with the 5-HT-IR neurons restricted to the central NS and the RF-IR neurons occurring both in the central NS and the peripheral NS (Fig. 27). Many other flatworms show a similar pattern (for references, see Reuter & Gustafsson 1995). The specific occurrence of RF-IR cells in the transverse ring commissures has been observed in the parasitic flatworms *Gyrodactylus salaris* (see Reuter 1987), *D. dendriticum,* and *Eubothrium crassum* (see Gustafsson 1991) and proseriates among the free-living flatworms (Joffe & Reuter 1993; Reuter et al. 1995). The single 5-HT-IR neuron in the brain commissure of *T. nodulosus* corresponds to the four 5-HT-IR neurons found in the brain commissure of *D. dendriticum* (see Gustafsson 1990).

Table 2. Concentration of 5-HT in the intestine of the pike.

5-HT content (u-g/g wet weight) determined with	
Butanol/o-phthaldialdehyde (Maickel et al. 1968)	Butanol-HCl (Udenfriend 1962)
0.560-2.850	0.837-2.854
(7)	(13)

Numbers in parentheses indicate number of experiments (fishes).

The number of cell bodies in a cestode brain has seldom been reported. We found 11 neurons in the commissure of *T. nodulosus,* a number within the same order of magnitude as the 13 in the brain commissure of *D. dendriticum* (Gustafsson 1990; Gustafsson & Eriksson 1992; Gustafsson et al. 1993, 1994).

The genital ducts of *T. nodulosus,* like those in other free-living and parasitic flatworms (Reuter et al. 1984, 1988; Gustafsson 1992; Joffe & Reuter 1993; Gustafsson et al. 1994), showed strong peptidergic innervation. Specifically, the muscular walls of its reproductive tract appeared innervated by RF-IR nerves. The gonads themselves, however, were not innervated by either RF-IR or 5-HT-IR nerves.

Ultrastructure

The nerve cells of *T. nodulosus* can be seen by electron microscopy to be of a highly secretory nature, as is typical for flatworm neurons (for references, see Gustafsson 1992). We found five different types of neurons in *T. nodulosus,* distinguishable by size, general cytoplasmic density, and type of vesicles they contain. Correlates of three of these—the "light" neurons, the large multipolar neurons, and the neurosecretory neurons—occur in *D. dendriticum* (Gustafsson & Wikgren 1981; Gustafsson, unpubl.) and in *Grillotina erinaceus* (see Biserova 1991). Similarly, correlates of the "light" and the large multipolar neurons have been found (Bockerman et al. 1994) in the free-living flatworm *Geocentrophora waginii.* Deep invaginations of the neuronal plasma membrane, as we found in the large multipolar neurons of *T. nodulosus* (as well as in such cells in *D. dendriticum* and in the neurons of the free-living flatworm *G. waginii),* are typical for neurons in cestodes (Golubev 1982).

A classification of neurons with respect to function and neuronal vesicle types is problematic or impossible on the basis of ultrastructure alone (Reuter 1991; Reuter & Gustafsson 1995). Vesicle ultrastructure appears to depend on developmental stage, processing of neuroactive substances, and co-occurrence of neuroactive substances. Thus we cannot equate unequivocally any of the five types of neurons identified by electron microscopy with neurons shown reactive to 5-HT or RFamide by light microscopy. Dense-cored vesicles like those in the dominant type of neuron in *T. nodulosus,* the light neurons, have been found in other flatworms to be reactive to anti-RFamide, however (Reuter et al. 1990; Brownlee et al. 1994); and our preliminary tests with immunogold-labeling on *T. nodulosus* point to the same identity (N. M. Biserova and M. Reuter, unpubl.).

The population of nerve cells increases continuously

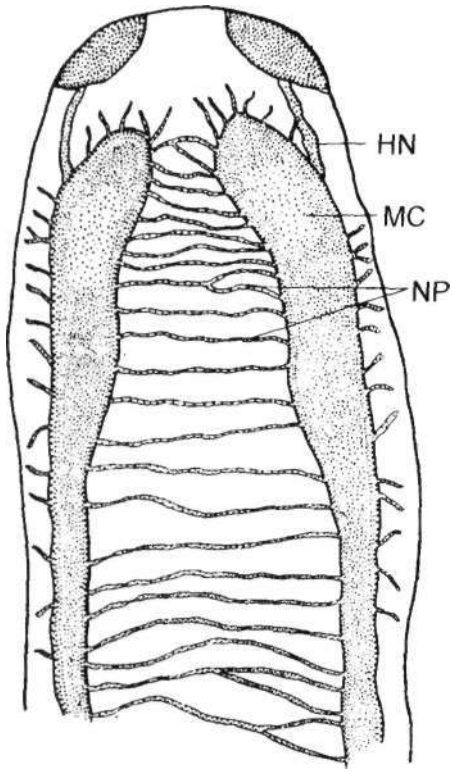

Fig. 26. Schematic drawing of the actylcholinesterase activity in the nervous system of adult worms, *T. nodulosus* (after Kotikova & Kuperman 1977). Main cord (MC), hook nerve (HN), neuronal plexus (NP). Worm is 0.5 mm wide at the neck.

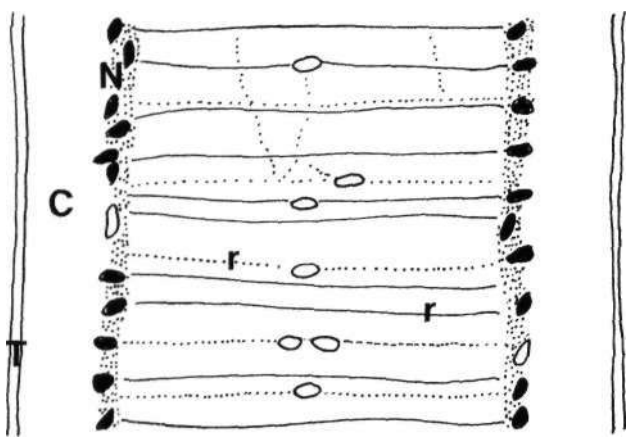

Fig. 27. Diagram showing distribution of 5-HT-IR cell bodies (filled cells) and RF-IR cell bodies (unfilled cells) in neck region of adult worms, *T. nodulosus.* The 5-HT-IR cell bodies are confined to the two main nerve cords (N), while the RF-IR cell bodies occur both in main nerve cords and in transverse ring commissures (r). Cortical parenchyma (C), surface tegument (T). Worm is 0.5 mm wide at the neck.

in tapeworms, which are characterized by never-ending growth. Germinative cells in the neck region of *D. dendriticum,* for example, migrate from the surrounding parenchyma into the MC's at a rate of 34% in two days (Gustafsson 1976). Such cells, which are at the beginning of their neuronal differentiation, are among the cells along the MC's of *T. nodulosus.* Their further differentiation takes place close to or within the MC's. Cells reactive for basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF) have been found in the brain and MC's of *D. dendriticum* by Gustafsson & Eriksson (1992) and Gustafsson et al. (1995b). One of the most prominent effects of bFGF on neurons is promotion of neurite outgrowth—i.e., neuronal differentiation (for references, see Westermann et al. 1990).

Several types of neurotransmitter-release sites, including true chemical synapses, have been identified in flatworms (for references, see Reuter & Gustafsson 1995); but the morphology of these sites varies significantly, and it is difficult to draw a distinction between even synaptocrine and paracrine release, particularly in light of the lack a circulatory system in flatworms. *T. nodulosus* has at least three types of synapses and a neuromuscular junction. Its synapses fullfill the criteria for conventional synapses and are similar to those described by Gustafsson (1984) from *D. dendriticum.* The paramembranous density that occurs in the presynaptic terminals with small clear vesicles is characteristic of synapses both in some flatworms and in arthropods, and this similarity has raised a question of their parallel evolution in these two groups (for references, see Bockerman et al. 1994; Reuter & Gustafsson 1995). The neuromuscular junctions in *T. nodulosus* are of the same type as those in *D. dendriticum* (see Gustafsson & Wikgren 1981) and *G. waginii* (see Bockerman et al. 1994). Release appears to take place toward both contractile and noncontractile parts of the muscle cell.

Spectrofluorimetry

Biogenic amines appear rather spottily among members of the phylum Platyhelminthes (Terenina 1984, 1991; Eriksson et al. 1993a,b; Eriksson 1995; Reuter & Gustafsson 1995; Terenina et al. 1995). In cestodes, however, 5-HT seems to be ubiquitous. It has been detected in all genera that have been tested through radioenzymatic, histochemical, and immunocytochemical methods (for references, see Terenina et al. 1995). The level of 5-HT in *T. nodulosus* (0.297-1.411 u,g/g wet wt) is of the same magnitude as that in *Mesocestoides vogae* (0.333-1.146 u,g/g wet wt; Terenina et al. 1995) and in larvae of *D. dendriticum* (6.0 ± 0.7 nmol/g fresh wt; Eriksson et al. 1993a).

Cestodes are able to actively absorb 5-HT from their environment (Hariri 1975; Gyr et al. 1983); thus, their 5-HT could be of host origin. The levels of 5-HT, in the rat-tapeworm *Hymenolepis diminuta,* for example, and in the intestine of its rat host are positively correlated (Cho & Mettrick 1982; Terenina et al. 1986). As the level of 5-HT in the intestine of the pike clearly exceeds that of its parasitizing tapeworm, the worm could conceivably rather easily absorb 5-HT from its host.

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