ELECTRON-MICROSCOPIC OBSERVATIONS ON THE CEREBRAL GANGLION OF THE FRESH WATER MUSSEL (*ANODONTA CYGNEA L.*)

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The nervous system of molluscs is in recent years much dealt with by physiologists (TAUC and GERSCHENFELD 1960, 1961, 1962). Besides a great number of physiological work comparatively few communications appeared on the ultrastructure of the nervous system of various mollusc species (SCHLOTE 1957, FÄHRMANN 1961, BATHAM 1961, ROSENBLUTH 1963, GERSCHENFELD 1963). No data are found in literature on the ultrastructure of the ganglions of *Anodonta cygnea* and from the results of electron-microscopic investigations on the ganglions of the related *Unio tumidus* mainly the data connected with neurosecretion were published by FÄHRMANN (1961).

The central nervous system of Lamellibranchiata consists of three pairs of ganglions arranged far from each other among which the connection is assured by the cerebrovisceral and cerebropedal pairs of connectives. This peculiar, only partly centralized construction of the nervous system is unique in molluscs and exhibits a similarity with the nervous system of phylogenetically more primitive animals. This peculiarity of the microscopic structure raises the question whether there are no differences also in the ultrastructure of the nervous system of mussels as compared with the nervous system of other molluscs.

Departing from these, we started investigations for the electronmicroscopic study of the nervous system of *Anodonta cygnea*. In the present communication we report on the data obtained so far in the investigation of the cerebral ganglion.

Material and method

The cerebral ganglions of 12 to 18 cm specimens of *Anodonta cygnea* were observed in the September to January period. The animals were kept in an aquarium, in streaming Lake Balaton water. Since the structure of materials fixed in 1 per cent OsO_4 solution buffered with veronal acetate was injured even after repeated attempts, presumably by anisotonia, we applied the following fixation process.

From the pericardium cavity of large mussels the blood was sucked off. This is a colourless water-clear liquid the pH of which is according to data of literature (PROSSER and BROWN 1962) and our own observations 7.2 on the average. The blood was filtered and mixed with the same amount of 2 per cent

OsO_4. The osmotic conditions of this mixture provided for optimum fixation results. Fixation lasted for 2 hours on 4° C. The pH of the fixation mixture was 6.9 to 7.2 and did not substantially change during fixation. The fixation process was followed by alcoholic or acetonic dehydration and subsequently the material was embedded in araldite in the usual way. Contrasting was performed after 70 per cent alcohol for 1 hour with uranyl acetate respectively after sectioning with lead citrate (REYNOLDS 1963) for 15 to 20 minutes. The sections were prepared with REICHERT's ultramicrotome and the photographs with JEM 6C electron microscope using 4500 to 25 000 × direct enlargement.

Results

The nerve cells are found on the peripheral part of the cerebral ganglion (CG) while the interior of the ganglion is occupied by the neuropile.

The neurons are mostly unipolar, without dendrites. Frequently two or more neurons contact each other and their cell membranes are often closely adjacent, while in other cases separated by a narrow intercellular gap. The nucleus is in most cases of excentric arrangement with fine chromatine structure. The nerve cells contain a rich endoplasmatic reticulum and GOLGI apparatus can be often observed in them *(Fig. 1.)*.

In the cells frequently 0.2 to 2 μ neurosecretory granules are seen which have their proper membrane. Part of the granules contain a homogeneous substance of great density while in others the dark substance is of inhomogeneous distribution *(Fig. 2.)* and again in others beside the inhomogeneous distribution a fine lamellar design appears *(Fig. 3.)*. Also such formations were observed in which the lamellar design was predominant. These "myeline figures" in some cases contained dense-core vesicles *(Fig. 4)*. Around the granules of inhomogeneous density masses of hyperdens granules of a size of 300 to 500 Å appear, some of which are surrounded by a separate membrane. These small granules often in a regular row are adjacent to the membrane of the large granule. In the vicinity of these large granules we regularly observed mitochondria *(Fig. 2.)*.

The axon departing from the cell contains neuroprotofibrils and sometimes a small number of dense-core vesiculum appears in it.

In the surroundings of the nerve cells nerve endings are found which contain 500 to 700 Å synaptic vesicles and 500 to 1500 Å sized dense-core vesicles *(Fig. 5.)*. Some terminal nerves join the surface of the nerve cells with a structure that can be regarded as a synapsis *(Fig. 6.)*. On sections contacting the cell tangentially it occurs that within the cell nerve endings forming axo-somatic synapses are seen. These endings probably invaginated in the surface part of the cell *(Fig. 7.)*.

In the vicinity of some neurons special lamellar glia cells are found *(Fig. 5.)*. Around each cell or cell group the glia lamellae can be observed in 8 to 14 layers; their thickness amounts to 0.1 to 0.6 micron but at places they broaden and there high density granules of 1 to 1.5 μ and stretched nuclei are arranged. The longer diameter of the nuclei is 5 to 7 μ. In the plasma of the glia cells an endoplasmatic reticulum of fine vesicular structure was observed. In the gaps between the lamellae a small amount of medium density substance of fine pubescent structure is found which does not entirely fill out the gaps *(Fig. 8.)*.

In the vicinity of some nerve cells a 4 to 6 μ large formation of unusual structure limited by a membrane is arranged, the interior of which is filled out by a labyrinth of intricate linear design. In the labyrinth there is a part which is apparently empty and a substance of quite loose structure containing a few vesicles. The exterior surface of this structure is contiguous both with nerve cell membranes and membranes of terminal nerves *(Fig. 9.)*.

The picture of the neuropile is rather diversified. The axons have neither myeline sheath nor neurilemma, the axolemmae touch each other and there is only a 200 to 250 Å wide gap between them. Greater axon-groups are surrounded by glia cell appendices.

In some axon sections a great number of synaptic vesicles and dense-core vesicles are seen, often separated, often the two mixed in a diversified way. According to the size of the dark centre and the external diameter (500 to 1500 Å) several variants of the dense-core vesicles are found *(Fig. 10.)*. In the neuropile frequently axo-axonic synapses are observed *(Fig. 10.)*. Also mitochondria are found in the axons, mostly 3 to 5 in a group.

Discussion

Evaluation of the structures found in the CG encounters great difficulties and can largely rely on assumptions only. The difficulty consists mainly in that on the nervous system of the Lamellibranchiata in general few data of structural relationship are available in literature and on the other hand such structural elements are found in this material which can be fitted in with difficulties into the general picture developed hitherto on the construction of the nervous system of the higher animals. For these reasons the evaluation of our material at present can only offer a hypothesis with the aid of which further examinations can help us to get nearer to the solution of these issues.

The various types of the high density neurosecretory granules seen in the nerve cells suggest that the hyperdens substance from these large granules gets evacuated in the form of tiny (300 to 500 Å) granules and hereby the "myeline figure" like skeleton of the large granule becomes visible. It can not be decided whether between the small granules and the dense-core vesicles visible in the nerve endings there is a connection, but it is remarkable that also in the resulting axons dense-core vesicles are seen. Thus it is not impossible that they wander from the cell towards the nerve endings and that there is a certain connection between them and the large neurosecretory granules. Also the possibility arises that the "myeline figures" correspond to degeneration products.

In the terminal nerves such vesicles are seen that correspond in shape and size to the classic synaptic vesicles (PALADE 1954, DE ROBERTIS and BENNETT 1955). Since it has been demonstrated by DE ROBERTIS and co-workers (1962 and 1963) that these vesicles contain acetylcholine and in the ganglions of molluscs acetylcholine is present (BACQ 1946), presumably also in *Anodonta*, not only in Pulmonata (GERSCHENFELD 1963) there are constituents of cholinergous synapsis.

Among the dense-core vesicles GERSCHENFELD (1963) in Pulmonates distinguished a smaller group named by him DSV (Dense Synaptic Vesicles) and a group of larger size called NSV ("Neurosecretory" Synaptic Vesicles.) According to this author DSV might correspond to the catecholamine granules

but it is not excluded that these vesicles contain serotonin. As to NSV it is involved in a way not cleared up as yet in the nervous activity. These assumptions are manifest also in our case; we merely raise the question whether it is justified to separate the dense-core vesicles according to size if we consider the following.

The diameter of the larger group is 1000 to 1400 Å, about one third of which finds place in the section of 400 to 500 Å thickness, thus if the plane of intersection attains the presumably globe-shaped granule not equatorially, then also segments with diameters less than the greatest diameter originate. Besides, also the possibility arises that the dense-core vesicles change their size in function and this is another reason why there can be a group of smaller and larger diameter among them.

It appears that the conclusion drawn from the observations conducted on Pulmonata according to which there are no axo-somatic synapses in molluscs (BULLOCK 1961, TAUC 1960, GERSCHENFELD 1963) does not hold, since in CG pertaining to Lamellibranchiata we could observe structures that can be regarded as axo-somatic synapsis. Besides a great number of axo-axonic synapses too can be found in the neuropile. It is interesting to note that some terminal nerves call forth axo-somatic synapses invaginated in the nerve cell.

Also the glia elements observed around the neurons are unusual formations. They are evidently involved in the nutrition of the nerve cell since there is no other structure in the vicinity of the neuron that would perform satellite-cell function. In the gap between the glia lamellae probably a bodily fluid flows the organic matters of which were precipitated in the gaps on the action of fixation. This lamellar glia is not the only kind of glia in the ganglion.

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Summary

In the cerebral ganglion of *Anodonta cygnea* unipolar neurons without dendrite are arranged in the peripherial part while in the interior of the ganglion the neuropile is seen. In the plasma of the nerve cells several transitory forms of the neurosecretory granules can be observed. On the surface of the nerve cells structures are found which can be regarded as axo-somatic synapses, but axo-axonic synapses also occur in a great number. The terminal nerves contain synaptic vesicles and dense-core vesicles, often separated but frequently mixed to a diversified proportion. Around certain neurons special glia cells of lamellar structure are arranged in 8 to 14 layers, between which there are gaps containing a substance of fine pubescent structure.

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ELEKTRONMIKROSZKÓPOS VIZSGÁLATOK AZ ÉDESVIZI KAGYLÓ (*ANODONTA CYGNEA* L.) CEREBRÁLIS GANGLIONJÁN

Zs.- Nagy Imre

Összefoglalás

Az *Anodonta cygnea* cerebrális ganglionjában unipoláris, dendrit nélküli idegsejtek foglalnak helyet a perifériás részen, a ganglion belsejében a neuropil látható. Az idegsejtek plazmájában a neuroszekrétum granulák több átmeneti alakja figyelhető meg. Az idegsejtek felszínén axo-szomatikus szinapszisnak tartható struktúrák helyezkednek el, de axo-axonikus szinapszisok is bőségesen előfordulnak. Az idegvégződések szinaptikus vesiculákat és dense-core vesiculákat tartalmaznak, sokszor szeparáltan, sokszor változatos arányban keveredve. Bizonyos idegsejtek körül speciális, lemezes szerkezetű gliasejtek helyezkednek el 8—14 rétegben, köztük finom pelyhes szerkezetű anyagot tartalmazó rés van.

ЭЛЕКТРОННОМИКРОСКОПИЧЕСКИЕ ИССЛЕДОВАНИЯ ЦЕРЕБРАЛЬНОГО ГАНГЛИЯ БЕЗЗУБКИ (*Anodonta cygnea L.*)

И. Ж.- Надь

На поверхности церебрального ганглия беззубки расположены униполярные, лишенные дендритов нервные клетки, а внутри ганглия расположен нейропиль. В плазме нервных клеток видны многочисленные переходы нейросекреторного зерна. На поверхности нервных клеток расположены структуры, которые можно принять за аксо-соматические с напсы, но видно и довольно много аксо-аксоных синапсов. Нервные окончания содержат синаптические везикулы и т. н. денскор-везикулы, которые расположены или раздельно или смешано. Вокруг определенных нервных клеток расположены специальные клетки глии, имеющие 8—14 слоев, а между отдельными слоями обнаруживаются щели, содержащие вещество тонкой флоккулярной структуры.
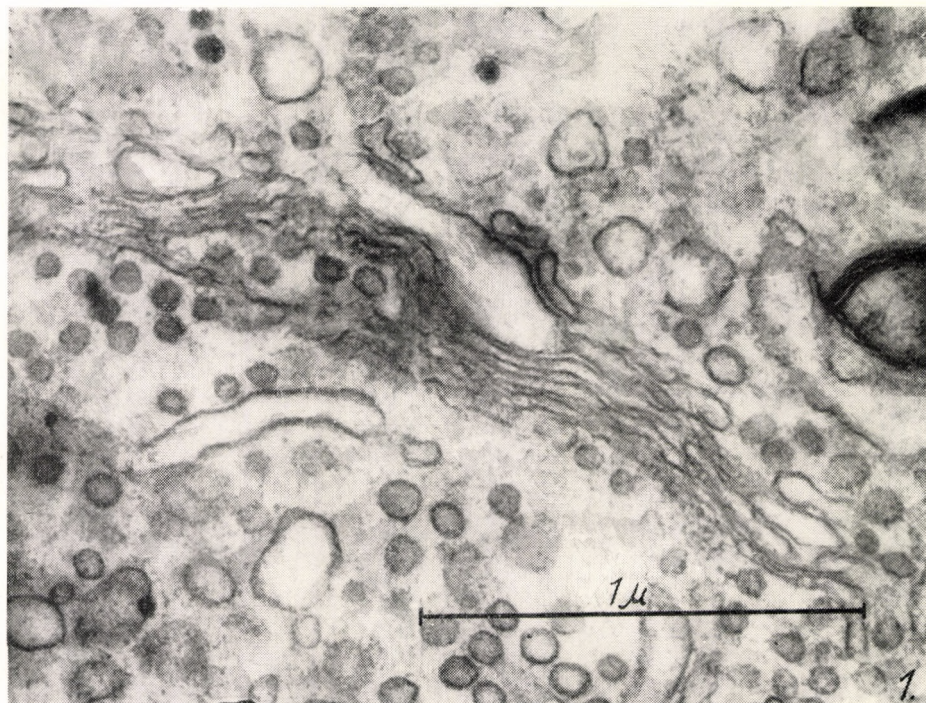
Fig. 1. GOLGI complex in a neuron. Lead-citrate after-contrasting
*1. ábra.* GOLGI-komplex egy idegsejtben. Ólomnitrát utókontrasztosítás
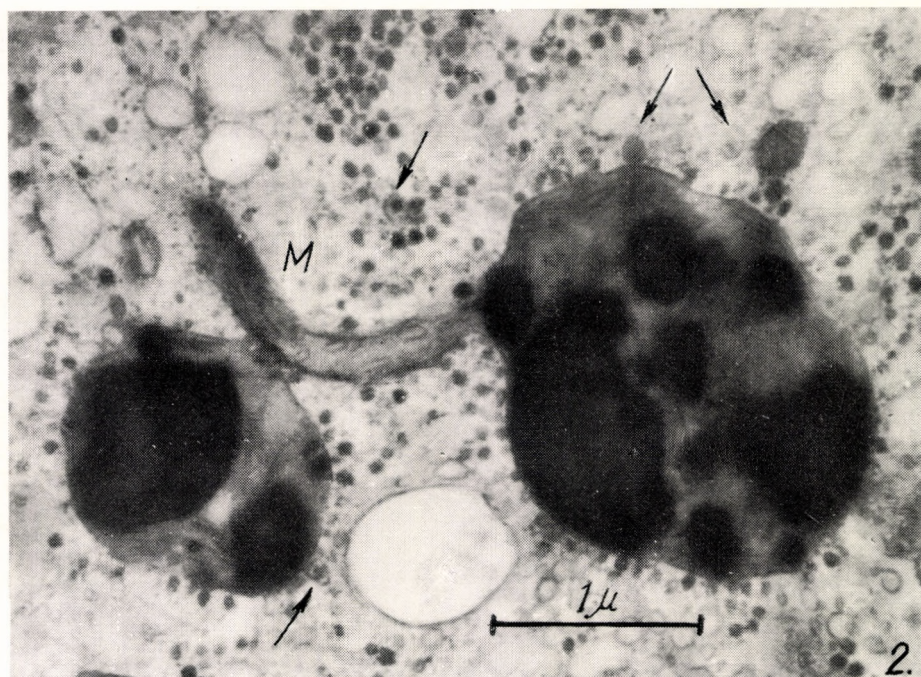
Fig. 2. Detail of a neuron. Neurosecretory granules of inhomogeneous density around which 300 to 500 Å small granules are seen. M-mitochondrium. The arrows point to small granules surrounded by membrane. Lead-citrate after-contrasting
2. ábra. Idegsejt részlete. Inhomogén denzitású neuroszekrétum granulák, melyek körül 300—500 Å nagyságú apró szemcsék láthatók. *M* — mitochondrium. A nyilak membránnal körülvett apró szemcsékre mutatnak. Ólomcitrát utókontrasztosítás
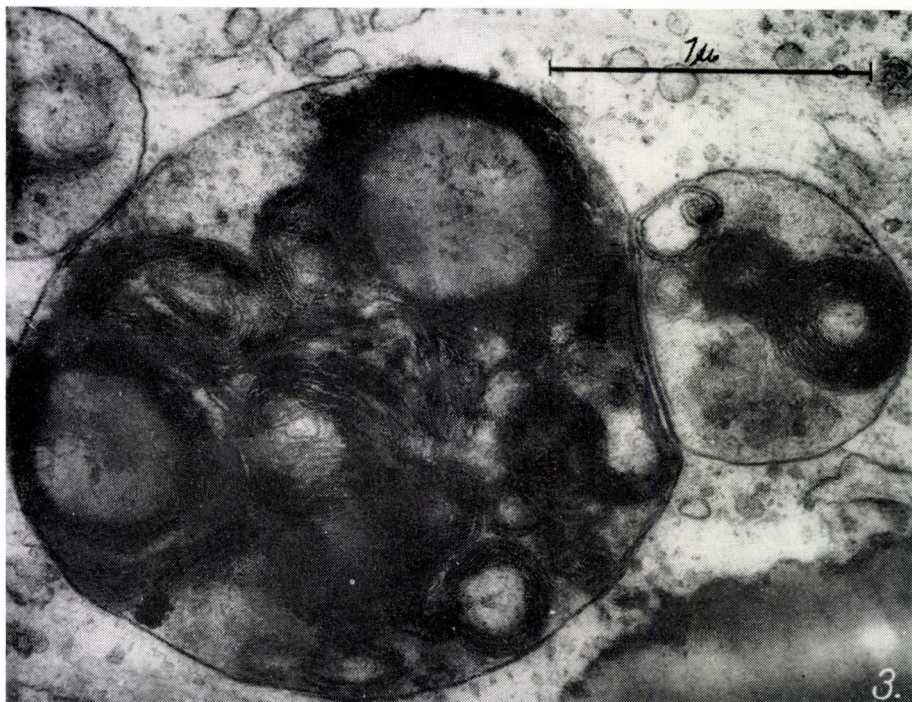
Fig. 3. Detail of a neuron. Neurosecretory granule of inhomogeneous density in which also the lamellar design appears. Lead-citrate after-contrasting.

3. ábra. Idegsejt részlete. Inhomogén denzitású neuroszekrétum szemcse, melyben a lemezes rajzolat is megjelenik. Ólomcitrát utókontrasztosítás
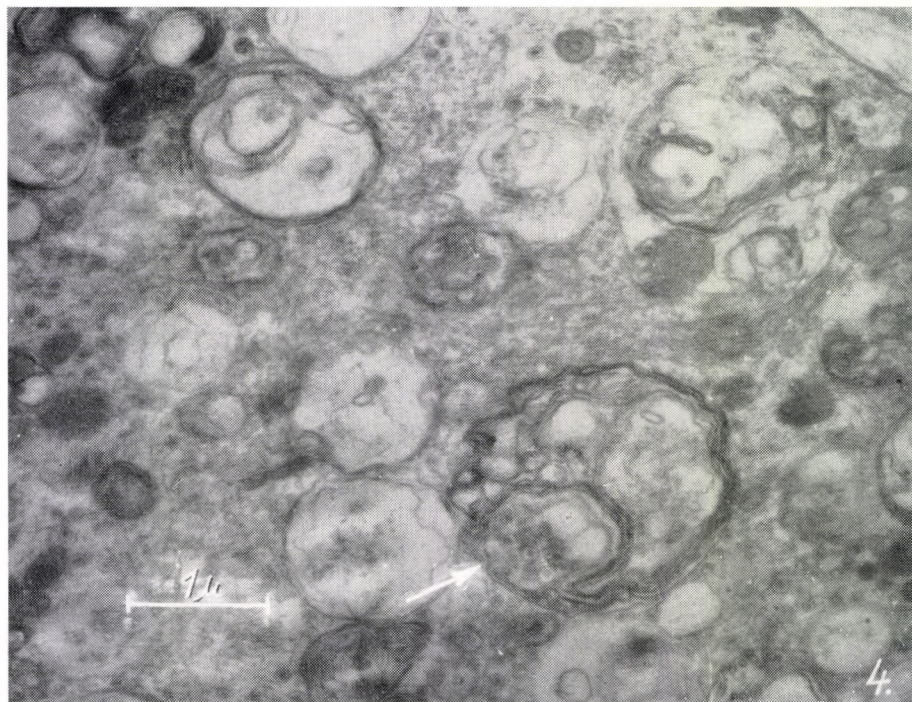

Fig. 4. Detail of a neuron. "Myeline figures" in which there is no more a substance of high density. Arrow: dense-core vesicles in the myeline figure. Uranyl acetate contrasting

4. ábra. Idegsejt részlete. „Myelinfigurák", amelyekben nagydenzitású anyag már nincs. Nyíl: denso-core vesiculák a myelinfigurában. Uranilacetát kontrasztosítás
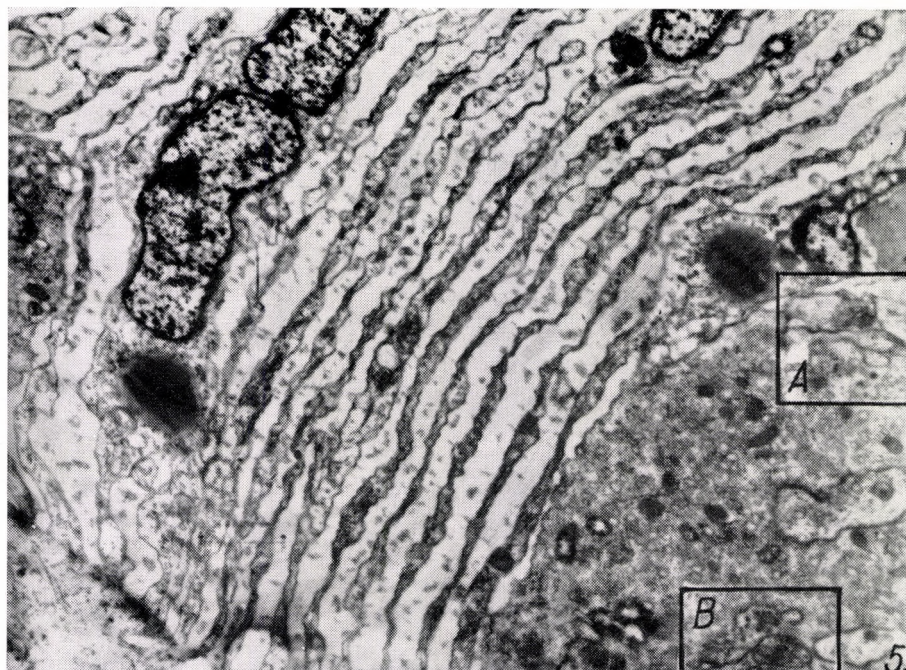
Fig. 5. Detail of a neuron surrounded by lamellar glia cells. Two encircled details of the cell are seen enlarged in *Figs. 6* and *7.* Uranyl acetate contrasting

5. ábra. Idegsejt részlete, melyet lemezes gliasejtek vesznek körül. A sejt két bekeretezett részletét látjuk a *6.* és *7. ábrán* nagyítva. Uranilacetát kontrasztosítás
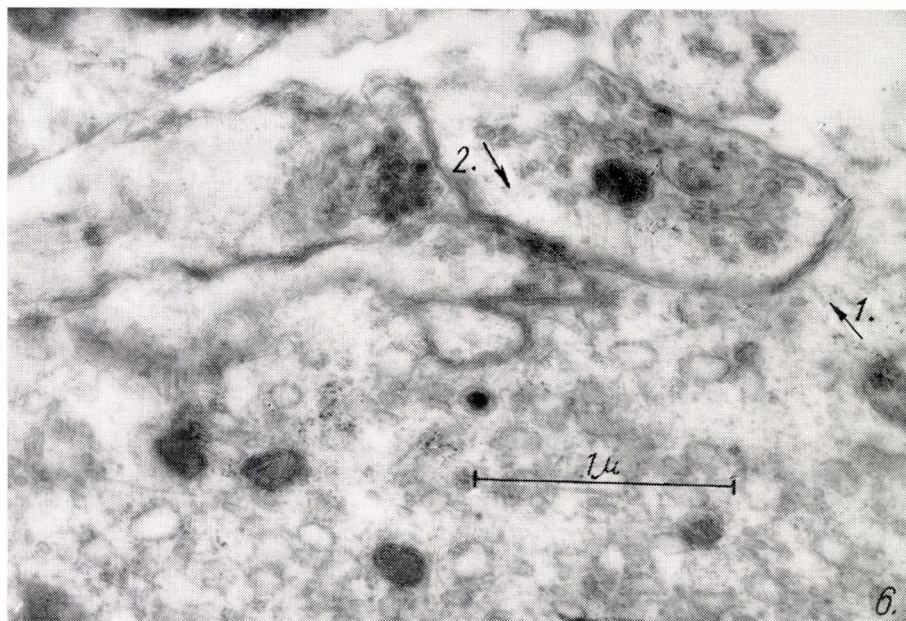
Fig. 6. Detail marked *A* of *Fig. 5,* enlarged. Nerve endings join the surface of the neuron. Arrow 1: axosomatic synapsis. Arrow 2: axo-axonic synapsis. Uranyl acetate contrasting

6. ábra. Az *5. ábra A*-val jelölt részlete nagyítva. Az idegsejt felszínéhez idegvégződések csatlakoznak. 1. nyíl: axo-szomatikus szinapszis. 2. nyíl: axo-axonikus szinapszis. Uranilacetát kontrasztosítás
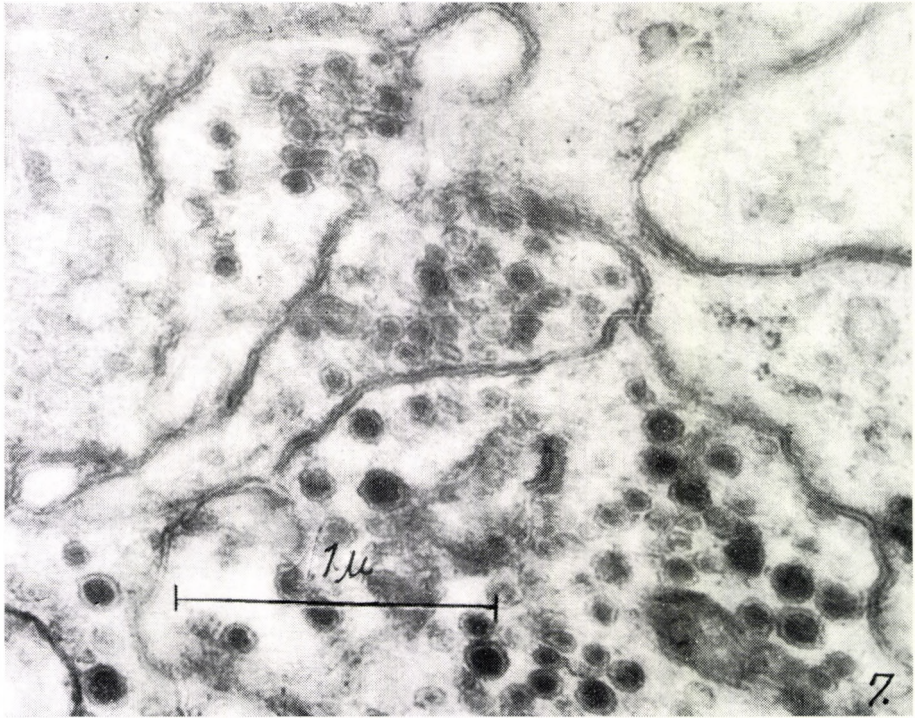
Fig. 7. Detail marked *B* of *Fig. 5* in greater enlargement. One of the nerve endings invaginated into the cell form an axo-somatic synapsis (arrow). Uranyl acetate contrasting
7. ábra. Az *5. ábra* B-vel jelölt részlete nagyobb nagyításban. A sejtbe invaginálódott idegvégződések egyike axo-szomatikus szinapszist képez (nyíl). Uranilacetát kontrasztosítás
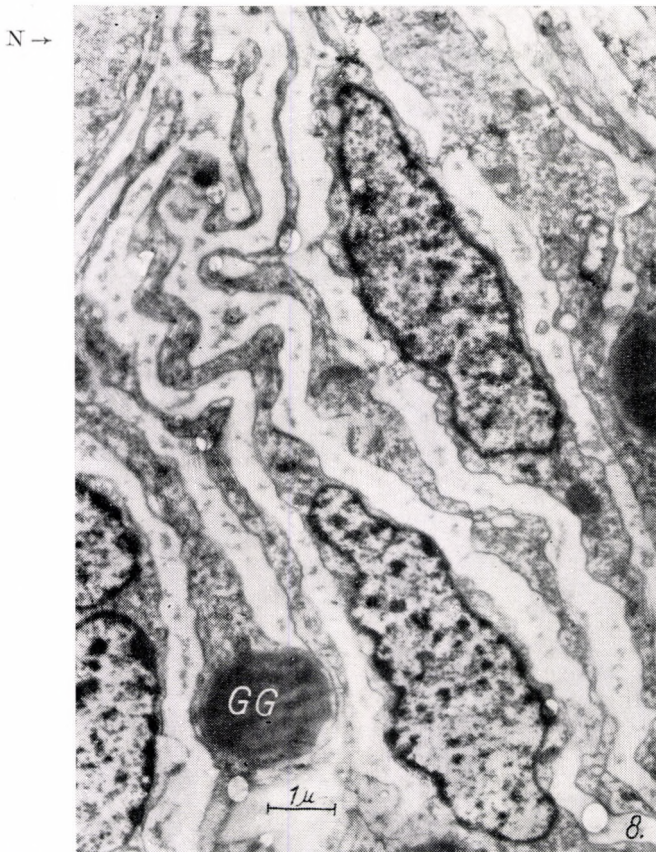
Fig. 8. Details of Lamellar glia cells arranged around the neurons. N.—edge of neuron.
GG — glia granules

8. ábra. Az idegsejtek körül elhelyezkedő lemezes gliasejtek részletei. *N* — idegsejt széle,
GG — gliagranulumok

Fig. 9. Labyrinth-like structure in vicinity of a neuron. Lead-citrate after-contrasting
9. ábra. Labirintusszerű képlet az egyik idegsejt szomszédságában. Ólomcitrát utó-
kontrasztosítás
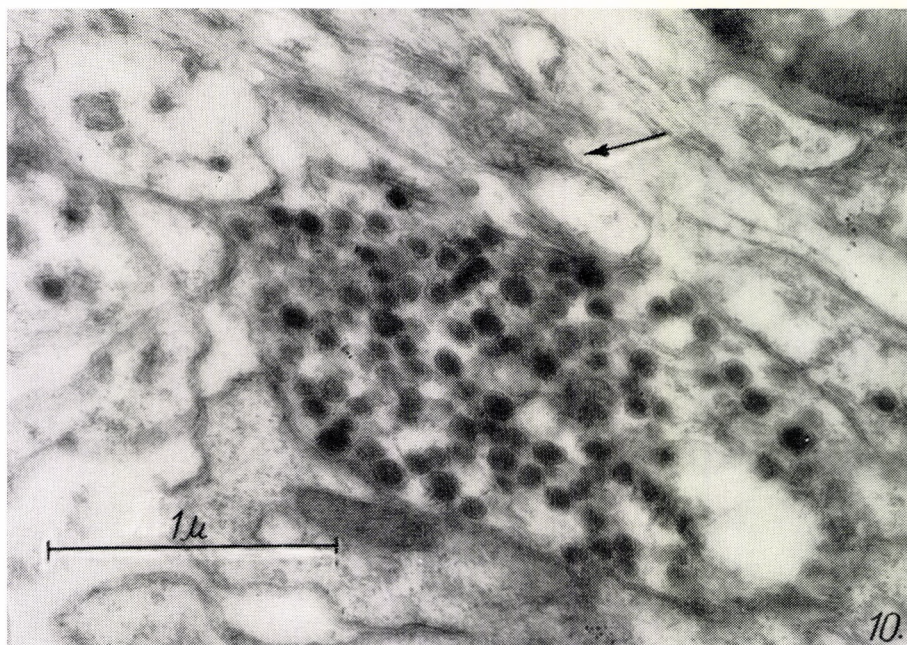
Fig. 10. Detail of neuropile. Dense-core vesicles of various size in the nerve ending.
Arrow: axo-axonic, presumably cholinergous synapsis. Uranyl acetate contrasting
10. ábra. Neuropil részlete. Különböző nagyságú dense-core vesiculák az idegvégződésben.
Nyíl: axo-axonikus, feltehetően cholinerg szinapszis. Uranilacetát kontrasztosítás