

The Study of Neurons Under an Electron Microscope. Review

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ARTICLE INFO

Received: 📅 December 11, 2023

Published: 📅 January 03, 2024

Citation: Bon LI, Maksmovich N Ye, Ostrovskaya OB and Moroz EV. The Study of Neurons Under an Electron Microscope. Review. Biomed J Sci & Tech Res 54(2)-2024. BJSTR. MS.ID.008536.

ANNOTATION

In many tissues, the morphological relationships between cells are not of particular importance, and the cytologist is mainly interested in the study of organelles found in a certain population of cells of the same type in structure and function. The situation is different in the nervous system, where the study of subtle cytoplasmic details must ultimately go hand in hand with the elucidation of relationships or connections between cells of different types. Since, however, the processes of neurons often stretch over considerable distances, the electron microscopic examination of even very small volumes of tissue is associated with incredible difficulties.

Keywords: Neurons; Electron Microscope; Methods

Abbreviations: DL: Dense Line; PS: Presynaptic Sacs; RNA: Ribonucleic Acid; IM: Inner Membrane; NF: Neurofilaments; SC: Synaptic Cleft; SV: Synaptic Vesicles

Introduction

In many tissues, the morphological relationships between cells are not of particular importance, and the cytologist is mainly interested in the study of organelles found in a certain population of cells of the same type in structure and function. The situation is different in the nervous system, where the study of subtle cytoplasmic details must ultimately go hand in hand with the elucidation of relationships or connections between cells of different types. Since, however, the processes of neurons often stretch over considerable distances, the electron microscopic examination of even very small volumes of tissue is associated with incredible difficulties. For example, in order to capture a structure with a volume of no more than 1 mm³ with the help of a photograph at such a relatively small magnification as 10,000 X, it would be necessary to take 4-108 photographs [1].

The processes of neurons, especially in the gray matter, can twist and bend in all directions, so that with the inevitable oblique sections of the membranes in this case, they would be invisible. When it comes to the nervous system as a whole, the methods of light and electron microscopy are of equal importance and must be continued

to improve and use with the same energy. As might be expected, many of the advances referred to in this chapter are related to a better understanding of the structures already known from examination of conventionally stained specimens under a light microscope, such as Nissl, Golgi apparatus, neurofibrils of the perikaryon and the myelin sheath of the axon. The thin spiny outgrowths on the arborizations of the dendrites seen on the Golgi-stained preparations can no longer be considered an artifact, since these are undoubtedly specialized synaptic structures, and it is quite possible that they play an important role in the excitability of the dendrites. Now we understand why it was not possible to detect «buttons» in the cerebral cortex on preparations impregnated with silver, which led some researchers to assume the presence in this area, where higher functions are carried out, of neuronal connections of an unusual type; in fact, there are many synaptic contacts of the usual morphology in the cortex. For us, the cytoplasmic structural basis of the ring-shaped, reticulate and bulbous buttons, especially characteristic of the spinal cord, has ceased to be a mystery.

At the same time, relatively few structures were revealed on electron micrographs that would not have equivalents on conventional histological preparations. The most obvious example of this is the presynaptic vesicles (the question of whether they contain a neurotransmitter is still very controversial). An important observation, available only for electron microscopy, concerns membrane thickenings, possibly representing the actual sites of impulse transmission through the synaptic cleft [2]. The width of this gap is now known, and as a result of its discovery, many researchers have concluded that the dispute about discontinuity (division into neurons) or transsynaptic continuity of the cytoplasm is over. Most of the observations described in our essay were made on the cerebral cortex of rats, cats, dogs, guinea pigs and humans, the cerebellar cortex of rats and various parts of the gray matter of the spinal cord of rats and cats.

Preparation Methods

Fixing

The areas of the brain to be examined were excised under ether anesthesia with a fine scalpel and immediately cut into smaller pieces (preferably less than 0.5 mm in all directions) in fixative. When making preparations of the cerebral cortex or cerebellum, it is convenient to use four or five razor blades, fixed in a clamp, with intermediate spacers 0.3 mm thick. With the help of such a device, a number of cuts were made in the surface of the cortex, then this area was cut off tangentially, and the resulting thin plates were separated by shaking in a fixative. The most suitable fixative was osmium tetroxide, although some of the material was fixed in potassium permanganate. Fixation lasted 3 mc at 4°C, a light mixing device was used [3].

Dehydration and Coloring

After fixation, tissue pieces were washed in 10% ethanol and dehydrated in a series of alcohols of increasing concentrations; The whole process took 15 minutes. The osmium-fixed material was then stained with a 1% solution of phosphotungstic acid in absolute alcohol, after which embedding was performed [4]. The material fixed with permanganate left unpainted. Other changes in methodology will be indicated at the appropriate places in the text.

Filling

After dehydration and staining, tissue pieces were washed in absolute alcohol and then immersed in araldite (SU 212) for 12-24 mc at 50 °C for soaking. In the first 5-10 minutes after immersion, they made sure that the pieces did not float to the surface and did not dry out, then they were transferred to a mixture of resin (50 ml), sealant (NU 964, 50 ml) and plasticizer (dibutyl phthalate, 2,5 ml) for 2-3 days at 50°. The final mixture consisted of resin (50 ml), densifier (50 ml), plasticizer (2.5 ml), and FU accelerator 0.64, 1.2 ml); left the resin with the pieces immersed in it to harden. Changes were made at intervals of 2 hours, the temperature of the immersed pieces was maintained at 50°C, while the rest of the mixture was kept at room

temperature to slow down the polymerization. Before each change, the excess of the old mixture was removed with filter paper. From the obtained blocks, sections were made, usually manually, which were placed in immersion oil and examined in a phase-contrast microscope in order to accurately determine the localization of the studied area. The block was given the appropriate shape.

General Remarks

The various methods described here have certain drawbacks, and it is quite possible that, as perfusion fixation techniques are improved, they will replace immersion fixation. In addition, a detailed study in relation to the central nervous system requires the role of various ions, especially Ca ++, and the osmotic concentration of fixatives. The infiltrating properties of pouring media also leave much to be desired. Although phosphotungstic acid, uranyl acetate, lead oxide hydrate, and potassium permanganate have been successfully used to "stain" the tissue of the central nervous system, their relative merits and the possibility of their selective affinity for various structures have hardly been studied. The method of negative staining with phosphotungstic acid has recently been applied to subcellular fractions of the brain. Researchers report very important data that salts of phosphotungstic acid are able to penetrate through gaps in the membrane surface and outline the contours of organelles (synaptic vesicles, mitochondria, etc.) in the axon. The negative contrast method is extremely promising for high-resolution work, as it allows you to do without fixing and filling. In this case, the fabric, probably, changes little, except for drying, and turns out to be enclosed in a phosphotungstate film with a high electron density. In addition, here you can fully use the advantages of the large depth of focus of the electron microscope - about 0.5 mm at the level of the object. The main disadvantage, of course, is that the fabric has to be mechanically or physically reduced into small fragments [2,5-7].

Descriptive Part

Membranes

In our review, the term membrane without any defining word will be used to describe the structure, which, on sections of material fixed with osmium, usually has the form of a single dense line 60-80 A thick [8]. The membrane limits the cell from the outside and surrounds double shell nucleoplasm; it divides the mitochondria into a series of «compartments» and forms the walls of the structures of the endoplasmic reticulum, synaptic vesicles and other vesicular components. The concept of an elementary membrane is used to designate those formations that, after being fixed with permanganate, show a three-layer structure on sections, consisting of two dense lines 25 A each, separated by a light gap of the same width, which is about 75 A in total. The term basement membrane is used to describe an extracellular layer of material 100-300 A thick or more, which is well fixed by osmium, but very poorly fixed by potassium permanganate and never exhibits a three-layer structure. Simply, the membrane and

the elementary membrane are the result of the interaction between the fixative and the bimolecular lipid film, which is adjacent to the protein or other material, while the basement membrane is the product of the reaction between the fixative and the mucopolysaccharide complex.

In the study of tissues in general, the first thing that usually needs to be established is the territories of cells and their organelles. In particular, in the nervous system it is necessary to know exactly the position of the excitable membrane (a physiological concept). In this regard, many disputes arose about what is its relationship to the corresponding morphological element - the membrane or the elementary membrane. It is likely that the outermost zone of the excitable membrane corresponds most closely to the elementary membrane of permanganate-fixed preparations, although much more research is needed to elucidate this issue. Until this is known with accuracy and, most importantly, until the artifacts associated with compression or swelling of the tissue during its processing are eliminated, it will not be possible to accurately determine the linear dimensions and volume of the extracellular space, synaptic clefts, synaptic vesicles, etc. e. Further details about membrane structures will be given in subsequent sections, where the various components of neurons and neuroglia are considered separately [9].

Nucleus of Neurons

The results of applying electron microscopy to the study of the structure of the interphase nucleus turned out to be generally disappointing; the nuclei of neurons and neuroglial cells were no exception in this sense - they differ little from the nuclei of other cells. Here you can see bunches of chromatin granules, but these granules, unlike cytoplasmic ribosomes, are never arranged in separate rosettes [10]. Thin filaments are also visible, but the relationship of both these structures to the location of interphase chromosomes and the distribution of deoxyribonucleoprotein in them remains unclear. The nucleolus containing RNA (photo 215) often has a mesh structure. The nuclear membrane consists of two closely spaced membranes. The inner membrane (IM) is denser than the outer one, and sometimes one can observe the continuous transition of the latter into the membranes of the granular endoplasmic reticulum. In places, both membranes are interconnected, forming «pores», separated by regular intervals. In a neuron of the anterior horn of the spinal cord, the nucleus, 10 microns in diameter, has up to 3-4 pores. The pore is obviously closed by a dense diaphragm, which is not visible on preparations fixed with permanganate. On the tangential section of the nuclear membrane (photo 209) one can see the hexagonal arrangement of pores. They form a typical ring, consisting of 7-10 subunits of a spherical or tubular structure. The central particle apparently lies on the diaphragm or inside it, and the latter appears pale on a tangential cut, since it weakly scatters electrons.

Obviously, understanding the mechanisms of transport of substances across the nuclear membrane would be very useful for us, but little is known about them, especially in the case of neurons. It is

possible that the exchange is carried out only through the pores and is somehow regulated by the protein membrane. The central particle of the diaphragm may be a fixed structure, or perhaps a unit of migrating material, delayed on the way between the nucleoplasm and cytoplasm [11]. A feature of mature neurons is their inability to mitotic division, even after damage. Another feature is the displacement of the nucleus to an eccentric position after the axon has been transected.

Granular Endoplasmic Reticulum and the Substance of Nissl

A characteristic feature of the perikaryons of many neurons, when examined under a light microscope, are accumulations of basophilic material, which are detected when stained with aniline dyes. Nissl divided neurons into different groups depending on the content, orientation and distribution of this material. The Nissl substance was identified with the granular endoplasmic reticulum, pointing out its similarity to the structures seen with a light microscope in the basal basophilic region of some cells of the glands that secrete proteins. Photo 210 shows a mass of granular (rough) reticulum lying in the peripheral region of a large neuron from the anterior horn of the cat's spinal cord. Presynaptic sacs (PS) are in contact with the surface membrane of the neuron. The mass of the reticulum consists of a number of flat cisterns limited by a membrane, twisted and separated by groups of small dense granules 100-300 Å in diameter. They are called ribosomes or RNP granules (ribonucleoprotein granules) [12]. It is believed that ribonucleic acid (RNA), which is in solution or bound in ribosomes, plays the role of a template for protein synthesis in the neuron, and this process, in turn, is controlled by nuclear DNA. These proteins include, for example, proteins involved in the construction and repair of membranes, metabolic enzymes of a general type, special neuron proteins, for example, enzymes involved in the synthesis of synaptic transmitters (for example, choline acetylase, etc.), and inactivators these transmitters (for example, cholinesterase), as well as neurofilament proteins. A significant part of the evidence in favor of the presence of RNA in ribosomes was obtained from the analysis of particles separated from membranes with detergents and separated by ultracentrifugation.

Although the ribosomes of gland cells that secrete proteins are the most studied, similar methods have shown that neuronal ribosomes also contain RNA. The brain fractions rich in ribosomes necessary for analysis can be prepared without the use of detergents, since many of these granules, lying in groups in the cytoplasm near the membranes of the endoplasmic reticulum, are not attached to the latter. In studies at high resolution, no indication has yet been obtained that protein and nucleic acid are separated from each other in mammalian ribosomes, in contrast to, for example, bacterial ribosomes. The membranes of the endoplasmic reticulum in the nerve cell are similar in their morphology to the surface membrane and on preparations fixed with permanganate, they show the characteristic structure of an elementary membrane. Morphological continuity between the surface membrane and the endoplasmic reticulum has not yet

been demonstrated, and it is possible that it does not exist. Perhaps many of the cavities of the reticulum are interconnected by canals, and possibly communicate with the smooth reticulum, but some of the cavities are probably isolated. Finally, in this context, mention should be made of hypotheses regarding the physical and chemical mechanisms of recording, storing and reproducing information that are associated with learning processes. It has been suggested that the transmembrane ion currents accompanying the pulse can lead to the appearance of altered RNA, on which, in turn, qualitatively altered proteins are synthesized, which are then included in the composition of the synaptic membrane [13]. At present, there is almost no experimental evidence to support this theory.

Chromatolysis

If the axon of the nerve cell is not cut too close to the perikaryon, so as not to cause irreversible damage, then often there will be a redistribution, reduction, or sometimes complete disappearance of the Nissl stain, accompanied by displacement of the nucleus into an eccentric position. After a few weeks, the axon regenerates, and the normal picture is gradually restored. Chromatolysis was studied in cells after transection of the vagus nerve using a light and electron microscope. A study in a light microscope showed that Nissl's substance disappeared completely in only about 10% of the affected cells [14]. For technical reasons, low magnifications proved to be the most suitable for elucidating the distribution of the granular reticulum. Photo 213 shows a normal ganglion cell with discrete masses of the granular reticulum, and Photo 215 shows a cell 11 days after axon transection with nearly obscured granular reticulum and an eccentric nucleus. Mitochondria are preserved, and their number increases [15]. However, after 11 days, most cells still contained clusters of granular reticulum structures, but the latter was reduced and redistributed. A study of damaged cells on the 11th day after axon transection at high magnification showed that masses of neurofilaments are located in the areas of the cytoplasm that were previously occupied by the granular reticulum. 25 days after axon transection, the granular reticulum was still reduced, but at the same time, few neurofilaments remained in the cytoplasm. Significant changes were found in the agranular reticulum and the Golgi apparatus. Its numerous elements could be seen throughout the cytoplasm, the cisterns looked like flattened and elongated contours, surrounded by many small spherical formations, many of which contained dense material [16].

It is likely that both the ribosomal and membrane elements of the granular reticulum disappear, but electron microscopy does not yet provide any information on how this occurs. Since at least part of the RNA is bound to ribosomes, a decrease in their number means the release of RNA in a soluble form, but whether this leads to a decrease in the total amount of RNA in the neuron or only to its redistribution is unclear. The fact that neurofilaments become more visible in electron micrographs is not unexpected, as it corresponds to the changes in neurofibrils that are observed with a light microscope

in silver-impregnated cells that have undergone chromatolysis. Significantly worse studied with the help of conventional and electron microscopes are the possible changes in those neurons in which the bodies and processes are entirely located within the central nervous system. In some of these cells, axon transection often does not result in chromatolysis; other cells no longer recover after cutting the axon.

Agranule Reticulum (Golgi Apparatus)

The second system of cytoplasmic membranes, which called the agranular reticulum due to the absence of ribosomes attached to it was described. This system was notable for variability, but it was based on densely packed contours with narrow gaps, expanded in some places (usually at the ends of the contours). The impression was created that these contours often break up into many bubbles. The described structure is now well known, and we will consider only two examples. Photo 214 shows a system of a rather simple type ER, represented by individual complexes in a relatively narrow rim of the cytoplasm of the pyramidal cells of the cerebral cortex. It consists of several non-pelletized tanks with extensions and accompanying bubbles. Under it lies the nucleus (N), and above it there are elements of the granular (rough) endoplasmic reticulum ER (gr) with characteristic ribosomes. For comparison, photo 212 shows a more complex set of a large nerve cell of the anterior horn of the cat's spinal cord. It contains bubbles with simple walls (500-1000 Å and more in diameter); one can also see vesicles whose walls contain structural subunits. On a cut of a small neuron, most often only one complex of the agranular reticulum can be found, but in a large perikaryon, several such complexes may appear in the cut plane.

Most of the available evidence strongly suggests that the Golgi apparatus is at least a marker of the localization of the agranular reticulum. For example, in each of the well-fixed Purkinje cells, a number of reticulum elements can be seen using an electron microscope, and this suggests that the entire agranular reticulum system is distributed in a perinuclear network, similar to the Golgi network [17]. In most glandular cells, the agranular reticulum is located between the nucleus and the secretory pole of the cell; it is in this area that the Golgi apparatus is detected with the help of a light microscope. In cerebellar granule cells at one pole, a single Golgi complex is detected by staining, and an agranular reticulum complex is detected in the same place using an electron microscope. In cells of several types, including neurons, the Golgi apparatus was stained for examination using a light microscope, and then the same material was examined using electron microscopy. Under these conditions, the fixation was poor, but nevertheless it was possible to see metal deposits in close association with the agranular reticulum. Finally, in the perikaryon, in addition to agranular membranes, there are only three common organelle systems - mitochondria, granular reticulum (Nissl substance) and neurofilaments. The agranular reticulum forms the basis for the metal deposits that form the Golgi apparatus visible under a light microscope, but opinions on the histochemical mechanism of this staining reaction are very controversial. In the cells

of the exocrine glands, the protein secretion is first synthesized in ribosomes, and then, moving through the channels of the endoplasmic reticulum, it finally reaches the Golgi apparatus and takes the form of granules, which are later released from the cell. Little is known about the functions of the Golgi apparatus in neurons.

Neurofilaments

As we know axoplasm has the birefringent properties, from which it was concluded that it contained longitudinally oriented filaments. In photo 217, bundles of neurofilaments (Nf) can be seen in the cytoplasm of the nervous body. Each strain is about 100 Å thick (arrow indicates their cross sections), appears solid at low magnification, has no ends, and probably does not branch. Neurofilaments can be seen in most large neurons, where they pass in bundles through the Nissl substance, but they are few or absent in the bodies of many neurons of the cerebral cortex and in the bodies of cerebellar granule cells. They are absent in some dendrites. In the dendrites of spinal cord cells, they can stretch for hundreds of microns, forming a zone where neurofilaments are mixed with dendritic tubules, but eventually, as the dendrite thins, they disappear and only tubules remain. Neurofilaments are almost always present in myelinated fibers, less often in the axons of nonmyelinated fibers, and in some cases they enter presynaptic sacs, where they can form rings or reticular masses.

On the basis of X-ray diffraction data, the theory that the neurofilament is built from globular protein molecules arranged in a spiral around a longitudinal cavity - similar to how they are located in the tobacco mosaic virus, only there is no evidence of the presence of nucleic acid was appeared. Indeed, a number of researchers using high-resolution electron microscopy have found a longitudinal internal cavity in the neurofilament. The presence and distribution of neurofilaments in the perikaryon, dendrite, axon, and presynaptic sac correlate with linear deposits of reduced silver detected in these parts of the cell after silvering. Deposits of silver visible under a light microscope are called neurofibrils. The relationship between neurofilaments and the neurofibrillary structure of synaptic buttons will be discussed later. The function of neurofilaments is currently unclear.

Mitochondria, Lysosome, Centriols and Lipid Drops

Mitochondria, structures in which enzymes of oxidative phosphorylation are localized, are found throughout the cytoplasm of a neuron. In dendrites and axons, they are usually elongated along the axis of the process and reach several microns in length. Most neuron cell bodies contain many mitochondria. During chromatolysis, their number increases. These ultracentrifuge-settling particles, similar in size to mitochondria, have been found in liver cells, but are also believed to be present in neurons. They are better studied from the biochemical than from the morphological side. Lysosomes contain hydrolytic enzymes that show their autolytic action only after cell death or in the presence of phagocytosed particles, when their activity is more localized. In the bodies of neurons, for example, in small cells-

grains of the cerebellum, centrioles are occasionally visible. They are probably absent in most neurons of the spinal cord and cerebral cortex, which is undoubtedly connected in some way with the inability of neurons to mitotic division. Nerve cells can sometimes even have cilia: they were described in the preoptic nucleus in silver carp, in the lizard forebrain. Perhaps they function as chemoreceptors. Finally, in the bodies of neurons, as in cells of another type, dense homogeneous bodies are often found. They usually do not have a boundary membrane and considered to be lipid droplets.

Dendrites

Dendrites in the central nervous system are never covered with myelin; in adult mammals, in those parts of the central nervous system where the blood-brain barrier operates, the surface membranes of dendrites - as well as the surfaces of the bodies of nerve cells and axons - never directly adhere to the basal membranes surrounding blood vessels: they are always separated by processes of glial cells. In larger neurons, the granular endoplasmic reticulum can enter the basal areas of the dendrites, which corresponds to the distribution of the Nissl substance according to light microscopy. The most characteristic feature of many dendrites is the presence of numerous, regularly arranged tubules of unlimited length, about 200 Å in diameter. Photo 216 shows a longitudinal section of a dendrite from the visual cortex of a rat. The tubules run longitudinally in the axial region of the dendrite; at the bottom left they are shown separately at a higher magnification. In the places marked with the letters x and y, they are directed perpendicular to the plane of the cut (apparently, these are the places of dendrite branching), and here you can see the regularity of their location and the annular shape in the cross section. It was suggested that these tubules are continuation of the tubules of the endoplasmic reticulum located in the perikaryon. However, at present it seems likely that this is not the case, because after fixation with potassium permanganate, the reticulum membranes are preserved and reveal a three-layer structure of the elementary membrane type, indicating their lipoprotein nature. Dendritic tubules, on the contrary, are poorly fixed with permanganate, and, since they do not have a three-layer structure, they cannot be a continuation of the membrane components of the endoplasmic reticulum. Some authors now believe that tubules, like neurofilaments, are fibrillar protein structures, but it has not yet been established whether they also have argyrophilic properties.

The tubules are not argyrophilic and thus should not give a picture of neurofibrils. Dendrites often contain some neurofilaments mixed with tubules. Similar tubules can be found in axons, and occasionally a small number of them can be seen in oligodendroglia. There is no indication that the tubules are connected to the surface membrane or pass into it without interruption. Dendrites also contain mitochondria (often elongated) and groups of cavities limited by membranes, often located in the peripheral zone of the dendrite cytoplasm (photo 216). In the sensorimotor area of the cortex, in the cytoplasm of the basal sections of the dendrites, structures resembling spiny

apparatuses were noted. Other dendrites can directly adjoin the dendrite, separated only by a gap 200 Å wide, in which there are no processes of glial cells; its surface may contain glial processes and axodendritic synapses. Many dendrites are equipped with thin spike-like protrusions that serve as sites of synaptic contact. The synaptic relationships of dendrites will be discussed in detail later.

Axon

In vertebrates, most neurons, with the exception of cells such as retinal amacrine neurons, are believed to have axons that usually originate from the perikaryon or from the basal portion of the dendrite, as, for example, in a small part of the cerebellar granule cells. Axons often branch at various distances from the perikaryon, sometimes forming recurrent collaterals. The axon hillock, the site where the axon originates from the perikaryon, has not yet been described in detail from electron microscopy data. It is often found on sections, but often remains unrecognized, as it is easily mistaken for a dendrite [18]. At present, its only distinguishing features are a decrease in the amount of granular endoplasmic reticulum and a rather strong narrowing towards the periphery, in contrast to the places where the dendrites originate. The axon hillock and axon origin deserve more detailed study with an electron microscope, since, according to physiology, it is in this area in the spinal motor neurons that the action potential arises.

As in the peripheral nervous system, the axons of the central nervous system may or may not be covered with myelin, although in the latter case there are no Schwann-like cells. The outer diameter of the myelin sheath can vary from 0.5 microns to several microns, while the thickness of non-myelinated fibers can be as little as 0.1 microns, but can reach 2, 3 or more microns, especially in the transition region between the axon and the presynaptic bag [19]. Axoplasm contains oval or elongated mitochondria, and in some places there are still light areas bounded by a membrane. Often, especially in myelinated fibers (photo 218), neurofilaments can be seen, which serve as the basis for the deposition of silver in staining methods that reveal neurofibrils. In myelin fibers one can also observe tubules 200 Å across, similar to tubules in dendrites. In some places they are also found in myelinated nerve fibers; for example, they are common in the axons of the Lissauer tract in the spinal cord. In both axons and dendrites, tubules probably do not have argyrophilic properties. Compact myelin of the pulpy fibers of the central nervous system detects the periodicity of the structure, similar to the periodicity of myelin in peripheral nerve fibers; this structure consists of main dense lines lying at a distance of 120-140 Å from each other, and intermediate lines, which look less dense and discontinuous on osmium preparations, but after fixing with permanganate they have the same density as the main dense lines. Photo 218 shows a myelinated axon from the rat cerebral cortex in cross section. The arrow points to the surface membrane of the axon. The axoplasm shows neurofilaments and one mitochondrion.

The myelin sheath has the same type of organization as that of

peripheral nerve fibers, only it is not enclosed in a sleeve from the cytoplasm of the mother (Schwann) cell, the edges of which close to form a mesaxon. Instead, the outer membrane, which belongs here to the oligodendrocyte, is included in the myelin sheath and forms its surface plate, so that only a small «tongue» of the cytoplasm remains (photos 217, 218). This tongue extends along the entire length of the internode in the form of a ridge on the outer surface of the myelin sheath. Since the cytoplasm does not surround the shell on all sides, it is clear that there cannot be a mesaxon here. Photo 218 shows dense granular material, which is very common in this strip of cytoplasm. The internal mesaxon (photo 217, 218) lies against the cytoplasmic tongue, and this position is very stable; this even suggests that during the laying of the myelin sheath in the process of ontogenesis, some mechanism operates, as a result of which the line of contact at the inner mesaxon in most cases “stops” against the tongue of the outer cytoplasm. Another typical feature (photos 217, 218) is the presence of dense radial stripes (x) in myelin. In photo 218, it can be seen that these radial stripes, apparently, often pass between dense granules lying in the outer tongue and in the inner cytoplasm of the oligodendrocyte.

In addition, in the myelin sheaths of axons located in the central nervous system, the inner layer of the glial cytoplasm is sometimes absent for a considerable extent (photo 218) and instead of it a dense line (DL) is formed, directly adjacent to the surface membrane of the axon. In other areas, the internal cytoplasm may be preserved. Finally, in contrast to the peripheral nervous system, the myelin sheaths in the brain often come into contact with each other, and in these places one continuous periodic structure is created. The mechanism of development of the myelin sheath in the central nervous system, according to some researchers, is similar to the mechanism of its formation in the periphery, where were observed various stages of myelinogenesis, allowing us to conclude that the Schwann cell first forms a loose helix around the axon from the cytoplasm [20].

Then, as a result of “squeezing out” of intermediate extracellular zones and intracellular cytoplasm and dense “closure” of extracellular and cytoplasmic surfaces of the Schwann cell membrane, compact myelin is formed. In the central nervous system, myelin is produced by oligodendrocytes, but the very mode of formation of the lamellar structure has not been fully elucidated. It was assumed that both during primary myelination and during remyelination after experimentally induced degeneration the myelin sheath is spirally twisted. Although the tongue of the glial cytoplasm has usually been considered an outgrowth of the oligodendrocyte, its direct relationship to the body of this type of cell has only recently been demonstrated.

As for the nodes of Ranvier, in this respect light microscopy gave much more information about the peripheral nerve fibers than about the nerve conductors of the central nervous system. As we know the internodes of myelinated fibers of the central nervous system are much longer and that the «naked» section of the axon at the nodes is relatively shorter. Indeed, the small number of nodes of Ranvier in

electron micrographs can be directly explained by the large distances between them. The "bare" section of the axon sometimes reaches 3 μ m or more in length, and processes of other neurons and glial cells adjoin its surface membrane, separated from it only by a gap 200 \AA wide. Inside the spiral layers and form tight contacts with the surface of the axon, isolating the extracellular zone between the axon and its Schwann sheath from the «naked» area of interception. In peripheral nerve fibers, the naked area is separated only by narrow (200 \AA) channels passing between the intertwined processes of adjacent Schwann cells.

Synapse

Sherrington used the term «synapse» to refer to the functional connections created by close contact between nerve cells. Light microscopy, with its limited resolving power, did little to refine this definition, although the results obtained supported the theory of discontinuity of the cytoplasm - they were consistent, for example, with differences in the tinctorial properties of the pre- and postsynaptic cytoplasm and the lack of apparent continuity between neurons. On Golgi-stained preparations. However, only the electron microscope made it possible to see the synaptic cleft 200-300 \AA wide, so that now the concept of «close contact» can be given greater accuracy. In the study of synapses from various regions of the central nervous system, a striking constancy in the width of this gap has been found so far; its similar dimensions are also observed in the synapses of the peripheral sections of the autonomic nervous system. The gap of the motor end plate is an exception - its width can exceed 600 \AA . We know a lot about the «functioning» of synapses from physiological observations; it is now believed that the mechanism of transmission of excitation in the synapses of mammals in the overwhelming majority of cases is associated with the release of a chemical substance. Since the neurotransmitter accumulates only in the presynaptic terminal and is released in effective amounts only upon stimulation of the presynaptic axon, synaptic transmission (as opposed to conducting an impulse along the axon) for this and, perhaps, other reasons, is possible only in one direction. Accordingly, electron microscopy also shows that the synapses are morphologically polarized. For example, on the presynaptic side there are bubbles of a more or less uniform size, suitable for the transmission of «packets», or «quanta», of the neurotransmitter released into the synaptic cleft. The asymmetry of those thickenings of the synaptic membranes, in which, possibly, the points of direct release and diffusion of the mediator are located, is also clearly visible [21]. Dense, hexagonally arranged protrusions are found only on presynaptic membranes, while the postsynaptic membrane, on its inner side, may have a particularly extensive and dense continuous thickening.

All these structures and their morphological modifications in the postsynaptic element, the functional significance of which is currently not so clear, will be described in the following sections. Much less attention has been paid to the question of the existence in mammals of synapses with electrical transmission not associated with the

release of any substance, and electron microscopy still does not provide any information on this matter. On the contrary, in relation to the lower vertebrates (gold carp) and invertebrates (crayfish), there are both physiological and electron microscopic data in favor of electrical transmission. The membranes in such synapses are not separated by a clearly visible gap, as in chemical transmission, but are very closely adjacent to each other. Finally, in the central nervous system of mammals, with the help of an electron microscope, one can often see dendrites, bodies of neurons, and unmyelinated axons of various nerve cells, which are not separated from each other by anything but a gap of 200 \AA . About possible interactions in these areas almost nothing is known, and electron microscopy has not yet revealed any specific structures in mammalian tissues comparable to the structures of «chemical» synapses that could indicate this kind of ephaptic transmission.

General Morphology of the Synapses of the Central Nervous System: When studying synapses with the help of a light microscope, methods based on the reduction of silver were usually used; while the buttons looked like neurofibrillary rings, bulbs or mesh extensions adjacent to the postsynaptic dendrite or the body of the nerve cell. The Golgi method also proved to be suitable for revealing the shape of some presynaptic processes, for example, the endings of the mossy fibers of the cerebellum and the hippocampus. In addition to neurofibrils, only mitochondria could be clearly recognized in "buttons" using light microscopy, and, as we have already said, it was sometimes possible to observe differences in the nature of staining of the cytoplasm of the presynaptic and postsynaptic apparatuses. Based on these and other data, various criteria have been established for the recognition of synaptic contacts on thin sections, for example, for the identification of a motor end plate, which can be indirectly recognized with certainty by presynaptic and postsynaptic components. In certain places, for example, on the surface of large cells of the anterior horns or in islets of the granular layer of the cerebellum containing mossy fibers, electron microscopy allows you to confidently determine the size and location of the «buttons». In some cases, neurofibrillary ring figures can be directly recognized by cytoplasmic neurofilaments lying within the presynaptic sac. The most thorough electron microscopic examination of the surface of glial cells failed to detect contacts with a fine structure of presynaptic sacs. Further confirmation of the correct identification of presynaptic sacs is provided by the study of degeneration processes that develop after axon transection at a great distance from the terminal apparatus. Degeneration of terminal devices was found in the zone of structures with signs of presynaptic sacs; moreover, the development of degenerative changes correlates with what is known about this from light microscopy data.

In very rare cases one can see the direct transition of the axon, reliably recognized by the myelin sheath, into the presynaptic sac, and the latter has all the signs of a terminal device, established indirectly from other observations. This kind of transition of a myelinated fiber into the presynaptic sac has been observed in the cerebral

cortex, the cerebellar cortex, and the cat's spinal cord. The terminal section of the myelin sheath (x) forms a neck around the axon, behind which the axon expands into the presynaptic sac. This sac contains the characteristic synaptic vesicles (SP) and mitochondria and is in contact with the surface of the anterior horn neuron. Membrane thickenings are observed in the contact area. Presynaptic sacs in the cerebral cortex are usually small - from 0.3 to 1.5 microns. They contain synaptic vesicles with a diameter of 300-500 Å and mitochondria; their membranes have thickened areas in the zone of contact with the postsynaptic membrane. There are two types of synapses. Synapses of the 1st type are found only on the trunks of dendrites and on their spines, and synapses of the 2nd type are found on the trunks of dendrites and perikaryons.

The same two types of synapses can also be distinguished in the cerebellar cortex and in the spinal cord, where there are other forms of synaptic devices. As already mentioned, in the cerebral cortex, the distribution of type 1 synapses is limited by dendritic trunks and their spines, and type 2 contacts are limited by dendritic trunks and perikaryons. In some areas of the brain, with the exception of the cerebral cortex, the presynaptic sacs are much larger, reaching 5 microns in one direction, and often have an irregular shape. An example is the endings of mossy fibers of the cerebellum and hippocampus - their shape is similar to that seen in a light microscope on Golgi-stained preparations. In the central nervous system, the majority of presynaptic sacs are round, oval, or pear-shaped. The synapses still considered have been axodendritic, in which the presynaptic sac is in direct contact with the dendrite shaft, or axosomatic. The first of these types is quite common in all parts of the central nervous system; the second is also quite widespread, but in some areas - for example, on the bodies of pyramidal cells of the cerebral cortex - axosomatic contacts are few, and in others - for example, on the bodies of granule cells in the cerebellar cortex - they are completely absent. The sites of synaptic contact are also dendritic spines, which will be described in the next section.

Spines: On Golgi-stained preparations, in many parts of the central nervous system, including the cerebral cortex, the caudate nucleus and the cerebellar cortex, one can see thin spike-like protrusions on the dendrites (photo 226, in the box). An electron microscopic study of the visual cortex in rats confirmed that these were indeed sites of synaptic contacts (Fig. 2b and photo 226). The presynaptic sac in photo 226 contains two mitochondria and synaptic vesicles (SV) and forms a type 1 synapse with a spine (S) of the dendrite. The length and shape of this spine correspond to what can be seen with a Golgi stain under a light microscope. In an electron microscope, only in rare cases, the spine and dendrite represent a continuous structure in the plane of the cut. Usually, the tip of the spine on sections looks like a separate pear-shaped body, with which the presynaptic sac is in contact. Some of the spines contain an organelle which called the spiny apparatus. It consists of several pouches alternating with hard plates, which sometimes appear to be double. In the zone of the hippocampus, the

spines of the dendrites are large and branched; they are introduced into the presynaptic sacs of mossy fibers containing mitochondria and synaptic vesicles. Each of the branches of the spine may contain a separate spine apparatus. The spiny apparatus, apparently, is most often found in the dendrites of the pyramidal cells of the cortex. It is absent in numerous spines of Purkinje cells and in short spines that are quite common on the dendrites of spinal cord neurons.

In photo 230 show a spine on the dendrite of a neuron from the anterior horn of the spinal cord of a cat; it is seen here in cross section inside the presynaptic sac. More recently, the spinous apparatus has been found in neurons of the reticular nucleus of the lateral geniculate body in the thalamus of the cat and has been seen in several cases in the dendrites of neurons from the posterior horn of the spinal cord of cats and rats. Until now, this apparatus has not been found in any of the classes of vertebrates, except for mammals. In the visual cortex of the rat, the spine apparatus is found approximately 16 days after birth. Apparently, first the bubbles approach each other, and then dense plates form between them. It would be interesting to find out whether the number of sacs and lamellae increases throughout life, or whether there is some factor that limits the growth of the spiny apparatus. The function of the spine apparatus is still unknown.

Function and Origin of Synaptic Vesicles: The appearance of synaptic vesicles has been described in previous sections. The contours of vesicles are often found in most parts of neurons and glial cells, but «synaptic vesicles», more or less uniform in size (300-500 Å) and forming characteristic groups, often concentrated near membrane thickenings, as far as is known, are present only in axons, in particular, in the presynaptic. It has not yet been clearly shown that synaptic vesicles contain a neurotransmitter, although there is evidence to support this. For example, in all synapses for which the chemical mechanism of excitation transmission has already been established, there are characteristic accumulations of presynaptic vesicles. The motor end plate data show that the neurotransmitter is released from the presynaptic process in «quanta» which, quite possibly, have previously been located in separate synaptic vesicles. There is some evidence that the number of vesicles in presynaptic sacs changes after axonal stimulation. However, direct data could only be obtained by isolating a pure sample of synaptic vesicles available for direct chemical analysis. Subcellular fractions isolated by ultracentrifugation from guinea pig brain homogenates using sucrose to separate the particles were examined. Three main fractions were isolated:

- Nuclei and cell residues,
- Crude mitochondrial fraction, and
- Microsomal fraction (membrane and ribosomes attached to them).

Active substances (acetylcholine, oxytryptamine and norepinephrine) were concentrated in the mitochondrial fraction.

This fraction was then subdivided into 3 subfractions by centrifugation in a sucrose density gradient. Samples of various subfractions were examined in detail in an electron microscope. Three subfractions of the crude mitochondrial fraction contained:

- Myelin fragments,
- Mitochondria, and
- Structures of nerve endings in the form of a clearly demarcated subfraction.

The presynaptic sacs here contained characteristic synaptic vesicles (SV) and mitochondria (M), and in some cases it was possible to distinguish non-separated postsynaptic thickenings. It was in this subfraction that a high concentration of chemical mediators (acetylcholine, etc.) was previously found. Thus, it is now possible to isolate samples of nerve endings, analyze mediators, which must first be converted by various methods from a bound form into a solution, and control this entire procedure by electron microscopic examination of the corresponding samples. The next step should be to isolate intact vesicles from the presynaptic sacs and try to show that they contain a neurotransmitter [5-7]. The origin of synaptic vesicles remains unclear. Thin tubules in axons, similar to tubules in dendrites were described and suggested that synaptic vesicles bud from these tubules, which in turn communicate with the endoplasmic reticulum of the perikaryon. However, at present this seems unlikely, since fixation with permanganate preserves the vesicles (photo 233) and the endoplasmic reticulum, but does not preserve the tubules: apparently, the latter, unlike the first two structures, do not contain lipoprotein membranes. This does not exclude the possibility that tubules in axons are involved in the formation of synaptic vesicles and their contents, but indicates that the boundary membranes of these vesicles must come from some other source - perhaps they are formed from large «empty» vesicles sometimes found on sections in the cytoplasm of presynaptic sacs.

They might even arise *de novo* from the cytoplasm. Possibly, vesicles bud from presynaptic mitochondria, but the content of mediators in mitochondrial fractions isolated from the brain is very low. It has also been suggested that synaptic vesicles are separated from the Golgi apparatus in the body of the neuron and move to the nerve ending with the current of the axoplasm. However, there is almost no evidence to support this. Finally, it is not excluded that vesicles form at the synaptic membrane and accumulate inside the synaptic sacs. Then they could either return to the membrane to release the mediator, or remain in the synaptic sac [16].

Neurofilaments of the Presynaptic Sac: On preparations of some parts of the central nervous system stained with reduced silver, neurofibrillary buttons may look like ring-shaped, mesh or bulbous structures. Photo 1 (light micrograph) shows such synaptic structures of a large neuron from the anterior horn of the spinal cord of a cat. Sometimes you can see a direct connection of such

rings with neurofibrils of the axon. In some parts of the brain and anterior horns of the spinal cord of the lizard, where, according to light microscopy, there are a lot of annular and bulbous buttons, the electron microscope revealed bundles of neurofilaments that form rings or networks and lie in the transparent zone of the presynaptic cytoplasm. These bundles undoubtedly serve as the basis for the silver deposits visible under a light microscope. Attempts were made to directly examine the material fixed with formalin and impregnated with silver salts using an electron microscope. In addition, the neurofilaments themselves, unfortunately, were poorly visible against the background of granular silver deposits [22]. However, the obtained pictures clearly showed that neither mitochondria, nor synaptic vesicles, nor surface membranes are involved in the staining reaction. Remains the question of whether the silver grains are deposited directly on the surface of the neurofilament (or inside it) or whether the argyrophilic material is located in the light zone of the cytoplasm where the neurofilaments lie, but remains invisible in the electron micrograph, has not yet been resolved.

A comparison of preparations stained by various methods showed that neurofibril-staining reagents reveal only a small proportion of presynaptic endings present in the tissue. It is now clear from electron microscopy data that the reason for the poor detection of buttons in neurofibril staining is that most presynaptic sacs do not contain neurofilaments and therefore remain invisible under a light microscope after silver impregnation. This applies in particular to the cerebral cortex, where there are almost no buttons with neurofibrils. This does not mean that there are no ordinary synapses in the cortex; using an electron microscope, it can be established that this area is replete with presynaptic sacs with characteristic organelles - they lack only argyrophilic neurofilaments. The function of neurofilaments in presynaptic sacs and elsewhere is unknown. It is also unknown why the relative number of buttons with neurofilaments varies in different sections of the nervous system, in the same section in different individuals of the same species and in the same section in animals of different species.

Synaptic Cleft and Thickening of Synaptic Membranes: We have already mentioned the synaptic cleft and specialized thickenings of pre- and postsynaptic membranes [23,24]. These thickenings are best seen on osmium-fixed preparations after heavy metal staining. In particular, they show a particular affinity for phosphotungstic acid, indicating that they are probably composed of proteinaceous material in the form of thin spiral filaments that reinforce the surfaces of the pre- and postsynaptic membrane. This is also supported by the absence of visible thickenings in the synapses of the cerebral cortex after fixation with permanganate. Permanganate has no or almost no affinity for most protein systems. After such fixation, it is impossible to detect the basic three-layer structure of the elementary membranes of the pre- and postsynaptic processes and no thickenings are visible; there is no and clearly visible layer of extracellular material in the synaptic cleft (SC).

After fixation with osmium and staining with phosphotungstic acid, the synapses of the central nervous system can usually be seen to have a series of dense protrusions on the cytoplasmic side of the presynaptic membrane, when the cut plane passes normally to its surface. On tangential sections, these protrusions look like dense star-shaped spots and are characterized by an approximately hexagonal arrangement, with distances between centers of about 1000 Å. At high magnification, it can be seen that they consist of intricately spiraled threads. These structures are characteristic of the presynaptic membrane and can serve as a criterion for its recognition.

Their discovery has fundamental importance for understanding the nature of synaptic function. Another structure is sometimes associated with the postsynaptic membrane of the synapses of the spinal cord - a narrow subsynaptic sac, often expanded at one end. Then the postsynaptic membrane is devoid of any noticeable thickening. A similar structure located opposite the glial terminal legs was observed on the neurons of the cerebral cortex.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2024.54.008536

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