Peculiarities of cerebellar cortex ultrastructure under the influence of opioid in experiment

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Abstract. The first signs of impairment of the structure of the rat's cerebellar cortex have been noted already after 2 weeks of the experimental effect of nalbuphine which is manifested by the change of the form of cerebellum cells, by clarified cytoplasm, formation of vacuoles as well as by the development of microangiopathies. Destructive changes in cerebellar cortex cells and in parts of hemomicrocirculatory bloodstream increase in the course of 6 weeks of the experiment, which leads to disorganization of the structure of cerebellar cortex.

Keywords: *cerebellum, ultrastructure, nalbuphine, experiment, hemomicrocirculatory bloodstream.*

Introduction. Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Chronic pain is pain that persists over a period of time,typically for at least three months [1]. More women than men reported chronic pain [2]. Overall, 31% of men and 37% of women reported this. The prevalence of chronic pain increased with age, from 14% of men and 18% of women aged 16-34 to 53% of men and 59% of women aged 75 and over [3]. There are four major classes of medications used in the treatment of chronic pain: non-opioids (Aspirin, and acetaminophen), opioids, also called narcotics (examples of opioids include but are not limited to morphine, codeine, hydrocodone, oxycodone, and Nalbuphine.), adjuvant analgesics, etc [4, 5]. The pressing problem today in modern medicine is the growing tolerance to the narcotic analgesics that induces patients take them in ever greater doses and such property of opioid drugs as causing euphoria in case of their systematic injection [6]. This sooner or later leads to the formation of psychic and then physical dependence, that is, drug addiction [7]**.** The final stage of any drug addiction is degradation of personality, dramatic trophic and functional changes in the entire organism, especially in central nervous system [8]. The mechanism of the analgesic effect of narcotic analgesics is explained by the fact, that they suppress the processes of interneuronic transmission of the pain impulses in the central part of the afferent paths, slow down conduction of the pain impulses in the reticular formation of the brain, thalamus and therefrom to the cerebral cortex. In most cases analgesic action of the opioids can be explained by the binding and activation of μ- or κ-opioid receptors [9, 10]. Narcotic analgesics alter the psychic appreciation of painful sensation, eliminating the fear of such sensation by way of disruption of the subjective-emotional perception [11]. A great number of problems pertaining to the structural changes in tissues in case of the use of narcotic drugs remain unresolved. The data presented in professional literature on these problems are contradicting and require further studies. As the function of each organ is based on its adequate structure and its impairment under the effect of pathogenic factors is the foundation for the development of a pathologic process that determines its character and peculiarities of clinical manifestations, there is undoubtedly the need for the study of morphological peculiarities of the organ.

Aim. The objective of our work was to determine ultra-structural peculiarities of the cerebellar cortex and its hemo-microcirculatory bloodstream components in the dynamics of 6-week long injection of nalbuphine in the experiment.

Materials and methods. The study was carried out on 24 mature white male rats aged 3.0-3.5 months and body weight 160-180 g. Nalbuphine (Rusan Pharma Ltd, India) was injected intramuscularly daily to the experimental animals of the first group (5 rats) during 2 weeks (1^{st} week - 8 mg/kg, 2^{nd} week - 15 mg/kg), of the second group (5 rats) during 4 weeks (1^{st} week - 8 mg/kg, 2^{nd} week - 15 mg/kg, 3^{rd} week - 20 mg/kg, 4^{th} week - 25 mg/kg, of the third group (5 rats) during 6 weeks (1^{st} week - 8 mg/kg, 2^{nd} week - 15 mg/kg, 3^{rd} week - 20 mg/kg, 4^{th} week - 25 mg/kg, 5^{th} week - 30 mg/kg, 6^{th} week - 35 mg/kg. 9 white rats to which saline solution was injected served as the control group. All animals were kept in the vivarium of Danylo Halytsky National Medical University of Lviv, and the experiments were conducted in compliance with the provisions of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986), European Council Directive 86/609/EEC (1986), the Law of Ukraine #3447-IV ''On protection of animals from cruel treatment'', general ethical principles of experiments on animals approved by the first National Congress of Ukraine on Bioethics (2001) [12]. Sampling of the material was made after 2, 4, 6 weeks of the experiment. The animals were withdrawn from the experiment by the use of thiopental sodium at the rate of 25 mg/kg of body weight. The research materials were presented by ultramicroscopic sections of the cerebellar cortex. Ultrastructural study of the rat's cerebellum was conducted with the aid of electron microscope UEMB-100K (Ukraine) at acceleration speed 75 kV and magnification on the microscope screen ×4000, ×8000. Ultrathin sections were prepared with the aid of ultramicrotome UMTP-3M with the help of glass knives produced on CCH-1 device.

Results. Changes in the cerebellar cortex after two weeks of nalbuphine injection. Morphological indications of pathological changes appear in the bodies of the neurons of all layers of cerebellar cortex after 2 weeks of injection of nalbuphine. Nuclei of most of the cells have a rounded form, contain no nucleoli. Occasionally occur the cells with the altered form of the nucleus containing both, condensed and non-condensed chromatin (Fig. 1a). A greater part of heterochromatin is concentrated near the internal surface of the nucleus membrane. Nucleolemma of these nuclei forms

invaginations. Their neuroplasm contains an insignificant quantity of organelles. Mitochondrions are not numerous, they have a clarified matrix and partially with degraded cristae (Fig. 1b).

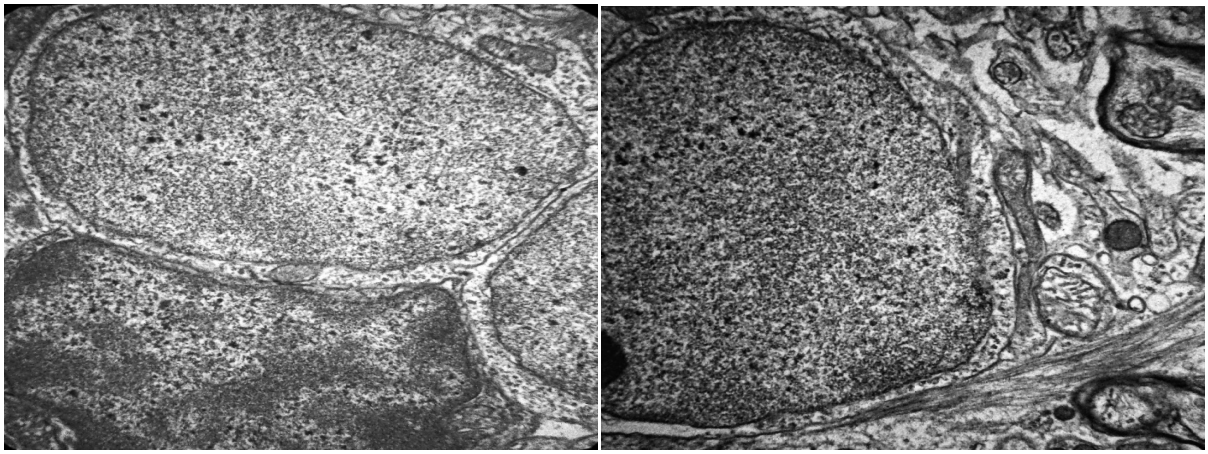

Fig. 1. Altered form of the nucleus (a), clarified matrix and partially degraded cristae of mitochondrions (b) of the white rat's cerebellar cortex after 2 weeks of injection of nalbuphine. Electron microphotograph. Magnification: a – × 4000, b – × 8000.

The first signs of angiopathy, perivascular edema (Fig. 2a) appear at this stage of the experiment in parts of hemomicrocirculatory bloodstream. Lumens of capillaries become constricted acquiring an irregular slit-like form (Fig. 2b).

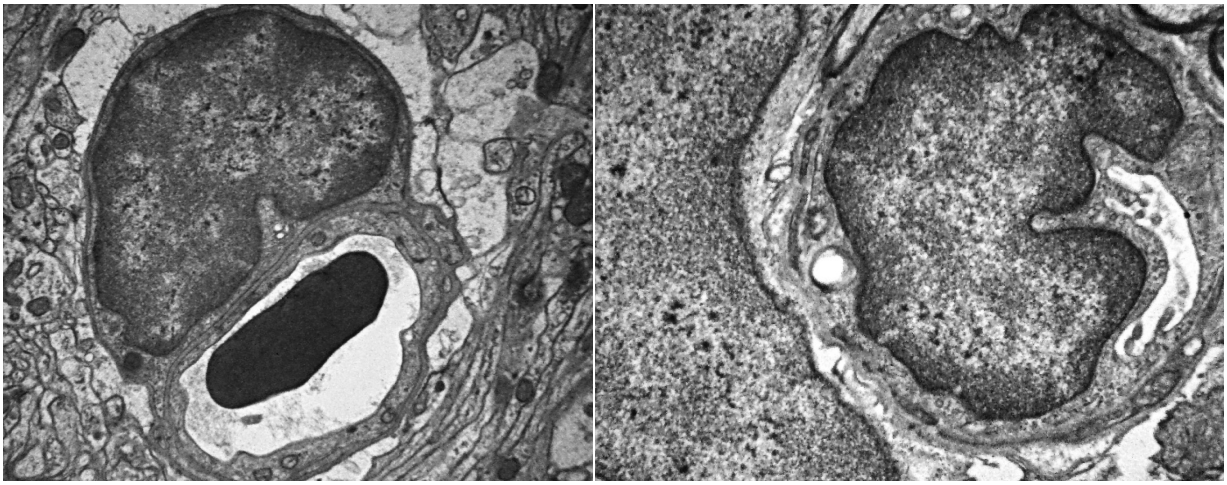

Fig. 2. Perivascular edema (a), slit-like form of lumen of the capillary (b) of the white rat's cerebellar cortex after 2 weeks of injection of nalbuphine. Electron microphotograph. Magnification: × 4000.

Excessively elongated electron-dense nuclei of endotheliocytes protrude into the vessels' lumen. Nucleolemma forms numerous protrusions and deep invaginations. Akaryotic regions of endotheliocytes are thinned. Occasional protrusions of plasmolemma are found in the vessels' lumen. A great number of destructively altered mitochondrions are present in the cytoplasm perinuclear region. Mitochondrions' cristae are shortened, often ruined. Lumens of the white rat's cerebellar cortex arterioles at this stage of the experiment are somewhat constricted. Basal membrane is thickened, its contours lose their sharpness. Thickening of the elastic membrane is also observed.

Changes in the cerebellar cortex after four weeks of nalbuphine injection. Distinct changes in the ultrastructural organizaton of endotheliocytes in hematoencephalic barier capillaries are observed after 4 weeks of injection of nalbuphine: ruined (Fig. 3a), local clarification and compaction of endothelial cells' matrix, condensation of chromatin take place, mitochondrions' cristae are ruined, basal membrane is fluffy and thickened, the phenomena of the vessels' walls swelling increase (Fig. 3b).

Nuclei of the cerebellar cortex neurons at this stage of the experiment acquire their polygonal form (Fig. 4a). Heterochromatin is present in the periphery of the nucleus, in the center – the zone of low electron density filled with occasional granules of non-condensed chromatin. Nucleolemma is thickened, fluffy. Perinuclear space is expanded. Cytoplasm contains no organelles in some areas. A great number of swollen mitochondrions whose size varies considerably (Fig. 4b).

Changes in the cerebellar cortex after six weeks of nalbuphine injection. After 6 weeks of experiment, electron microphotographs of ultrathin sections of the white rats' cerebellar cortex show morphological signs of

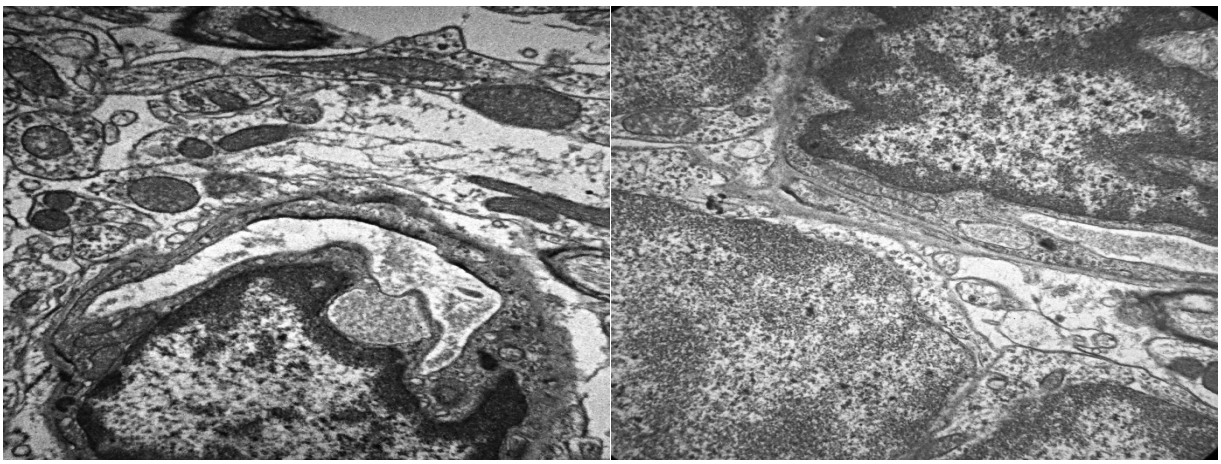

Fig. 3. Deformed nuclei of endotheliocytes protrude into the capillaries' lumen (a), destructurized hematoencephalic barrier (b) of the white rat's cerebellar cortex after 4 weeks of nalbuphine injection. Electron microphotograph. Magnification: × 4000.

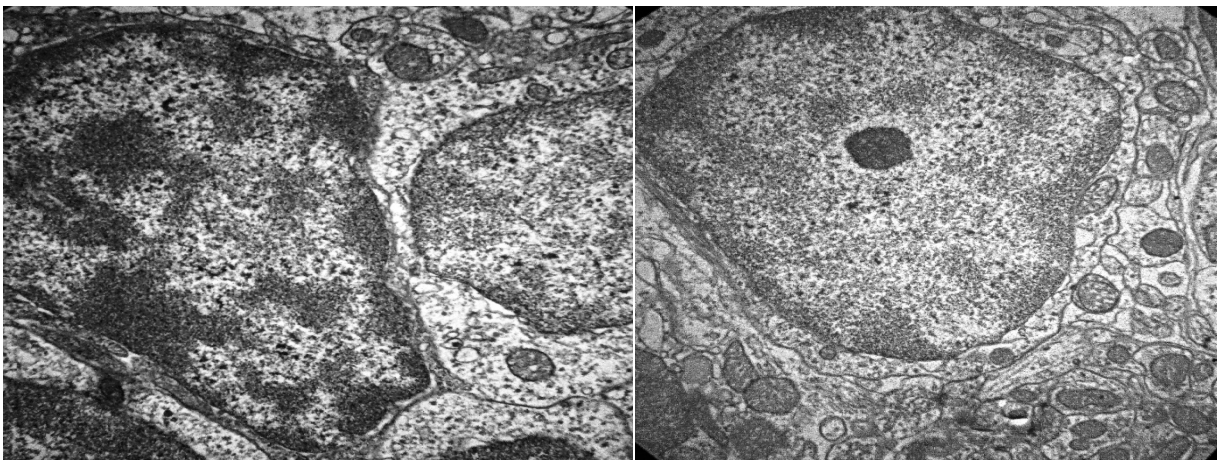

Fig. 4. Nucleus of polygonal form (a), edema of the mitochondrions (b) of the rat's cerebellar cortex neurons after 4 weeks of nalbuphine injection. Electron microphotograph. Magnification: × 8000.

degenerative changes, in particular, the nerve cells are disorganized, their form is altered, the boundaries between them are blurred (Fig. 5a). Occasional neurocytes occur with an increased electron density of nucleo- and neuroplasm. The nuclei are of irregular form, occupy the entire cell. Nucleolemma forms protuberances that have a prepyknotic appearance. Nucleoli are often missing. Few round-shaped mitochondrions with a lucid matrix can be seen against the background of neuron cytoplasm compaction and vacuolization. The contours of internal mitochondrial membrane are blurred, cristae disrupted, spaces between them dilated. Canaliculi of granular endoplasmic reticulum are dilated unevenly, fragmented. Lumens of the cerebellar cortex arterioles are dilated, nuclei of endothelial cells have large sizes relative to cytoplasm, their nucleolemma forms finger- and cupola-shaped protrusions. The wall of the arterioles and precapillary arterioles is thickened, sclerotized. Intracellular membrane structures, particularly, mitochondrions and the endoplasmic reticulum have no distinct contours. Luminal surface of endotheliocytes forms a considerable number of minute microvilli, and cytoplasm contains vacuolized mitochondrions, it contains few ribosomes and polysomes, pinocytic vesicles. Mural thrombi have been found in the arterioles' lumens. Lumens of the cerebellar cortex capillaries are constricted because of swelling of the cytoplasm of endotheliocytes and protrusions of cytoplasm into the lumen. Lumens of hemocapillaries are filled with the accumulations of erythrocytes, their adhesion was noted in places of erytrocytes plasmolemmas fluffing on the luminal surface of endothelial cells (Fig. 5b). Cytoplasm of endotheliocytes is filled with precipitates and coagulates. Basal membrane of hemocapillaries is thickened, laminated, contains electron-dense deposits. Venules are hyperemic. Acidophilic leukocytes, thrombocytes, erythrocytes are in the venules' lumens. Interendothelial contacts are expanded, which indicates diapedesis of leukocytes through the venules' walls. Basal membrane the venules is fluffy.

Discussion. The results of the present work are a fragment of the project scientific research «The structure of organs and their bloodstream in ontogenesis under the effect of laser irradiation and pharmaceutical agents in cases of blood supply disorders, reconstructive surgeries and diabetes mellitus" (state registration number 0110U001854), being conducted at the Department of General Anatomy of Danylo Halytskyy National Medical University of Lviv in accordance with the state plan and program.

Drug addiction has become a serious problem not only

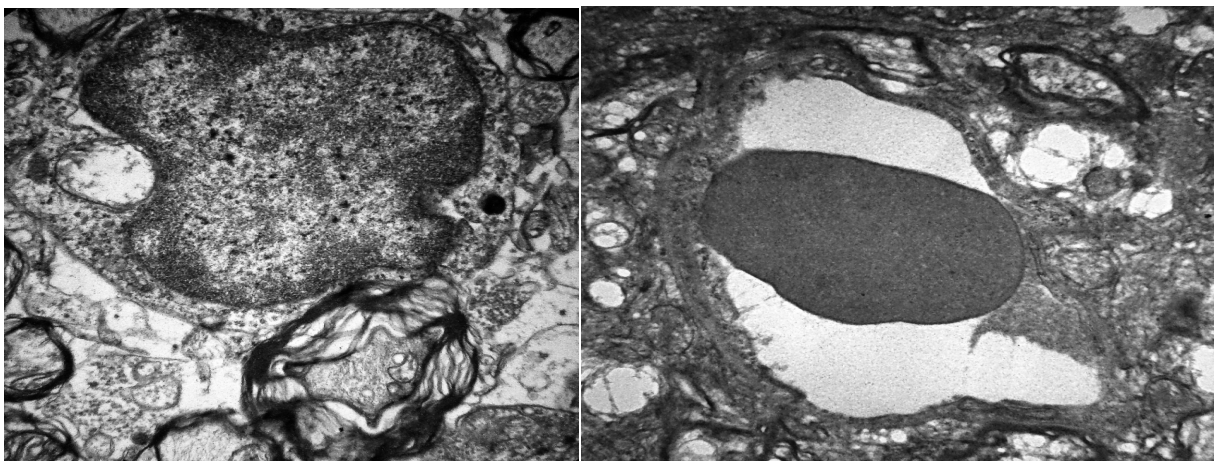

Fig. 5. Degenerative changes in the neuron (a) and in the hemocapillary wall (b) of the white rats' cerebellar cortex after 6 weeks of nalbuphine injection. Electron microphotograph. Magnification: a – × 8000, b – × 4000

because of the progressing pathopsychological changes in drug using patients, but also because of the development of multiple polyorgan comorbid pathology, that together with the great economic and moral losses place the problem of drug addiction in the range of the most important problems in many countries of the world.

Despite the wide range of modern methods of research on the morphological level in the mechanisms of lesions of various organs and system that appear under the effect of opioids, the results of ultramicroscopic investigations that could fully explain this problem practically have not yet been described. Data on the development of pathological changes of the cerebellum under the influence of drugs are insufficient and need to be studied further on. That is why the study of the ultrastructure of the cerebellar cortex in dynamics of a long lasting effect of the opioid is undoubtedly of an essential practical importance both, in medical and in social aspects. The studies that we have carried out have shown, that already after 2 weeks of nalbuphine injections to the white rats there appear the first signs of impairment of the ultrastructural organization of the cerebellar cortex and its angioarchitecture. First of all there have been detected intra- and extravascular changes in the elements of cerebellar cortex hemomicrocirculatory bloodstream. Similar changes in the ultrastructural organization of the rat's optic nerve microvessels have been observed in the case of a short-lasting duration of streptozotocin-induced diabetes mellitus that kept on increasing in subsequent stages of the experiment [13]. Angiopathy, in the author's opinion, became the triggering mechanism for the development of the optic nerve neuropathy which confirms the opinion that hemomicrocirculatory bloodstream vessels are among the first to react to the pathogenic factors by structural changes that appear to be the soil for the development of a pathological process and determine its character and the peculiarities of its clinical manifestations [14]. This is why the study of the structure of vessels, ultrastructure of their walls under normal conditions and under the effect of various factors allows to examine importance of the vascular factor in morphofunctional insufficiency of internal organs.

A long-term injection of nalbuphine causes appearance of morphological signs of pathologic changes in the perikaryons of neurons of all cerebellar cortex layers. The same situation was after the long-term effect of morphine sulphate treatment [15]. The light microscopy revealed that the molecular layer of cerebellum showed vacuolation. The Purkinje cells lost their specific shaped appearance, decreased in size and numbers. The granular cells highly degenerated. Electron microscopy revealed fragmentation of the cisterns of the both types of endoplasmic reticulum, resulted in a progressive depletion of total protein contents as well as general carbohydrates in all treated groups as supported by histochemical observation. Obvious destruction of mitochondrial inner membrane and cristae mediate cell death. Also, abnormal nucleus with deformed perforated nuclear membrane and deformation of the plasma membrane with degeneration of the synapses could interpreted as a sign of necrosis.

Changes in the cerebellar cortex cells were studied also under the effect of ethanol [16]. The ultrastructural examination of the cerebellar cortex of ten-day-old rat pups of ethanol-treated dams during pregnancy (1 group), pregnancy and lactation (2 group), and lactation (3 group) revealed that alcohol administration leads to a delayed maturation of Purkinje cells. It was most strongly manifested in the pups of dams treated with ethanol during pregnancy and lactation. Moreover, this study showed degenerative changes in Purkinje cells as well as in granular layer cells in all experimental groups. There was a difference in the ultrastructural picture of both types of dying cells, which might result from different time frame of their sensitivity to ethanol administration. The quantitative analysis showed the most pronounced decrease in the density of Purkinje cells in the posterior superior fissure of cerebellar cortex in the pups of dams treated with ethanol during pregnancy.

The changes we have discovered in the cerebellar cortex structure after 4 and 6 weeks of injection of nalbuphine in the experiment, in our opinion, can be explained by the data of professional literature that antibodies to the opioids have specific sites of binding in certain structures of the brain, particularly, in the cerebellar cortex granular layer [17]. Reports in the professional literature show, that signs of edema and swelling of its tissues and arachnoid membraine have been found in the brain under the effect of narcotic

substances [18]. This is explained by the combination of impairment of microcirculation, which is characteristic of the acute intoxication by drugs. Histologically, aside from the edema of drainage glia that is manifested microscopically by perivascular and pericellular edema, there have been found various impairments of microcirculation in the form of erythrocyte aggregations stasis in the capillaries, general venous hyperemia, paresis of microcirculation resistant link, sludge of erythrocytes, sometimes formation of fibrin-erythrocytic thrombi and multiple small diapedetic hemorrhages in the subcortical regions and the brainstem. Sometimes hemorrhages encroach pia matter of the brain. Hemorrhages in the brainstem are linked to the acute venous hyperemia of hypoxic genesis and impairment of rheological properties of blood.

This project research work differs from other already known works of this kind by the fact that new data on the effect of opioid on the peculiarities of ultrastructure of the cerebellar cortex of the white rat have been obtained on the basis of a complex of micro-, macro- and electron microscopic studies. For the first time we have investigated dynamics of ultrastructural changes of the cortex of the rat's cerebellum under the effect of injection of opioid. The data obtained enable us to extend our notions and resolve the disputable problems of the effect of opioid on the cerebellar cortex ultrastructure, which will create the morphological basis for understanding pathogenesis of nervous system diseases of drug users and patients who have to take opioids for an extended period of time, and for finding optimal methods of treatment. The obtained data are important for both, morphologists and clinicists.

Conclusions. Phenomena of angiopathy have been observed already after 2 weeks of nalbuphine injections which testifies to the reaction of hemomicrocirculatory bloodstream elements to the injection of opioid as primary that serves as soil for the development of system structural changes. Destructive changes in the cells of all cerebellar cortex layers in the dynamics of the long-term opioid injection increase, which is manifested by the alteration of the form and size of the nucleus, by vacuolization of cytoplasm, destructive changes in the organelles.

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Особенности ультраструктуры коры мозжечка под влиянием опиоида в эксперименте

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Аннотация. Первые признаки нарушения структуры коры мозжечка крысы обнаружено уже через 2 недели течения экспериментального воздействия налбуфина, что проявляется изменением формы клеток коры мозжечка, просветлением их цитоплазмы, формированием вакуолей, а также развитием микроангиопатий. В процессе эксперимента в течение 6 недель нарастают деструктивные изменения клеток коры мозжечка, и звеньев гемомикроциркуляторного русла, что приводит к дезорганизации структуры мозжечка.

Ключевые слова: *мозжечок, ультраструктура, налбуфин, эксперимент, гемомикроциркуляторное русло.*