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Synapses in Diphyllobothrium dendriticum (Cestoda). An electron microscopical study

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The ultrastructure of the synapses in *Diphyllobothrium dendriticum* (Cestoda) was studied. Three main types of vesicles were observed: 1) dense-core vesicles regarded as aminergic, 2) small clear vesicles regarded as cholinergic and 3) large dense vesicles regarded as peptidergic. On the basis of vesicle content and presynaptic morphology the synapses were divided into two main classes: A) Aminergic synapses characterized by dense-core vesicles and pronounced postsynaptic densities (asymmetrical). Single, shared and multiple synapses occur. Several types of postsynaptic elements were observed. B) Cholinergic synapses characterized by small clear vesicles and pre- and postsynaptic densities (symmetrical). Single and shared synapses occur.

The central position of the aminergic synapses in relation to other nervous and non-nervous elements is discussed.

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1. Introduction

The nervous system (NS) of platyhelminths has been studied both at the light-microscopic and electron-microscopic levels. The pertinent literature has been reviewed extensively with the emphasis on the Classes Turbellaria, Trematoda and Cestoda by Reuter and co-workers (Reuter & Lindroos 1979a, b; Reuter et al. 1980, Reuter 1981), Shaw (1981, 1982) and Gustafsson & Wikgren (1981a, b, c) respectively. The ultrastructure of synapses in cestodes has, however, received little attention.

The present paper is a detailed study of the fine structure of the chemical synapses in the central (CNS) and peripheral nervous system (PNS) of the pseudophyllidean tapeworm *Diphyllobothrium dendriticum*. An attempt at a classification of the synapses has been made. The classification is based on the characteristic type of vesicle in the terminal and follows the principles discussed by Gray (1959), Colonnier (1968) and Uchizono (1976).

2. Material and methods

Plerocercoid larvae of *Diphyllobothrium dendriticum* Nitzsch, 1824 (Cestoda, Pseudophyllidea) were obtained from whitefish (*Coregonus lavaretus*) from Lake Pyhäjärvi, SW Finland. The larvae were excised from the stomach wall and fixed at 4° C in either 3 % glutaraldehyde in 0.3 M cacodylate buffer, pH 7.4 and postfixed in 1 % osmic acid in cacodylate buffer; or in 2.5 % glutaraldehyde in 0.2 M collidine buffer, pH 7.4 and postfixed in 1.33 % osmic acid in collidine buffer, dehydrated, and embedded in epon. Thin sections were stained with uranyl acetate and lead citrate and examined with a UEMV-100V (USSR) electron microscope or a Zeiss EM 109 electron microscope.

3. Results

The gross anatomy of the NS of *D. dendriticum* has been described by Gustafsson & Wikgren (1981a, b) and is schematized in Fig. 1. The neuropile is composed of a dense network of unmyelinated nerve fibres. No extracellular stroma is present between the fibres (Fig. 2). The nerve fibres are tightly packed in the longitudinal nerve cords extending posteriorly in the

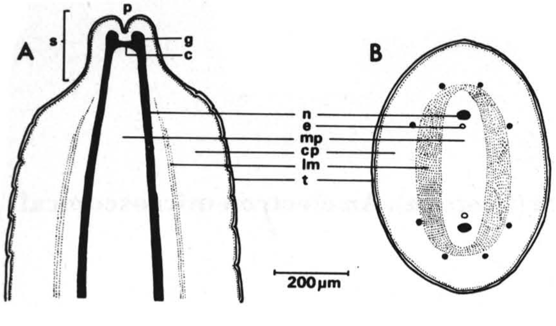


Fig. 1. Diagram showing the nervous system in the scolex (s) and the neck region of a *Diphyllobothrium dendriticum* plerocercoid.
A. Longitudinal section showing the brain consisting of two cerebral ganglia (g) and a connecting commissure (c). From the ganglia the two main nerve cords (n) extend through the medullary parenchyma (mp). Frontal pit (p).
B. Cross section through the neck region showing the eight peripheral nerve cords (●) in the cortical parenchyma (cp). Excretory duct (e), tegument (t), longitudinal muscle layer (lm).

medullary and cortical parenchyma. In the nerve fibres the following types of vesicles occur:

1. dense-core vesicles (dcv), diameter 60–120 nm.
2. small clear vesicles (sv), diameter 30–50 nm.
3. large dense vesicles (ldv), diameter 80–320 nm.
4. large lucent vesicles (llv), diameter 60–120 nm.

The vesicles occur in different combinations in the nerve fibres. The following types of nerve fibres and synapses were observed.

3.1. Types of nerve fibres

Nerve fibres containing dense-core vesicles

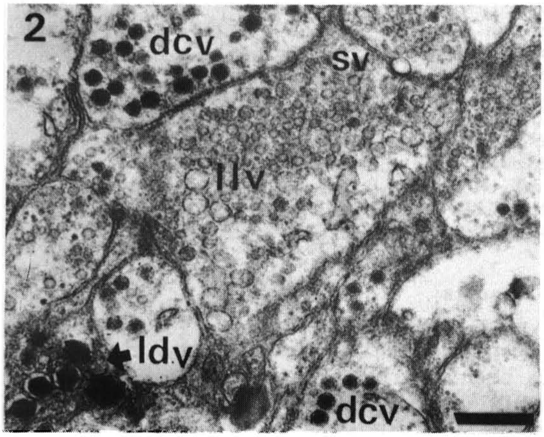
Nerve fibres containing dense-core vesicles dominate the CNS and the PNS of the worm. Alongside the dense-core vesicles small clear vesicles occur (Fig. 2). These nerve fibres belong to the aminergic neurons described by Gustafsson & Wikgren (1981a).

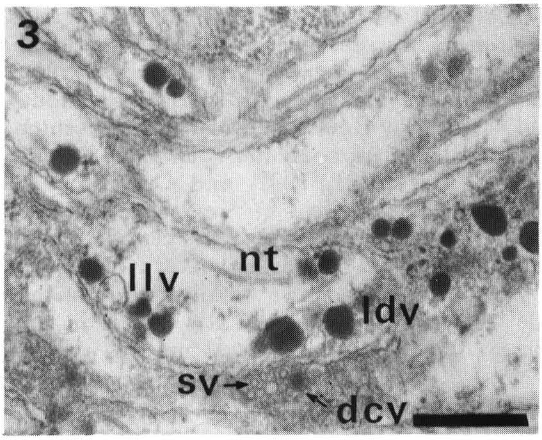
Nerve fibres containing small clear vesicles

Small clear vesicles are frequently present in the nerve fibres of the neuropile and in the nerves of the PNS. They constitute the sole population of vesicles in part of the fibres and tightly fill the profile of the fibre in the vicinity of, and at, a synapse (Fig. 2).

Nerve fibres containing large dense vesicles

Nerve fibres densely packed with large dense vesicles occur frequently in the neuropile (Fig. 2). They also constitute a considerable part of the PNS and innervate the muscle layers and the subtegumental region. Small clear vesicles occur alongside the large dense vesicles and are especially prominent in the nerve terminals.





Figs. 2–3. Electron micrographs of the nervous system in *Diphyllobothrium dendriticum*. Bar = 400 nm.
Fig. 2. Neuropile with tightly packed nerve fibres containing different types of vesicles. Dense-core vesicles (dcv), small clear vesicles (sv), large dense vesicles (ldv), large lucent vesicles (llv).
Fig. 3. Large lucent nerve fibres with mixed vesicle content and of presumed sensory nature. Neurotubules (nt).

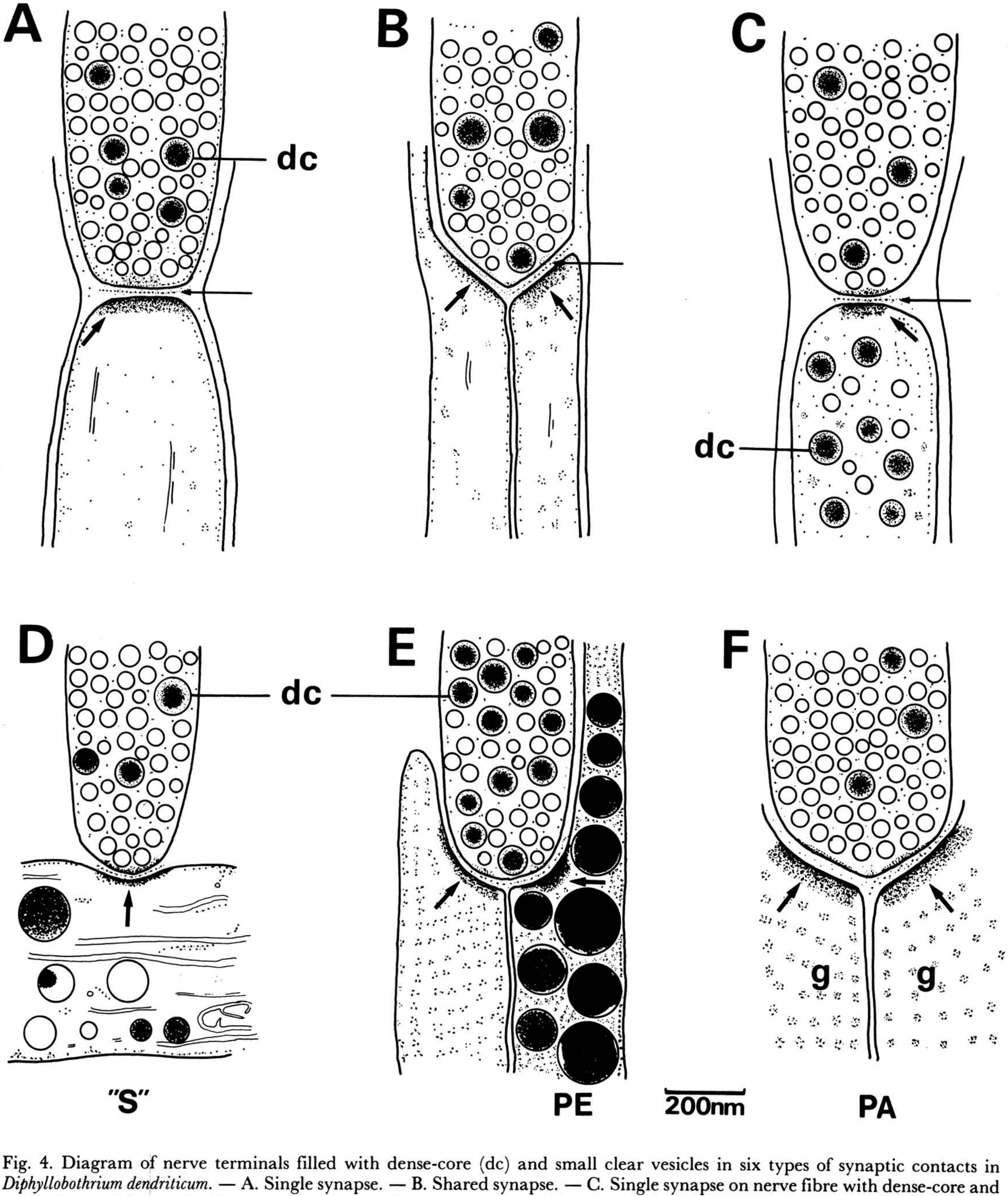
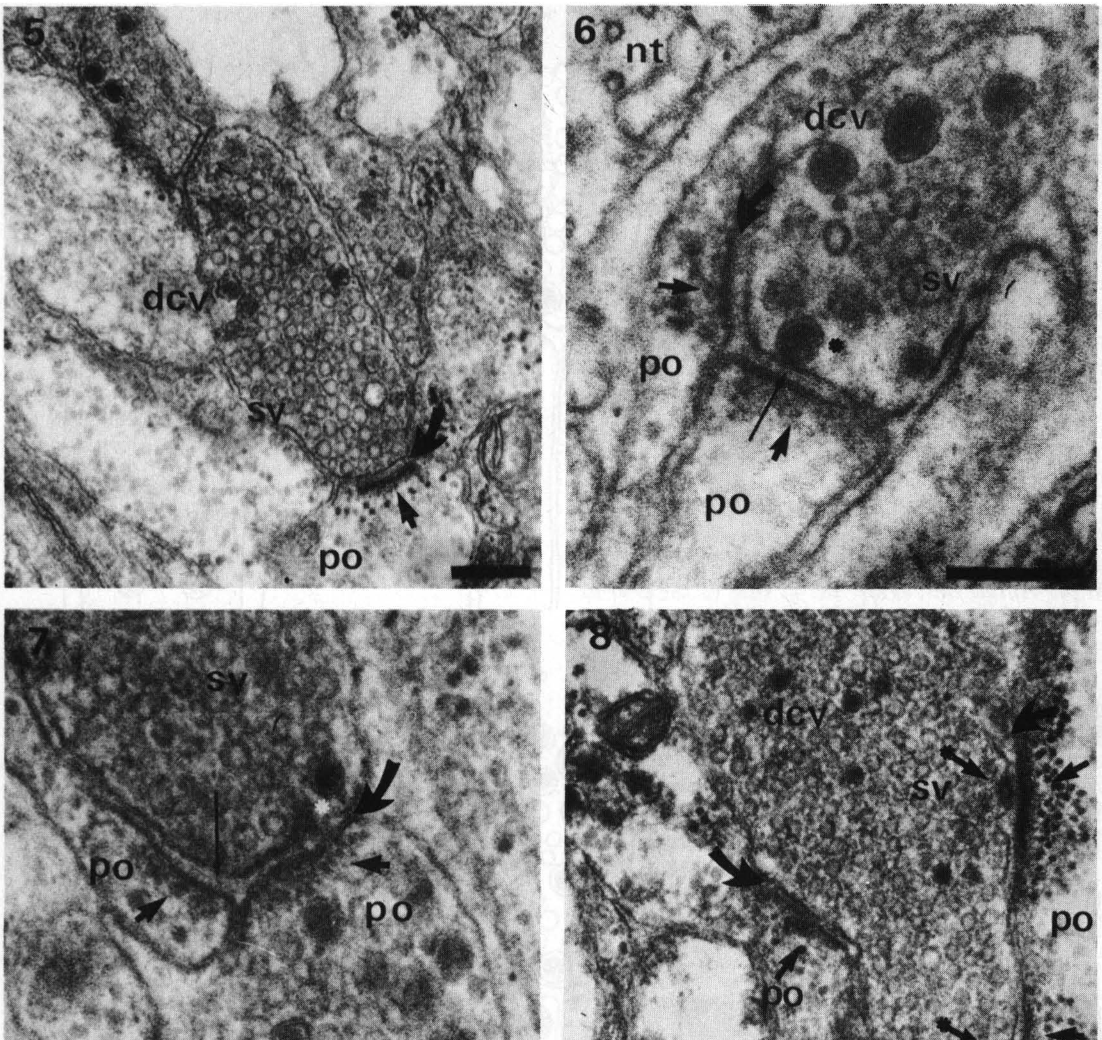


Fig. 4. Diagram of nerve terminals filled with dense-core (dc) and small clear vesicles in six types of synaptic contacts in *Diphyllobothrium dendriticum*. — A. Single synapse. — B. Shared synapse. — C. Single synapse on nerve fibre with dense-core and small clear vesicles. — D. Single synapse on large lucent nerve fibre with mixed vesicle content and of presumed sensory nature ("S"). — E. Shared synapse on peptidergic nerve fibre (PE) and fibre lacking special characteristics. —F. Shared synapse on glycogen-rich (g) parenchyma cells (PA). The postsynaptic densities (arrows) and the synaptic clefts (thin arrows) are well developed in all synapses.



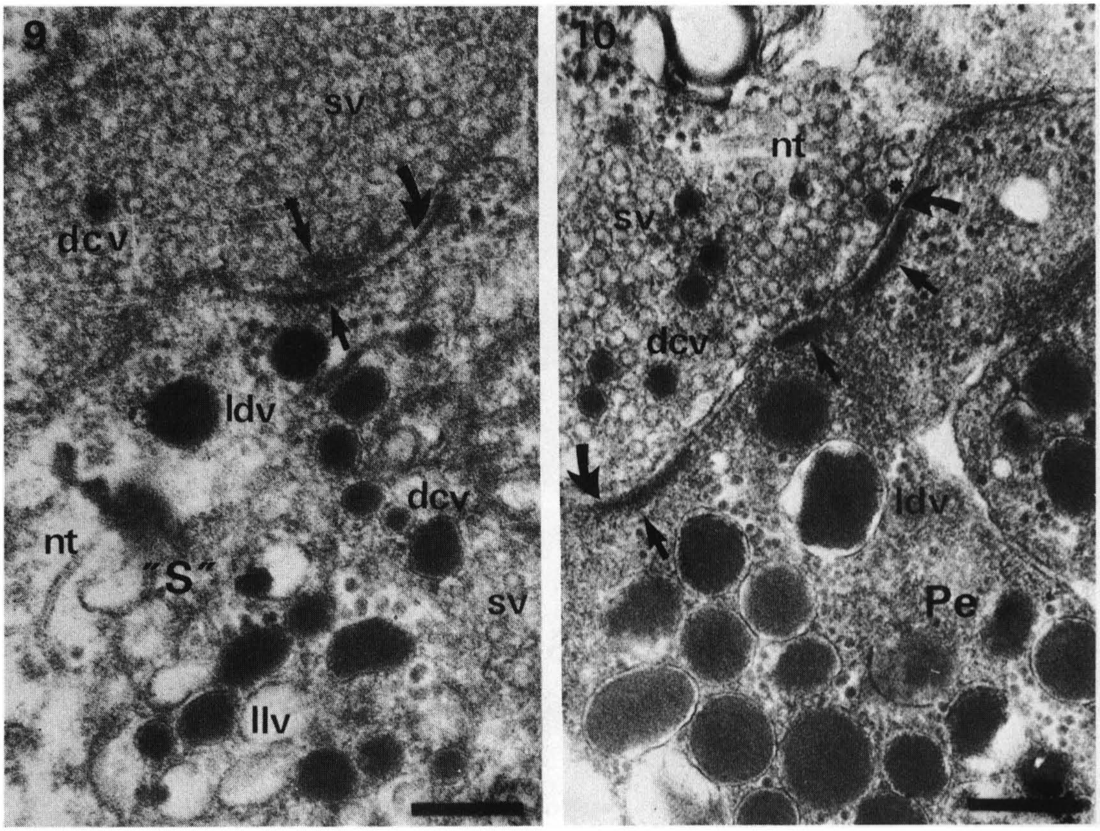
Figs. 5–8. *Diphyllobothrium dendriticum.* Electron micrographs of presynaptic terminals containing dense-core vesicles (dcv) and small clear vesicles (sv). Postsynaptic fibre (po). Bar = 200 nm in all figures.

Fig. 5. Single synapse. Note the well developed postsynaptic density (arrow) and the synaptic cleft (bent arrow).

Fig. 6. Shared synapse. Note the dense-core vesicle (*) close to the synaptic site and the thin membrane (thin arrow) in the middle of the synaptic cleft (bent arrow). The postsynaptic membranes of both nerve fibres are thickened and postsynaptic densities occur (arrows).

Fig. 7. Shared synapse. One of the postsynaptic fibres contains dense-core and small clear vesicles. Note the dense-core vesicle (white *) close to the synaptic site and the thin membrane (thin arrow) in the middle of the synaptic cleft (bent arrow). Both the pre- and the postsynaptic membranes are thickened and postsynaptic densities occur (arrows).

Fig. 8. Multiple synapses. Note the prominent postsynaptic densities (arrows), the smaller presynaptic densities (arrow-star) and the distinct synaptic clefts (bent arrows).



Figs. 9-10. *Diphyllobothrium dendriticum.* Electron micrographs of presynaptic terminals containing dense-core (dcv) and small clear vesicles (sv). Neurotubules (nt). Bar = 200 nm.

Fig. 9. Synapse on a large lucent nerve fibre of presumed sensory nature ("S"). The postsynaptic fibre containing large dense vesicles (ldv), large lucent vesicles (llv), small clear vesicles (sv) and dense-core vesicles (dcv). The postsynaptic membrane is thickened (arrow) and a presynaptic density (arrow-star) occurs. The synaptic cleft is distinct (bent arrow).

Fig. 10. Serial synapse on peptidergic nerve fibre (Pe) containing large dense vesicles (ldv). Note the dense-core vesicle (*) close to the synaptic site, the prominent postsynaptic densities (arrows) and the synaptic clefts (bent arrows).

These nerve fibres belong to the peptidergic neurons described by Gustafsson & Wikgren (1981a, b, c).

Nerve fibres with mixed vesicle content

The fourth type of nerve fibre is characterized by its large size (profiles measure 500-1000 nm in cross section) and very electron-lucent content. Neurotubules were often observed in them. Along the plasma membrane denser material occurs, including a few ribosome-like particles. In these fibres occur large lucent vesicles, small clear vesicles, large dense vesicles and a few dense-core vesicles (Figs. 2-3). This type of nerve fibre is common in the center of the neuropile but also occurs in the PNS associated with sense organs (Gustafsson, unpublished) and is therefore presumed to be sensory.

In addition to the above mentioned nerve fibres, fibres lacking special characteristics occur.

3.2. Types of synapses

The nerve fibres make several types of synaptic contacts. The following pattern was discerned.

Synapses with dense-core vesicles

The most common type of synapse in *D. dendriticum* contains dense-core and small clear vesicles (Fig. 4A-F). The MD of the dense-core vesicles is 70 nm and that of the clear vesicles is 40 nm. The small clear vesicles outnumber by far

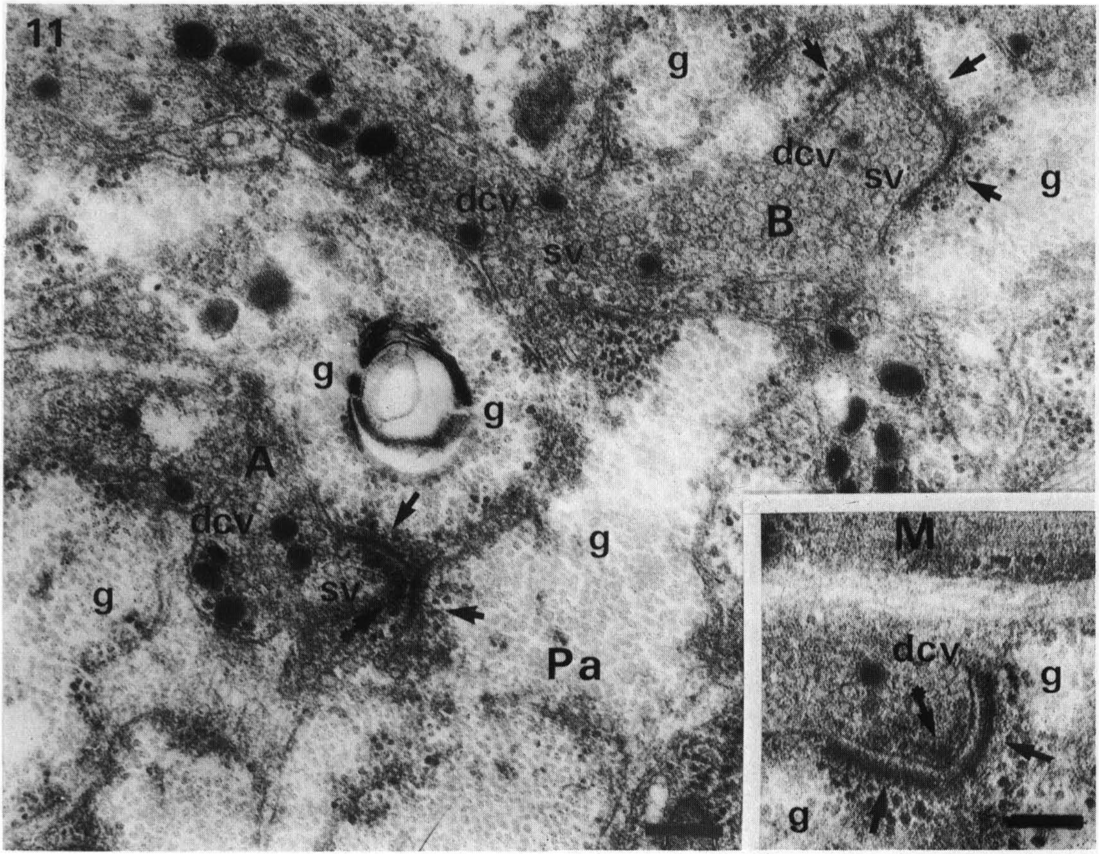


Fig. 11. *Diphyllobothrium dendriticum.* Electron micrograph of two presynaptic terminals (A and B) containing dense-core vesicles (dcv) and small clear vesicles (sv). The synapses are directed towards glycogen-rich (g) parenchyma cells (Pa). Note the prominent postsynaptic densities (arrows). In the shared synapse A a presynaptic density (arrow-star) is present. Inset. Shared synapse with presynaptic density (arrow-star) and postsynaptic densities (arrows) close to muscle fibre (M). Bar = 200 nm.

the dense-core vesicles. Both types of vesicles are tightly accumulated on the presynaptic side. Generally, but not always, the dense-core vesicles are located farther from the synaptic site than the small clear vesicles (Fig. 5). The synaptic cleft is 20 nm wide and filled with material of moderate electron density. Occasionally a thin membrane was observed in the middle of the synaptic cleft (Figs. 6–7). The majority of the synapses lack a presynaptic density and are classified as asymmetrical (Colonnier 1968). If present, the presynaptic density is usually shorter than the postsynaptic density (Figs. 8–9). The postsynaptic density is consistently present and measures approximately 40 nm in width and 100–360 nm in length. Single, shared and multiple synapses were observed (Figs. 5–8).

The postsynaptic elements vary from nerve fibres lacking special characteristics (Figs. 4A–B, 5, 6, 8) to nerve fibres containing dense-core vesicles (Figs. 4C, 7), large lucent fibres with mixed vesicle content and of presumed sensory nature (Figs. 4D, 9) and peptidergic nerve fibres containing large dense vesicles (Figs. 4E, 10).

Synaptic contacts between dense-core terminals and non-nervous elements were also observed. In the parenchyma they were frequently seen directed towards glycogen-containing cytoplasmic areas. The synapses have characteristics common to conventional synapses — i.e. large accumulations of small clear and dense-core vesicles, distinct postsynaptic densities and occasionally presynaptic densities. The synaptic cleft is 20 nm wide. Single, shared and multiple synapses occur (Figs. 4F, 11).

Synapses with small clear vesicles

Synapses characterized by a content of solely small clear vesicles occur both in the CNS and

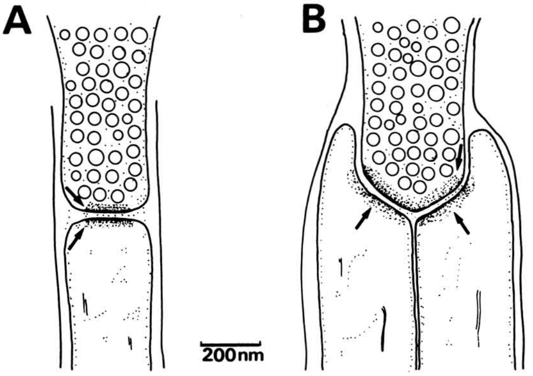


Fig. 12. Diagram of nerve terminals filled with small clear vesicles in two types of synaptic contacts in *Diphyllobothrium dendriticum*: A. Single synapse, B. Shared synapse. Note pre- and postsynaptic densities in both synapses.

PNS. The MD of the vesicles is 40 nm and they are tightly packed on the presynaptic side. The synaptic cleft is approximately 20 nm wide and filled with material of moderate electron density. Pre- and postsynaptic densities occur and are of equal width (Figs. 12A–B, 13). These synapses are classified as symmetrical synapses (Colonnier 1968). Occasionally the postsynaptic density is more pronounced and of greater length than the presynaptic. Single and shared synapses occur at equal frequences. The postsynaptic fibre generally lacks special charateristics.

4. Discussion

The results of this study indicate a high degree of complexity in the NS of *Diphyllobothrium dendriticum*.

4.1. The aminergic synapses

In *D. dendriticum* aminergic neurons characterized by large amounts of dense-core vesicles were described by Gustafsson & Wikgren (1981a). The dense-core vesicles of the synapses in the same worm correspond closely to the dense-core vesicles of the aminergic neurons and these synapses are thus tentatively classified as aminergic synapses. Microspectrofluorometric analysis indicated the presence of 5-HT in *D. dendriticum* (Gustafsson & Wikgren 1981a). Unpublished immunocytochemical studies show a strong reaction to anti-serotonin in the CNS and PNS of the worm. Ribeiro & Webb (1983) have demonstrated the enzymatic capacity of *Hymenolepis diminuta* for the synthesis of 5-HT from both tryptophan and 5-HTP. They discuss the function of 5-HT as a possible excitatory neurotransmitter in the worm. The ultrastructural studies clearly show the domination of the dense-core nerve fibres and synapses in *D. dendriticum.* Most of the synapses have pronounced postsynaptic densities and are classified as asymmetrical (Colonnier 1968). According to Colonnier (1968) asymmetrical synapses generally are excitatory in nature. The questions whether all the dense-core synapses in *D. dendriticum* contain 5-HT and whether they are all excitatory remain to be settled.

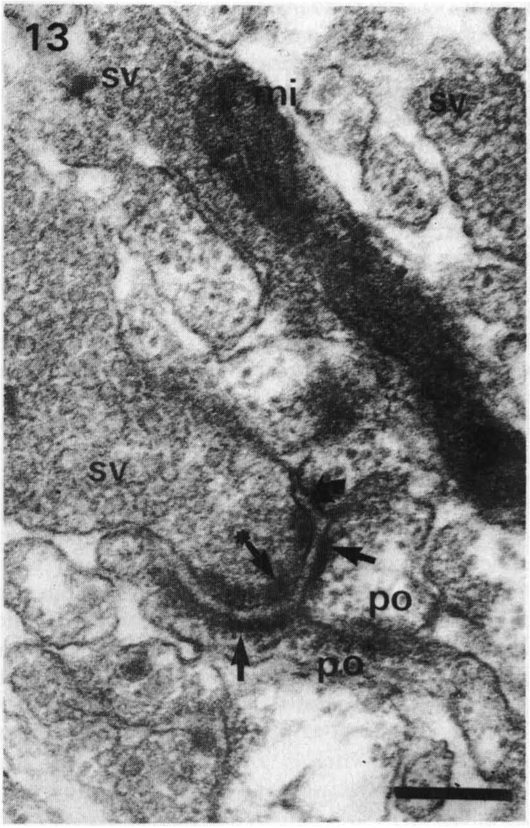


Fig. 13. *Diphyllobothrium dendriticum.* Shared synapse between presynaptic terminal containing small clear vesicles (sv) and two postsynaptic fibres (po). Note distinct pre- (arrow-star) and postsynaptic densities (arrows) and the synaptic cleft (bent arrow). Bar = 200 nm.

The dense-core synapses of *D. dendriticum* show a high degree of diversity and occupy a strategic position in relation to other nervous and non-nervous elements. Dense-core synapses were often observed on the large lucent nerve fibres with a mixed content of vesicles. Nerve fibres with similar characteristics have been described from *H. microstoma* (Webb & Davey 1976) and from the turbellarian *Gyratrix hermaphroditus* (Reuter 1975, Reuter & Lindroos 1979a). In both cases they were regarded as belonging to sensory neurons. Reuter & Lindroos (1979a) describe synapses between dense-core fibres and sensory fibres. Further research into the NS of *D. dendriticum* is needed to certify the status of the large lucent fibres. Dense-core synapses were also seen in connection with the neurites of the peptidergic neurons (Gustafsson & Wikgren 1981a). A similar type of contact between dense-core fibres and peptidergic fibres has been reported from the turbellarian *Microstomum lineare* (Reuter 1981). The peptidergic neurosecretory system of *D. dendriticum* is very prominent and undergoes rapid activation in connection with the transfer of the plerocercoid larvae from the poikilothermic fish host to the homeothermic final host (Gustafsson & Wikgren 1981b, c). An interaction between the aminergic and the peptidergic NS seems to prevail. The dense-core synapses on the glycogen-rich cytoplasmic areas in the parenchyma of *D. dendriticum* fulfill the criteria for conventional synapses (Manolov & Ovtscharoff 1982). In *M. lineare* the dense-core nerve fibres make synapse-like structures with stroma channels in the vicinity of muscle fibres (Reuter 1981). In *D. dendriticum* dense-core synapses directed exactly towards muscle fibres were not observed. However, the muscle cells of *D. dendriticum* have large cytoplasmic areas, which contain α- and β-glycogen (Bonsdorff et al. 1971). It is thus difficult to tell with certainty whether a glycogen-rich cytoplasmic sac belongs to a muscle cell or to a glycogen-storing parenchyma cell.

The very large number of small clear vesicles alongside the dense-core vesicles in the presynaptic terminal is characteristic of the synapses of *D. dendriticum.* The coexistence of small clear and dense-core vesicles in the same nerve cell has been discussed by Patterson et al. (1978). They have studied the development of cholinergic neurons from adrenergic neurons.

Dense-core vesicles have been mentioned in connection with synapses in other flatworms (see f.i. Webb 1976, Dei-Cas et al. 1980, Reuter & Lindroos 1979b, Reuter 1981, Fairweather & Threadgold 1983). The occurrence of more than one synaptic site in the same presynaptic terminal towards several postsynaptic fibres is rather common in *D. dendriticum* and has been interpreted as an indication of integrative processes taking place. Synaptic convergence was not observed in *D. dendriticum.*

4.2. The cholinergic synapses

The presence of AChE in *D. dendriticum* was histochemically demonstrated by Öhman-James (1968). The small clear vesicles which constitute the sole population of vesicles in part of the synapses of the worm are morphologically identical with vesicles regarded as containing ACh (Manolov & Ovtscharoff 1982) and these synapses are tentatively regarded as cholinergic. Ultrastructural localization of AChE in the worm has not yet been made.

The classification of the cholinergic synapses in *D. dendriticum* is difficult. On the basis of well developed pre- and postsynaptic densities and spherical vesicles, they can be referred to the type-I of Gray (1959) and the type-S of Uchizono (1976), both of which are supposed to be excitatory. However, according to Colonnier (1968) the symmetrically arranged synaptic densities are characteristic for inhibitory synapses. Support for an inhibitory function is gained from *H. diminuta* in which ACh serves as an inhibitory neurotransmitter (Wilson & Schiller 1969).

The cholinergic synapses of *D. dendriticum* conform with synapses from other flatworms. Minor differences can be discerned. The synapses of *Echinococcus granulosus* have a rather wide synaptic cleft (approx. 30 nm) and lack distinctive pre- and postsynaptic densities (Morseth 1967). In *H. microstoma* a presynaptic network is reported and the presynaptic density seems to be shorter than that of *D. dendriticum* (Webb 1976). According to Fairweather & Threadgold (1983) the most common synapses of *H. nana* contain small clear vesicles (MD=32 nm). However, occasionally large clear vesicles (MD=59 nm) were observed in the presynaptic terminals. In the Class Turbellaria the ultrastructure of the NS of both primitive and advanced groups has been studied (for references see Reuter & Lindroos 1979b). In the primitive Catenulidae the synapses are unpolarized and slightly differentiated (Moraczewski et al. 1977). In the more advanced *G. hermaphroditus* four kinds of synapses were found (Reuter & Lindroos 1979b). This multiplicity indicates a high organizational level. Scattered data about the structure of synapses containing small clear vesicles in trematodes are available (for references see Shaw 1981). He points out that variation in the prominence of paramembranous synaptic densities is common within this group and discusses its possible causes — variation in preparation procedures, developmental stages or synaptic function.

In a recent review, Lumsden & Specian (1980) discuss neurotransmitters and neuromodulators in *H. diminuta*. They suggest that 5-HT serves as an excitatory neurotransmitter, ACh as an inhibitory neurotransmitter and neurosecretory substances as general modulators for metabolic processes. The results of this study hint in the same direction.

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