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LEARNING ABILITY AND SPATIAL MEMORY STATUS IN RATS DURING THE ACUTE PERIOD OF MILD BLAST-INDUCED TRAUMATIC BRAIN INJURY

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The mild blast-induced brain traumatic injury like a battle fatigue, leads to neurological disability, including due to a violation of spatial memory. The presented work is the result of a blast-induced brain traumatic injury study on a self-developed device. For the experiment, 15 white Wistar rats were used and were divided into 3 groups: rats of Experimental group exposed by air shock wave (26–36 кPa); Sham group (anesthesia with halothane and fixation in a horizontal position) and Intact group. The ability to learning (food reinforcement) and the spatial memory state (time to search for food, vertical locomotor activity) were studied in the complex maze during the first 5 days of the posttraumatic period. To assess the emotional state, we counted the number and observed the quality of grooming acts. All researches were conducted in compliance with modern standards for humane attitude of animals. The results of our study clearly indicate a violation of spatial memory, which was manifested in the time of searching lengthening of the feeder and in the increase of vertical locomotor activity in the rats of the experimental group, while the animals clearly remembered that they were looking for food. The analysis of grooming showed qualitative changes in the form of incomplete and prolonged acts. Such changes indicate the development of anxiety in rats with mild blast-induced traumatic brain injury. This, in turn, led to deterioration of the spatial orientation and memory formation. Thus, the consequence of a mild blast-induced traumatic brain injury in the acute period is changes in the cognitive function of the brain in the form of deterioration of spatial memory, which was aggravated by a disturbance of emotionality in the form of anxiety. At the same time, the memory regarding the presence of food was formed adequately.

Keywords: *explosion, central nervous system, cognitive function, memory, maze.*

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Introduction

Blast-induced traumatic brain injury (bTBI) is a major hazard for people in active combat, resulting in long-term neurological disability [1]. High prevalence, loss of working capacity even with light damage, lack of studied pathogenesis of bTBI makes this problem not only medical, but also socio-economic [2].

The known consequences of bTBI are impairment of cognitive functions of the brain, in particular various types of memory, which are based on diffuse axon damage directly due to the blast wave and damage to nervous tissue by secondary factors, in particular free radicals, disruption of homeostasis due to damage to blood-brain barrier, leading to long-term functional imbalance [3; 4]. In previous studies, signs of the oligodendrocytes death were established [5], and also microglial activation in the hippocampus and corpus callosum negatively correlated with spatial learning and memory [6–8]. It is also known about the dysfunction of the limbic system, which is involved in the formation of orientational research activities [9]. This leads to a deterioration of relational memory when a simple task is performed with increased stress [10].

Thus, the negative consequences caused by bTBI directly affect spatial memory, but the pathogenesis of these changes has not been definitely established. Currently, in addition to axonal injury as a result of the action of the blast wave, many different directions of both primary and secondary damage are considered from the side in various ways of experimental reproduction [4]. Therefore, the presented work is the result of a bTBI study on a personally developed device [11], and the complex of obtained data will allow to reveal the yet unexplored links of pathogenesis.

The purpose of the study is learning process and the state of spatial memory in rats with blast-induced brain injury.

Material and methods

The study was carry out on 15 white Wistar rats aged 7–8 months, weighing 220–270 g in the laboratory of the Department of Pathological Anatomy, Forensic Medicine and Pathological Physiology of the Dnipro State Medical University (DSMU). The animals were kept under standard conditions and on a standard diet in the vivarium of the DSMU. All studies were conducted according to a previously developed and approved plan, and in compliance with the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1986), The procedures followed the recommendations of the NIH Guide for the Care and Use of Laboratory Animals and the ARRIVE guidelines, the Resolution of the First National Congress on Bioethics (Kyiv, 2001), the Law of Ukraine "On the Protection of Animals from ill-treatment" No.3446-IV dated February 21, 2003.

All animals were divided into 3 groups: Experimental (n=5), rats exposed to air shock wave; a Sham group (n=5), rats that were only anesthetized with halothane and fixed in a horizontal position, and an Intact group (n=5) that were not affected by any of the above factors. Sham and Intact groups were created to estimate the action of additional pathogenetic factors (anesthesia, fixation).

Animals of the Experimental group were subjected to inhalation anesthesia with halothane (Halothan Hoechst AG, Germany), were softly fixed in a horizontal position on the abdomen with the head to the muzzle end at a distance of 5 cm and a one-time simulation of bTBI by generating an air shock wave with an excess pressure 26–36 кPa [11].

The main requirement during the research was the minimization of pain sensations in Experimental animals. The bTBI was reproduced using inhalation anesthesia with halothane, as a result of which the animals did not feel pain and quickly recovered.

The study of the ability to learn and the state of spatial memory was carried out in the complex maze, which is made of Plexiglas and consists 9 compartments: the starting zone, two T-shaped branches on the right and left sides, connected by a common corridor, and 2 side compartments right and left for exit from general part to the maze starting zone. The entrance from the starting zone to the branches is carried out through the passage, which is located in the center of the common corridor. T-shaped branches to the right and left end with feeders, the same one of which always contained feed. Production of complex closed feeding behavior in rats was carried out by the method of giving hungry animals a free choice of movements in a maze. For this, the animals had to complete the following steps:

1. enter to the maze from the starting area;
2. explore the maze;
3. find one of the feeders with food and get reinforcements.

To evaluate the learning ability and memory development in the complex maze, throughout 5 days after bTBI simulation, the time during which each animal found the feeder, as well as its own desire to find food, was recorded. An indicator of the activity of researching a new territory – the number of vertical racks – was also analyzed. To assess the emotional state, we counted the number and observed the quality of grooming acts.

The numerical results were performed using STATISTICA 6.1 software (serial No.AGAR909E415822FA, StatSoftInc.). Means and standard deviations values were calculated. Intergroup differences were estimated using the Student`s t-test and considered statistically significant with $p \le 0.05$.

Results and discussion

To establish the presence and modifications of learning ability and spatial memory impairments in rats with mild bTBI in the acute period when using self-developed device, we used the complex maze, the technique of which is based on orienting-exploratory motor activity reinforced by the food reflex. Positive food reinforcement in the complex maze stimulates hungry rats to remember the location of palatable food, which is usually seen in the acceleration of goal-directed search for the feeder.

The differences between the rats of the Experimental and Sham groups indicate the absence of an analgesic effect of halothane against the background of injury and an increase in the functional and adaptive resources of the brain in rats with bTBI. Therefore, a detailed comparison of the results was performed between the Experimental and Intact groups.

Clinical observations indicate the presence of people affected by the explosion with various types of memory impairment, lack of learning ability, changes in emotionality, which are directly related to the specific mechanism of brain damage as a result of the action of the blast wave [12–14]. The our study results clearly indicate an impairment of spatial memory in particular. A comparison of the time of searching for a feeder between rats of the Experimental and Intact groups (*Tab.*) showed a significant ($p \le 0.05$) prolongation in the rats of the Experimental group on the 1st day by 67%, on the 2nd day by 46%, on the 3rd day by 57%, on the 4th day by 83% and on the 5th by 81%. We also noticed that rats with bTBI spent the least amount of time searching for a feeder on the 3rd day of the post-traumatic period.

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Table. Indicators of spatial memory and behavior in complex maze

Title of study	Days	Groups		
		Experimental (M±m)	Sham (M±m)	Intact (M±m)
Time to find the feeder (sec)	1	361.2±6*	307.2±5**	120±3
	2	227.7±6*	479.2±4**	122.2±4
	3	206±6*	45.7±4	88.8±4
	4	240.8±11*	44±3	41±2
	5	157.8±4*	119.8±4	30.2±2
Vertical locomotor activity (number)	1	32.3±3*	17.5±1	12.8±1
	2	18.3±3*	34.8±3**	9.5±2
	3	11.5±1*	2.8±0.6	8±0.5
	4	14±1*	3±0.5	3±0.5
	5	14±1*	3.8±0.7	1.5±0.2
Number of grooming	1	2.7±0.5*	0.7±0.3	0.2±0.1
	2	1.2±0.4	2.7±0.4**	1±0.3
	3	0.8±0.4	1±0.1	0.7±0.3
	4	1.2±0.3	1.2±0.1	1±0.1
	5	0.7±0.2	0.7±0.3	1±0.1
Duration of grooming (sec)	1	34.3±4*	6.7±3**	0.8±0.1
	2	8.5±2	25.8±2**	11.7±3
	3	15.5±11*	1.8±0.1	1.7±0.8
	4	23±6*	1±0.1	1±0.1
	5	5.5±1*	1.7±0.8	1±0.1

Note: * – $p \le 0.05$ statistically significant between Experimental and Intact groups;
** – $p \le 0.05$ statistically significant between Control and Intact groups.

This coincides with the experimenttal data of modern literary sources using other methods of studying spatial memory in rats with bTBI, where a significant impairment of rats spatial memory was observed in the form of an extension of the seeking shelter time [15].

Analysis of vertical locomotor activity (VLA) showed high motor mobility in exploring a new territory in rats with bTBI during the 5 days of the acute posttraumatic period in comparison with animals of the Intact group (*Tab.*).

Thus, on the 1st posttraumatic day VLA was significantly ($p \le 0.05$) higher by 40%, on the 2nd day by 48%, on the 3rd by 30%, on the 4th by 79% and in the 5th – by 89%. This increase in VLA suggests that spatial orientation in the maze was impaired in rats with bTBI, as they constantly explored the maze by standing on their hind paws. Such behavior indicates that the memory of food reinforcement in rats was formed adequately, because each animal was looking for food. There was also a general trend toward decreased locomotor activity in bTBI rats over the course of the study. Minimal VLA in bTBI rats was observed on the 3rd day and then increased.

To determine the influence of the emotional component on the learning process and spatial memory, the amount, duration and quality of grooming were additionally analyzed.

During the analysis of the quality of grooming, the acts of comfortable grooming were taken into account, i.e. washing the animal's muzzle, nose, behind the ears and cleaning the whole body, and uncomfortable grooming, when the animal washed inconsistently and intermittently or cleaned only one part of the body. As a result of observations, it was established that grooming was uncomfortable in rats with bTBI, the animals constantly looked around during this act, were extremely worried, which indicates an anxiety-like state. Given the previously established negative effect of emotional state on spatial memory in rats with different emotional profiles or in diseases [16] we consider that in rats with bTBI, who experienced anxiety according to the grooming index, emotional disturbance affects the process of spatial orientation and the formation of spatial memory. And the increase in the impairment of cognitive functions indicates the exhaustion of compensatory mechanisms and the deterioration of the course of bTBI from the 4th day of the posttraumatic period.

Conclusions

Thereby, the consequence of mild bTBI in the acute period is changes in the brain cognitive function in the form of a deterioration of spatial memory, which was aggravated by a disturbance of emotionality in the form of anxiety. At the same time, the memory regarding the presence of food was formed adequately.

Prospects for further research

It is perspective to study other types of memory and compare the results with pathomorphological, biochemical and spectrographic data, which will contribute to the establishment of new links of pathogenesis and the development of new methods of bTBI treatment and prevention of complications.

The authors declare **no conflict of interest.**

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ЗДАТНІСТЬ ДО НАВЧАННЯ І СТАН ПРОСТОРОВОЇ ПАМ'ЯТІ У ЩУРІВ В ГОСТРОМУ ПЕРІОДІ ЛЕГКОЇ ВИБУХО-ІНДУКОВАНОЇ ТРАВМИ ГОЛОВНОГО МОЗКУ

Вибухо-індукована травма головного мозку легкого ступеня, як травма воєнного часу, призводить до неврологічної інвалідності, в тому числі за рахунок порушення просторової пам'яті. Представлена робота є результатом дослідження вибухо-індукованої

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травми головного мозку на особисто розробленому пристрої. Для експерименту використали 15 білих щурів лінії Wistar, яких поділили на 3 групи: експериментальна (щури, що піддавалися дії повітряно-ударної хвилі тиском 26-36 кПа), контрольна група (наркотизація галотаном та фіксація у горизонтальному положенні) та інтактна група. Здатність до навчання (харчове підкріплення) і стан просторової пам'яті (час пошуку їжі, вертикальна рухова активність) досліджували в Ж-подібному лабіринті протягом 5 перших діб посттравматичного періоду. Для оцінки емоційного стану рахували кількість та спостерігали за якістю актів ґрумінгу. Усі дослідження проведені із дотриманням сучасних нормативів щодо гуманного ставлення до тварин. Результати нашого дослідження чітко вказують на порушення саме просторової пам'яті, що проявлялось у подовженні часу пошуку годівниці, і у підвищенні вертикальної рухової активності у щурів експериментальної групи. При цьому тварини чітко пам'ятали, що шукають саме їжу. Аналіз ґрумінгу показав якісні зміни у вигляді неповних і тривалих актів. Такі зміни вказують на розвиток тривожності у щурів з легкою вибухо-індукованою травмою головного мозку. Це, в свою чергу призводило до погіршення процесу просторової орієнтації і формування пам'яті. Таким чином, наслідком легкої вибухо-індукованої травми головного мозку у гострому періоді є зміни когнітивної функції головного мозку у вигляді погіршення просторової пам'яті, що посилювалось порушенням емоційності у вигляді тривожності. При цьому пам'ять щодо наявності їжі формувалась адекватно.

Ключові слова: *вибухо-індукована травма, головний мозок, щур, когнітивна функція, пам'ять, лабіринт.*

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