Cytoarchitectonic features of the neocortex, archicortex and amygdala of white rats after a 20-minute occlusion of the common carotid arteries

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ABSTRACT

Purpose. The study is focused on glioarchitectonics of the neocortex, archicortex and amygdala of Wistar white rats in normal state and after a 20-minute occlusion of the common carotid arteries.

Materials and methods. Light (stained with hematoxylin and eosin) microscopy, immunohistochemistry (GFAP), and morphometry were used to study the distribution, shape, and area of GFAP-immunopositive brain cells in the normal range \(n = 5\) and at days 1, 3, 7, 14, 30 \(n = 25\) after acute ischemia.

Results. Focal changes were found in the density of the glial network: decrease and increase in the local content of GFAP-positive material. Reactive, dystrophic and necrobiotic changes in neurons after acute ischemia were accompanied by reorganization of neuroglia and increase in the neuroglia index in certain zones by 1.2–1.5 times. The surface area of the particles in GFAP-positive astrocytes in sections of the neocortex in the control was 8.4–18.1, but after 3 days after ischemia this rate in some parts of the neocortex rose to 45.0–59.3%. In the hippocampus this rate was 8.1% and 16.2%, and in the amygdala it was 12.6% and 21.2%. Hypertrophy of mature astrocytes was manifested by the increase in the diameter, degree of branching and length of their processes.

Conclusion. The obtained data are considered as a phenomenon of ischemic preconditioning and activation of protective processes in neuro-glio-vascular microcomplexes.

Key words: acute ischemia, neocortex, hippocampus, amygdala, glia, GFAP.

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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INTRODUCTION

The study of neurogliacyte reaction to acute ischemia and brain reperfusion is relevant, prospective and practically important [1–3]. Astrocytes, oligodendrogliacytes and microgliacytes are regarded as an integrated cellular system providing the protection, restoration and restructuring of damaged neural tissue [4, 5].
Earlier studies of neurogliia have shown that its cells provide metabolic support to neurons and play a key role in the regulation of blood flow in the areas of active brain neurons (release of a neurotransmitter). Astrocyte networks, being an analogue of the lymphatic system, perform draining and detoxifying functions in the central nervous system. These functions of astrocytes are of great importance in the acute postischemic period, when the maximum manifestations of cell swelling are revealed [5].

The effect of ischemia on brain neurogliia of experimental animals was also studied [5, 6]. However, the purpose, objectives, experimental models, research methods, brain areas, and the duration of the observation period in those studies were different from those in our work. No data on comparison of the neocortex, archicortex and amygdala glioarchitectonics within 30 days after reperfusion are present. Mainly, gliocytes in the necrotic focus and perinecrotic zone were studied during long-term carotid artery ligation, i.e. irreversible changes in neurons. Thus, the details of the reorganization of glioarchitectonics in the neocortex, archicortex and amygdala have not been compared. Therefore, the existing morphological studies are insufficient for understanding the impact of global ischemia on the glioarchitectonics of these brain areas.

The purpose of this study was to carry out a comparative analysis of the structural basis of the glial cell reorganization in the neocortex, different regions of the hippocampus and nuclei of the amygdala after a 20-minute occlusion of the common carotid arteries.

**MATERIAL AND METHODS**

The work was carried out at Omsk State Medical University and was approved by the local Ethics Committee (Protocol No. 83 of October 14, 2016). White rats \((n = 30, \text{males})\) of the Wistar line weighing 180-200 g were used as experimental animals. The studies were conducted in accordance with the recommendations of the International Committee for Laboratory Animal Science, supported by WHO, directive of the European Parliament No. 2010/63/EU of 22.09.10 “On the protection of animals used for scientific purposes”.

Acute 20-minute cerebral ischemia induced by common carotid artery occlusion (CCA0), two-vessel model of incomplete global ischemia without hypotension) was modeled with premedication (atropine sulfate 0.1 mg/kg, subcutaneously) and general anesthesia (Zoletil 100, 10 mg/kg). The general condition of animals in the postoperative period was estimated in points taking into account the principles of investigating the brain physiology and pathophysiology [7].

Biopsy of the material was performed at days \(1 (n = 5), 3 (n = 5), 7 (n = 5), 14 (n = 5) \text{and} 30 (n = 5)\) after ischemia. False-operated (without arterial occlusion) animals of the same age served as a control \((n = 5)\). The brain was fixed by perfusion of 4% paraform solution on 0.1 M phosphate buffer (pH 7.4) through the ascending part of the aortic arch. Serial frontal sections \((2-4 \mu m)\) at the level of sensorimotor cortex, hippocampus and amygdala (MT) [8] were stained with hematoxylin and eosin, and with the immunohistochemical method for gliarial fibrillary acidic protein (GFAP, Leica Biosystems Newcastle Ltd., UK). Verification was performed according to the recommendations of the reagent manufacturer, finishing staining was done with hematoxylin. The Leica DM 1000 microscope was used for digital microphotography \((200 \text{fields of view from each studied area for a period})\).

Morphometric analysis was performed on binary images using ImageJ 1.46 software. The relative area of GFAP-positive material in the field of view was determined. The nature of the distribution of the specified value was evaluated using the Kolmogorov-Smirnov criterion. Statistical hypotheses were tested using the \(\chi^2\) test (StatSoft Statistica 8.0) [9]. The results were presented as a percentage \((95\% \text{confidence interval} – 95\% \text{CI})\) for 200 fields of view for each region for a period of time, StatSoft Statistica 8.0; MedCalc 11.6.1.0). In the course of statistical analysis, the null hypothesis was rejected at \(p \leq 0.05\).

**RESULTS**

In the brain of control animals, the GFAP-positive material was localized in the bodies and processes of astrocytes between normochromic neurons (Fig. 1).

Due to local features of cytoarchitectonics and relative density of cells in the nervous tissue, the studied brain areas differed in the proportion of GFAP-positive material per one neuron. This was due to the fact that in the neuron area (layers of pyramidal cells) the minimum relative area of GFAP-positive material (cytoplasm and processes of astrocytes) per unit area of the slice was in the neocortex and CA1, the maximum – in CA4 and amygdala (MT). Statistically significant differences in paired comparison are shown in the table (Table 1).
Table 1

| Brain region | Relative area and results of comparison |
|--------------|----------------------------------------|
| SMC          | 16.2% (95% CI: 11.4–22.1%)              |
| CA1          | 11.5% (95% CI: 7.4–16.8%)               |
| CA2          | 19.7% (95% CI: 14.4–25.9%)              |
| CA3          | 25.5% (95% CI: 19.4–31.9%)              |
| SMK          | 21.4% (95% CI: 15.9–27.7%)              |

Note. SMC – sensorimotor cortex, DF – dentate fascia, CA – carotid artery, AN – amygdaloid nucleus, CI – confidence interval.

Thus, despite the uniform distribution of glial cells in normal conditions, neuroglia relations in the neocortex, hippocampus and amygdala had features associated with the density of neurons.

It was found that after 20 minutes of CCAO, diffuse reversible microfocal ischemic changes of the neurons and the corresponding local response of neuroglia in the neocortex, hippocampus and amygdala dominated. In the studied brain parts, areas with low and high density of processes were identified, that is not typical of the normal state (Fig. 2).

That is, reactive, dystrophic and necrobiotic changes in neurons after acute ischemia were accompanied by reorganization of neuroglia and increase in the neuroglia index in certain zones by 1.2–1.5 times ($\chi^2 > 8.2; p < 0.01$).

Thus, the area of GFAP-positive astrocyte material on SMC sections in the control was 8.4–18.1, and 3 days after ischemia this index in some areas of the neocortex increased to 45.0–59.3% ($\chi^2 = 10.2, p < 0.001$). In the hippocampus, it was 8.1% (95% CI: 4.7–12.8%) and 16.2% (95% CI: 4.8–12.8%; $\chi^2 = 3.4; p = 0.02$). In the amygdala – 12.6% (95% CI: 8.3–18.0%) and 21.2% (95% CI: 16.6–28.6%; $\chi^2 = 3.2; p = 0.03$).

Hypertrophy of mature astrocytes was manifested by an increase in the diameter, branching degree and length of their processes (Fig. 2–4).

At the same time, the average relative area of GFAP-positive material (taking into account high and low density zones) calculated on 200 random
Fig. 2. Glial cells (brown bodies and processes) of layer III of the sensorimotor cortex (a – 6 h, b – 3 days) and dentate fascia (c – 6 h, d – 3 days) of the white rat brain in the post-ischemic period: hypertrophy of astrocytes and their processes after 3 days of reperfusion. Staining: immunohistochemistry, glial fibrillary acidic protein. The lens ×40, scale 100 µm

Fig. 3. Glial cells (brown bodies and processes) of the dental fascia (a, b), CA3 (c) and CA1 (d) of the white rat brain in the post-ischemic period, 1 day: different density of GFAP-positive material in the zone of granular and pyramidal neurons (arrows). The relative area of GFAP-positive material in DF – 15.9%, CA3 – 8.8% and CA1 – 7.7%. Staining: immunohistochemistry, glial fibrillary acidic protein (GFAP). The lens ×40, scale 100 µm
fields of view in the neocortex frontal sections, hippocampal formation and amygdala remained stable at the control level in the postischemic period for 30 days of observation.

Only in the neocortex, at days 1 and 3 after ischemia, a statistically significant difference by the Min–Max difference was revealed. This was explained by focal changes (Tables 2, 3).

Thus, using the immunohistochemical study of the GFAP distribution, common regularities and features of the glioarchitectonics of the neocortex, hippocampus and amygdala in normal conditions and

Fig. 4. Sensorimotor cortex (a, layer III), dentate fascia (b), CA1 (c), CA3 (d), CA4 (e) of the hippocampus and the central nucleus of the amygdala (f) of the white rat brain, day 7 of reperfusion: different density of cut processes of the glial cells in the neuropyl and around the neuron bodies in the presented brain regions. Arrows are neuron bodies.

Staining: immunohistochemistry, GFAP. The lens ×40, scale 50 µm
after CCAO were found. Focal changes in the glial network density – a decrease and an increase in the local content of GFAP-positive material - were revealed. It is likely that the former is associated with swelling of the neuroglia, and the latter – with its local compensatory hypertrophy and proliferation.

Table 2

| Group | Comparison area |
|-------|-----------------|
| day 1 | 11,6 (CI: 7,5–16,9); χ² = 0,03; p = 0,85 |
| day 3 | 9,4 (CI: 5,7–14,3); χ² = 0,8; p = 0,37 |
| day 7 | 13,4 (CI: 9,0–18,9); χ² = 0,0; p = 0,95 |
| day 14 | 10,2 (CI: 6,4–15,3); χ² = 0,4; p = 0,52 |
| day 30 | 12,5 (CI: 8,3–17,9); χ² = 0,0; p = 0,93 |

Note. CI – 95% confidence interval. In comparison with the control, the differences are statistically significant at p ≤ 0.05 (χ² test).

Table 3

| Group | Comparison area |
|-------|-----------------|
| day 1 | 9,8 (CI: 6,1–14,8) |
| day 3 | 11,0 (CI: 7,0–16,2) |
| day 7 | 9,6 (CI: 5,9–14,6) |
| day 14 | 10,1 (CI: 6,3–15,1) |
| day 30 | 10,7 (CI: 6,8–15,8) |

Note. CI – 95% confidence interval. In comparison with the control, the differences are statistically significant at p ≤ 0.05 (χ² test). No significant differences were found.
CONCLUSION

The obtained data on the insignificant small-focal damage to the neocortex, hippocampus and amygdala should be considered as a phenomenon of ischemic preconditioning. In the implementation of this phenomenon in the post-ischemic period, the processes associated with the activation of the natural protective mechanisms of the CNS nervous tissue are put to the forefront. [10].

After a 20-minute CCAO, numerous processes in neuro-glio-vascular microcomplexes start already in the first minutes or hours of reperfusion, preventing the development of irreversible neuron changes [4]. These processes may be associated with activation of neuroglia (drainage, trophic inactivation of toxins), as well as with activation of receptors, intracellular kinase cascades, transcription factors, certain mitochondrial proteins and nuclear neuron effectors [11, 12].

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Authors contribution

All the authors have personally and evenly contributed to the study by applying the integrated methodological approach, including experimental, anatomical, histological, morphometric and information and mathematical methods as well as methods of observation, description and analysis.

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