The increase in the number of astrocytes in the total cerebral ischemia model in rats

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Abstract. Astrocytes are the most abundant cell class in the CNS. Astrocytic therapies have a huge potential for neuronal repair after stroke. The majority of brain stroke studies address the damage to neurons. Modern studies turn to the usage of morphological and functional changes in astroglial cells after stroke in regenerative medicine. Our study is focused on the changes in the number of astrocytes in the hippocampus (where new glia cells divide) after brain ischemia. Ischemia was modeled by occlusion of tr. brachiocephalicus, a. subclavia sin., a. carotis communis sin. Astrocytes were determined using immunohistochemical labeling with anti GFAP antibody. We found out that the number of astrocytes increased on the 10th and 30th days after stroke in the CA1, CA2 fields, the granular layer of dentate gyrus (GrDG) and hilus. The morphology of astrocytes became reactive in these regions. Therefore, our results revealed long-term reactive astrogliosis in the hippocampus region after total ischemia in rats.

1. Introduction
Stroke is one of the leading causes of death and disability of the population around the world. Elimination of the consequences of this disease is an important task for medicine. The primary attention in the study of ischemic injury mechanisms is directed to neuronal loss and reparative neurogenesis [1-4], while this disease affects absolutely all types of brain cells including astrocytes. Nowadays studies on the development of drugs for the treatment of stroke more and more often become aimed at the support and regulation of astrocytes in this pathology. Astroglia plays an important part in the regulation of post ischemic inflammation [5-8]. This study aimed to investigate the effect of total cerebral ischemia (TCI) on the reactivation of astrocytes.

2. Materials and methods
The experiment was performed on 20 adult male Wistar rats weighing 250-300 g. During the study, the animals were kept in standard vivarium conditions. The model of total ischemia was performed by a surgical occlusion of vessels (left common carotid artery, left subclavian artery and brachycephalic trunk), using ligature [1,3]. The ischemic episode lasted 7 minutes. After the removal of ligatures, reperfusion of vessels ensued. Chloral hydrate anesthesia with artificial pulmonary ventilation was used during the operation, and ether anesthesia – for transcardial perfusion. The tracking of changes was made at several time points. For this purpose, the animals were divided into 4 groups: 10 days after the ischemia – "Control-1" and "Ischemia-1", after 30 days – "Control-2" and "Ischemia-2". The control groups underwent the same manipulations except for the ligature tightening and the creation of an ischemic episode. The fixation of tissues was carried out with a 4% formalin solution. For cryoprotection, the brain was placed in a 10% sucrose solution for 24 hours, followed by a 20% sucrose solution for another 24 hours. After cryoprotection, the tissues were frozen in nitrogen vapors.
and placed in a freezer for storage at -80°C. Sections of the frozen tissues were prepared using a cryotome (Thermo Scientific HM 525, Germany), at a step of 10 μm. Immunohistochemical analysis of the tissues was carried out using specific antibodies, such as antibodies to GFAP (Rabbit anti GFAP, Alexa Flour, USA) in order to evaluate the reactivation of glia. To perform a fluorescent survey, an antibody marker was used, such as Alexa Flour®488 Donkey anti Rabbit. During the analysis, the number of cells in the hippocampal regions was counted: fields CA1, CA2, layers, granular layer of dentate gyrus (GrDG) and hilus. When carrying out data analysis by means of the STATISTICA 10 software, the t-test for unrelated samples was used.

3. Results
Statistical analysis of the data showed an increase in the number of astrocytes in the "Ischemia-1" group in the CA2 zone, hilus (p<0.01), CA1 and GrDG (p<0.05), as compared to the "Control-1" group. The reactive proliferation of astrocytes around damaged neurons more than doubled in the fields CA1 and CA2. Anatomically, the fields CA1 and CA2 are characterized with a high density of neurons and a small amount of glia. The migration and proliferation of glia in this zone indicates the presence of inflammation. The least significant changes on day 10 were observed in the GrDG layer, where not only adult astrocytes but also radial glia expressing GFAP underwent the ischemic effect (Fig. 1). These changes indicate the induction of stem cell division.

Figure 1. The increase in the number of astrocytes in the layers of the hippocampus after ischemic injury on days 10 and 30. The number of cells per μm² is shown. The means for the group with the standard error of the mean are presented. Statistical differences are marked with the following symbols: * – p<0.05, ** – p<0.01.

Differences between the groups intensified by day 30. There was a significant increase in the number of astrocytes in the layers GrDG, CA1, CA2 (p<0.01) and in hilus (p<0.05) in the "Ischemia-2" group in comparison with the "Control-2" group. The number of astrocytes in the field CA2 exceeded the control indicators by more than 3 times. The increased differences as compared to the control value in the GrDG layer indicate the presence of a peak in the division of stem cells in the granular layer in the period of about 30 days after ischemia.

In both the ischemic groups, astrocytes retained a morphology characteristic of the state of reactivation, as evidenced by the characteristic 'swelling' of the cells, an increased branching and lengthening of the processes identified by an enhanced expression and GFAP distribution (Figure 2). This morphology persisted at all time points of the study after ischemia.

4. Conclusion
Pyramidal neurons of the CA1 field are most sensitive to ischemic effects [1], and a decrease in blood supply leads to the death of neurons of this layer. The damaged cells of the CA1 field and the signals emitted by them induce the reactivation of astrogia [1,9]. Our previous results showed an increased level of neurogenesis 10 days after total ischemia that however, falls one month after surgery [1-3]. On the contrary, reactive astrogliosis intensifies within a month after ischemia. Based on our data proving the persistence of glial reactivation one month after the ischemic episode, it can be assumed that post-ischemic reactivation of astrogia changes its functions in support of the metabolism of mature neurons.
in the CA1 layer. The postnatal division of stem cells persists in the GrDG zone of the hippocampus and is a physiologically significant process. Recent studies suggest that the death of neurons is an inducer of proliferation of stem cells which can be differentiated into neuronal progenitors [10]. Our data confirmed that ischemia is a factor stimulating the division of GFAP-positive stem cells.

**Figure 2.** The change in the number of astrocytes in the CA1 field of the hippocampus of rats. The left shows the group "Control-1", and the right – the group "Ischemia-1". The cell nuclei (DAPI) are colored in blue, and the astrocyte processes (GFAP) are colored in green. Image size: 100 μm. Image magnification: × 20.

**Acknowledgements**
The study was supported by the Russian Science Foundation, project No.14-45-00040-P.

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