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Fine Structure of Ganglion Cells in the Central Nervous System of Neptune Whelk (*Neptunea arthritica*)

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Abstract

Light microscopically stainable ganglion cells, possibly of neurosecretory nature, in the central nervous system of neptune whelk, *Neptunea arthritica*, were studied electron microscopically. AF-positive type A cells are characterized by the occurrence of a prominent mitochondrial lump, numerous Golgi bodies with electron-dense contents, and numerous vesicles measuring 750–2300 Å, in the central region of the perikaryon. A granular endoplasmic reticulum is conspicuously developed in an earlier stage of secretory activity, while it is observable only in a small amount in later stages, assuming an appearance of fragmented vesicles. On the other hand, the organelles in other nerve cells are not conspicuous on account of a huge number of secretory materials in the cytoplasm. AF-positive type B-I cells are packed with vesicular secretory products of 1300–2000 Å in size with finely granular matrices, while acidophilic type B-II cells are filled with electron-dense granules of 1600–2000 Å in size. AF-positive types C and D cells are also provided with electron-dense granules of 900–1300 Å and about 1100 Å, respectively. The granules and vesicles of these nerve cells seem to derive from the granular endoplasmic reticulum-Golgi body complex. These products occur in the axons in the neuropile and some of them appear also in the axon endings terminating to the blood lacunae in the perineurium serving as a neurohemal organ.

In the preceding report[1], the writer reported the occurrence of five kinds of possible neurosecretory cells in the ganglia of the central nervous system of neptune whelk, *Neptunea arthritica*. These cells are all provided in the cytoplasm with a remarkable amount of materials which have a staining affinity either to aldehyde fuchsin or to acidic dyes. Moreover, it was demonstrated that most of the cells showed a histological change with the seasons.

However, many cytoplasmic inclusions and organelles such as lysosomes, mitochondria, lipofuscin granules and the lamellar system can be mimic secretory products, which often mislead the histological determination of neurosecretory cells as pointed out by many workers such as Bern[2] and Scharrer and Brown[3]. An electron microscopic study on presumed neurosecretory cells seems to be helpful to lessen this apprehension. The present paper deals with the results of electron microscopic observations of the aforementioned ganglion cells of possible neurosecretory nature in the central ganglia of neptune whelk, *Neptunea arthritica*.

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Material and method

The material used in this study was neptune whelk, *Neptunea arthritica*, collected from Kamiiso in the suburbs of Hakodate. Four to six animals of 7–12 cm in shell length were sampled each month during the period from August 1971 to February 1972. Blocks of the central ganglia were dissected out immediately after the animals had been killed, and were usually immersed in Millonig's solution for 2 hours. Concurrently, some of them were placed in 2% paraformaldehyde combined with 2.5% glutaraldehyde in 0.12 M phosphate buffer (pH 7.2) for 2 hours followed by post fixation with Millonig's solution for 2 hours. After the fixation, the specimens were dehydrated by graded ethanol series and two changes of n-buthyl glycidyl ether, and embedded in Epoxy resin mixture. Sections were cut with glass knives on a Porter-Blum microtome at a thickness of about 500–800 Å, stained with uranyl acetate in combination with lead citrate, and examined with a Hitachi HS-7 electron microscope. Besides, adjacent sections of the Epon-embedded specimens of about 1 μ thick were stained with methylene blue- azur II mixture or with aldehyde fuchsin, and light microscopically examined for the identification of ganglion cell types to be observed by the electron microscope.

Result

As described in the preceding report[1], the central ganglion system of *Neptunea arthritica* contains four types of possible neurosecretory cells. Light microscopically, these cells have a large amount of secretory granules in the perikarya and axons which show an intense staining affinity for AF, with the exception of the type B-II cells of which the secretory product is evidently of acidophilic nature. The fine structural characteristics of these cells are as follows.

Type A cells

The cytoplasm of cells of this type is as a whole less opaque in electron density as compared with that of the other cell types, especially in earlier stages of their secretory activity. A marked mass of mitochondria which are round or rod-like in shape and 300–600 mμ in size occupies the center of the cytoplasm, making the nucleus push in the region of axon hillock. A number of Golgi bodies with electron-dense contents are distributed around the mitochondrial lump (PL. I-1).

Numerous vesicles of 750–2300 Å in size, possibly originating from the Golgi vesicles, and found crowding nearby the Golgi bodies. Most of the vesicles are electron-lucent but those with varying electron densities are also encountered (PL. I-2). Among those the electron-lucent vesicles, which are light microscopically AF-positive in character, shown an obvious increase in number coincidentally with the secretory activation of the cell advancing during the period from December to February. An associated development of Golgi bodies is also evident in their increase in number and in the amount of the electron-dense contents. In association with this phenomenon, a granular endoplasmic reticulum of lamellar features is very conspicuous throughout the perikaryon during an earlier stage of secretory activity in late autumn (PL. I-3), while an endoplasmic reticulum assuming an appearance of fragmented vesicles is observable only in a small amount during later stages in late winter. Besides, large electron-dense granules, reaching a maximum size of 1200 mμ, are seen situated usually in the peripheral cytoplasm encircling the Golgi zone as well as in other types of cells. These granules seem to be lysosomes or lipofuscin granules as judged by their structural aspects and their positive reaction to Schmorl staining.

Type B cells

The cells of this type can be light microscopically subdivided into AF-positive B-I cells and acidophilic B-II ones. Secretory materials of the B-I cells are recognizable as small vesicles of 1300–2000 Å in diameter containing finely granular matrices. The perikaryon of the cells is packed thickly with the vesicles. Other cytoplasmic inclusions and organelles reveal only a poor development: ovoid or rod-shaped mitochondria are dispersed among the vesicles or are pressed against the periphery of the cytoplasm; lysosomal bodies and Golgi bodies are also seen scattered among the vesicles (PL. II-4).

On the other hand, the acidophilic B-II cells have numerous granules of various sizes ranging from 1600–2000 Å, most of the granules measuring approximately 1600 Å. Each of the granules contains a homogeneous electron-dense material enclosed with a limiting membrane. The granules are distributed rather compactly through the whole perikaryon, which results in a thin dispersion of other cytoplasmic inclusions and organelles, as in the B-I cells (PL. II-5). Rod-like mitochondria and Golgi bodies, both being small in number, are present rather in the central cytoplasm, while a granular endoplasmic reticulum in lamellar type is seen in the periphery. Axons and their endings containing granules, which correspond to those found in the perikaryon of the B-II cells, are frequently observed in the medullary neuropilar zone of ganglia and also in the region abutting on the perineurium (PL. II-6).

Type C cells

AF-positive materials in the C cells appear as electron-dense, oval or round granules of 900–1300 Å. Electron-lucent vesicles of similar size are also encountered diffusely among the granules and in the medullary portion of the cytoplasm. The distribution of these granules and vesicles is not so compact as in the B-I and B-II cells, and they mostly occur as clusters existing in the peripheral zone of the perikaryon. The other cytoplasmic inclusions and organelles show a moderate development in the perikaryon. The mitochondria are mostly round in shape and appear here and there. The Golgi bodies with electron-dense material are located in the central region of the cytoplasm. The granular endoplasmic reticulum, though few in number, is observed near the nucleus. Lysosomal bodies and lipid droplets are also present (PL. III-7).

Type D cells

These cells are filled with electron-dense granules like the B-II cells (PL. III-8). Under high magnification, the granules have a mean diameter of 1100 Å, and appear to be irregularly bound each by a membrane (PL. III-8, inset). The mitochondria, round in contour, and the Golgi bodies with electron-dense contents are relatively conspicuous in the central portion of the perikaryon, which is reminiscent of the feature found in the A cells.

Discussion

Histological studies made by Yahata and Takahashi[1] have revealed that, in the neptune whelk, 4 kinds of basophilic cells (types A, B-I, C and D) and one kind of acidophilic cell (type B-II) are distinguishable in the central ganglia. In fair accordance with these findings, the present electron microscopic study could demonstrate the occurrence of 5 kinds of cells with different types of granules and vesicles, probably of secretory nature, in the central ganglia of the neptune whelk.

Ultrastructural characteristics of the type A cells seem to bear a slight resemblance to those of "ordinary" neurons[4][5], but the former differ from the latter in that they are characterized by having numerous vesicles of 750–2300 Å in diameter in the perikaryon. Furthermore, especially prominent in the A cells, there is a change in the condition of the cytoplasmic organelles in possible association with the seasonal change in tinctorial response of the cells reported in the preceding paper[1]. That is, a granular endoplasmic reticulum is very conspicuous during the earlier stages of secretory activity when the cytoplasm stains weakly with AF, while it is detectable as fragmented vesicles merely in the regions adjacent to the nucleus and to the plasma membrane in the later stages when the cell stains intensely with AF. On the contrary, the vesicles, which have been

scanty in number in those earlier stages, become more numerous to be distributed compactly over the perikaryon in the later ones. These facts, together with the existence of well-developed Golgi bodies in the cells, may explain that some proteinous substance(s) is produced and secreted in the manner general to most neurosecretory cells[3].

Light microscopically basophilic, type B-I cells are provided with abundant vesicles having finely granular contents. On the contrary, presumed secretory products of light microscopically acidophilic, B-II cells are electron-dense granules with which the perikarya are packed as in the B-I cells. The granules are almost similar in size and fine structure to those found in the "Gomori-negative", CDC cells of *Lymnaea stagnalis*[4]. Furthermore, the axons of the B-II cells containing the neurosecretory granules seem to reach the perineurium where they terminate with the endings bordering on the blood lacunae. Different axons containing other types of granules are also found in the perineurium. These granules seem, however, to differ in size and shape from the secretory granules present in the perikarya of ganglion cells of the other types observed in the present study, and remain to be identified at present. The perineurium of the central ganglion in the neptune whelk is evidently comparable to the so-called neurohemal organ of other molluscs[4)5)6)7)8)9)10].

The granules in the cells of types C and D, both having an AF-positive property, also fall within the range of size and ultrastructural aspects of neurosecretory granules described in other species[11)12].

As was observed typically in the A cells, the above described secretory materials in the ganglion cells appear to originate in the granular endoplasmic reticulum-Golgi body complex as suggested previously[3]. This may be supported by the fact that, as is shown typically in the A cell, an active granular endoplasmic reticulum appears in the ganglion cells prior to the occurrence of numerous Golgi bodies with electron-dense contents and finally the secretory materials accumulate in the perikaryon.

The results of the present observations may provide an additional support to the existence of neurosecretory cells in the central ganglia of the neptune whelk, though considerations must be paid to the repeatedly insisted note made by many researchers such as Bern[2], Andrews[5] and Simpson et al.[13] that the presence of so-called neurosecretory granules is not in itself sufficient evidence to establish the neurosecretory nature of the neuron. Further studies including an experimental analysis of the neurosecretory phenomena are now being carried out to establish the physiological significance of the neurosecretion in the neptune whelk.

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Explanation of Plates

PLATE I

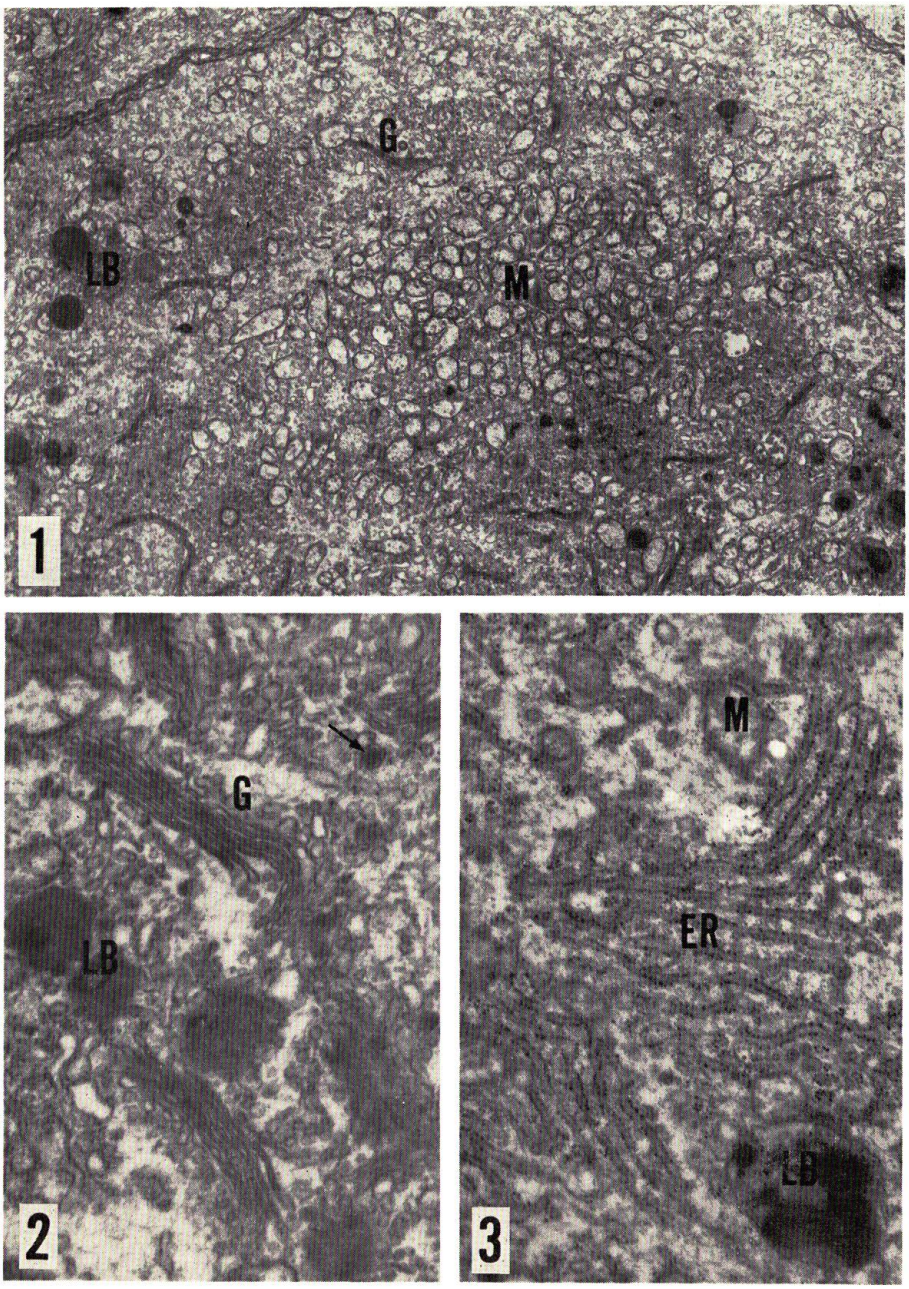
All figures are electron micrographs of the type A cells of the neptune whelk.

ER, endoplasmic reticulum; *G*, Golgi body; *LB*, lysosomal body: *M*, mitochondria.

Fig. 1. Fine structure of the perikaryon, showing a prominent lump of mitochondria and peripherally arranged Golgi bodies accompanied with electron-lucent vesicles. Lysosomal bodies are also noticed rather dispersedly. Millonig, × 6,000.

Fig. 2. Prominent Golgi bodies and associated vesicles. Note the presence of some of the vesicles with electron-dense contents (arrow). Millonig, × 6,000.

Fig. 3. Well-developed granular endoplasmic reticulum in the perikaryon of a cell in an earlier stage of secretory activity, examined in October. Paraformaldehyde-glutaraldehyde and Millonig, × 30,000.



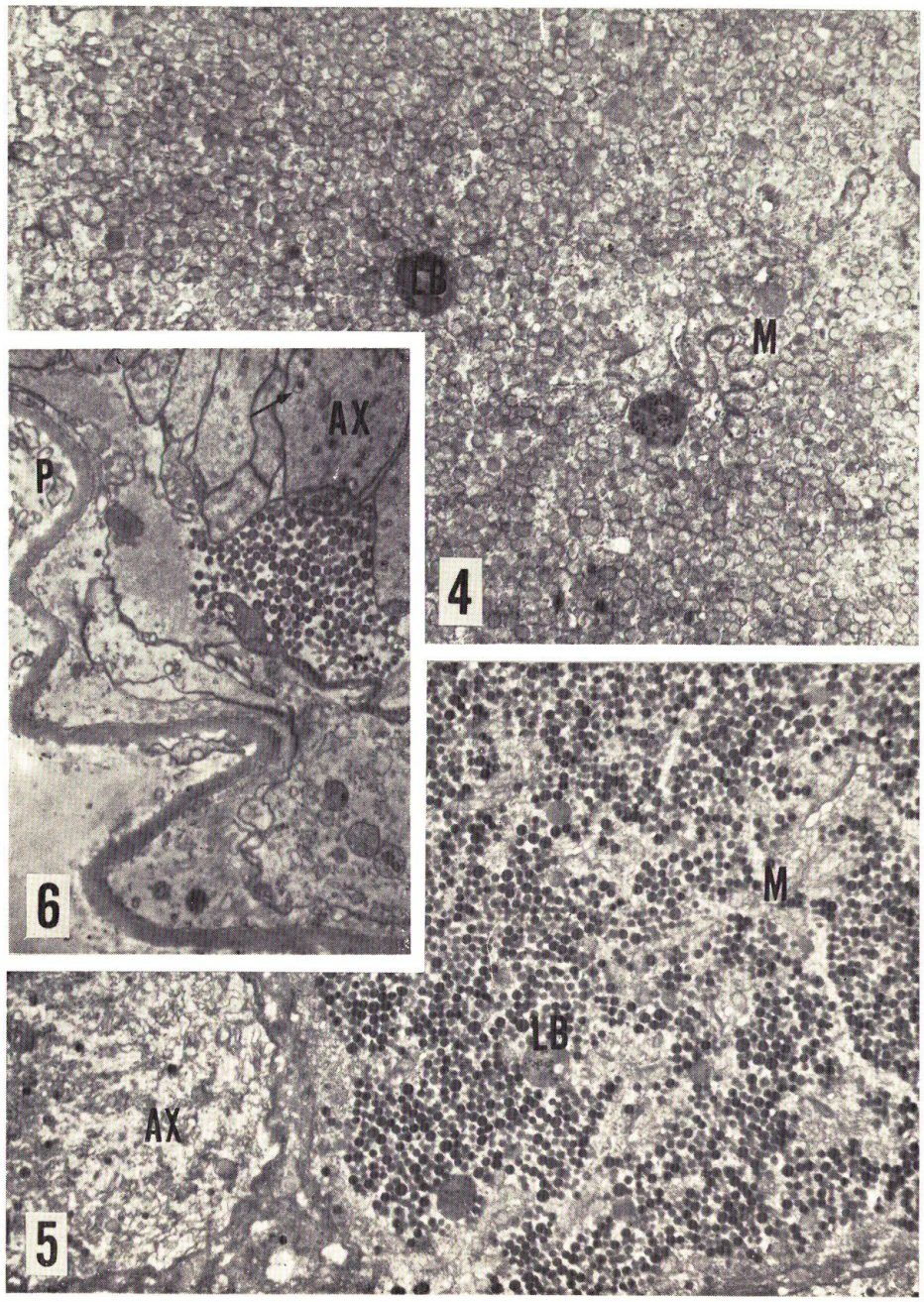
YAHATA: Fine structure of ganglion cells in *Neptunea*

PLATE II

Fig. 4. The perikaryon of the type B-I cell, filled with vesicles containing finely granular matrices. *LB*, lysosomal body; *M*, mitochondrion. Paraformaldehyde-glutaraldehyde and Millonig, × 15,000.

Fig. 5. The perikaryon of the type B-II cell packed with electron-dense granules. The granules are also observed in an axon (*AX*) running nearby the cell. *LB*, lysosomal body; *M*, mitochondrion. Paraformaldehyde-glutaraldehyde and Millonig, × 7,500.

Fig. 6. Axons (*AX*) abutting on the perineurium (*P*). Secretory granules of the type B-II cell are seen accumulated in an axon. A different kind of granules is also noticeable in different axons (arrow). Millonig, × 9,000.

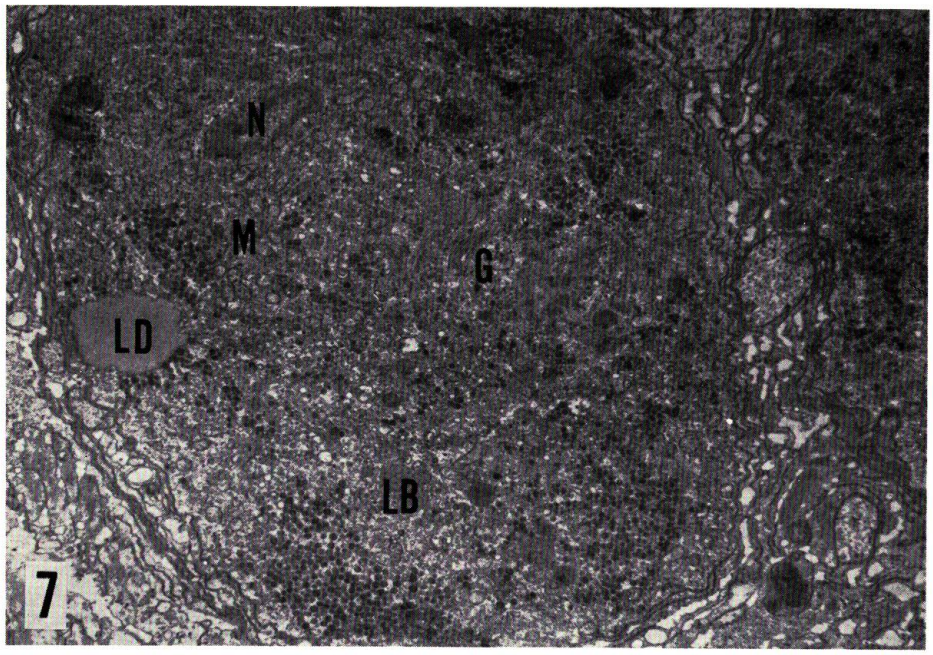


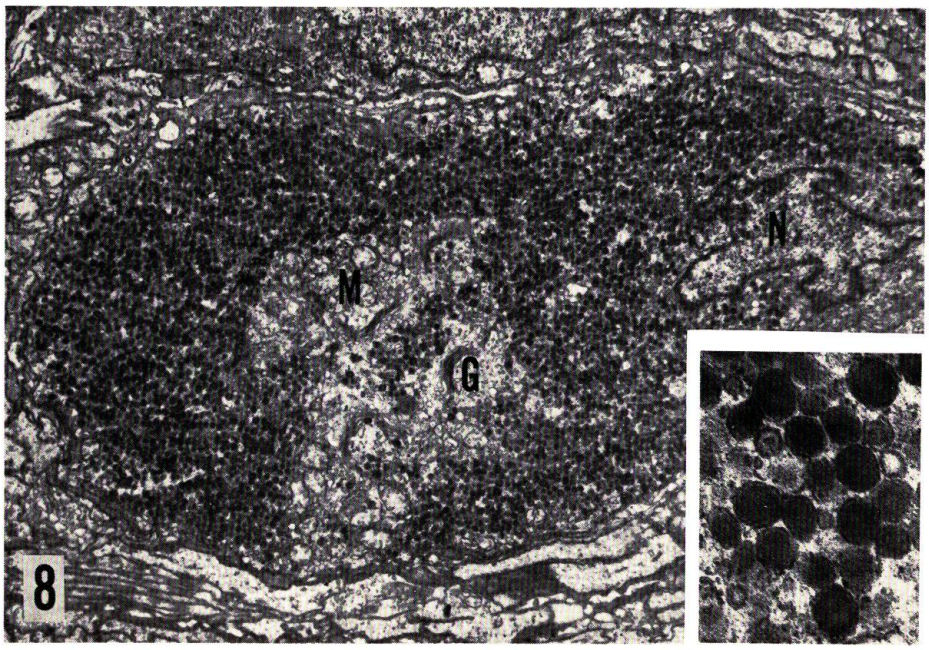
YAHATA: Fine structure of ganglion cells in *Neptunea*

PLATE III

Fig. 7. Type C cell, showing cell organelles and secretory granules arranged typically in its perikaryon. *G*, Golgi body; *LB*, lysosomal body; *LD*, lipid droplet; *M*, mitochondrion; *N*, nucleus. Millonig, × 10,000.

Fig. 8. Type D cell filled with electron-dense granules. Cytoplasmic organelles such as mitochondria (*M*) and Golgi bodies (*G*) are located in the central region of the perikaryon. *N*, nucleus. Millonig, × 8,000. Inset: High magnification of granules found in the type D cell. Millonig, × 50,000.





YAHATA: Fine structure of ganglion cells in *Neptunea*