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ULTRASTRUCTURAL STUDY OF TYPE "B" NEUROSECRETORY CELLS IN THE EARTHWORM NERVOUS SYSTEM

By

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ABSTRACT

Neurosecretory cell types B_1-B_5 are widely distributed throughout the cerebral, subesophageal, nerve cord and peripheral ganglia of the oligochaete *Aporrectodea caliginosa, Lumbricus terrestris, Eisenia foetida, Octolasion cyaneum, Dendrobeona subrubicunda* and *Allolobophora longa*. Cell types are distinguished by certain cytological and ultrastructural features. Nerve fibres of each cell type extend centrally and/or peripherally in each ganglion where release of neurotransmitters/neuromodulators take place by exocytosis. Elements of neuromuscular junctions, within the perineurium, are compared with the body wall and heart muscle junctions to detect the origin of neuromuscular fibres. Other ultrastructural elements were also investigated by using zinc iodide osmium tetroxide reagent.

INTRODUCTION

Neurosecretary (Nsy) cell types A_1—A_5, SEF and C have only been found within the cerebral ganglia of a variety of earthworm species and exhibit special cytological and ultrastructural features (Aros *et al.*, 1977; De Morais *et al.*, 1979; Al-Yousuf, 1987b and 1988a). In contrast, divergent opinions have been expressed about the distribution of type B cells, "the large and medium-sized neurones" which are less superficial in position and larger in size than "A" cells. (Herlant-Meewis 1966, 1967). These cells correspond to "B" or "Large" cells which were described by Aros *et al.* (1965) as unipolar cells containing Gomori-positive granules, although they were not specialised for a secretory function as the "A" cells. In a further study, the same authors referred to type "B" cells as Gomori-negative cells (Aros *et al.*, 1977). In *Dendrobaena otheca cernosvitae*, "B" cells were shown to be distributed in all parts of the central nervous system and corresponded to the large and medium-sized cells of Herlant-Meewis (1966, 1967), (Baid and Gorgees 1977).

Ultrastructurally, there are few presented electron micrographs of unclassified "B"

cells (Rehlich *et al.*, 1962, Pellegrino De Irladi and Roberts, 1962; Takeuchi, 1967; Berjon and Meunier, 1968; Gallissian and Girardie, 1972). In *pheretima hilgendorfi,* the observations carried out by transmission electron microscopy (TEM) showed that the granules of type "A" cells were larger and less electron dense than those of type "B" cells which were Anti-AVP-reactive cells (Kinoshita and Kawashima, 1986). In this study "B" cells were differentiated from "A", SEF and "C" cells on the basis of their location, stain affinity and ultrastructural characteristics.

Hubl (1956) first described "U" cells in the subesophageal ganglion and suggested that they constitute a homogeneous population situated ventrally in the anterior region, close to the origin of the circumesophageal connective. Corresponding cells were described by several authors, (Takeuchi; 1967, Baid and Gorgees 1977).

In the nerve cord, Nsy cells were described in each segmental ganglion. Gomori-positive cells have also been observed in the peripheral ganglia of the vegetative nervous system (Aros *et al.*, 1965; Teichman *et al.*, 1966; Baid and Gorgees, 1977; Zahid; 1977). With exception of this study, no attempt has been made to correlate these cells with the brain-cell types.

Within the cerebral ganglion of *L. terrestris,* Aros *et al.* (1977) observed that Nsy cell processes project centrally into the storage zone, while other peripheral Nsy fibres form a primitive neurohaemal area or function as vasomotor nerves, but no clear distinction emerged from their study. In the present study, Nsy cells of the subeosophagael, nerve cord and peripheral ganglion are compared with those of the cerebral ganglion. Release sites and neuromuscular junctions are also described and compared with those of body wall and heart junctions.

MATERIALS AND METHODS

The earthworms *Aporrectodea caliginosa were collected from Rowdat Al-Faras, State of Qatar, The earthworm Lumbricus terestris, Eisenia foetida, Allolobophora longa, Dendrobeona subrubicunda,* and *Octolasion cyaneum* were kindly supplied by the Rothamstead Research Centre, England. Freshly dissected cerebral subesophageal, nerve cord and peripheral ganglia and body wall and heart muscles were fixed in different fixatives at 4^0C as follows: (a) 2.5% glutaraldehyde in 0.1 M, 0.075 M or 0.05 M phosphate (PO_4) buffer at pH 7.2 for 1 hr, rinsed in 0.1 M (PO_4) for 15 min and post-fixed in 1% OsO_4 in 0.1 M (PO_4) at pH 7.2, for 1 hr. (b) 0.8% glutaraldehyde + 1% OsO_4 in 0.05 M (PO_4) buffer at pH 7.2 for 1/2 hr, rinsed in 0.05 M PO_4 buffer at phH 7.2 for 15 min and fixed in 1% OsO_4 for 1 hr. (c) 1% OsO_4 in 1 M (PO_4) buffer at pH 7.2 for 1 hr, then rinsed in 0.1 M (PO_4) for 15 min. (d) 1% OsO_4 in sodium veronal acetate buffer at pH 7.4 for 1 hr. and then rinsed in the same buffer for 15 min. The results obtained with the different

fixatives are similar to those reported by (Al-Yousuf, 1988a). The zinc iodide-osmium terioxide (ZIO) staining method was applied according to the method of Akert and Sandri (1968).

Subsequently, the specimens were washed in buffer, dehydrated in an ethanol series and processed for epoxy embedding. Semi-thin sections (1 μm) some times serially prepared, were cut on LKB Ultratome, stained with 1% toludine blue (TB) in 1% borax solution, rinsed, dried and examined with the light microscope (LM) for a general tissue survey. Semi-thin sections (0.5μm) were also stained with paraldehyde fuchsin (PAF) after partial resin removal with saturated sodium methylate in methanol and oxidation in peracetic acid as described by Steel and Morris (1977). Conventional histological sections stained with PAF were also prepared as suggested by Gabe (1966). Sections mounted on slides were examined by LM and adjacent ultrathin sections were double stained with uranyl acetate and lead citrate and examined by TEM/KORA (Kratos Ltd.) or TEM/JOEL operating at 80 Kv or 120 Kv respectively.

The study of release sites within the perinerium involved examination of serial thin sections of the neuromuscular junctions of the body wall and heart muscles and comparing them with the neuromuscular junctions of the cerebral, subesophageal and nerve cord ganglia.

RESULTS

Description of B_1-B_5 Nsy cells within the cerebral ganglion:

Type B_1-B_5 Nsy cells were widely distributed throughout the nervous system with a highly distinctive ultrastructural appearance from other elements. The criteria used to distinguish the various types of cells were: (1) staining affinity for TB and PAF stains; (2) position, form and average size of the cell and (3) size and appearance of the Nsy granules. The equivalence between cell types described with LM and TEM, respectively, was established, by TB staining performed on semi-thin sections for the LM adjacent to thin sections for the TEM. The standard used in this description was the morphology of Nsy cells within *A. caliginosa* fixed in 1% OsO_4 in veronal acetate buffer where cell type identification was made then a comparative survey was made for *L. terrestris, E. foetida, A. longa, D. subrubicunda* and *O. cyaneum*. The sizes of perikarya and Nsy granules vary from one species to another (Table 1).

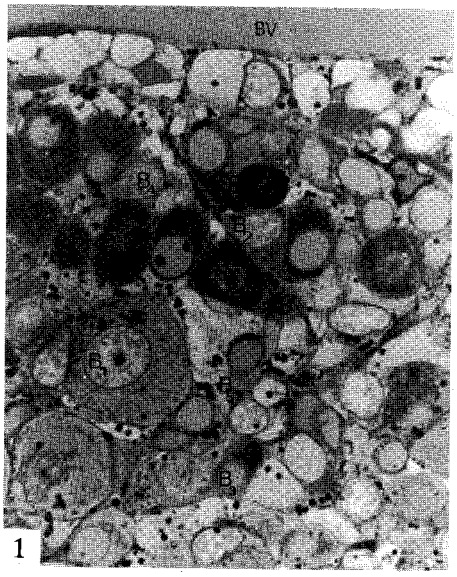
Type B_1 cells are rounded bipolar cells, measuring about 38 x 42 μm, (Figs. 1-2). They stained lightly with TB, and were partially PAF-positive in semi-thin sections. Profiles bearing inclusions of the appropriate granules occur within both the neuropile and perineurium. These cells possess large cytoplasmic areas occupied by

Table 1

Cell size range (μm) of (B_1–B_5) cell types in the cerebral ganglia in various species.

Species	B_1 cells	B_2 cells	B_3 cells	B_4 cells	B_5 cells
A. calignosa	38 x 42	22 x 33	18 x 26	14 x 24	13 x 20
L. terrestris	38 x 41	22 x 31	16 x 25	14 x 22	13 x 20
E. foetida	30 x 34	17 x 25	12 x 20	11 x 19	10 x 16
O. cyaneum	38 x 40	21 x 31	16 x 24	15 x 22	12 x 19
D. subrubicunda	31 x 35	20 x 27	14 x 23	13 x 20	9 x 17
A. longa	39 x 43	23 x 37	17 x 26	14 x 23	13 x 21

a rough endoplasmic reticulum (RER) which forms huge vacuoles enclosing a fine material of low electron density (Fig. 2). The Nsy granules measured about 900-2100^0 A in diameter and contained moderately dense, homogenous cores surrounded by irregular narrow halos. They occur in prominent rounded clusters (Fig. 3).



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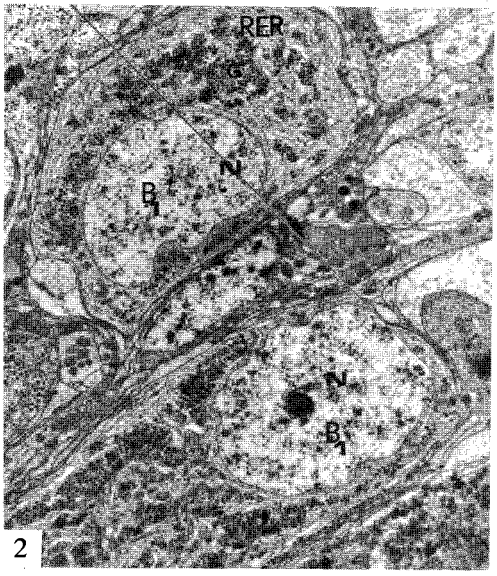

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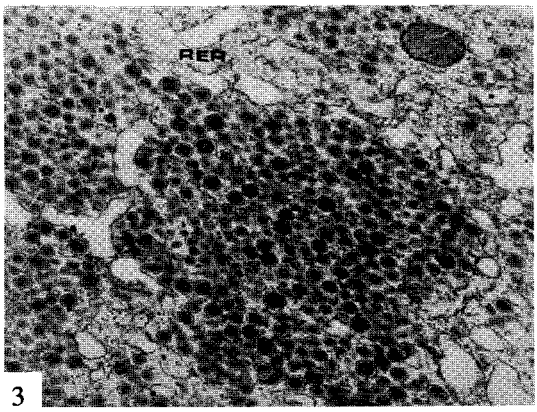
Fig. 1: Sagittal semi-thin section stained with TB showing variations of staining affinity and dorso-posterior locations of NSY (B_1–B_5) cell types. Note blood vessel (BV) outside the cerebral ganglion. X. 240.

Fig. 2: Type B_1 cells with distinctive vacuolation of rough endoplasmic reticulum (RER). Nsy granules (G), Nucleus (N). X. 11.000.

The B_2 cells are unipolar with variable form, measuring about 22 x 33 μm, and stained lightly with TB and negatively with PAF stain. They are monopolar with axons extending to the neuropile (Fig. 1). The perikarya shows prominent glial inpushings — the "trophospongium" effect (Fig. 4a). The Nsy granules are typically irregular in shape with dense cores surrounded by large halos, measuring about 800-2000^0 A in diameter (Fig. 4b).



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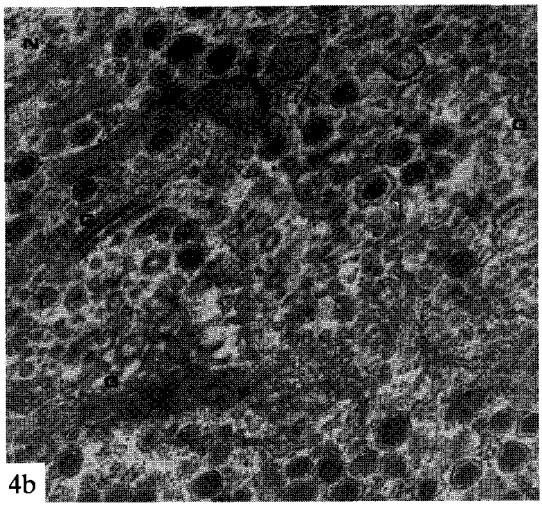


4b

Fig. 3: Type B_1 cell showing distended RER, the medium size and density of the Nsy granules. X. 33.000.

Fig. 4a: Type B_2 cell showing the "trophospongium" (Arrowes). Note elementary granules (E6). X. 33.000.

Fig. 4b: Type B_2 cell showing wide halo surrounding the dense Nsy granules. Golgi bodies (G), nucleus (N). X. 55.000.

Type B_3 cells are medium sized, measuring about 18 x 26 μm, typically pear shaped (Fig. 1) and stained darkly with TB and PAF stains. They are monopolar with axons extending to the neuropile. They contain numerous rounded Nsy granules, 1100-2400^0 A in diameter, with extremely dense homogenous cores surrounded by very regular narrow halos (Fig. 5).

Type B_4 cells are pear shaped, and measuring about 14 x 24 μm. They are PAF-negative and stained very slightly with TB (Fig. 1), They are bipolar with axons extending to the perineurium and neuropile. They contain Nsy granules of variable shape, often being elongate. The Nsy material is of low electron opacity and fills disrupted enclosing membrane. Many small granular vesicles (600-800^0 A in diameter) intermingle with larger ones (1200-1900^0 A in diamter) (Fig. 6).

Type B_5 cells are so small that they may be spotted only by TEM, measuring about 13 x 20 μm. They contain a small number of rounded or oval granules (900-1200^0 A in diameter) with intensely dense homogenous cores surrounded by wide halos (Fig. 7).

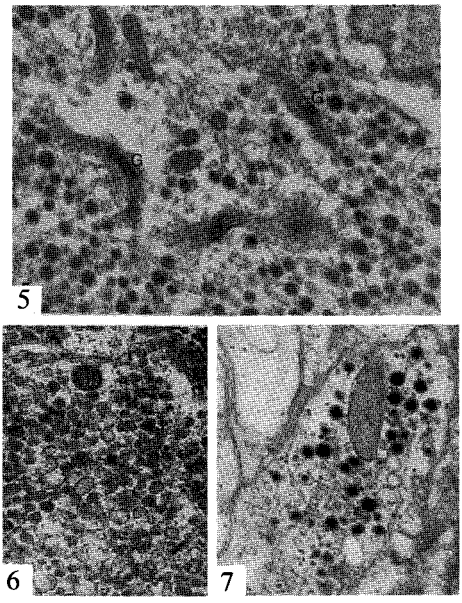


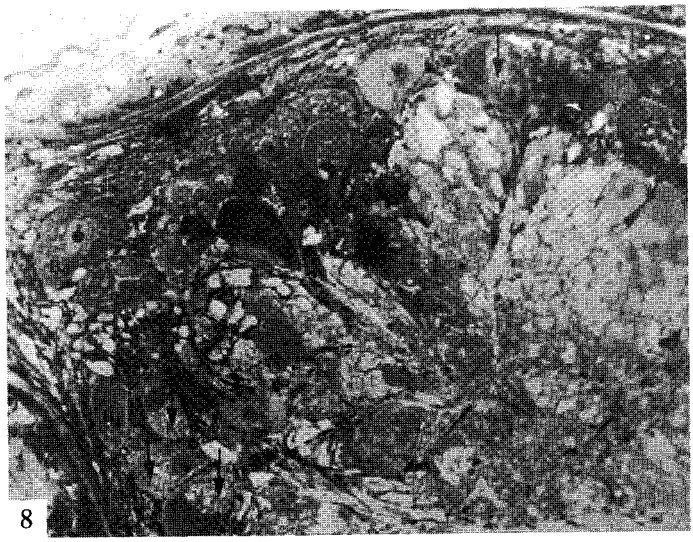
Fig. 5: Type B_3 cell (OsO_4/PO_4 fixation) showing peptidergic appearance of the dense granules with the surrounding halo, Nucleus (N), Golgi body (G). X. 55.000.

Fig. 6: Type B_4 cell showing low electron density of the irregular shaped granules and small granulated vesicles. X. 33.000.

Fig. 7: Part of type B_5 cell with extremely dense granulated vesicles surrounded by a halo. Note endocytatic profile (★). X. 33.000.

Cell B_1-B_5 types within the subesophageal and nerve cord ganglia:

In the 1 μm sections, only one cell type with a pronounced affinity for TB and PAF stains is observed in the nerve cord ganglia and measure 20 x 39 μm (Fig. 8). In the subesophageal ganglion only a few of these are observed and measure 14 x 30 μm. These cells have numerous Nsy granules measuring 1200-2300° A in diameter in the subesophageal ganglion, (Fig. 9), while those in the nerve cord measure 1100-1800° A in diameter. These granules are ultrastructurally similar to those of type B_3 cells in the cerebral ganglion (Fig. 10).



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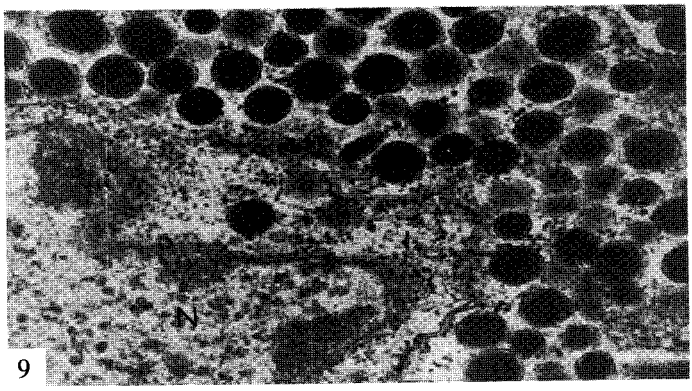

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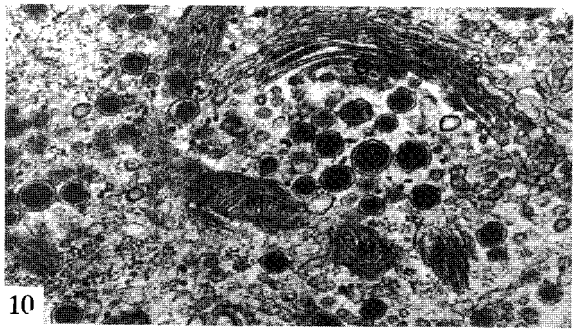
Fig. 8: Semi thin sections of the *L. terrestris* nerve cord showing type B_3 cell (large arrow) with strong affinity for TB stain and B_1 cell with vaculated perikarya (small arrows). Note also type B_4 cells. X. 600.

Fig. 9: Type B_3 cell of the subesophageal ganglion showing the high density of Nsy granules surrounded by thin halos. Nucleus (N). X. 55.000.

Other cells similar in size, staining affinity and ultrastructure to types B_1, B_2, B_4, and B_5 respectively, in the cerebral ganglion; are also observed in the nerve cord and subesophageal ganglia.

Cell types within the peripheral nervous system:

In the ganglia associated with the circumesophageal connective, forming part of the "vegetative nervous system" three cell types similar to types B_1, B_4 and B_5, respectively, are observed (Fig. 11). A new cell type with a strong affinity for TB and containing numerous large granules without halos, are also observed in (Fig. 12). (See Fig. 29) for comparison of cell types).



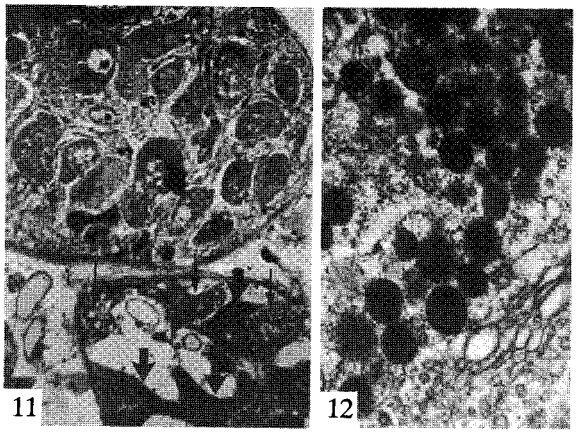


Fig. 10: Type B_3 cell of the nerve cord showing ultrastructure similarity to type B_3 of the brain and subesophageal ganglion. Mitochondria (M), Golgi body (G). X. 55.000.

Fig. 11: Semi thin section of the *L. terrestris* vegetative system. Large arrows indicates cells staining darkly with TB, small arrows indicate vacuolated cells similar or type B_1 cells. X. 600.

Fig. 12: Vegetative system. Ultrastructure of cells which is stained darkly with TB. Note the elementary granules (EG). X. 55.000.

Organization and ultrastructure of release sites within the neuropiles of various ganglia:

Using serial sections, processes were traced from their somata to their axon terminals of cell types B_1, B_2, B_3 and B_4 but not in case of type B_5 due to their small size.

Fibres of cell types B_1 and B_3 are abundant within the storage zone, the dorsoposterior surface layers of the neuropile (Figs. 13-18). Deeper regions of the neuropile also contain terminals of type B_2, B_4 and B_5 fibres. (Figs. 19-20). All fibre types form synapses with characteristic clusters of small vesicles associated with thickened membrane regions and differentiated intercellular clefts (Figs. 13, 15, 18, 20).

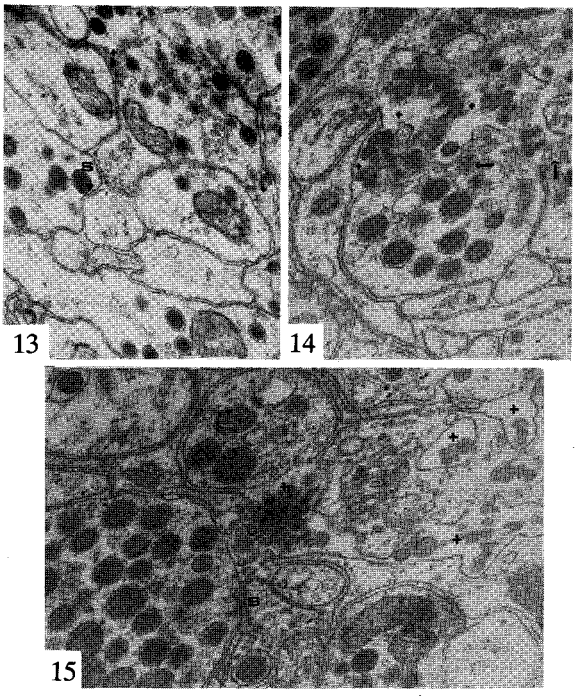


Fig. 13: Type B_1 fibre of *L. terrestris* showing synapse (S) and many exocytotic profiles (+). X. 55.000.

Fig. 14: Type B_3 fibre of *A. longa* showing pools of exocytotic materials (+). Small arrows indicate membrane retrieval by possible coated vesicles. X. 55.000.

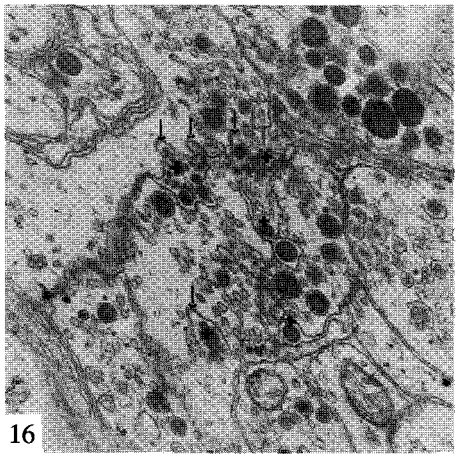
Fig. 15: Type B_3 terminal of *A. longa* cerebral ganglion showing many pools of discharged material (+) note adjacent synapse (S). X. 33.000.

Release sites of the Nsy granules by exocytosis, occur frequently in all of the examined species (Figs. 13-19). Compound exocytosis, involving fusion of two or more Nsy granules at a single site, or scattered exocytotic profiles involving up to five distinct sites are frequently observed within a single nerve terminal, forming large quantities of released material as intercellular pools (Figs. 13-18).

Microvesicles, measuring 300-400^0 A in diameter and large vesicles, measuring 600-800^0 A in diameter, possess prominent coats and often attach to exocytotic profiles (Figs. 14, 16). Release sites by synapse and exocytosis may occur in the same profile (Figs. 13, 15, 18). On the other hand synapses may observed alone in various fibres (Fig. 20).

Application of the zinc iodide-osmium tetroxide (ZI0) reagent:

A single type of Nsy cells is filled with ZI0-positive Nsy granules, with similar characteristics to type B_1 cells. Their fibres contain clusters of ZI0-positive vesicles and granules (Fig. 21). The other types of terminals had ZI0-positive synaptic vesicles only. Almost all of the rounded or flattened vesicles are positive; while dense-cored vesicles were ZI0- negative in most cases (Fig. 22).



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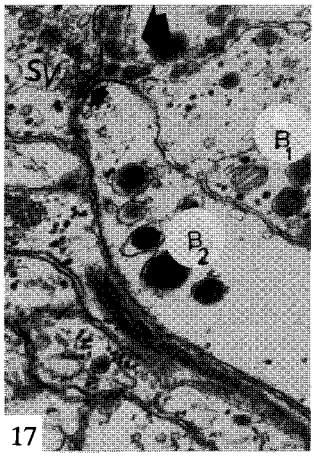

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Fig. 16: Type B_3 terminal of *L. terrestris* cerebral ganglion showing pools of discharged material (+). Small arrows indicate coated profiles involved in endocytosis. X. 55.000.

Fig. 17: Type B_2 terminal of *L. terrestris* showing a wide regular synaptic cleft (small arrow) and a cluster of synaptic vesicles (S). Large arrow indicate, pool of discharged materials possibly produced by adjacent B_1 fibre. X. 55.000.

Peripheral release sites:

1. **Endolamella release sites:**

Type B_1 fibres with clusters of small lucent vesicles (300-400 A in diameter) resembling synaptoid vesicles are observed in contact with the inner surface of the capsule (Fig. 23). Granules release by exocytosis is observed in an endolamella position in various examined ganglia.

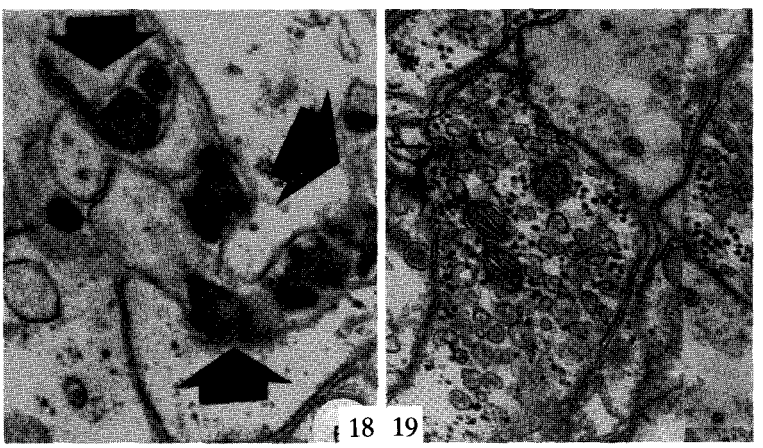

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Fig. 18: Type B_1 fibres within the cerebral ganglion of *D. subrubicunda*. Note: Extensive pools of exocytotic material (large arrow) (+), possibly produced by a number of terminals. X. 55.000.

Fig. 19: Type B_4 terminal of *L. terrestris* (OsO_4/PO_4) showing material discharge by exocytosis (arrow). X. 55.000.

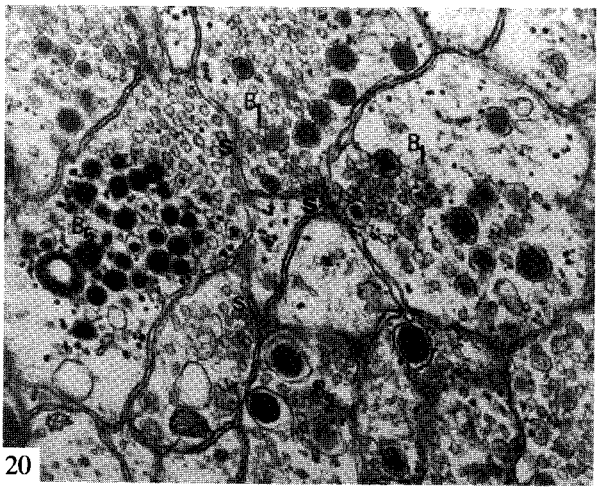
2. **Perilamellar release sites:**

Bundles of type B_1 axons are observed leaving various ganglia through the capsule foramina and form well defined synaptoid complexes against scattered collagen fibres. Some B_1 fibres were traced from the cell bodies to the periphery, other fibres were identified on the basis of size, appearance and Z10 staining affinity of their secretory inclusions (Fig. 21).

Neuromuscular junctions within the cerebral perinerium:

Within the perinerium, type B_4 fibres are often associated with muscle fibres with definite neuromuscular junctions (type 1). These junctions contain Nsy granules and large clusters of small lucent vesicles (250-400^0 A in diameter) adjacent to the muscle body or tail (Fig. 24). In both cases, the junction is separated from the muscle by a collagenous lamina surfaced or by an amorphous membrane forming a

wide cleft between the fibre and the muscle.



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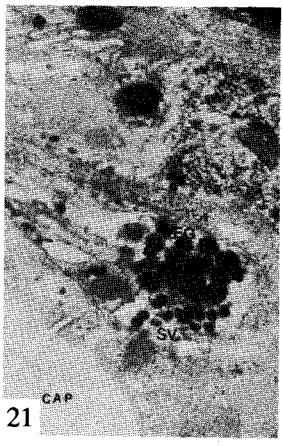

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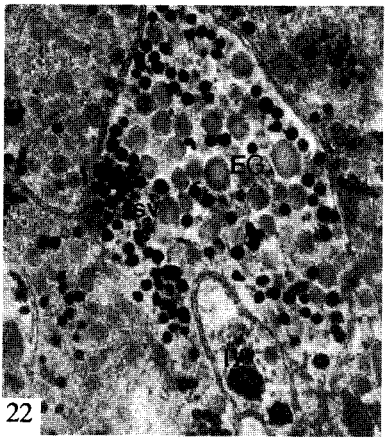

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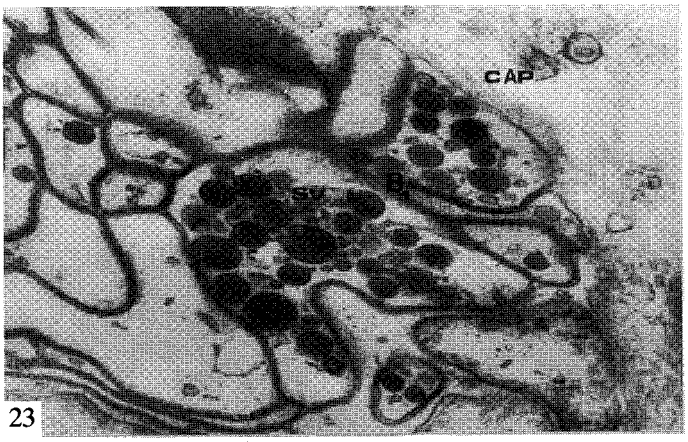
Fig. 20: Type B_5 fibre showing granulated vesicles and synaptic vesicles (S). Note adjacent B_1 and B_2 fibres. X. 55.000.

Fig. 21: Type B_1 fibre of *L. terrestris* showing ZI0-positive elementary granules (EG) within the peripheral nerve terminal against the brain capsule (CAP). Note cluster of ZI0-positive synaptoid vesicles (SV). X. 33.000.

Fig. 22: Type B_3 fibre with ZI0-positive spherical and flattened synaptic vesicles (SV). The majority of elementary granules (EG) react negatively, but the lysosome (LY) is densely stained. X. 33.000.



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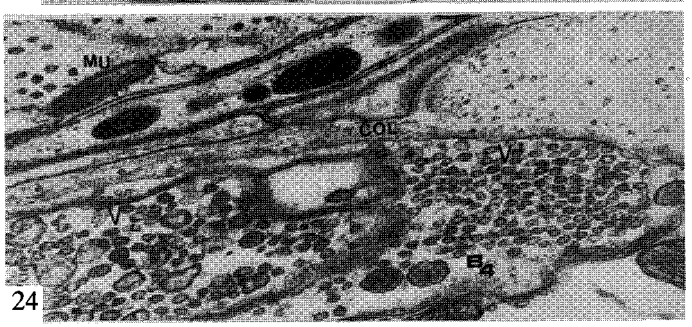

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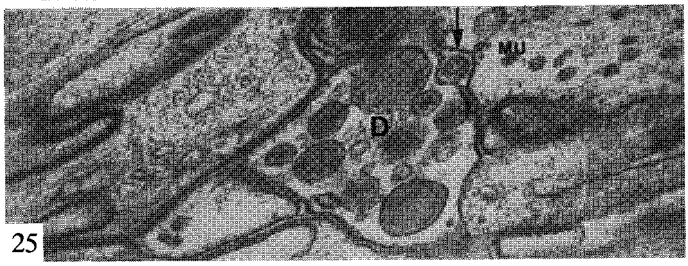

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Fig. 23: Type B_1 terminal within the cerebral ganglion; showing a cluster of synaptoid vesicles (SV) adjacent to the brain capsule (CAP), in endolamella position. X. 55.000.

Fig. 24: Type B_4 fibres within the perinerium of *L. terrestris*; forming type I neuromuscular junction with a cluster of lucent vesicles (V). Note separation of nerve terminal from muscle fibre (MU) by a collagenous lamina (COL). X. 55.000.

Fig. 25: Type D fibre within the perinerium of *L. terrestris*; forming type II neuromuscular junction. Note direct contact of terminals with the muscles (MU). Arrow indicates exocytosis of the granule. X. 55.000.

Type 2 neuromuscular junction contain only large dense granules (type D 1200-2300^0 A in diameter) and observed in direct contact with the muscle body or tail, with a narrow cleft (300^0 A) occuring between the junction and the muscle. At such junctions, granule exocytosis is observed (Fig. 25). However, the perikeria of these fibres was not detected.

Observations on the perinerium of the subesophageal and the nerve cord ganglia:

Bundles of axons of type B_1 and B_4 penetrate through the capsules of the subesophageal and nerve cord ganglia (Fig. 26); and are distributed over a large area of the perinerium. Type B_1 fibres terminate against collagenous strands where they form clusters of synaptoid vesicles. Type I and II neuromuscular junctions involving the type B_4 fibres (Fig. 27) described above are observed in both ganglia.

Heart and body wall neuromuscular junctions:

A close similarity is found between the previously described neuromuscular junctions involving type B_4 fibres and those within the heart and body wall muscles (Fig. 28). The second type of junctions is also identified in both the heart and the body wall muscles.

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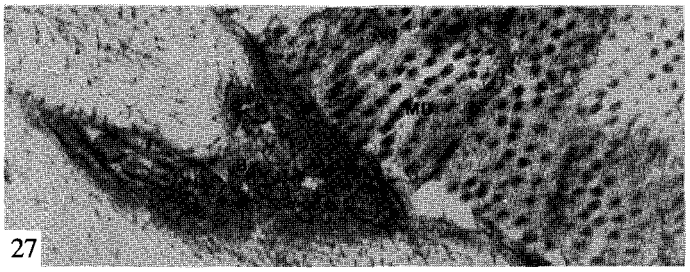

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Fig. 26: Type B_1 and B_4 fibres penetrating the subesophageal ganglion capsule (CAP) on their way to the perinerium. X. 55.000.

Fig. 27: Type B_4 fibre makes type I neuromuscular junction within the subesophageal ganglion of *L. terrestris*. Note muscle (MU) and lucent vesicles (V). X. 55.000.

Table 2

Neurosecretory cell types throughout the nervous system of earthworm *A. caliginosa* and their characteristics.

Cell type	Cell shape	Cell size (μm)	Affinity for TB[1]	Affinity for PAF[2]	Shape of the Elem. gran[3]	Size of the Elem. gran	Electron density of the Elem. gran	Other special features
B_1	Rounded cells	Large, range about 38 x 42	Light affinity	Moderate affinity	Round with thin clear halos	900-2100	Homogenous; moderately electron opaque	Highly vaculated perikarya with Z10-positive reaction. Bipolar cells with fibres distributed within the perinerium forming clusters of synaptoid vesicles against collagenous strands.
B_2	Irregular	Medium, range about 22 x 33	Light affinity	Negative affinity	Irregular with large halos	800-2000	Homogenous; highly electron opaque	The perikarya show prominent glial impushings
B_3	Typical pear shape	Cerebral ganglion 18 x 26 subesophageal ganglion 14 x 30 Nerve cord ganglion 20 x 39	Strong affinity	Strong affinity	Rounded with regular thin halos	Cerebral ganglion 1100-2400 subesophageal ganglion 1200-2300 Nerve cord ganglion 1100-1800	Homogenous; highly electron opaque	Produce large number of exocytatic profiles within the neuropile.

Table 2 Contd.

Neurosecretory cell types throughout the nervous system of earthworm *A. caliginosa* and their characteristics.

Cell type	Cell shape	Cell size (μm)	Affinity for TB[1]	Affinity for PAF[2]	Shape of the Elem. gran[3]	Size of the Elem. gran	Electron density of the Elem. gran	Other special features
B_4	Variable	Medium range about 14 x 24	Very weak affinity	Negative affinity	Variable often elongated with encloning disrupted membrane	1200-1900	Very low electron density mixed with granular vesicles	Bipolar cells with fibres associated with muscles to form definite neuromuscular junctions
B_5	Fusiform	Small range about 13 x 20	Light affinity	Negative affinity	Rounded or oval with wide halo	900-1200	Homogenous: highly electron opaque	Very wide spread. large in numbers

[1]TB, toluidine blue.

[2]PAF, paraldehyde-fuchsin.

[3]Elem. gran, elementary granules.

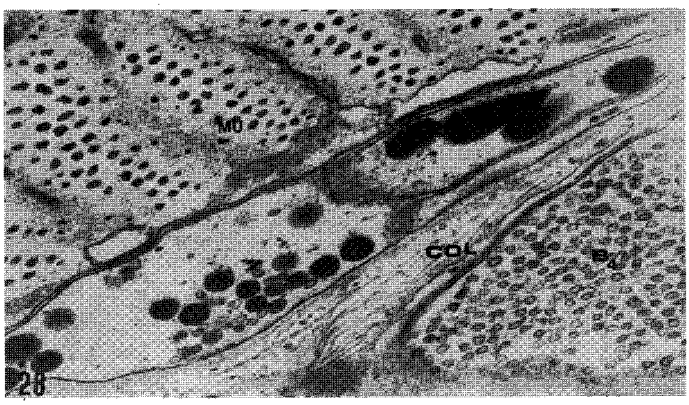

Fig. 28: Type B_4 fibre makes type I neuromuscular junction within the body wall muscles. Note collagenous fibre (COL) between the muscle (MU) and the junction. X. 55.000.

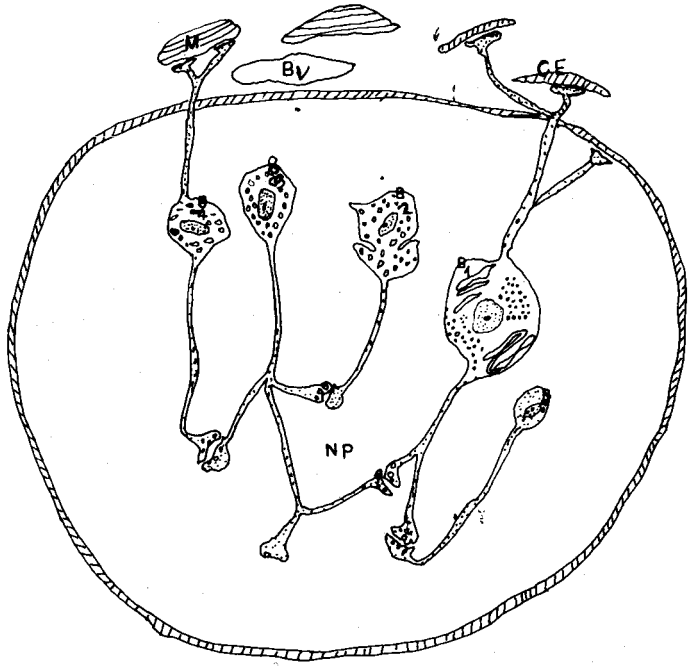

Fig. 29: A summary diagram shows the location of cell types B_1-B_5 and fibres distribution at the −dorsoposterior region in each ganglion throughout the nervous system. Muscle (M), Collagen fibres (CF), Neuropile (NP), Blood vessels (BV).

DISCUSSIONS

Classification of Cell Types:

This is the first ultrastructural classification of the B cells which are the most common Nsy cells through the earthworm nervous system. However, Oosaki (1966) attempted to classify cell types in the cerebral ganglion of *E. foetida* using the TEM. He distinguished three types of 'peptidergic' Nsy cells by the size and character of their Nsy granules. He also detected three possibly aminergic cell types. According to his micrographs, cell types 3, 4, 5 and 6 appear to be equivalent to cell types B_1, B_2, B_4 and B_5 respectively of the present studies respectively. In 1979, De Morais *et al.* described $(a)_3$ cell type probably equivalent to the B_3 cells in this study. It is interesting to note that type B_3 granules are ultrastructurally similar to the secretory granules in the alpha cell of an islet of Langerhans in the human pancreas (Fawcett, 1981). In this system, as in other systems (Zahid, 1977; Maddrell and Nordman, 1979), a multiplicity of cell types may be correlated with a diversity of hormones. Lattaud and Marcel (1989) established that the earthworm cerebral ganglion is the source of hormone controlling sexual maturation and anterior regeneration. Type A cells may control reproduction while B cells may be involved in other activity aspects (Al-Yousuf, 1991a).

U-cells in the subesophageal ganglion were PAF positive (Dogra, 1968) but differed histochemically from other Nsy cells within the Nsy system of earthworms (Teichmann *et al.*, 1966). Other authors described lightly stained cells as stages in the secretory cycle of U-cells; these cells may be equivalent to types B_1, B_2 and B_4 in this study. (Dogra, 1968; Zahid, 1977; Baid and Gorgees, 1977). Ultrastructurally, U cells contain Nsy granules of 1300-2500^0 A in diameter (Aros *et al.* 1975), these cells are equal in size, shape, position and in the appearance of their Nsy granules to type B3-cells in this study. Furthermore, PAF positive Nsy cells in the subesophageal ganglion were designated as B-like cells, because they were similar to B cells present in the cerebral ganglion, with respect to tinctorial property and cellular size (Kinoshita and Kawashima, 1986).

Teichmann *et al.* (1966) found those histochemical resembling between Nsy cells in the earthworm nerve cord and of the cerebral ganglion, which was confirmed ultrastructurally in this study. Herlant-Meewis (1966, 1967) described four groups of Nsy cells C_1-C_4 of which only C_3 cells are active throughout the life-cycle. According to her description, C_3 cells are probably equivalent to type B_3 cells in this study. De Veris-Schoumacker (1977) distinguished five types of aminergic cells in the nerve cord according to their location and staining affinity. However, as only low power micrographs were provided of those cells it was impossible to correlate them with this study.

Gomri-positive cells were also observed in the ganglia of the vegetative nervous system (Aros *et al.*, 1965; Teichmann *et al.*, 1966; Baid and Gorgees, 1977). Zahid (1977) reported only one type of Nsy cells which have oval or spherical perikarya and possess a week to moderate staining affinity for PAF. This type is probably equivalent to TB positive cells in this study.

The role of the neuropile zone:

In this study, many different types of Nsy granule were found to be involved in synaptic connections throughout the whole nervous system. In contrast, Aros *et al.* (1977) suggested involvement of only one type. Fusion of a synaptic vesicle with the presynaptic membrane observed in this study supports the "vesicle hypothesis" (review by Meldolesi *et al.*, 1978), according to which exocytosis is the exclusive method of discharge of neurotransmitters.

In the present study evidence of Nsy granule discharge by exocytosis was produced. Golding and May (1983) interpreted materials released by granule exocytosis as neuromodulators, which have longer lasting and more diffuse influence on neural activities than those produced by synaptic transmitters. The most massive release within the neuropile of several species were associated with type B_3 cells terminals, which appear to be typically peptidergic. Furthermore, the number of B fibres within the neuropile are much more numerous than type A fibres in general. According to Pickel *et al.* (1979), the same peptides may act as a hormone when released into the blood system, but as a neurotransmitter modulator when released in the neuropile (reviewed by Hokfelt *et al.*, 1980). A similar interpretation may be applied to the peripheral B_1 fibres which form synaptoid complexes and sites of granule exocytosis within the perinerium and the neuropile of the cerebral ganglia. Since these fibres are also present around the ganglia of the ventral chain and within the vegetative system, it seems likely that at least one hormone has a multiple origin within the central nervous system. The possibility of the endocrine function of the ganglia of the ventral chain has been suggested by several authors (Hubl, 1956; Herlant-Meewis, 1966; Kinoshoita and Kawashima; 1986). Such systems are common in invertebrates (Zahid, 1977; Maddrell and Nordmann, 1979).

Neuromuscular junction:

In annelids, all of the muscles are obliquely striated and synaptic contact is made either with the main body of the muscle or with the muscle tail (reviewed by Mill, 1982). Similar contacts were observed in this study in both the body wall and heart muscles. Type I junctions described in the present study probably corresponds to the type I terminals of other workers, since both contain an abundance of synaptic vesicles and small granules. Type II junctions resemble those of Rosenbluth (1972) in containing mainly (or only) dense-cored granules. Similar junctions were

observed within the body wall of a leech (Yaksta-Sauerland and Coggeshall; 1973). Of the three types of junctions described in the body wall of *Nereis* (Dhainaut-Courtois *et al.* 1979), two of them resemble the terminals in earthworms.

Acetylcholine, the probable exactitory neurotransmitter at neuromuscular synapses in the earthworm, may be associated with type I junctions in vertebrates (Rosenbluth, 1972) and *Nereis* (Dhainaut-Courtois *et al.*, 1979), whereas dense cored granules were regarded as containing 5-HT in the leech neuromuscular junctions (Yaksta-Sauerland and Coggeshall, 1973). Rosenbluth (1972) suggested that type II junctions in polychates represent an inhibitory catecholaminergic junctions.

In this study evidence for the origin of type I fibres from type B_4 cells is presented and the histological and ultrastructural characters of the cell bodies are described, which may help in clarifying the identity of the neuromuscular junctions in the perineurium. In contrast, Aros *et al.* (1977) found no way to differentiate between these structures.

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التركيب الفوق مجهري الدقيق لنمط الخلايا العصبية الإفرازية «ب» في الجهـاز العصبي لدودة الأرض

شعـــاع الســـيد اليوســـف

بعد الدراسة المجهرية الدقيقة للخلايا العصبية الإفرازية للأنواع الخمسة التابعة للنمط « ب » اتضح أنها الخلايا الأكثر انتشاراً في العقد المخية والتحت بلعومية والحبل العصبي وكذلك في العقد الطرفية المنتشرة حول الجهاز العصبي لدودة الأرض .

تمت هذه الدراسة في ستة أنواع مختلفة من ديدان الأرض وهي :
أبوريكتودا كاليجينوسا - لمبركس تريسترس - ايسينيافوتيدا - أُكتولاشن سيانيام - دندروبينا سبروبيكوندا ثم **أللولوبوفورا لونجا** .

وقد حددت هذه الأنواع الخمسة من الخلايا « ب » بمميزات مورفولوجية خلوية وتراكيب دقيقة محددة . كما أمكن تتبع الألياف العصبية لكل نوع على حدة مع رصد ميكانيكية الإفراز العصبي بالاستخلاء الخارجي . كذلك تم دراسة التشابكات العصبية العضلية المتواجدة حول العقد المخية ومقارنتها بتلك المتواجدة في عضلات القلب وعضلات الجسم وذلك لتحديد أنواع الخلايا « ب » المشاركة في مثل هذه التشابكات .

تم استخدام التكنيك الخاص بعقار (الزنك أيودين رابع أكسيد الأزوميك) وذلك لتوضيح مناطق محددة من التراكيب الدقيقة .