MODELING OF FOCAL INJURY IN THE LEFT HEMISPHERE OF RAT BRAIN AND FUNCTIONAL ASSESSMENT DEPENDING ON THE SEVERITY OF DAMAGE IN THE POSTTRAUMATIC PERIOD

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Traumatic brain injury (TBI) is one of the major causes of disability and high mortality of the population, especially in young people, and important health and social problem in view of the weight of the health, social and economic consequences. The consequence of TBI is often damage to the blood vessels of the brain, skull, which is accompanied by the deterioration of cerebral blood flow and metabolism, decrease in oxygen consumption of brain cells. Vascular changes can be extremely variable in characteristics and severity [2]. Diagnosis and the provision of skilled care at TBI is the leading problem of modern neuroscience with such a high prevalence of this disease. It is important to study in vivo models of mechanisms of the pathogenesis of brain injury, particularly in comparing the severity of the harmful effects and development of neurological deficit using advanced behavioral tests. This study is based on these principles of modeling and assessing.

MATERIALS AND METHODS

We used the previously proposed [6] and the modified model of focal open head injury in rats [7]. The study was performed on male Wistar rats weighing 160 to 220 g. Animals were anesthetized by introducing 3% solution of chloral hydrate at a dose of 330 mg / kg intraperitoneally before surgery. We placed a teflon movable piston diameter of 4 mm and a stroke of 2.5 mm in a hole drilled in the skull over the sensorimotor cortex area. 50 gram weight hit this piston with an free fall acceleration from different heights (5, 10 and 15 cm) to simulate traumatic damage to the left hemisphere. The localization of the sensorimotor cortex was determined from the data presented in the literature [8, 10].

Animals were divided into 3 groups:

1) injured with weight falling from a height of 5 cm (11 animals);

2) from a height of 10 cm (13 animals);

3) with a height of 15 cm (18 animals).

Behavioral tests with limb stimulation were carried out on the day prior to surgery and then at 1, 3 and 7 days after application of the TBI. We used our [15] 12-point scale [5] in modification Jolkkonen et al [9] to assess the neurological disorders caused by head injury.

Installation for test "tapered track" is raised above the floor to a height of 90 cm horizontal rail (track) 165 cm long, 6 cm tapering at the beginning and to 1.5 cm at the end. The ending of track is the dark compartment, which is attached to a narrow end path. Throughout the track 2 cm below the level, the 2 cm tabs are located on both side which allows the animal to put weakened front or hind leg, so as not to fall off the track. At the beginning of the test animal are always placed in the dark compartment for 1 min. Before baseline recording animal training required to properly perform the test. The testing procedure is to rat ran the entire length of the converging paths and hid in the dark compartment. We recorded video with high resolution on two sides a track limbs to assess function on both sides for further analysis. We assessed these parameters: the passage of the race before entering the dark compartment; the distance at which the first indent from the track has been committed; number of misses (stumbles), committed both contralateral and ipsilateral to damage limb. Control study and confirmation of the presence in the desired area lesion was performed on the seventh day after the injury. Animal brains were fixed by immersion in a mixture of formalin-ethanol-acetic acid in the ratio 2: 7: 1 for 24 hours, then transferred to 70% in ethanol for one day and photographed. Treatment of animals and experimental procedures were performed in accordance with the directives of the European Community Council of 86/609 / EEC on the use of animals for experimental research. All experimental protocols were approved by the Ethics Committee of Research Center of Neurology (protocol 2-5 / 16).

RESULTS AND DISCUSSION

The used model of TBI allowed us to get a standard size and localization of cortical lesion, which is accompanied by severe neurological deficit corresponding clinical manifestations of brain injury as was previously shown [1, 7].

Compared with the rats of the first and second groups, third group of animals throughout the postoperative week visual inspection had exhausted appearance: they were more sluggish, slow, some of animals refused from food. At the same time, the rats of the first and second group on the next day after surgery were active and mobile, actively foraged. It is worth noting that on the third day after modeling TBI rats traumatized with weight falling from 15 cm, had labored breathing (some

hissing and wheezing), sat hunched, their hair was ruffled, contaminated under the tail, there was discharge from the eyes and nose (porphyrin), many of them were uncoordinated, some collapsed on its side, did not hold the pose.

Before modeling TBI intact healthy rats performed the test with stimulation of the limbs without deviations or minor deviations and gained the maximum possible number of points for both the left (11,9 ± 0,03, n = 42, n - number of animals) and right limbs (11,98 ± 0,01, n = 42, n - number of animals). The very next day after the injury in the left hemisphere in the sensorimotor cortex area function of the right limbs, subordinate to this hemisphere, had a noticeable functional impairment in all groups of animals. However, by day 7 there is a slight spontaneous improvement in function of the right limbs. In the first group for the first day after the injury test result on the right side was an average of 9.22 ± 0.29 points, for the third - 9.31 ± 0.37, for the seventh - 9,77 ± 0,22 (n = 11, n - number of animals). In the second group for the first day test result was an average of 6,53 ± 0,3 points, the third - 7,3 ± 0,29, the seventh - 7,57 ± 0,29 (n = 13, n - number of animals). In the third group on the first day test result was of an average of 5,22 ± 0,19 points, the third - 7,01 ± 0,26, the seventh - 5,97 ± 0,29 (n = 18, n - number of animals). Fig. 1a shows the average number of points scored by the right side of the rats from different groups, on the seventh day after the head injury simulation, showing a clear correlation between dysfunction of contralateral limb and the height of dropping weight (5, 10, 15 cm), reflecting the severity of the damage.

A similar assessment of left limb function, independent from the damaged hemisphere, identified in the first and second groups 30% of the animals with a minimum functional deviations from normal results. On average, these animals on the seventh day after the injury in the test gained 11.77 ± 0.1 points (the first group of animals, n = 11, n - number of animals) and 11.81 ± 0.07 points (the second group, n = 13, n - number of animals). At the same time only 12% of the animals of the third group have shown no dysfunction of the left limb - on the seventh day with left side testing they gained 11,06 ± 0,1 (n = 18, n - number of animals) (Figure 1b). There is a clear dependence of the severity of dysfunction of the left limbs on the degree of damage of the right ones. We can assume that the left hemisphere is dominant in rats with disabilities of both right and left limbs, since it is known that damage of the premotor cortex in humans manifests in the contralateral hand, and in case of damage of the left (dominant) hemisphere - in work of both arms [3, 4].

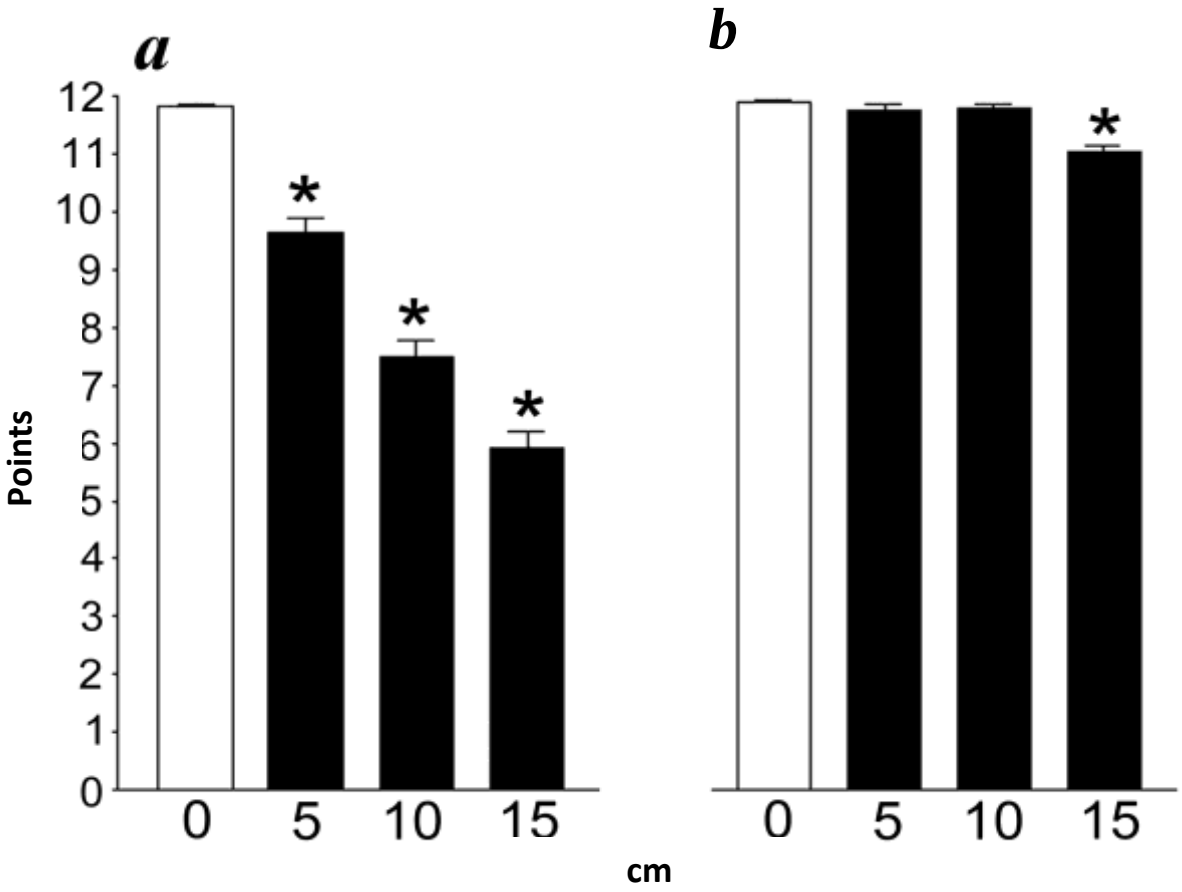
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Fig.1 Dysfunction of the right (a) and left (b) limb in simulation traumatic injury in the area of the sensorimotor cortex of the left hemisphere.
* p <0,01 as compared to the intact animals prior to surgery (the first white column).

Animals adapt to life with a weakened or paralyzed limb with one-sided brain injury [11]. In order to detect possible changes, not identified in the previous test we used the test of "tapered track", providing an increase in the complexity of the task [12]. Outs method are the need of training the rats before surgery and the inability to eliminate all environmental factors that distract the rat from purposeful moving forward along the path.

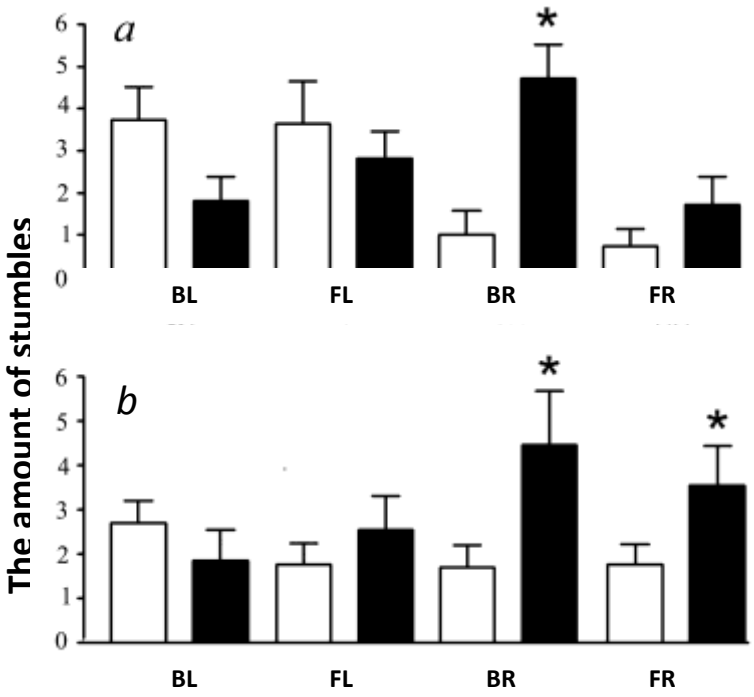

Fig. 2. The amount of stumbles of each limb in the test "tapered track" compared to intact animals (white bar).
BL - the back left, FL - the front left, BR - the back right and FR – the front right. a) the first group of animals, injured when the weight drops from a height of 5 cm; b) the second group of animals, injured when the weight drops from a height of 10 cm. * p <0,01 as compared to the intact animals (white column for the same limbs).

There was no significant difference in propagation time paths 7 days after TBI simulation. Rats from the first group made larger number of stumbles with back right limb which is contralateral to the lesion (Fig. 2a), whereas in the second group with more severe disorders number of stumbles was significantly higher in both the front and back limbs (Fig. 2b) compared to the baseline (before surgery, intact animals). At the same time, the path to the first stumble significantly decreased in both the first and second groups on the right side limbs, which were contralateral to the damage. Way

without stumbles decreased for front limb from 143,6 ± 12,67 cm to 95 ± 21.31 cm and from 113,8 ± 13,53 cm to 68,46 ± 16,64 cm, for the back limb - from 139, 5 ± 13,42 cm to 54.09 ± 14.81 cm and from 124.2 ± 13.17 cm to 70 cm ± 16,48 to the first and second groups, respectively (Fig. 3 a, b). Thus, if the damage is not so significant, this test is more sensitive to detect hidden dysfunction of the back limb only, which is consistent with the literature [13]. In addition, the more pronounced decrease limb motor function in modeling TBI, the topical application of this test to evaluate the function performance, not only back, but also the front legs. The more reduced motor function of limbs in modeling TBI, the more relevant the use of this test to evaluate the function of not only back but the front limbs also.

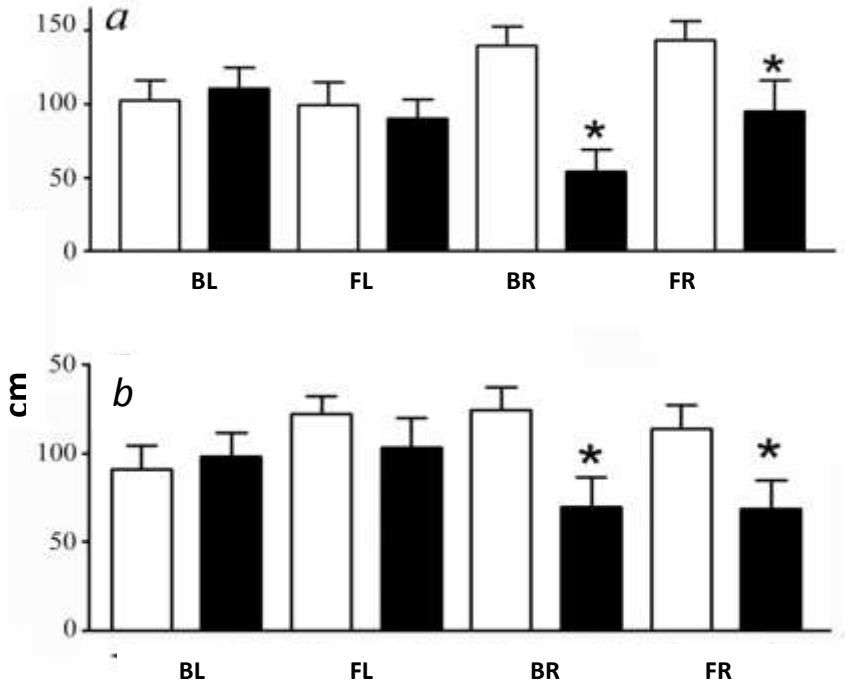

Fig. 3. Changing of the distance to the first stumble from a track on the 7th day after TBI simulation.
a) The first group of animals that received damage from 5 cm; b) the second group of animals received a damage from 10 cm. * p <0,01 as compared to the intact animals (white column for the same limbs).

We revealed a direct correlation of the function of the right limb with the left limb, especially for the transfer of body weight while moving, hold the posture and adequate motor behavior. While the main role in the formation of motor asymmetry belongs to the limbs which are contralateral to the lesion,

increasing attention is paid to processes in the intact hemisphere, especially in the homotopic to each other or associated areas of the cortex which are responsible for the control of undamaged intact limbs, due to which most of the behavioral work is produced in cases of significant motor disorders. Thus motor task is much more complicated for the ipsilateral paw as well as for contralateral to the brain damage limb. Perhaps, changes may occur in the intact hemisphere in order for the animal to use the appropriate limb most effectively, allowing with an expending of more time more efficient use of the affected limb with intact in the complex movements. We have shown that the percentage of rats with significant functional damage not only the right but also left paw increases depending on the severity of damage in the left-side focal injury of the sensorimotor area of the cerebral cortex. This model can be used to test new substances and study the stages of pathogenesis of the focal cortical damage.

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