

METHODS FOR ESTIMATING NEUROLOGICAL DISTURBANCES IN EXPERIMENTAL CEREBRAL ISCHEMIA

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Investigation of the brain pathology in experimental ischemia requires adequate methods for assessing the neurological deficit that occurs in laboratory animals, including sensory-based and behavioural disorders. In this research, we aimed to compare motor and behavioural disorders in rats with partial and subtotal experimental cerebral ischemia. The rats modelled with cerebral ischemia are found to exhibit a decrease in muscle strength, resistance to hypoxia, motor and emotional activity. The animals with incomplete cerebral ischemia demonstrated more pronounced sensory-based motor and behavioural disorders compared both with those modelled with partial cerebral ischemia and, in particular, with the control group.

Keywords: cerebral ischemia, sensomotor and behavioural disorders, rats

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МЕТОДЫ ОЦЕНКИ НЕВРОЛОГИЧЕСКИХ НАРУШЕНИЙ ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ЦЕРЕБРАЛЬНОЙ ИШЕМИИ

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Изучение патологии головного мозга при экспериментальной ишемии обуславливает потребность в адекватных способах оценки возникающего у лабораторных животных неврологического дефицита, включающего сенсомоторные и поведенческие нарушения. Целью работы явилось сравнительное изучение двигательных и поведенческих нарушений у крыс с частичной и субтотальной экспериментальной церебральной ишемией. Установлено, что крысы после экспериментальной церебральной ишемии обладали меньшей мышечной силой, были менее устойчивы к гипоксии, проявляли меньшую двигательную и эмоциональную активность. У животных с субтотальной церебральной ишемией наблюдались более выраженные сенсомоторные и поведенческие нарушения по сравнению с крысами, которым моделировали частичную церебральную ишемию и, особенно, по сравнению с контрольными животными.

Ключевые слова: церебральная ишемия, сенсомоторные и поведенческие нарушения, крысы

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Introduction

The expediency of studying the pathology of the brain in experimental ischemia necessitates adequate methods for assessing the neurological deficit that occurs in laboratory animals, including various sensory and behavioural disorders. A number of methods can be used to study the degree of sensory-based motor and behavioural disorders in animals, such as the forced swimming, muscle strength, open field tests, as well as those for evaluating the modified indicators of the depth of a neurological deficit [3]. Among other popular methods are the Bederson, gas exposure, angular, paw-pulling tests.

This article sets out to generalize the available literature data on contemporary approaches to assessing sensory-based motor reflexes, learning and memory abilities in experimental animals at different ages. In the early postnatal period, reflexes are assessed using such methods as the slip on the surface, negative geotaxis, avoidance of falling, reaction to acoustic stimuli, olfactory reaction and muscle strength tests.

Sensory-based motor and behavioural disorders have been extensively studied using various models of cerebral ischemia (CI) [7–10]; however, the data obtained in these works has not thus far been properly generalized.

Research works devoted to the investigation of CI effects have identified a number of sensory-based motor disorders, including a decrease in motor activity when an animal is suspended by the tail, walking along a circle on a horizontal surface, dis-coordination of movements during walking along the bar, a decrease in the expression of unconditioned reflexes,

animals' inability to navigate through space. Local ischemic damage to the anterior parts of the frontal cortex of the rat brain leads to disruption in the production, preservation and reproduction of conditioned reflexes, while damage to the posterior parts of the frontal cortex is accompanied by a loss of the ability to navigate in T-shaped labyrinths [8–10].

The purpose of this research was a comparative study of motor and behavioural disorders in rats with partial and subtotal experimental cerebral ischemia.

Tests for studying the maturation of the nervous system in newborns

In new-born rat pups, methods for assessing the development of sensory-based motor reflexes comprise the slip on the surface, negative geotaxis, avoidance of falling, reaction to acoustic stimuli, olfactory reaction and muscle strength tests. These measurements can be carried out both multiple times to trace dynamic changes and on a single occasion, e.g. on the supposed day of maturation of the studied reflex in intact animals [1, 26–28].

Tests for studying sensory-based motor disorders in adult animals

The degree of sensory-based motor disorders in adult rats is identified using a diversity of methods, including the Bederson, Garcia, angular, pulling-the-paw, open-field tests, as well as that for assessing the modified depth indices of a neurological deficit [3, 5–7, 11, 14, 15, 17–25].

Materials and methods

The experiments were performed on 30 female non-native white rats weighing

230±20 g. All the requirements of the 2010/63/EU Directive of the European Parliament and the Council of 22.09.2010 on the protection of animals used for scientific purposes were observed during the experiments [16]. The animals were kept in an air-conditioned room (22°C) with mixed lighting under standard vivarium conditions implying free access to feed and water. One vivarium cage housed no more than 5 animals. The choice of experimental animals was determined by the similarity between rats and humans in terms of the brain angioarchitectonics. Before the research, all the necessary conditions were met. Thus, 60 minutes before testing, the animals were kept in a quiet, poorly lit place, without any regrouping and changes in feeding and other conditions [4]. Subtotal cerebral ischemia (SCI) was modelled by a stepwise ligation of a carotid artery, which manipulation promoted the survival of animals compared to those with simultaneous bandaging. Partial cerebral ischemia (PCI) was modelled by ligation of one common carotid artery (CCA) under intravenous thiopental anaesthesia (40–50 mg/kg).



Fig. Muscle strength evaluation.

Рис. Оценка мышечной силы.

The second group consisted of rats, which were simulated with SCI 7 days following PCI by ligation of the second (right) CCA [4]. The control group (control) comprised false-operated animals. The tests were performed 5 days following the surgery.

The ischemic damage to the brain was estimated by assessing the animals' emotional state, behaviour and motor activity. To this end, the open field, muscle strength and forced swimming tests were applied.

Muscular strength was assessed by placing a rat on a metal mesh with a length of 60 cm and a centimetre scale, and determining the time when the animal falls off after lifting the mesh to a horizontal position (by 90°).

In the forced swimming test, the animals were placed in a glass tank filled with water (21°C) for determining the time during which the animal can maintain swimming and floating behaviour.

Statistical processing of the obtained data was carried out using the Statistica 10.0 program for Windows (StatSoft, Inc., USA). The values obtained were analysed by non-parametric statistics. The quantitative data were presented as Me (LQ; UQ), where Me was the median, LQ — the value of the lower quartile; UQ — the value of the upper quartile. Differences between the control and experimental groups were considered significant at $p < 0.05$ (the Kruskal — Wallis and Mann — Whitney tests with the Bonferroni correction) [1].

Results

A significant decrease in muscle strength was observed in both groups of the animals with CI compared to the control group. Thus, this indicator decreased by 75% ($p < 0.05$) and 95% ($p < 0.05$) in the PCI and SCI groups, respectively. Muscular strength in rats with PCI was 5 times more pronounced than in those with SCI (Table).

In addition, the rats with PCI showed a greater resistance to the load-induced hyp-

Table. Indicators of sensory-based motor and behavioural tests. The quantitative data are presented in the form of Me (LQ; UQ)

Таблица. Показатели сенсомоторных и поведенческих тестов. Количественные данные представлены в виде медиана (нижний квартиль; верхний квартиль)

Group	Muscle strength	Forced swimming test	Indicators			
			Number of crossed squares	Open field test		Number of defecation and urination acts
				Number of short washings	Climbing	
Control	20 (15; 24)	21 (18; 23)	67 (64; 72)	6 (5.1; 7.2)	9 (8; 9.4)	5 (4.6; 6.5)
PCI	5 (4.7; 5.9)*	12 (15; 12.3)*	54 (52; 59)*	4 (3.5; 4.3)*	6 (5.8; 6.4)*	3 (2.5; 3.6)*
SCI	1 (0.5; 1.2)*#	5 (4.5; 5.7)*#	24 (22; 28)*#	2 (1.6; 2.4)*#	3 (2.6; 3.2)*#	2 (1.2; 2.3)*#

Note: * — $p < 0.05$ compared to the control, # — $p < 0.05$ compared to the PCI.

Примечание: * — $p < 0.05$ по сравнению с контролем, # — $p < 0.05$ по сравнению с частичной церебральной ишемией.

oxia in the forced swimming test. The time of their floating on the surface exceeded that in the animals with SCI by 58% ($p < 0.05$). Compared to the control group, the floating time was by 57% ($p < 0.05$) and 76% ($p < 0.05$) lower in the PCI and SCI groups, respectively.

The assessment of motor activity by the open field test also revealed the presence of motor deficiency in rats with CI. In comparison with the control group, the rats with PCI and SCI demonstrated a decrease in crossed squares by 20% ($p < 0.05$) and 64% ($p < 0.05$), respectively. The motor activity in the horizontal plane test was higher in rats with PCI by 44% ($p < 0.05$) than in the SCI group. In comparison with the control group, the number of short washings in the rats with PCI and SCI was 33% ($p < 0.05$) and 67% ($p < 0.05$) lower, respectively. The rats with PCI performed 50% ($p < 0.05$) more washings compared to the SCI group.

Compared to the control group, the weight loss in the rats with PCI and SCI was 33% ($p < 0.05$) and 67% ($p < 0.05$) lower. The rats with PCI made 50% more racks in comparison with the SCI group ($p < 0.05$).

The rats with PCI and SCI demonstrated a 40% ($p < 0.05$) and 60% ($p < 0.05$) decrease in the number of defecation and urination acts

compared to the control. In terms of this indicator, the rats with PCI differed from animals with SCI by 33% ($p < 0.05$).

Long-term washings and rearing posts were observed only in intact animals.

Conclusions

The rats after experimental CI have demonstrated a decrease in muscle strength, resistance to hypoxia, motor and emotional activity. In animals with SCI, more pronounced sensorimotor and behavioural disorders have been observed compared to those modelled with PCI. The morphological basis of the revealed disorders is found to be damage to the neurons of the brain, leading both to the destruction of physiological functional connections and physiological systems, as well as to the emergence of pathogens. The latter causes the destabilization of nervous processes (coordinated activity of excitation and inhibition), which subsequently results in the disruption of brain cognitive functions. Another reason for these disorders is an imbalance between the levels of biogenic amines and neuromediators in brain structures, which act as endogenous pathogenic factors and determine the nature and severity of ischemic damage [2, 12, 13].

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