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ANALYSIS OF THE CORPUS CALLOSUM MYELOARCHITECTONICS OF MATURE PEOPLE

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Serial semi-thin sections of laminated preparations of the corpus callosum of mature people were studied. It was found out that there is a tunnel network of interstitial fissures between the blood exchange microvessels and myelinated nerve fibres, which closes around the cellular portions. This interstitial network is an intermediate compartment between the blood flowing through the microvessels and the cells in which the interfascicular oligodendrocytes are cellularly distributed. In the corpus callosum, the entire transport system is completely aimed at ensuring the structural and functional stability of only the myelin sheaths of nerve fibres due to the secretory activity of interfascicular oligodendrocytes.

Key words: corpus callosum, myeloarchitectonics, commissural cords, interfascicular oligodendrocytes, fibrillar astrocytes.

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АНАЛІЗ МІЄЛОАРХІТЕКТОНІКИ МОЗОЛИСТОГО ТІЛА ЛЮДЕЙ ЗРІЛОГО ВІКУ

Було досліджено серійні напівтонкі зрізи пластинованих препаратів мозолистого тіла людей зрілого віку. Встановлено, що між кровоносними обмінними мікросудинами та мієлінізованими нервовими волокнами знаходиться тунельна мережа інтерстиційних щілин, яка замикається навколо стільникових порціонів. Ця інтерстиційна мережа є проміжним відсіком між кров'ю, що протікає мікросудинами, та комірками, у яких розосереджені у стільниковому порядку інтерфасцикулярні олігодендроцити. У мозолистому тілі уся транспортна система повністю спрямована на забезпечення структурно-функціональної постійності тільки мієлінових оболонок нервових волокон за рахунок секреторної діяльності інтерфасцикулярних олігодендроцитів.

Ключові слова: мозолисте тіло, мієлоархітектоніка, комісуральні канатики, інтерфасцикулярні олігодендроцити, фібрилярні астроцити.

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Glial cells are specialized cellular elements in the white matter of the brain. They differ not only in cytophysiological properties but also in a well-defined constant place in the structure of myelinated nerve fibers [7]. Even though the cytophysiological properties of glial cells of white matter are mostly known [2, 4], their topology, in our opinion, requires some more significant clarifications. In addition to microglial elements, which are phagocytic cells of nervous tissue, the cells that determine the structure of the white matter include, as we know, two populations of macroglia – fibrillar astrocytes and interfascicular oligodendrocytes [10]. According to the literature, they can be found in the structure of corpus callosum in the ratio of 1: 4, respectively [3]. Besides, the former has been found to be closely related to blood vessels and the latter to be associated with nerve fibres. Thus, it may seem that the issue of their disposition is resolved. However, their interconnection in general myeloarchitectonics remains unclear as well as the way this interconnection is carried out.

The purpose of the study was to establish the relationship of structural elements in the general myeloarchitectonics of the corpus callosum.

Materials and methods. The material obtained at Kharkiv Regional Bureau of Forensic Medical Examination (within the framework of the concluded cooperation agreement) was the brain of men and women (10 specimens each) of the second period of adulthood, who died for the causes not related to the pathology of the central nervous system. The research methods described in the publication were applied in compliance with human rights in accordance with the legislation in force in Ukraine, meet international ethical requirements and do not violate ethical norms in science and standards of biomedical research.

After two weeks of fixation in a 10 % formalin solution, the brain was prepared by separating the cerebral hemispheres and other parts of the brain from the corpus callosum. Histological methods in the modern modification [1] were used in the work, which allowed to increase the resolution of light microscopy by obtaining semi-thin sections of tissue placed in epoxy resin.

Laminated preparations of the corpus callosum were used as the initial material for the production of serial semi-thin sections. For this purpose, the required sections in the form of rectangular flat pieces with a size of not more than 4x4 mm were purposefully cut from thick sections of the corpus callosum tissues both treated with osmium and non-treated. Then they were glued with epoxy resin to the end of a pre-prepared short epoxy rod made by polymerizing epoxy resin in a gelatin capsule. The latter (with the glued sample) was firmly fixed with a special cartridge in the collet holder of the microtome. Finally, we stained the obtained 2–3 mm thick serial sections with methylene blue and polychrome dye, containing Azur-1 and basic magenta in addition to methylene blue.

The study of the obtained preparations, as well as their photographic documentation were carried out with the help of binocular magnifier MBS-9 and a light microscope "Konus", equipped with a digital photo set-top box.

Results of the study and their discussion. First, let's get acquainted with what the tissue elements of the corpus callosum look like on semi-thin sections of tissue that were not treated with osmium when they are stained with a monochrome of methylene blue. To distinguish the smallest structures of the corpus callosum, which are nerve fibers, we need to magnify the microscope with at least 40 times, when they in their mass represent basophilic fields separated by interstitial lumens of different configurations. These fields consist of an extremely dense mass of innumerable short, crooked and cross-cut rod structures with a light circle. There are nerve cell processes that act as basophilic lines, giving the appearance of a dotted pattern to the general, moderately basophilic background (fig. 1). It is clear that the light circles that frame them are cavities of the former myelin sheaths of nerve fibres.

Histological sections of the corpus callosum prove an extremely dense arrangement of a huge number of nerve fibres, which cannot be distinguished by thickness in the survey microphotographs. It is noticeable that against their background there are scattered cellular elements secreted by the intensity of basophilia. They mostly belong to the interfascicular oligodendrocytes. This can be seen at a maximum magnification of the microscope on the following cytological basis: in the reaction with methylene blue, their cytoplasm acquires intense basophilia, which indicates the presence of granular endoplasmic reticulum, which is known to be responsible for the synthesis of substances necessary for the renewal of myelin sheaths of nerve fibres. (fig. 2).

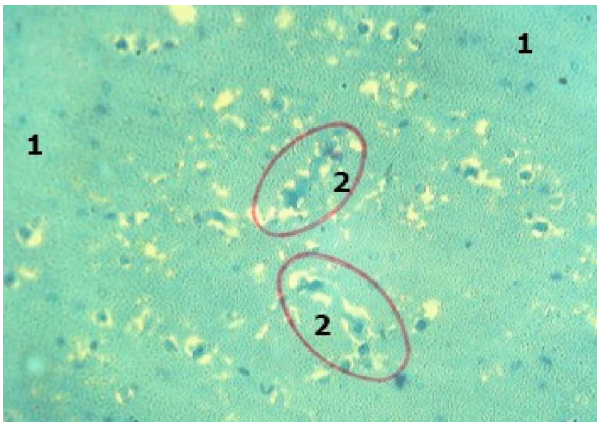

Fig. 1. The microstructure of the corpus callosum commissural cords (in cross-section) of a mature man. Semi-thin slice of tissue (without osmium treatment). Stained with methylene blue. 40x lens. 1 – general granular background, represented by a dense arrangement of nerve fibres, with interfascicular oligodendrocytes scattered among them in the form of blue specks; 2 – interstitial layers, which contain blood microvessels and fibrillar astrocytes (circled in red).

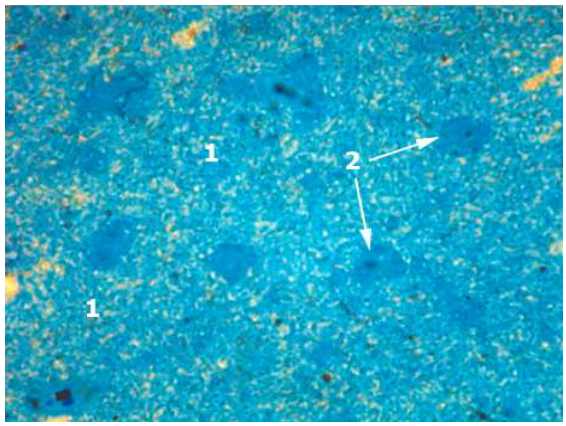

Fig. 2. The microstructure of the corpus callosum commissural cords of a mature man. Semi-thin slice of tissue (without osmium treatment). Stained with methylene blue. 100x lens (immersion). 1 – general coarse-grained background, represented by a dense arrangement of myelinated nerve fibers, with interfascicular oligodendrocytes (2) scattered among them; interfascicular oligodendrocytes are characterized by increased basophilia of the cytoplasm.

Besides, in these microphotographs, one can distinguish the processes of the same basophilia, which, emerging from these cells, are introduced into the bundles of nerve fibres. The striking difference between the structures on the semi-thin sections from the same ones on the thin sections is, first of all, the fact that clear cells with interfascicular oligodendrocytes can not be distinguished on them, but become clearer. The described effect is explained by the fact that osmium treatment leads to an increase in the contrast of those areas in the corpus callosum substance where myelinated nerve fibres are concentrated; whereas when the sections that were not treated with osmium are stained with metylene blue this effect is lost due to the relatively uniform distribution of the reagent substance in the fascicular portions leading to the monochrome colour of these structures. Some selectivity among them is achieved by using a

polychrome dye consisting of a mixture of basic fuchsin, Azure-1 and methylene blue, when the entire field occupied by dense sets of nerve fibres, acquires a pink background where cells containing a cellular element recognized by the basophilic colour of the nucleus are scattered in the form of light blue, somewhat hidden spots. It can be seen that the intensity of this colour is given by chromatin, whose distribution in these cells is typical for interfascicular oligodendrocytes (fig. 3).

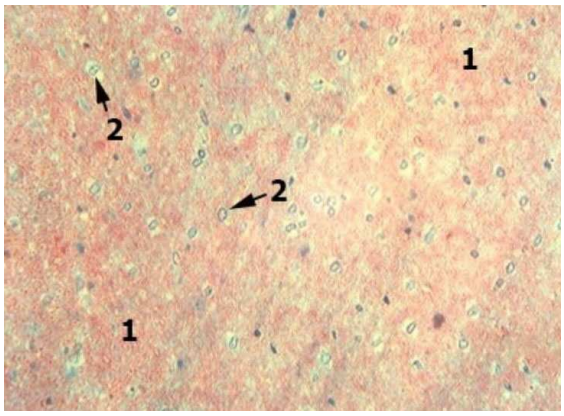

Fig. 3. The microstructure of the corpus callosum commissural cords of a mature woman. Semi-thin sections of tissue (not treated with osmium). Polychrome staining (basic fuchsin + Azure 1 + methylene blue). 40x lens. 1 – general granular-pink background, represented by a dense company of myelinated nerve fibres with interfacial oligodendrocytes scattered in the cells among them (2).

In addition, there are single small cells outside the cells with oligodendrocytes among myelinated nerve fibres on such preparations. These small cells undoubtedly belong to the microglial elements, i.e. they are nerve tissue macrophages that perform the utilization of myelin "waste" in the process of myelin sheath renewal. Thus, there is a synthetic (secretory) activity of interfascicular oligodendrocytes at one pole of this process whereas the phagocytic activity of microglial elements is at the other.

But the polychrome staining of semi-thin sections of tissue that were not treated with osmium has one drawback. The fact is that when polychrome staining is applied, interfascicular interstitial layers are stained as well. As a result, they become poorly visible against other structures. This disadvantage is absent in monochrome staining of sections with methylene blue, where the interstitial layers look like branched light slits of different configurations. In these layers, blood microvessels are in close contact with another type of glial cells – fibrillar astrocytes. They are the organizing structures of the corpus callosum myeloarchitectonics.

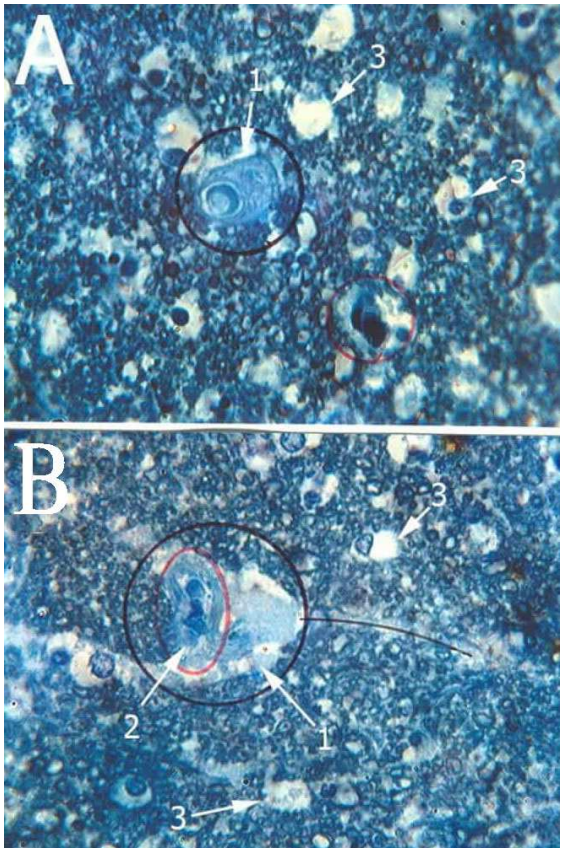

Fig. 4. The microstructure of fascicular portions of the corpus callosum of a man (A) and a woman (B) of mature age. Semi-thin sections of osmium treated tissue. Methylene blue staining. 100x lens (immersion). 1 – interfascicular layers of the interstitium (red and black circles outline the location of blood vessels with adjacent bodies of fibrillar astrocytes); 2 – arterioles; 3 – interfascicular oligodendrocytes among myelinated nerve fibers of different thickness.

A significant advantage of semi-thin sections of osmium-treated tissues in comparison with the ones that were not treated with osmium is the clear, legible nature of myelinated nerve fibres detection in them that enables not only to distinguish these units but also to judge their thickness taking into consideration the extraordinary density of their concentration.

It should also be noted that there is some order in the arrangement of interfascicular oligodendrocytes with bundles of nerve fibres among them that are represented fractionally and in most cases in oblique section. Interfascicular interstitial layers containing microvascular-astrocytic complexes can also be seen in fragments. It should be noted that it is not always possible to visualize them together, because it all depends on the case if these formations occur on the plane of the section. But numerous observations including looking at the depth provide evidence that if we see the body of a fibrillar astrocyte within the interfascicular layer, it definitely indicates the location of a nearby blood vessel and vice versa. This position is illustrated by the photographs in fig. 4.

The upper micrograph (outlined in black circle) shows the body of an astrocyte whose cytoplasm completely covers the transverse profile of the blood capillary partially visible on the section. Besides, several processes can be seen coming from its body in different directions and penetraing among the bundles of nerve fibres to the nearest foci of interfascicular oligodendrocytes. At some radial distance from astrocyte body underneath it in the cross-section there

are two blood capillaries localized in cross intrafascicular spurs of interstitial layers with no obvious signs of an astrocytic cover around them.

This calls into question the existing assertion that the perivascular limiting membranes in the cerebral substance are continuous everywhere, in other words, the ones that completely exclude direct contact of the vascular wall with nerve elements. This position is amply demonstrated by the lower photomicrograph of the same picture, where there is an arteriole within the interfascial layer in cross-section. On one side only its wall is adjacent to the body of the fibrillar astrocyte with several processes emerging from it in different directions. Moreover, one of them penetrates the transverse intrafascicular layer and is visible there at a relatively large distance (indicated by a black thin line). Meanwhile, the opposite wall of the arteriole from the adjacent body of the astrocyte appears to be in direct contact with the bundle of nerve fibres. However, such visibility is created due to insufficient resolution of the light microscope, which does not enable visualizing the microstructures that are between them. In this case, primarily basal membranes separating them are meant. They can only be seen with a transmission electron microscope.

However, it can be argued that fibrillar astrocytes are an essential attribute of the immediate environment of blood microvessels in the corpus callosum and their bodies are closely connected with their wall, and the lamellar processes have a different polar orientation; some of them extend through the wall of this microvessel while others penetrate between the bundles of nerve fibres [5]. This feature of the fibrillar astrocytes shape and their widespread orientation between metabolic blood vessels, on the one hand, and bundles of nerve fibres on the other, greatly complicates the possibility of creating a clear picture of the spatial relationship between them in the general myeloarchitectonics of the corpus callosum. The following statements established by us will help to understand this issue: 1 – according to our data mainly resistive blood microvessels (arterioles and precapillary arterioles) are localized in interfascicular interstitial layers having vertical orientation between fascicular portions of commissural cords; 2 – the transverse spurs of the interfascicular layers separating the bundle sets of nerve fibres (called subfascicular portions) are the location of exchange blood microvessels (blood capillaries); 3 – exchange microvessels are absent in the territorial boundaries of subfascicular portions; 4 – interfascial layers of the interstitium are the places where fibrillar astrocytes bodies (perikaryons) are located; here they are closely spaced at equal distances from the wall of resistive microvessels, forming a kind of perivascular couplings around them. In the gaps between them, the walls of these arterial microvessels are covered with perivascular processes of fibrillar astrocytes; 5 – other lamellar processes of the latter together with blood capillaries and separately from them get into cross spurs of interfascicular layers where it is very difficult to detect them on sections.

It is necessary to find out where exactly the processes of astrocytes are directed and which intrafascicular structures they are connected with. Numerous observations suggest that the intrafascicular cells are interconnected by the thinnest tunnel fissures, forming a kind of cellular network inside the subfascicular portions, whose individual cells (in some sections) have a polygonal shape. There is no doubt that in such tunnel fissures there are processes which in the opposite direction depart from the interfascicular oligodendrocytes located in corners of separate cells. Based on this, we consider it to be appropriate in the decomposition of the general myeloarchitectonics of the corpus callosum to distinguish a minimal subset of nerve fibres called "cellular portions" with oligodendrocyte processes located in them, which are marginally separated by microscopic tunnel fissures. Interstitial fluid coming from the interfascicular and transverse intrafascicular layers, where blood microvessels, donors of this fluid are localized, passes through such tunnel fissures.

It is obvious that the connection between the interstitial compartments and the branched intrafascicular cellular network of tunnel fissures is carried out by lateral processes of perivascular astrocytes, which penetrate through narrow slits into the peripheral zone of fascicular portions where they are in contact with the processes of oligodendrocytes which are in the marginal position.

In the corpus callosum there is a tunnel network of interstitial fissures, between the blood microvessels of the capillary type and myelinated nerve fibres [6]. It is quite extended by adjacent district microregions and, becoming narrower, it closes around the intrafascicular cellular portions with the help of interstitial cells containing interfascicular oligodendrocytes [2, 4 , 5]. We believe that the interstitial fluid circulating in this transport system cannot penetrate the thickness of the cellular portions themselves due to the extremely tight closure of the myelinated fibres contained in them, which excludes the possibility of the free circulation of fluid between them. It should be assumed that myelinated nerve fibres of the corpus callosum have two sources of power, namely: the metabolic processes of nerve processes are carried out by axonal transport directed from the body of the nerve cell, while the myelin sheaths are constantly

renewed entirely due to productive activity of interfascicular oligodendrocytes [8] that is made possible because of the presence of developed granular endoplasmic reticulum in their cytoplasm, which is well detected by staining with basophilic dye.

Thus, in the corpus callosum, the entire transport system, carrying out the processes of microcirculation of fluid containing nutrients, is fully aimed at functional and structural stability of only the myelin sheaths of nerve fibres [9]. Ultimately, this process is provided by the synthetic activity of interfascicular oligodendrocytes. The connecting link between them and the exchange microvessels are perivascular (fibrillar) astrocytes, which do not have barrier functions. In our opinion, they are the organizing elements that ensure the structural integrity of a single system of microcirculatory processes. Presence of fibrillar structures belonging to the cytoskeletal formations in their cytoplasm, especially in the processes, can be considered indirect evidence of this fact. These structures are known to belong to the cytoskeletal formations that give such cells a stable shape.

Conclusions

1. Between the limiting membranes of the corpus callosum there are direct connections in the form of wider – interfunicular and narrow – interfascicular connective tissue layers, where blood microvessels with surrounding perivascular astrocytes are localized. The latter occupy a mediated position between blood microvessels and interfascicular oligodendrocytes, forming within the individual fascicular portions (with the help of their processes) a spatially branched cellular network.

2. In the cells of this network, which have a predominantly hexagonal shape, there are minimal sets of myelinated nerve fibres, whose sheaths are the product of the secretory activity of interfascicular oligodendrocytes located in the corners of such cells. In order to record the nomenclature of hierarchical levels of the corpus callosum organization, we call these minimal, uniform in terms of structure sets of nerve fibres fitting within individual cells of the oligodendrocyte network "cellular portions".

3. In the general constructive plan between blood exchange microvessels and myelinated nerve fibers there is a quite extended by adjacent district microregions tunnel network of interstitial fissures that, becoming narrower, closes around the intrafascicular cellular portions. This interstitial network is an intermediate compartment between the blood flowing through the microvessels and the cells in which the interfascicular oligodendrocytes are cellularly dispersed.

4. In the corpus callosum, the entire transport system is completely aimed at ensuring the structural and functional stability of only the myelin sheaths of nerve fibres by means of the secretory activity of interfascicular oligodendrocytes.

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