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A CYTO-TOPOGRAPHIC STUDY IN THE GANGLIA OF *ANODONTA CYGNEA L.*

A. GUBICZA and I. ZS.-NAGY

Biological Research Institute of the Hungarian Academy of Sciences, Tihany, Hungary

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In the last years the fine structure of the nervous system of molluscs was standing in the forefront of interest (SCHLOTE 1957, BULLOCK 1961, ÁBRAHÁM 1963, ROSENBLUTH 1963, ZS.-NAGY 1964, SUGAWARA 1964, etc.). This interest is due primarily to the fact that the giant nerve cells present in the nervous system of the gastropodes provide excellent possibilities for the use of electrophysiological methods. The molluscs which present an important group in the course of phylogenesis dispose also — as regards their nervous system — of several important features which little attention has been given to until now, further their specific anatomical structure provides a chance to approach problems that cannot be studied suitably in other animals.

In the mussels a neuromuscular system exists consisting of one ganglion, a nerve centre and the adductors. It is possible to examine the activity of this system also under completely physiological conditions by recording the movement of the adductors. Nevertheless, in studies of this kind it is important to learn what kind of nerve cells are localized in the different areas of the various ganglia. In earlier works a suitable impregnation technique has been developed (GUBICZA and ZS.-NAGY 1964) on *Anodonta cygnea*. In other works the chief histological features of the ganglia (GUBICZA and ZS.-NAGY 1965) and the relationship between the nerve cells and the dimension of the animal (GUBICZA 1965) is presented. The objective of the work reported here is the investigation of the cell-topography of the nerves primarily with the view to establish whether micro electrophysiological methods may be sucessfully used and in which area in the ganglia. The clearing of this problem may be of importance also in the application of micromanipulation exstirpation techniques.

Material and methods

The experiments were performed in the cerebral, visceral and pedal ganglia of 12—18 cm long specimens of *Anodonta cygnea L.* In agreement with the objective of the examinations complete serial sections were made. A modification (GUBICZA and ZS.-NAGY 1964) of the CAJAL I. block-impregnation technique which proved most suitable for this purpose was used for the demonstration of nerve cells and fibres. Embedding of ganglia was controlled

under stereoscopical microscope, whereby with regard to the in situ position of ganglia always sagittal and horizontal plain of intersection could be secured in case of cerebral ganglia and in the case of visceral and pedal ganglia respectively. Serial sections of 10 μ were prepared. To facilitate the reconstruction of serial sections a projector similar to the so-called "PALKOVITS-table" (1962) used in variation statistical studies on nuclei was utilized. On the sheet of paper placed on the screen the structural elements of several subsequent sections are outlined and the dimension of cell groups and the direction of processes and fibre fascicles of cells may be recorded in a relatively simple way.

Results

Cerebral ganglion

The two cerebral ganglia are connected with each other by the cerebral commissure, and with the other two pairs of ganglia by the cerebropedal and cerebrovisceral connectives (CVc). Besides the connectives the chief branches as nervus pallialis anterior I, II, III, and several side branches proceed from the ganglia (SPLITTSTÖSSER 1913).

The nerve cells form a 100—150 μ thick cortex on the surface of the ganglion. In the area between the site of origin of cerebropedal connective and cerebral commissure this cortex is only 50 μ thick *(Fig. 1)*. Next to the place, of origin of the commisure and the connectives, however, this cortex is thicker, about 200 μ. Groups of large cells are observable in the space between the

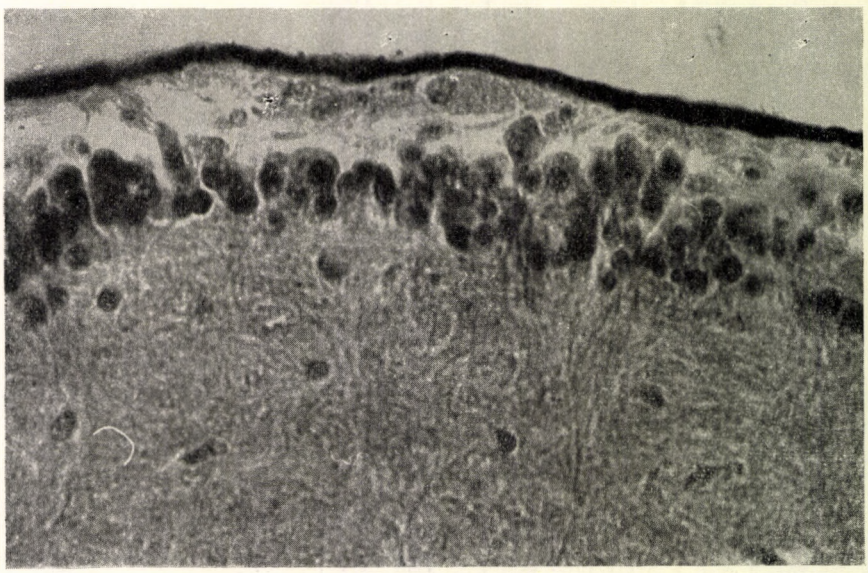

Fig. 1. Cerebral ganglion. Thin (40 μ) portion of cortex from the area between the cerebral commissure and the cerebropedal connective. × 500

1. ábra. Cerebrális ganglion. Vékony kéregrész (40 μ) a cerebrális commissura és a cerebropedalis connectivum közötti területről. Nagyítás: 500 ×.

site of origin of cerebral commissure and nervus pallialis. It has been also observed as a rule, that emerging nerves, as nervi adductoris anterioris, nervi retractoris anterioris and nervi protractoris are surrounded by the cells *(Fig. 2)*. It is seen in *Fig. 2* that there are several large cells among the cells localized at the cross-section of the emerging side branch. It is possible by serial sections to demonstrate the processes of these great cells in the initial part of emerging nerves.

Several large-sized multipolar cells independent of the cortex are also visible in the neuropile *(Fig. 3)*.

Thin and thicker (crisp) fibres are located in the neuropile. The thick fibres processing from the giant cells leave the ganglia through the nerve branches. The majority, about 60—70% of the robust fibres enter the cerebropedal connective. Only few such robust fibres entered the side branches as for instance nervus adductoris.

Large cells are very often surrounded by several layer of flat glia cells.

Visceral ganglion

The visceral ganglia which are medially completely fused are localized under the posterior adductor. The visceral ganglion is flattened in dorsoventral direction. CVc reaches it on its anterior tip and on its posterior tip originates nervus pallialis posterior major. Lobus branchialis (Zs.-Nagy 1966) originates on both sides of the anterior part of the ventrolateral surface. Besides these several thin nerve branches emerge from the ganglion (Splittstösser 1913).

The cortex is 150—200 μ thick in average, and is generally thickened close to the emerging nerves as in the case of the other two ganglia. The average dimension of cells is 15—40, μ large cells of 40—55 μ are relatively few and they are localized also here, near the emerging nerves. In lobus branchialis the dimension of nerve cells is less than 30 μ. These nerves are uni- or bipolar and are forming a cortex of varying thickness.

In the area between the spots from where the two pallial nerves originate a group of cells is observable which is wide in its middle and is narrowed laterally and separated from the cortex a by considerable neuropile area. Because of its localization it can be designated as nucleus posterior *(Fig. 4)*. This nucleus is constituted predominantly from uni-and multipolar cells, and close to the nucleus many robust fibres are observable in the neuropile *(Fig. 5)*. A cell group of similar appearance is localized in front of the area between the two CVc, this, however, is not separated from the cortex and may consequenly by regarded as its thickening.

In the neuropile of the visceral ganglion groups of thin and thick fibres passing in various directions are observable. The thick fibres may generally be traced until the initial section of emerging nerves. Groups of thin fibres passing unilaterally and also transversally between the sites of origin of nervus pallialis and the CVc are also observable. These paths are often recognizable also under stereoscopic microscope on the dorsal surface of the visceral ganglion. Minor or major groups of fibres from other nerve branches are also joining these tracks. Besides these, thin fibre groups of most varying directions are also demonstrable in the neuropile.

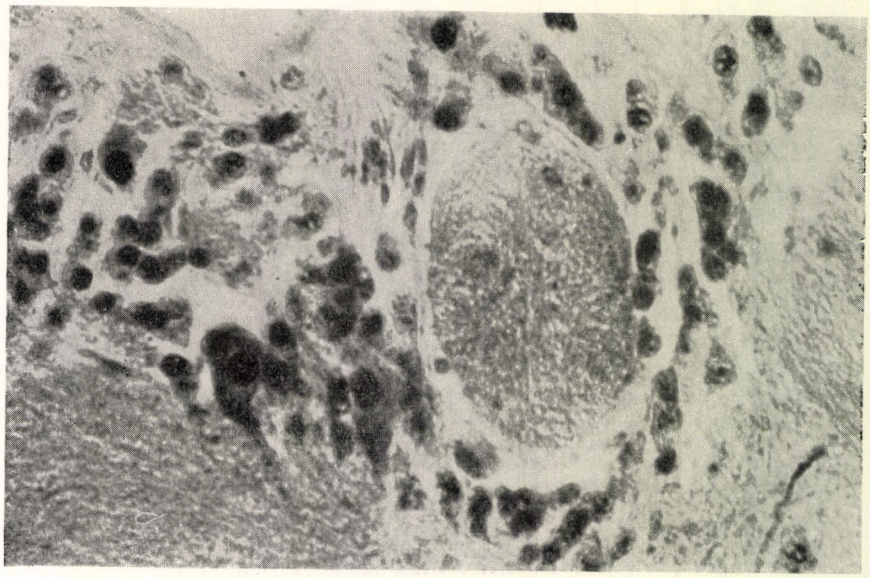

Fig. 2. Cerebral ganglion. Cross-section (N) of n. adductoris anterior surrounded by nerve cells — multipolar nerve cells. × 500

2. ábra. Cerebrális ganglion. A n. adductoris anterioris egy kilépő ágának keresztmetszete (N), amelyet idegsejtek vesznek körül — multipoláris idegsejtek. Nagyítás: 500 ×.

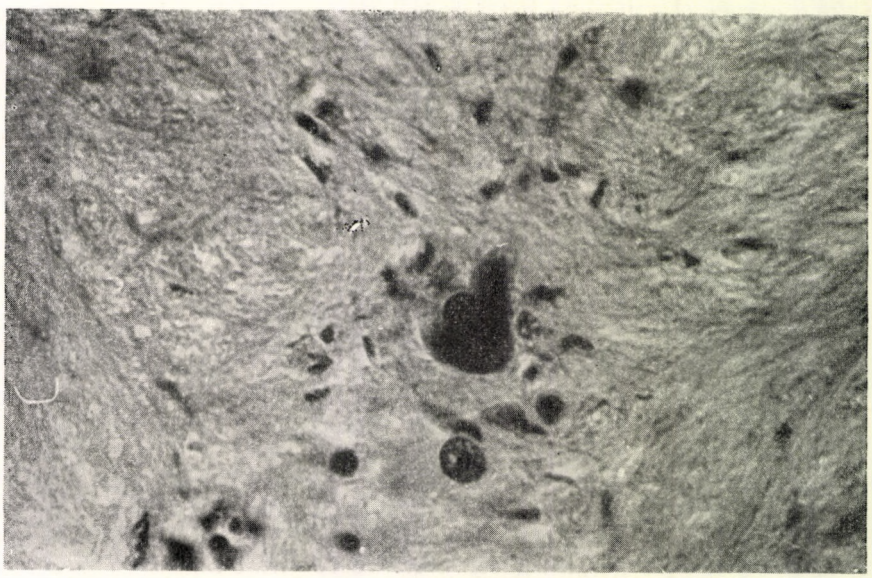

Fig. 3. Cerebral ganglion. Isolated multipolar cell in the neuropile. (The processes are readily observale in the serial sections). + 500

3. ábra. Cerebrális ganglion. Magános multipoláris sejt a neuropilben. (A nyúlványok sorozatmetszeten jól követhetők). Nagyítás: 500 ×.

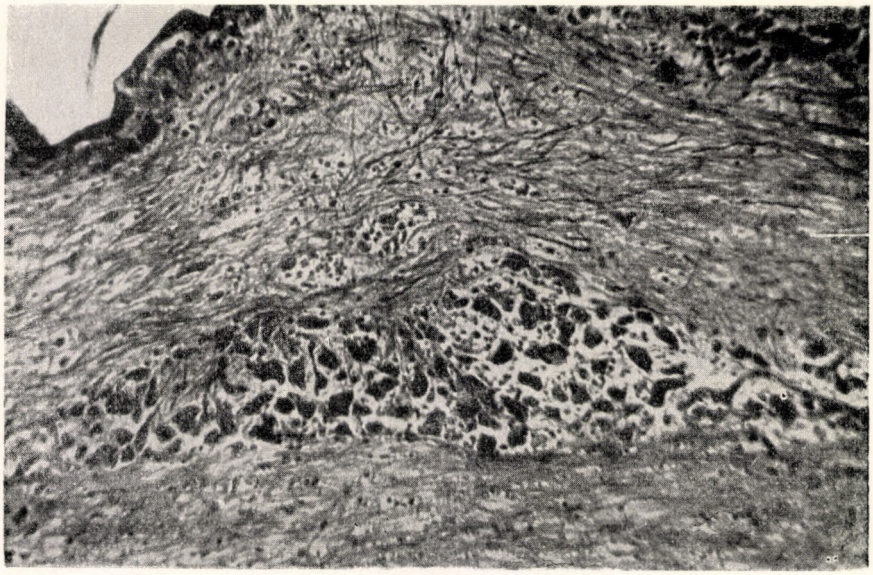

Fig. 4. General picture of the nucleus posterior of the visceral ganglion. × 160
4. ábra. A viscerális ganglion nucleus posteriorjának átnézeti képe. Nagyítás: 160 ×

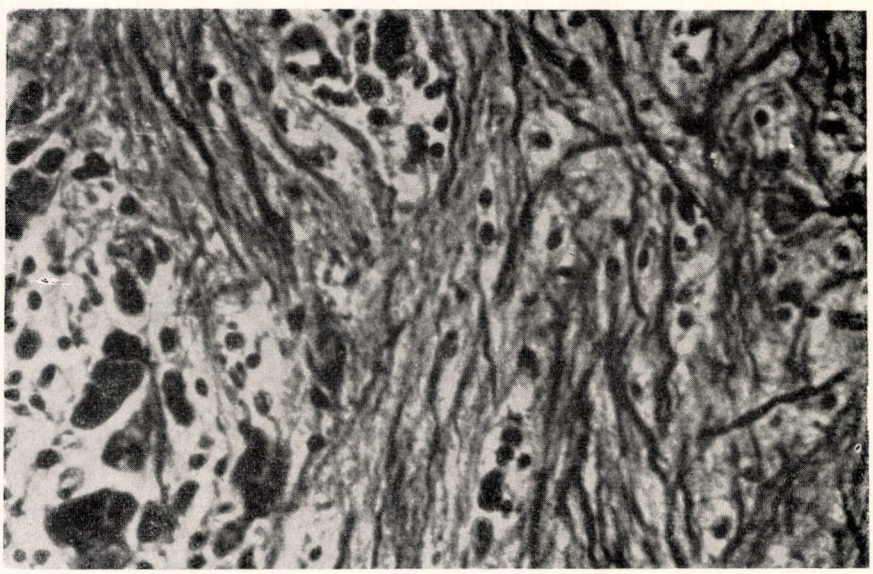

Fig. 5. Enlargement of the section of Fig. 4. The large numbered fibres are readily visible. × 500
5. ábra. A 4. ábra részlete. Jól láthatók a tömegben előforduló vastag rostok. Nagyítás: 500 ×.

Pedal ganglion

The bilateral lobes of pedal ganglion are not completely fused. Their medial surfaces are very close to each other, but organic contact exists only in the form of short commissure-kind connectives between them. On the dorsal and lateral surfaces of this pair of ganglia only very thin nerve branches originate. The cerebropedal connective arrives at the anterior tip. Nerve pedals I, II, III, and IV originate from the ventral side, moreover, from its lateral edge and from the posterior tip (SPLITTSTÖSSER 1913). It is worth mentioning that many individual variations both in number and in pattern of branching are observable in these nerves and very often differences exist also between the two sides.

On the dorsal and lateral surfaces where only thin nerves pass out from the ganglion a cortex is observable constituted chiefly of medium sized and smaller cells. A thicker cortex is found on the medial surface where large cells occur in relatively great umber. Their processes pass also to the opposite side across the connectives. The cortex is generally thicker and the emerging nerves are surrounded by coronary groups of large cells on the ventral surface, near the site of origin of the chief nerves. The thick axons of the large cells are passing towards the emerging nerves.

On this place large cells with light plasm are often observable. This area is extraordinarily rich in lamellar glia cells *(Fig. 6)*.

It should be noted that in the case of all three ganglia the thick fibres can be followed up to the initial section of the emerging nerves. Fibres of this kind are not demonstrable in sections of nerves lying at a greater distance from the ganglia. In some nerve branches, however, as for instance in the CVc *(Fig. 7)* and in nervus pallialis posterior maior these nerve cells appear solitarily or in small groups also at a greater distance from the ganglia.

Discussion

It may be established on basis of the experimental results that the cellular cortex of ganglia is far from being homogeneous everywhere. There are places where large cells are dominating, and in other parts of the cortex they are not demonstrable. A stratification, however, similar to that described by NAGY (1962) was not found in the cortex.

The structural differences in the various areas of cortex suggest that different functions are bound to its different areas. The thick axons visible around the place from where the nerves originate are observable also in the initial section of the nerves, which point to the fact, that these cells represent cetrifugal pathways i.e. they are of motoric nature. Thus, it is inferred that the areas where these giant cells and thick axons do not occur are most probably sensorial or associative areas.

No reference is made in earlier works to the nucleus posterior of visceral ganglion. This nucleus is probably of motoric character.

It would be decisive to explain the fact that in the peripherial nerves only thin fibers could be found. According to earlier electron microscopic observations of the CVc 75—80 per cent of the axons are thinner than 0.5 μ (LÁBOS et al. 1963). At the same time, it is worth mentioning that the axons of CVc are branching off in different directions (ZS.-NAGY and BENKŐ 1965).

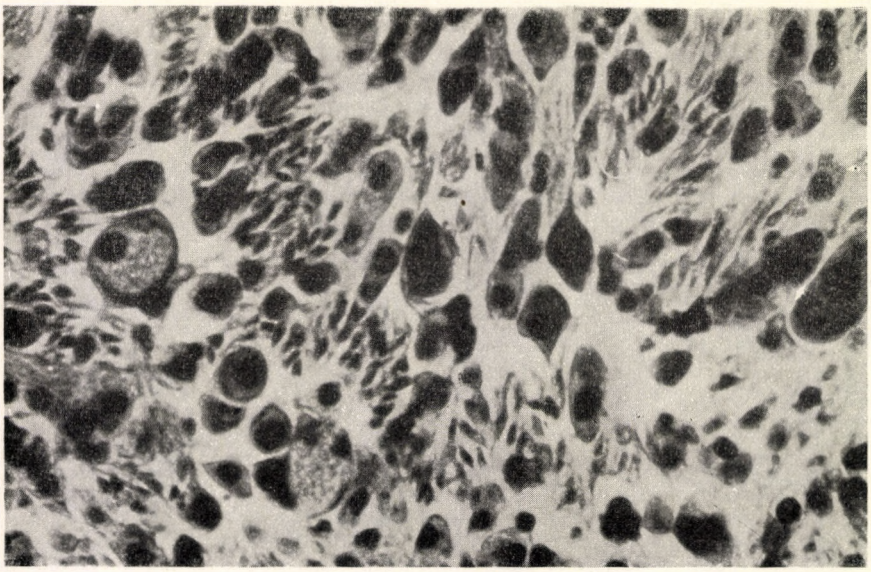

Fig. 6. Section of the ventral surface of pedal ganglion. × 500
6. ábra. A pedális ganglion ventrális felszínének részlete. Nagyítás: 500 ×.

On basis of these findings it is assumed that the thick axons are divided into thinner ones and this is why they are not demonstrable in the nerves in peripheral areas far from the ganglia.

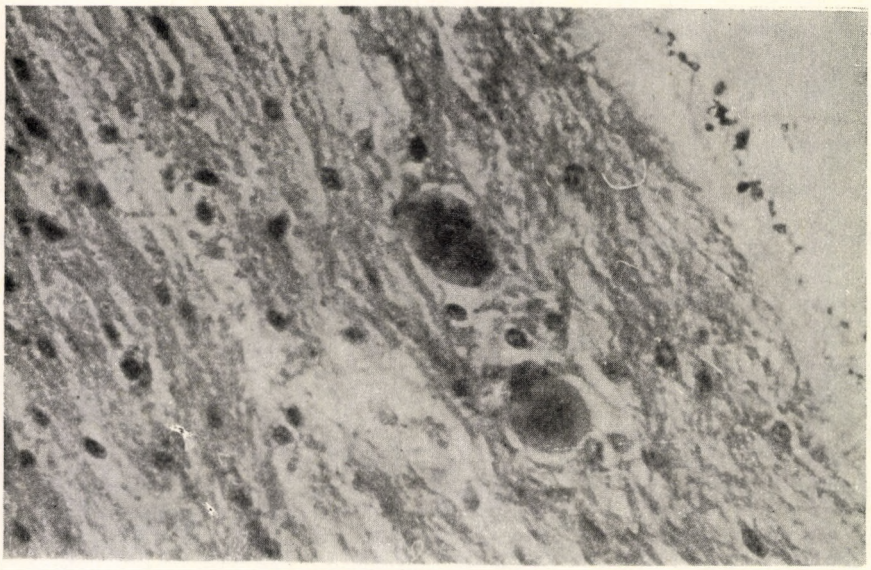

Fig. 7. Nerve cells in the CVc at 10 mm distance from the cerebral ganglion. × 500.
7. ábra. Idegsejtek a CVc állományában a cerebrális gangliontól kb. 10 mm-re. Nagyítás: 500 ×.

Summary

Authors investigated the cyto-topographic construction of ganglia of *Anodonta cygnea* by the impregnation technique. It was established that the large cells are present primarily in the immediate neighbourhood of the nerves, that originate from the ganglia and that their thick (2—3 μ) axons may be followed up until the initial section of the branches of nerves. In the cortex there are areas which contain only medium-sized or small nerve cells and are in contact only with thin axons. In the posterior part of the visceral ganglion groups of motor-like cells are observable, which are separated from the cortex and may be taken for nucleus posterior. In the visceral ganglia unilateral and transversal pathways are also observable.

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CYTO-TOPOGRÁFIAI VIZSGÁLATOK AZ *ANODONTA CYGNEA L.* GANGLIONJAIBAN

Gubicza András és Zs.-Nagy Imre

Összefoglalás

Szerzők impregnációs módszerrel vizsgálták az *Anodonta cygnea* ganglionjainak cyto-topográfiai szerkezetét. Megállapítást nyert, hogy a nagy sejtek, főleg a ganglionból kilépő idegek közvetlen környékén találhatók, vastag axonjaik (2—3μ) követhetők az degágak kezdeti szakaszáig. Vannak olyan kéregterületek, amelyek csak közepes vagy kisméretű idegsejteket tartalmaznak és csak vékony axonokkal állnak kapcsolatban. A viscerális ganglion hátsó részén motoros jellegű sejtek csoportja található, amely a kéregből lefűződött, s nucleus posteriornak nevezhető. A viscerális ganglionban azonos oldali és keresztezett átfutó pályák is találhatók.

ЦИТОТОПОГРАФИЧЕСКОЕ ИССЛЕДОВАНИЕ ГАНГЛИЕВ *ANODONTA CYGNEA L.*

Андраш Губица и Имре Ж.-Надь

Изучали цитотопографию ганглиев беззубки импрегнационным методом. Крупные нейроны обнаруживаются в непосредственной близости от места выхода нервов из ганглия, их толстые аксоны (2—3 μ) можно наблюдать до разветвления нервных стволов. В ганглиях имеются кортикальные участки, содержащие только мелкие и средние нейроны, которые связаны только тонкими аксонами. В задней части висцерального ганглия обнаруживается группа клеток моторного характера, отделяющаяся от корковых клеток; ее можно назвать nucleus posterior. В висцеральном ганглии можно видеть и прямые, и перекрещенные нервные пути.