Simulation of Unilateral Ischemic Injury to the Striatal Neurons Inflicted by Short-Term Occlusion of the Middle Cerebral Artery

D. E. Korzhevskii, O. V. Kirik, A. E. Baisa*, and T. D. Vlasov*

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The reproducibility of brain injury was evaluated by simulating ischemia in rats by 30-min occlusion of the middle cerebral artery. The selected ischemia-reperfusion protocol was characterized by high reproduction of the striatal neuron injury, which fact suggests this model for studies of nerve tissue reactions to injury and for evaluation of the efficiency of neuroprotective drugs.

Key Words: *brain; focal ischemia; neurons; striatum*

The problems of prevention and therapy of acute cerebrovascular disorders acquired special importance in recent years because of high prevalence of cerebrovascular diseases [1]. Creation of easily reproducible adequate experimental models of brain ischemia for development and trials of new therapeutic and preventive approaches is an important task of modern experimental medicine.

We evaluated the reproducibility and determined the adequate morphological criteria for evaluation of brain injury during simulation of brain ischemia in rats by means of transitory occlusion of the middle cerebral artery (MCA).

MATERIALS AND METHODS

Experiments were carried out on 16 adult male Wistar rats. Ischemia was induced by the endovascular method [7] under Nembutal narcosis. The duration of circulation arrest in the left MCA basin was 30 min in each case, followed by 48-h reperfusion. The brain of intact animals (n=5) and structures of the right hemispheric endbrain of experimental animals serves as the control. The material was processed by dehydrating fixation [2] and embedding in paraffin. The morphology of serial frontal sections at the level of the bregma (±0.5 mm) has been examined; this level corresponds to the zone of an extensive injury in permanent occlusion of MCA [3]. The neurons were detected by staining after Niessle and by immunocytochemical visualization of NeuN (nerve cell nuclear marker) in accordance with a previously reported protocol [6]. The cells were counted using ImageJ software (NIH). The results were compared using Student's t test and the sign number.

RESULTS

In contrast to permanent focal ischemia [3], transitory 30-min cerebral ischemia in the MCA basin did not lead to development of a large focal ischemic infarction in the brain in the majority of cases (n=13). Predominant involvement of the striatal neurons (nucleus caudatus/putamen) was seen in the ischemic hemisphere. Niessle's staining showed disappearance of chromatophilic substance from the cytoplasm of the majority of viable striatal

Department of Common and Special Morphology, Institute of Experimental Medicine, the Russian Academy of Medical Sciences, St. Petersburg; *Department of Pathological Physiology, St. Petersburg State Medical University, Russia. ***Address for correspondence:*** dek2@yandex.ru. D. E. Korzhevsky

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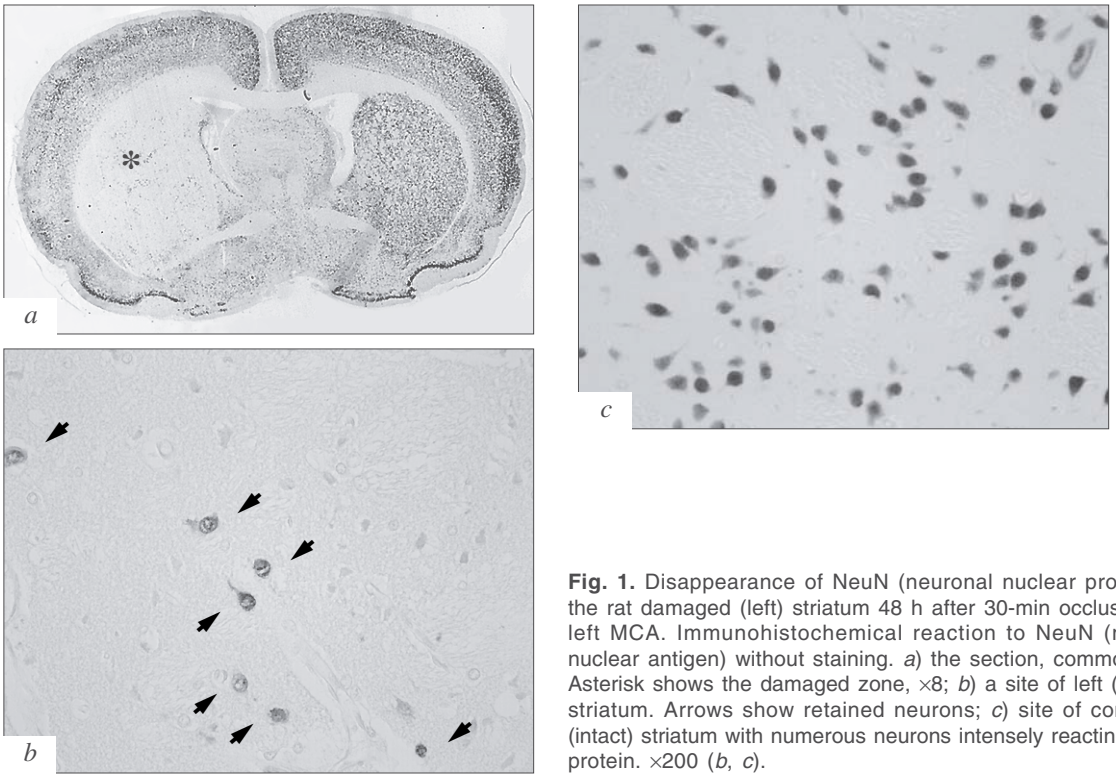

Fig. 1. Disappearance of NeuN (neuronal nuclear protein) from the rat damaged (left) striatum 48 h after 30-min occlusion of the left MCA. Immunohistochemical reaction to NeuN (nerve cell nuclear antigen) without staining. *a*) the section, common aspect. Asterisk shows the damaged zone, ×8; *b*) a site of left (damaged) striatum. Arrows show retained neurons; *c*) site of contralateral (intact) striatum with numerous neurons intensely reacting to NeuN protein. ×200 (*b*, *c*).

neurons on the involved side; degenerating (mainly by the "clear" type) neurons were noted. The remaining neurons were often hypertrophic. Small blood vessels are dilated. No clear-cut injuries to the neurons and changes in the vascular status were detected in the contralateral striatum.

The reaction to NeuN nuclear neuronal marker disappeared in the left (ischemic) striatal area (Fig. 1, *a*). This can be attributed to a significant reduction of the neuron population in the damaged area (Fig. 1, *b*, *c*). Quantitative analysis showed that the striatal neuronal population usually decreased 4-10-fold ($p<0.01$) in comparison with the contralateral area. Comparison of the population density of NeuN-positive neurons in symmetrical brain zones in intact animals showed that inter-hemispheric asymmetry by this parameter usually varied from 2 to 8% for the striatum. These data suggest using the contralateral hemisphere as the internal control in evaluation of the severity of unilateral ischemic injury to the endbrain structures. Using this approach, it is possible to compare the percentage of lost neurons in the hemispheres irrespective of the thickness of analyzed sections.

These data together with the results of previous studies [4,5] indicate that use of the NeuN neuronal marker is an adequate approach, providing rapid and reliable identification of neurons for quantitative analysis of their population.

Hence, the chosen ischemia-reperfusion protocol is characterized by a high reproducibility of striatal neuron injury in rats and can be used for studies of the basic aspects of nerve tissue reaction to ischemia and for evaluating the efficiency of new neuroprotective drugs.

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