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HISTOLOGICAL VIOLATIONS IN THE RAT HIPPOCAMPUS AND PARIETAL CORTEX IN TOTAL CEREBRAL ISCHEMIA

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Objective. To analyze the changes in the size and shape of perikaryons and the degree of cytoplasm chromatophilia of the rat hippocampal and parietal cortex neurons at different periods after the modeling of total cerebral ischemia.

Material and Methods. The experiments were performed on 42 male outbred white rats with an initial weight of 240 ± 20 g. Total cerebral ischemia in white outbred rats was modeled by decapitation. The material for further histological examination was taken at the 1st, 5th, 15th, 30th and 60th minutes, as well as 5 and 24 hours after decapitation. The study of histological preparations was carried out using an Axioscop 2 plus microscope, a digital video camera and the ImageWarp image analysis program. Among the total number, the cells were isolated by the intensity of cytoplasm staining (chromatophilia). After a preliminary check for the normal distribution of indicators, the data obtained were analyzed by non-parametric statistics.

Results. With total cerebral ischemia, a decrease in the size of neurons and deformation of perikaryons were observed. Normochromic neurons completely disappeared at the 60th minute. The number of hyperchromic neurons increased, and then progressively decreased. Shrunken neurons made up the majority of cells in the studied cortical sections at the 30–60th minutes, and then, after 5 and 24 hours, cells with pericellular edema prevailed in the neuron population.

Conclusion. The obtained data on histological changes in neurons of phylogenetically different parts of the cerebral cortex in the dynamics of total cerebral ischemia provide the basis for further detailed study of post-mortem changes of the brain as well as determining the time of death, thus creating a fundamental basis for studying the properties of neurons, including their transition from one functional state to another.

***Keywords**: rats, cerebral ischemia, cerebral cortex.*

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Introduction

Currently, in medicine, the concept of death is based on evidence of a persistent lack of brain function. A number of methods are used to diagnose brain functioning: electroencephalography, assessment of cranial nerve reflexes, and cerebral blood flow studies. In a histopathological examination, post-mortem changes include edema, hemorrhages, necrosis, ischemic softening, wrinkling and deformation of neurons, pycnosis of their nuclei. In the cerebral hemispheres, swelling and venous congestion are often found, in the subthalamic region and the optic tubercle - the area of spotted lysis. The most typical histological change is considered to be edema of its tissues with subsequent rupture of blood vessels [1]. Previous studies on the morphological changes in the neurons of the parietal and cortex and hippocampus with subtotal cerebral ischemia of the brain showed a decrease in the size of pericarions and an increase in the number of hyperchromic and hyperchromic shrunken neurons [2, 3]. At the same time, a quantitative study of changes in the size, shape, and degree of chromatophilia of the cytoplasm of neurons in different periods after total experimental cerebral ischemia is of interest. The aim was to analysis of changes in the size and shape of pericarions and the degree of cytoplasm chromatophilia of the rats hippocampal and parietal cortex neurons at different periods after modeling of total cerebral ischemia.

Material and methods

The experiments were performed on 42 males of outbred white rats with an initial weight of 240±20 g, in compliance with the requirements of the Directive of the European Parliament and the Council No. 2010/63 / EU of 09.22.2010 on the protection of animals used for scientific purposes. Animals were kept in an air-conditioned room (22° C) under mixed lighting on a standard diet of vivarium and free access to food and water, in groups of no more than 5 individuals in a vivarium cell [4].

The use of rats as experimental animals is due to the similarity of angioarchitectonics and morphology of the cerebral cortex in rats and humans [5]. Total cerebral ischemia in outbred white rats was modeled by decapitation [6]. Material sampling was carried out at 1, 5, 15, 30 and 60 minutes, as well as 5 and 24 hours after decapitation. After decapitation, the brain was quickly removed, pieces of the anterior cortex of the cerebral hemispheres were fixed in Carnoy fluid. Serial paraffin sections were stained with 0.1% toluidine blue according to the Nissl method and for the detection of ribonucleoproteins according to Einarson.

The study of histological preparations, their microphotography, morphometry and densitometry of chromogen sediment in histological preparations was carried out using an Axioscop 2 plus microscope (Zeiss, Germany), a digital video camera (LeicaDFC 320, Germany) and ImageWarp image analysis program (Bitflow, USA). The localization of the parietal cortex and hippocampus of the cortex in rat brain histological preparations was determined using a stereotactic atlas [7]. At least 30 neurons of the fifth layer of the parietal cortex and the pyramidal layer of the CA1 field of the hippocampus were evaluated in each animal, which provided a sufficient sample

size for subsequent analysis. On paraffin sections, the number of large pyramidal neurons per unit area of sections of the cerebral cortex was determined. Among the total number, cells were isolated by the intensity of cytoplasm staining (chromatophilia). Several types were distinguished: normochromic - moderately colored; hyperchromic - dark; hyperchromic shrunken - very dark, with deformed pericarions; hypochromic - light colored; shadow cells are almost transparent. The number of each type of cell was counted.

After a preliminary check on the normality of the distribution of indicators, the data obtained were analyzed by non-parametric statistics using the Statistica 10.0 software for Windows (StatSoft, Inc., USA). The results are presented in the form of Me (LQ; UQ), where Me is the median, LQ is the value of the lower quartile; UQ is the value of the upper quartile. Differences between the indices of the control and experimental groups were considered significant at $p < 0.05$ (Mann-WhitneyU-test) [8].

Results and discussion

At the 15th minute of total ischemia, the neurons of the parietal cortex and hippocampus significantly decreased in size - by 47 and 22%, respectively ($p < 0.05$). By 30 minutes of total ischemia, the size of the pyramidal neurons of the parietal cortex decreased by 74% ($p < 0.05$), compared with the control, and the size of the hippocampal neurons by 51% ($p < 0.05$). By 5 o'clock the area of pericarions of the neurons of the parietal cortex was only 1/6 ($p < 0.05$) of normal, and the hippocampus neurons decreased by 3.5 times, compared with the control ($p < 0.05$) (Table 1).

The shape of the neurons significantly changed already by the 15th minute - they became more elongated (25%, $p < 0.05$). By the 60th minute, the elongation factor of neurons of the parietal cortex and hippocampus increased by 35% ($p < 0.05$) compared to the control, while the form factor (the indicator of roundness of pericarions) decreased by 34% ($p < 0.05$).

At the 30th minute, the number of normochromic neurons decreased by 80% ($p<0.05$) compared to 1 minute, and at the 60th minute they completely disappeared. The number of hyperchromic neurons increased by 15 minutes by 3 times, and then progressively decreased. Hyperchromic shrunken neurons made up the majority of cells in the studied cortical regions at the 30-60th minute, and then, after 5 and 24 hours, cells with pericellular edema predominated in the neuron population (Figs. 1,2,3). Similar to the change in the number of hyperchromic neurons, the concentration of ribonucleoproteins in the cytoplasm of the cells also changed, reaching a maximum by 60 minutes of total ischemia and decreasing by 1 day, which is explained by a large number of neurons with pericellular edema, which have a low degree of cytoplasm chromatophilia.

Hyperchromic neurons are regarded as ischemic-altered cells [9]. The appearance of shriveled dark cells under hypoxic and anoxic conditions is the universal and most severe form of reactive and pathological changes in neurons, accompanied by changes in the level of metabolism, tinctorial properties of the cytoplasm, karyoplasm of cells and various degrees of ultrastructural changes in cytoplasmic organelles. RNA synthesis proceeds intensively in dark, non-wrinkled neurons, and pycnomorphic cells contain destructive organelles, their nuclei and cytoplasm become indistinguishable [10].

At the electron microscope level, organelle compaction is observed in their cytoplasm. At the same time, the cytoplasm and the nucleus of hyperchromic shrunken neurons are reduced in volume, which led to an increase in the density of ribosomes (respectively, ribonucleoproteins) and hyperchromatosis. The number of ribosomes on the outer membrane of the karyolemma is significantly greater than in animals of the control group. A shift in the nucleolus to the periphery of the nucleus and an increase in the concentration of ribonucleoproteins due to their exit from the nucleolus and a significant increase in the number of free ribosomes in the cytoplasm of rat experimental neurons are noted [9]. In shriveled necrotic neurons, clumps of tigroid material and neuro-fibrils usually stick together, and then the cells begin to diffuse and very intensely stain with thionine and silver [11].

Table 1. – The morphometric analysis of the neurons of the 5th layer of the parietal cortex and the pyramidal layer of the CA1 field of the hippocampus

Таблица 1. – Морфометрический анализ нейронов 5-го слоя теменной коры и пирамидного слоя поля CA1 гиппокампа

	area, mkm ^{2}		elongation factor, units		form factor, units	
Groups	parietal cortex	hippocampus	parietal cortex	hippocampus	parietal cortex	hippocampus
1 minute	153 (126; 165)	99 (92; 102)	1,3 (1,2; 1,3)	1,2 (1,2; 1,2)	0,9 (0,8; 0,9)	0,9 (0,9; 0,9)
5 minutes	157 (136; 159)	99 (95; 102)	1,2 (1,2; 1,3)	1,2 (1,1; 1,2)	0,9 (0,9; 0,9)	0,9 (0,9; 0,9)
15 minutes	81 (77; 86)*	78 (71; 89)*	1,6 (1,4; 1,7)*	1,6 (1,5; 1,7)*	0,8 (0,7; 0,8)	0,8 (0,8; 0,9)
30 minutes	40 (33; 44)*	49 (47; 52)*	1,8 (1,7; 1,9)*	1,8 (1,8; 1,8)*	0,6 (0,6; 0,6)*	0,6 (0,5; 0,6)*
1 hour	37(27;47)*	54(50;60)*	1,8 (1,7; 1,8)*	1,8 (1,8; 1,9)*	0,6 (0,6; 0,6)*	0,6 (0,5; 0,6)*
5 hour	24(22,5;26,5)*	28(26;31)*	2,1 (2; 2,1)*	1,9 (1,8; 2)*	0,6 (0,6; 0,7)*	0,6 (0,6; 0,6)*
1 day	24,5(23;25)*	26(24;28)*	2,4 (2,3; 2,5)*	2,3 (2,1; 2,4)*	0,6 (0,5; 0,6)*	0,5 (0,5; 0,6)*

*Примечание: data are presented as Me (LQ; UQ); * – p < 0.05, as compared to 1 minute*

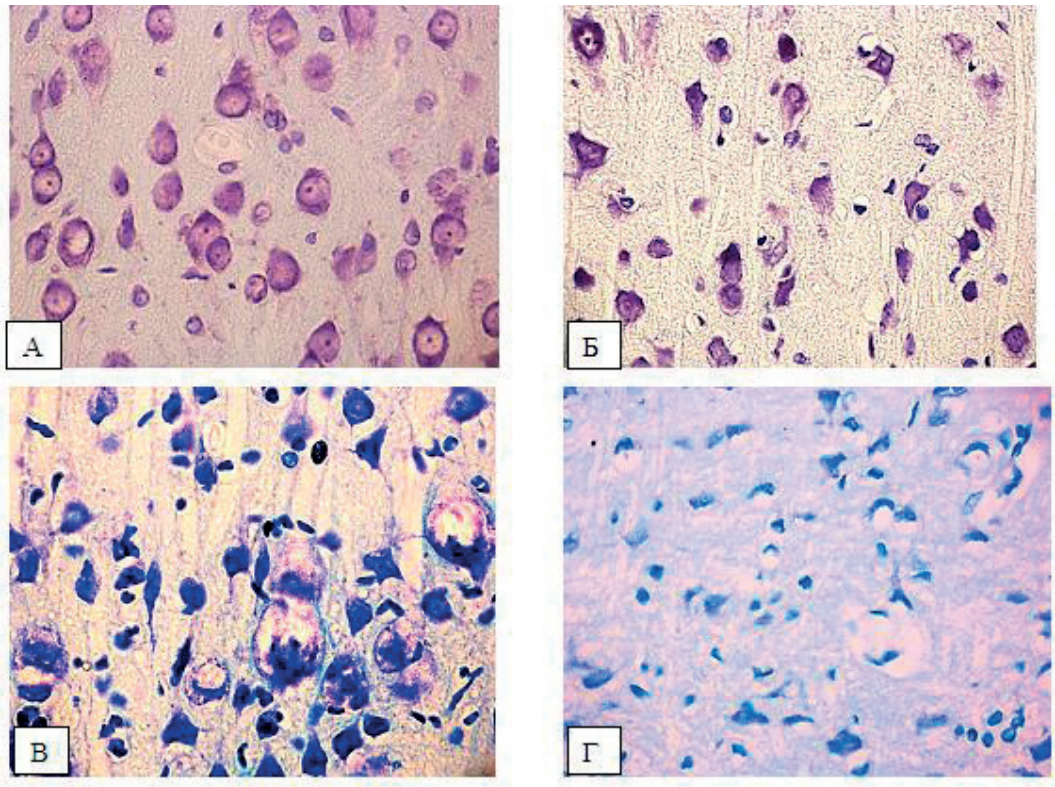

Figure 1. – Neurons of the fifth layer of the parietal cortex. A - 1 minute (predominance of normochromic neurons), B - 30 minutes (predominance of hyperchromic and hyperchromic shrunken neurons), C - 1 hour (swollen and hyperchromic shrunken neurons), D - 1 day (predominance of cells with pericellular edema). Stained by the Nissl method. Digital microphotography. Magnifications – ×40

Рисунок 1. – Нейроны пятого слоя теменной коры. А – 1 минута (преобладание нормохромных нейронов), В – 30 минут (преобладание гиперхромных и гиперхромных сморщенных нейронов), С – 1 час (набухшие и гиперхромные сморщенные нейроны), D – 1 день (преобладание клеток с перицеллюлярным отеком). Окрашено по методу Ниссля. Цифровая микрофотография. Увеличение – объектив ×40

It is believed that the intense staining of the neuronal cytoplasm characterizes the predominance of protein formation over its utilization [12]. But there is evidence that the hyperchromic neuron, through superexpression of amplified genes, is a cell that intensively synthesizes proteins. Some researchers regard hyperchromic neurons as hyperfunctional and believe that the protein synthesized by them goes to their own needs [13]. Shrunken neurons are cells with inhibition of functional activity. Their characteristic form is associated with pathological irreversible changes in water-salt metabolism [12, 13].

Depending on the operating conditions, neurons with the initial signs of hyper- and hypochromia either turn into shadow cells (hypochromic) or shrunken hyperchromic neurons with subsequent collision and coagulation necrosis or apoptosis [10].

In hyperchromic shrunken neurons, metabolic processes decrease, the decay of nucleoproteins, especially nuclear ones, prevails over their synthesis. Stocks of ribonucleoprotein particles in the nucleus are preserved, but their excretion into the cytoplasm is blocked.

According to the literature, in the late stages of ischemia, neuronal swelling is observed, accompanied by dissolution of the chromatophilic substance, coarsening, decay and melting of neurofibrils, pycnosis of the nuclei, thickening and decay of the processes [10, 11]. The neuropil is vacuolized and fragmented, undergoing granular-block decomposition, and myelin is dissolved, as a result of which droplets of lipids begin to appear along the nerve fibers. Synapses swell, collapse, and disappear [11].

The changes observed at the 15th minute of total cerebral ischemia are similar to those described at the 60th minute of subtotal ischemia, namely, the predominance of hyperchromic and hyperchromic shrunken neurons. Cells decreased in size, becoming more elongated due to deformation of the pericaryons [2, 3]. At the same time, changes at the 60th minute of total cerebral ischemia reflected a deeper destruction of the brain - normochromic neurons were absent, swollen neurons appeared. Hyperchromic neurons were almost not found, but wrinkled made up the majority of cells in the studied parts of the cerebral cortex.

Conclusion

The data on histological changes in neurons of phylogenetically different parts of the cerebral cortex in the dynamics of total cerebral ischemia provide a basis for further detailed study of post-mortem changes in the brain, determining the time of death, creating a fundamental basis for studying the properties of neurons, including their transition from one functional state to another.

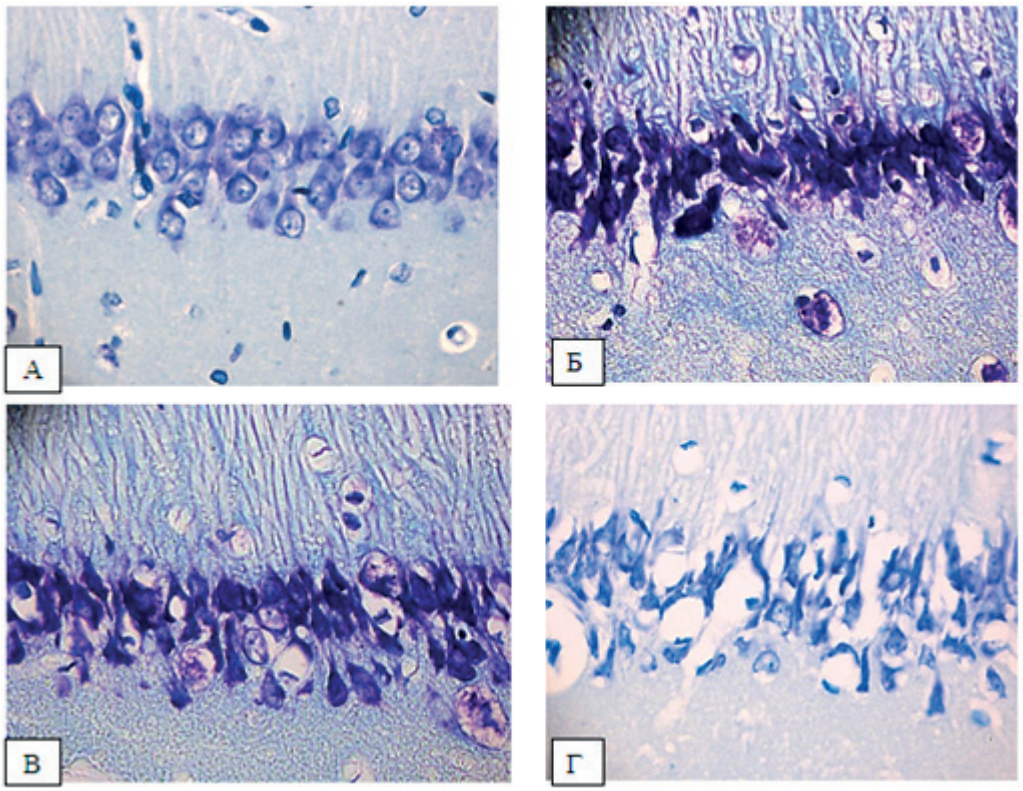

Figure 2. – Neurons of the pyramidal layer of the CA1 field of the hippocampus. A - 1 minute (predominance of normochromic neurons), B - 30 minutes (predominance of hyperchromic and hyperchromic shrunken neurons), C - 1 hour (swollen and hyperchromic shrunken neurons), D - 1 day (predominance of cells with pericellular edema). Stained by the Nissl method. Digital microphotography. Magnifications - ×40

Рисунок 2. – Нейроны пирамидного слоя поля CA1 гиппокампа. А – 1 минута (преобладание нормохромных нейронов), В – 30 минут (преобладание гиперхромных и гиперхромных сморщенных нейронов), С – 1 час (набухшие и гиперхромные сморщенные нейроны), D – 1 день (преобладание клеток с перицеллюлярным отеком). Окрашено по методу Ниссля. Цифровая микрофотография. Увеличение – объектив ×40

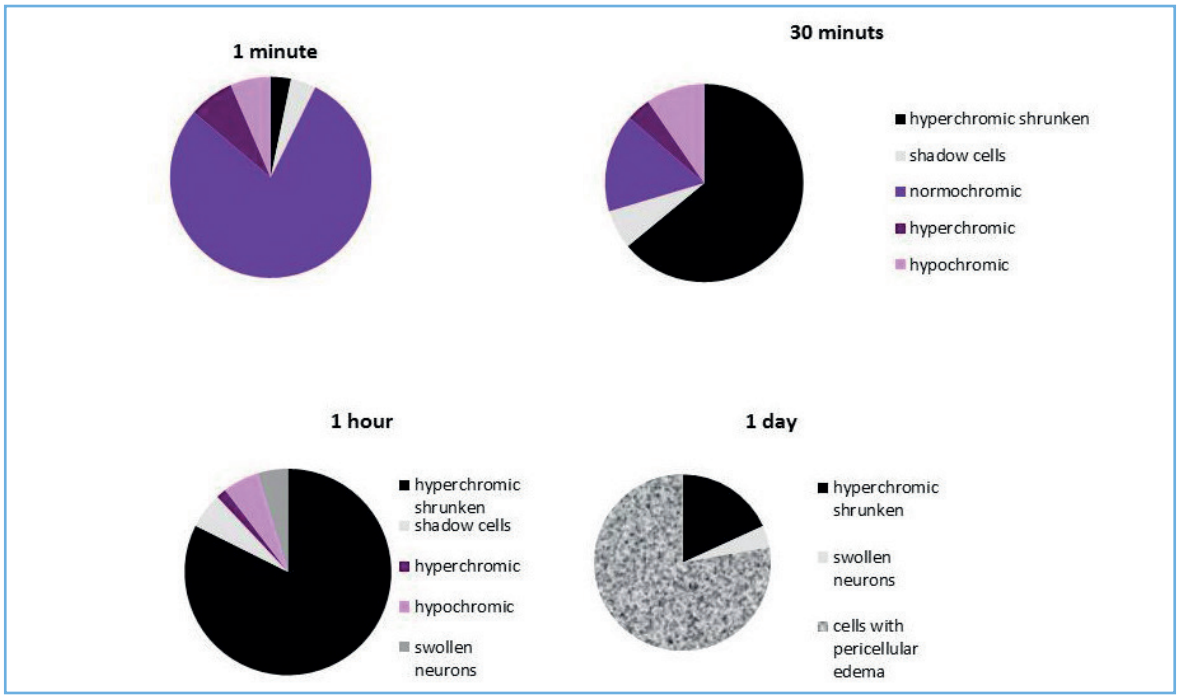

Figure 3. – The ratio of neurons with varying degrees of cytoplasm chromatophilia in the parietal cortex

Рисунок 3. – Соотношение нейронов с разной степенью хроматофилии цитоплазмы в теменной коре

Литература

1. Уолкер, А. Э. Смерть мозга / А. Э. Уолкер. – Москва : Медицина, 1988. – 198 с.
2. Бонь, Е. И. Морфофункциональные нарушения в гиппокампе крыс при субтотальной ишемии / Е. И. Бонь, Н. Е. Максимович, С. М. Зиматкин // Вестник Смоленской государственной медицинской академии. – 2018. – Т. 17, № 1. – С. 24-29.
3. Bon, L. I. Effects of experemental cerebral ishemia on metabolic characteristics of parietal cortex neurons / L. I. Bon, N. Ye. Maksimovich, S. M. Zimatkin // Bioprocess Engineering. – 2018. – № 2. – P. 1-5.
4. Руководство по лабораторным животным и альтернативным моделям в биомедицинских исследованиях / под ред. Н. Н. Каркищенко, С. В. Грачева. – Москва : Профиль-2С, 2010. – 241 с.
5. Бонь, Е. И. Микроскопическая организация изокортекса крысы / Е. И. Бонь, С. М. Зиматкин // Новости медико-биологических наук. – 2017. – № 4. – С. 80-88.
6. Бонь, Е. И. Способы моделирования и морфофункциональные маркеры ишемии головного мозга / Е. И. Бонь, Н. Е. Максимович // Биомедицина. – 2018. – № 2. – С. 59-71.
7. Paxinos, G. The rat brain in stereotaxic coordinates / G. Paxinos, C. Watson. – 6th ed. – London : Academic Press, 2007. – 448 p.
8. Компьютерный статистический анализ данных : учеб.-метод. пособие / Н. В. Батин [и др.]. – Минск : Ин-т подгот. науч. кадров НАН Беларуси, 2008. – 160 с.
9. Попова, Э. Н. Ультраструктура нейронов и межнейрональных связей сенсомоторной коры у потомства алкоголизированных крыс : монография / Э. Н. Попова. – Москва : Научный мир, 2010. – 158 с.
10. Морфологические изменения нейронов головного мозга крыс при двух-, четырёх сосудистой моделях ишемического повреждения головного мозга крыс и их коррекция тадалафилом в эксперименте / О. В. Мартынова [и др.] // Современные проблемы науки и образования. – 2016. – № 6. – С. 56-61.
11. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury / B. C. White [et al.] // Neurological Science. – 2000. – Vol. 179, № 1-2. – P. 1-33. – doi: 10.1016/s0022-510x(00)00386-5.
12. Baraskay, P. Generalization of seizures parallels the formation of “dark” neurons in the hippocampus and pontine reticular formation after focal-cortical application of 4-aminopyridine (4-AP) in the rat / P. Baraskay, Z. Szepesi, G. Orban // Brain Research. – 2008. – Vol. 1228. – P. 217-228.
13. Зиматкин, С. М. Темные нейроны мозга / С. М. Зиматкин, Е. И. Бонь // Морфология. – 2017. – № 6. – С.81-86.

References

1. Uolker AJe. Smert mozga. Moskva: Medicina; 1988. 198 p. (Russian).
2. Bon EI, Maksimovich NYe, Zimatkin SM. Morfofunkcionalnye narushenija v gippokampe krys pri subtotalnoj ishemii [Morphofunctional disorders in the hippocampus of rats with subtotal ischemia]. *Vestnik Smolenskoj gosudarstvennoj medicinskoj akademii* [Vestnik of the Smolensk State Medical Academy]. 2018;17(1):24-29. (Russian).
3. Bon LI, Maksimovich NYe, Zimatkin SM. Effects of experemental cerebral ishemia on metabolic characteristics of parietal cortex neurons. *Bioprocess Engineering*. 2018;2(1):1-5.
4. Karkishchenko NN, Gracheva SV, editors. Rukovodstvo po laboratornym zhivotnym i alternativnym modeljam v biomedicinskih issledovanijah. Moskva: Profil-2S; 2010. 241 p. (Russian).
5. Bon EI, Zimatkin SM. Mikroskopicheskaja organizacija izokorteksa krysy [Microscopic organization of the rat isocortex]. *Novosti mediko-biologicheskih nauk* [News of Biomedical Sciences]. 2017;4:80-88. (Russian).
6. Bon EI, Maksimovich NYe. Sposoby modelirovanija i morfofunkcionalnye markery ishemii golovnogo mozga [Modeling methods and morphofunctional markers of cerebral ischemia]. *Biomedicina.* 2018;2:59-71. (Russian).
7. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. London: Academic Press; 2007. 448 p.
8. Batin NV, Bogdanova IF, Kovalchuk AM, Lipnickij SF, Shkurko VV. Kompjuternyj statisticheskij analiz dannyh. Minsk: Institut podgotovki nauchnyh kadrov NAN Belarusi; 2008. 235 p. (Russian).
9. Popova EN. Ultrastruktura nejronov i mezhnejronalnyh svjazej sensomotornoj kory u potomstva alkogolizirovannyh krys. Moskva: Nauchnyj mir; 2010. 158 p. (Russian).
10. Martynova OV, Tverskoi AV, Pokrovsky MV, Martynov MA, Shkileva IJu, Sheljakina EV, Antsiferov OV, Mukhina TS. Morfologicheskie izmenenija nejronov golovnogo mozga krys pri dvuh-, chetyrjoh sosudistoj modeljah ishemicheskogo povrezhdenija golovnogo mozga krys i ih korrekcija tadalafilom v jeksperimente [Morphological changes in brain neurons of rats with two-, four-cavity models of ischemic damage to the brain of rats and their correction by tadalafil in the experiment]. *Sovremennye problemy nauki i obrazovanija* [Modern problems of science and education]. 2016;6:56-61. (Russian).
11. White BC, Sullivan JM, DeGracia DJ, O’Neil BJ, Neumar RW, Grossman LI, Rafols JA, Krause GS. Brain ischemia and reperfusion: molecular mechanisms of neuronal injury. *Neurological Science*. 2000;179(1-2):1-33. doi: 10.1016/s0022-510x(00)00386-5.
12. Baraskay P, Szepesi Z, Orban G. Generalization of seizures parallels the formation of “dark” neurons in the hippocampus and pontine reticular formation after focal-cortical application of 4-aminopyridine (4-AP) in the rat. *Brain Research*. 2008;1228:217-228.
13. Bon EI, Zimatkin SM. Temnye nejrony mozga [Dark brain neurons]. *Morfologija* [Morphology]. 2017;6:81-86. (Russian).

ГИСТОЛОГИЧЕСКИЕ НАРУШЕНИЯ В ГИППОКАМПЕ И ТЕМЕННОЙ КОРЕ ГОЛОВНОГО МОЗГА КРЫС ПРИ ТОТАЛЬНОЙ ЦЕРЕБРАЛЬНОЙ ИШЕМИИ

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Цель. Анализ изменений размеров и формы перикарионов и степени хроматофилии цитоплазмы нейронов гиппокампа и теменной коры крыс в разные периоды после моделирования тотальной церебральной ишемии.

Материал и методы. Эксперименты выполнены на 42 самцах беспородных белых крыс с начальной массой 240±20 г. Тотальная церебральная ишемия у белых беспородных крыс моделировалась путем декапитации. Забор материала для дальнейшего гистологического исследования осуществлялся на 1, 5, 15, 30 и 60-й минуте, а также спустя 5 и 24 часа после декапитации. Исследование гистологических препаратов проводилось с помощью микроскопа Axioscop 2 plus, цифровой видеокамеры и программы анализа изображений ImageWarp. Среди общего количества выделяли клетки по интенсивности окрашивания цитоплазмы (хроматофилии). После предварительной проверки на нормальность распределения показателей полученные данные были проанализированы методами непараметрической статистики.

Результаты. При тотальной церебральной ишемии наблюдались уменьшение размеров нейронов и деформация перикарионов. Нормохромные нейроны на 60-й минуте полностью исчезали. Количество гиперхромных нейронов возрастало, а затем прогрессивно снижалось. Сморщенные нейроны составляли большинство клеток в изучаемых отделах коры на 30-60-й минутах, а затем, спустя 5 и 24 часа, в популяции нейронов преобладали клетки с перицеллюлярным отеком.

Выводы. Полученные данные о гистологических изменениях нейронов филогенетически разных отделов коры головного мозга в динамике тотальной церебральной ишемии дают основу для дальнейшего детального изучения посмертных изменений головного мозга, определения времени смерти, создавая фундаментальную базу для изучения свойств нейронов, в том числе перехода их из одного функционального состояния в другое.

Ключевые слова: *крысы, церебральная ишемия, кора головного мозга.*

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Conflict of interest. The authors declare no conflict of interest.

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Financing. The study was performed without external funding.

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Conformity with the principles of ethics. The study was approved by the local ethics committee.

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