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Research Article

Post-Morther Changes in Rat Paretial Cortic and Hippocampus Neurons. Morphological Characteristics

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Abstract

Target: Analysis of changes in the morphological characteristics of neurons in such phylogenetically different parts of the rat cerebral cortex in different periods after total cerebral ischemia.

Methodology: Experiments were performed on 42 male outbred white rats with an initial weight of 240±20 g. Total cerebral ischemia in outbred white rats was modeled by decapitation. The material was taken at the 1st, 5th, 15th, 30th, and 60th minutes, as well as 5 and 24 hours after decapitation.

Results: With total cerebral ischemia, a significant decrease in the size of neurons and deformation of the perikarya were observed. Normochromic neurons completely disappeared at the 60th minute. The number of hyperchromic neurons increased and then progressively decreased. Shriveled neurons accounted for the majority of cells in the studied areas of the cortex at 30-60 minutes, and then, after 5 and 24 hours, cells with pericellular edema predominated in the neuron population.

Conclusion: The obtained data on histological changes in neurons of phylogenetically different parts of the cerebral cort in the dynamics of total cerebral ischemia provide the 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 other.

Key words: rats; cerebral ischemia; cerebral cortex

Introduction

Currently, in medicine, the concept of death is based on evidence of a permanent absence of brain function. A number of methods are used to diagnose the functioning of the brain: electroencephalography, assessment of cranial nerve reflexes, and studies of cerebral blood flow. On histopathological examination, post-mortem changes include edema, hemorrhages, infarcts, necrosis, ischemic softening, wrinkling and deformation of neurons, and pycnosis of their nuclei. In the hemispheres of the cerebellum, swelling and venous plethora are often found, in the subthalamic region and the optic tubercle – an area of spotted lysis. The most typical histological change in brain death is edema of its tissues followed by rupture of blood vessels [2-12]. Previous studies on the study of morphological changes in neurons of the parietal and cortex and hippocampus in subtotal cerebral ischemia of the brain showed a decrease in the size of perikarya and an increase in the number of hyperchromic and hyperchromic shriveled neurons [6,5]. At the same time, it is of interest to quantitatively study changes in the size, shape, and degree of neuron cytoplasm chromatophilia in different periods after total experimental cerebral ischemia.

The aim was to analyze changes in the morphological characteristics of neurons in such phylogenetically different parts of the cerebral cortex (parietal cortex and hippocampus) in rats in different periods after total cerebral ischemia.

Methodology

The experiments were performed on 42 male outbred white rats with an initial weight of 240 ± 20 g in compliance with the requirements of the Directive of the European Parliament and of the Council No. 2010/63/EU of September 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 vivarium diet and free access to food and water, in groups of no more than 5 individuals in a vivarium cage.

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

The study of histological preparations, their microphotography, morphometry and densitometry of the chromogen sediment in histological preparations were performed 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 cortex in histological preparations of the rat brain was determined using a stereotaxic atlas [12]. At least 30 neurons of the fifth layer of the parietal cortex and the pyramidal layer of the field CA1 of the hippocampus were evaluated in each animal, which provided a sufficient sample size for subsequent analysis. The number of large pyramidal neurons per unit area of sections of the cerebral cortex was determined on paraffin sections. Cells were isolated from the total number of cells according to the intensity of cytoplasm staining (chromatophilia). There were several types: normochromic – moderately colored; hyperchromic – dark; hyperchromic – very dark, with deformed perikarya; hypochromic – light colored; shadow cells are almost transparent. The number of each cell type was counted.

After a preliminary check for the normality of the distribution of indicators, the obtained data were analyzed by the methods of nonparametric statistics using the Statistica 10.0 program for Windows (StatSoft, Inc., USA). The results are presented as 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 indicators of the control and experimental groups were considered significant at $p<0.05$ (Mann-WhitneyU-test) [1].

Research results

In rats with total cerebral ischemia (TCI), changes in the size (S) and shape (form factor, elongation factor) of neuronal perikarya and the degree of chromatophilia of their cytoplasm were studied at certain time intervals (5 minutes, 15 minutes, 30 minutes, 1 hour, 5 hours, 1 day) in the parietal cortex (PC) and hippocampus (Hp). After 5 minutes of TIGM, there were no changes in the size, shape, and ratio of neurons in terms of the degree of cytoplasmic chromatophilia in both studied sections of the cerebral cortex, compared with those in the control group.

At the 15th minute of TCI, there was a decrease in the size of the PC and Hp neurons – by 47% ($p<0.05$) and 22% ($p<0.05$), and at the 30th minute – by 74% ($p<0, 05$), and by 51% ($p<0.05$), respectively.

At the same time, compared with changes at 15 minutes of TCI, after 30 minutes of the ischemic period, S neurons decreased in PC by 51% ($p<0.05$), and in Hp by 37% ($p<0.05$).

By the 60th minute of TCI, in comparison with the indicators in the control group, S of PC neurons decreased by 74% ($p<0.05$), and in Hp by 50% ($p<0.05$).

By 5 hours of TCI, the area of perikaryons of PC neurons in experimental rats was only 1/6 part ($p<0.05$) of S PC neurons in control group rats, having decreased 6 times, and in Hp – 4 times, amounting to 1/4 of area of neurons in rats Hp of the control group ($p<0.05$). Compared to 1-hour ischemia, by 5 hours TCI S of neurons decreased in PC by 35% ($p<0.05$), and in Hp by 48% ($p<0.05$).

By 24 hours of TCI, changes in the size of neurons in both studied structures, compared with manifestations of brain ischemia, was not observed ($P> 0.05$).

The shape of the neurons changed already by the 15th minute of TCI. Neurons became more elongated in both studied areas – the elongation factor (elongation index) increased by 25% ($p<0.05$), and by the 60th minute of ischemia – by 35% ($p<0.05$), while form factor (indicator of roundness of the perikarya) decreased by 34% ($p<0.05$) in both studied departments. By 5 and 24 hours of TCI, the form factor, compared with the value after 1 hour, did not change ($p>0.05$), and the elongation factor increased by 50% after 24 hours of the ischemic period ($p<0.05$) in both studied patients. structures of the cerebral cortex **(Table 1.).**

Animal groups	Areas of the cerebral cortex	
	parietal cortex	hippocampus
	area, μm^2	
control	144,6(130,5;153,8)	108,6(99,7;122,3)
5 minutes TCI	157(136;159)	99(95;102)
15 minutes TCI	81(77;86)*	78(71;89)*
30 minutes TCI	40(33;44)*+	49(47;52)*+
1 hour TCI	37(27;47)*+	54(50;60)*+
5 hours TCI	24(22,5;26,5)*+#	28(26;31)*+ #
1 day TCI	24,5(23;25)*+ #	26(24;28)*+ #
elongation factor, units		
control	1,2(1,1;1,2)	1,2(1,2;1,2)
5 minutes TCI	1,2(1,2;1,3)	1,2(1,1;1,2)
15 minutes TCI	1,6(1,4;1,7)*	1,6(1,5;1,7)*
30 minutes TCI	1,8(1,7;1,9)*	1,8(1,8;1,8)*
1 hour TCI	1,8(1,7;1,8)*	1,8(1,8;1,9)*
5 hours TCI	2,1(2;2,1)* +#	1,9(1,8;2)* +
1 day TCI	2,4(2,3;2,5)* +#	2,3(2,1;2,4)* +#
form factor, units		
control	0,9(0,9;0,9)	0,9(0,9;0,9)
5 minutes TCI	0,9(0,9;0,9)	0,9(0,9;0,9)
15 minutes TCI	0,8(0,7;0,8)	0,8(0,8;0,9)
30 minutes TCI	0,6(0,6;0,6)* +	0,6(0,5;0,6)* +
1 hour TCI	0,6(0,6;0,6)* +	0,6(0,5;0,6)* +
5 hours TCI	0,6(0,6;0,7)* +	0,6(0,6;0,6)* +
1 day TCI	0,6(0,5;0,6)* +	0,5(0,5;0,6)* +

Note: * – $p<0.05$ compared to the control group, + – $p<0.05$ compared to 15-minute TIBT, # – $p<0.05$ compared to 1-hour TCI

Table 1: Changes in the size and shape of the perikaryons of neurons in the parietal cortex and hippocampus of the brain of rats with its total ischemia (TCI) of different duration, Me (LQ; UQ)

In PC, there was an earlier, compared with Hp, decrease in the size of neurons. So, in PC at the 15th minute there was a decrease in S neurons by 49% ($p<0.05$), compared with TCI duration of 5 minutes, and at the 30th minute – by 51% ($p<0.05$), compared to the 15 minute TCI.

In Hp, the decrease in S at the 15th minute of TCI was 21% ($p<0.05$), compared with 5-minute cerebral ischemia, and at the 30th minute – 37% ($p<0.05$), according to compared to the 15 minute TCI.

When comparing changes in the size of neurons in the studied areas of the cerebral cortex, the decrease in S in the PC was more pronounced – by 72% with a 15-minute TCI ($p<0.05$) and by 29% with a 30-minute TCI ($p<0.05$), compared to Hp.

When studying the distribution of neurons according to the degree of cytoplasm chromatophilia, the following changes were noted **(Tables 2, 3, Figures 1).**

Animal groups	Areas of the cerebral cortex	
	parietal cortex	hippocampus
	number of normochromic neurons / mm^2	
control	3283(3216;3283)	3216(3149;3283)
5 minutes TCI	3098,8(2680;3216)	3065,3(2612;3149)
15 minutes TCI	1206(1206;1273)*	1474(1340;1608)*
30 minutes TCI	670(670;737)*+	804(804;871)*+
1 hour TCI	0(0;0)*+	67(0;134)*+
5 hours TCI	0(0;0)*+	0(0;0)*+
1 day TCI	0(0;0)*+	0(0;0)*+
number of hyperchromic neurons / mm^2		
control	201(201;268)	167,5(134;201)
5 minutes TCI	502,8(269;603)	302(134;536)
15 minutes TCI	804(804;938)*	871(804;938)*
30 minutes TCI	167,5(134;402)*+	234,5(134;268)*+
1 hour TCI	67(0;134)*+	100(0;134)*+
5 hours TCI	134(134;134)*+	134(134;134)*+
1 day TCI	0(0;0)*+	0(0;0)*+
number of hyperchromic shriveled neurons / mm^2		
control	134(67;134)	33(0;134)
5 minutes TCI	134(134;134)	134(134;134)
15 minutes TCI	536(402;536)*	402(402;469)*
30 minutes TCI	2680(2613;2747)*+	2814(2479;2948)*+
1 hour TCI	3450(3350;3551)*+	3618(3551;3685)*+
5 hours TCI	1206(1072;1273)*+	1139(1072;1206)*+
1 day TCI	201(134;268)+	234(201;268)+
number of shadow cells / mm^2		
control	134(0;134)	0(0;134)
5 minutes TCI	167,5(134;201)	134(67;134)
15 minutes TCI	268(0;335)*	134(134;134)
30 minutes TCI	268(268;335)*	268(268;335)*
1 hour TCI	234,5(201;268)*	201(134;335)
5 hours TCI	335(268;335)*	268(268;335)*
1 day TCI	134(134;201)	134(134;201)
number of neurons with pericellular edema / mm^2		
control	0(0;0)	0(0;0)
5, 15, 30 minutes, 1 hour TCI	0(0;0)	0(0;0)
5 hours TCI	1239,5(1206;1608)*	1072(1005;1139)*
1 day TCI	2579,5(2412;2747)*	2680(2479;3082)*

Note: * – $p<0.05$ compared to the control group, + – $p<0.05$ compared to 15-minute TCI

Table 2: Change in the number of different forms of neurons according to the degree of chromatophilia of the cytoplasm of the parietal cortex and hippocampus of rats with total cerebral ischemia (TCI) of different duration, Me (LQ; UQ)

After 15 minutes TCI, the number of normochromic neurons decreased by 63% ($p<0.05$) in PC and by 54% ($p<0.05$) in Hp, with their complete disappearance in PC after 1 hour of TBI and in Hp after 5 hours ischemic period.

The number of hyperchromic neurons increased to the maximum at 15 minutes of TCI (3 times, $p<0.05$) in both studied regions, and then progressively decreased by 1 hour ($p<0.05$). Hyperchromic shriveled neurons made up the majority of cells in the studied cortical regions at 30-60 minutes of TCI, and then, after 5 and 24 hours of the ischemic period, cells with pericellular edema prevailed in the neuron population ($p<0.05$).

Along with the change in the number of hyperchromic neurons in the cytoplasm of the studied cell population, the concentration of ribonucleoproteins increased, reaching a maximum by the 60th minute of TCI, subsequently decreasing by the 1st day of ischemia, which may be caused by the appearance of a large number of neurons with pericellular edema and a low degree of cytoplasm chromatophilia .

Changes in both studied brain regions were similar except for the absence of normochromic neurons in the parietal cortex after 1 hour of TCI. Also in the parietal cortex, the number of cells with pericellular edema after 5 hours of TCI was 14% higher than in the hippocampus ($p<0.05$).

Groups of animals	Areas of the cerebral cortex	
	parietal cortex	hippocampus
control	0,16(0,15;0,17)	0,18(0,17;0,18)
5 minutes TCI	0,2(0,14;0,22)	0,22(0,13;0,24)
15 minutes TCI	0,28(0,27;0,3)*	0,27(0,26;0,27)*
30 minutes TCI	0,35(0,3;0,37)*	0,37(0,35;0,39)*+
1 hour TCI	0,4(0,35;0,43)*+	0,38(0,37;0,4)*+
5 hours TCI	0,25(0,2;0,28)*	0,26(0,25;0,29)*
1 day TCI	0,23(0,23;0,24)*	0,26(0,25;0,27)*

Note: * – p<0.05 compared with the control group, + – p<0.05 compared to 15-minute TCI; units – units of optical density

Table 3: Changes in the content of ribonucleoproteins in rats with total cerebral ischemia (TCI) of different duration, (Me (LQ; UQ), units

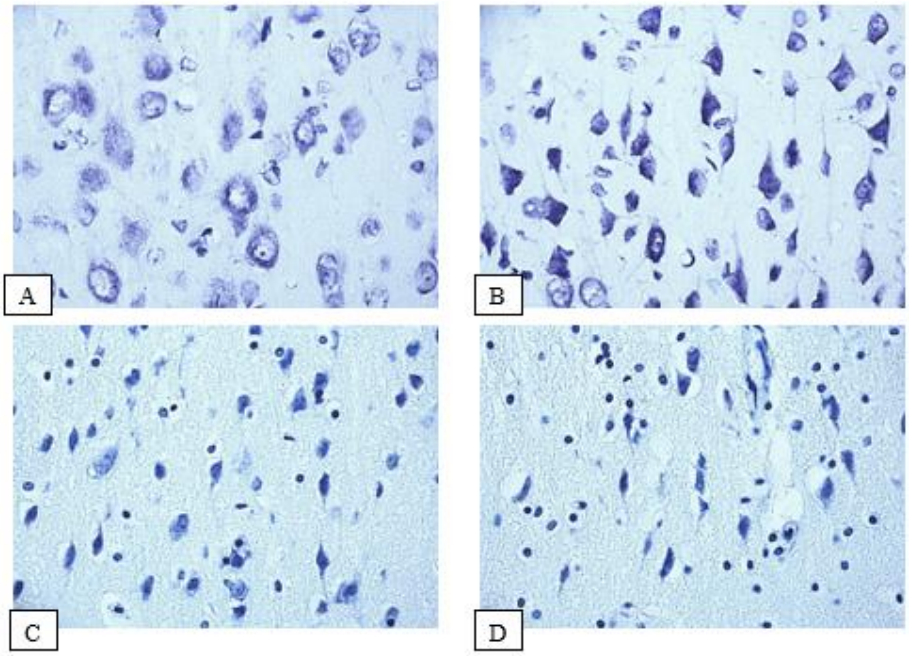

Figure 1. Neurons of the fifth layer of the parietal cortex of rats with TCI. A – 1 minute (the predominance of normochromic neurons), B – 30 minutes (the predominance of hyperchromic and hyperchromic wrinkled neurons), C – 1 hour (the presence of hyperchromic wrinkled neurons), D – 1 day (the predominance of cells with pericellular edema). Digital micrograph. Nissl coloring.

Zoom lens x 40 Thus, total cerebral ischemia is manifested by pronounced changes in the morphological characteristics of neurons (their size, shape and degree of cytoplasmic chromatophilia) in the cerebral cortex due to complete circulatory arrest. Moreover, in the parietal cortex, the degree of their severity is somewhat greater than in the hippocampus.

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