**Influence of trace elements changes in the cerebellum on the rat’s behavior in elevated plus maze in the early period of mild blast-induced traumatic brain injury**

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**Keywords:** traumatic brain injury, trace elements, elevated plus maze, cerebellum.

**Materials and methods.** The study was carried out on 126 sexually mature male Wistar and were divided into 3 groups: Experimental – exposed to a shock wave 26.4 ± 3.6 kPa (n = 42), Sham (n = 42), the animals of which were subjected to inhalation anesthesia with halothane and fixation in a horizontal position; and Intact (n = 42). Behavior was studied in elevated plus maze. The duration of presence in the open and closed arms, the number of stands, the duration of grooming was recorded in all groups of rats for 3 minutes. After, the animals were euthanized with halothane, followed by removal of the brain. The cerebellum was completely separated for spectral analysis using energy dispersive X-ray fluorescence analysis (EDRFA) on the analyzer EXPERT 3 XL.

**Results.** Results showed significant changes of cognitive activity in experimental group which are indicate functional disorders of the cerebellum in the form of maladaptation in space with subsequent inhibition of motor centers. Cu/Fe ratio was decreased in the 14th and 21st days and increased in the 28th. Cu/Zn ratio was decreased on the 14th day. Zn/Fe ratio was higher on the 14th and 28th days. The existence of correlations between changes in trace elements and behavioral disorders in experimental rats was established.

**Conclusions.** In the early period of blast-induced traumatic brain injury, cerebellar dysfunction in the form of spatial maladaptation with subsequent depression of motor centers was observed in the experimental rats. Correlation analysis showed the presence of different strengths and directions of relationships between the ratios of Cu/Fe, Cu/Zn and Zn/Fe in the cerebellum and behavioral indicators in the elevated plus maze (duration of stay in open and closed arms, grooming and vertical motor activity) of experimental rats.

The aim of the current study was to determine whether there are changes in brain trace elements of rats with blast-induced trauma and if these changes affect behavior in the elevated plus maze.

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burns, shrapnel injuries). But it is bTBI leads to behavioral and cognitive impairments both in an acute and an early, as well as in remote posttraumatic periods. And the pathogenesis of these changes is currently being actively researched and discussed by both clinicians and scientists around the world [3,4].

The issues of primary alteration of the brain by an explosive wave and mechanisms of secondary alteration are contemplated separately. Mechanisms of damage to certain structures of the brain, particularly the cerebellum, which participates not only in the implementation of orientation and motor activity, but also in behavioral-cognitive processes, remain unexplored. Also, the participation of trace elements (Fe, Cu, Zn) in secondary brain damage, which normally involved in the processes of myelination of nerve fibers, transmission of nerve impulses, synthesis of neurotransmitters, processes of energy exchange and are part of the antioxidant system, has not been established [5,6,7]. Therefore, the aim of the current study was to determine whether there are changes in trace elements in the brain of rats with blast-induced trauma and if these changes affect behavior in the elevated plus maze.

The obtained results will deepen the knowledge about the mechanisms of the development and course of bTBI and will contribute to the development of pathogenetically based modern methods of diagnosis and methods of treatment of this injuries type.

Aim

The aim of the current study was to determine whether there are changes in brain trace elements of rats with blast-induced trauma and if these changes affect behavior in the elevated plus maze.

Materials and methods

The study was carried out on 126 healthy, sexually mature male Wistar rats, body mass 220–270 g, aged 6–7 months in the laboratory of the Department of Pathological Anatomy, Forensic Medicine, and Pathological Physiology of the Dnipropetrovsk Regional Council, calculations were made in automated mode. The results are presented in the form of peak spectra and tables for each sample.

Regardless of the object shape, without using recalibration and special sample preparation, direct express analysis with a wide range of the bioelements spectrum measurement (from Na to U) allows you to quickly and highly accurately determine the elemental composition of any sample, in particular in brain tissues.

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the corresponding mass concentrations. After obtaining the quantitative mass fractions of biometals, the ratios of Cu/Fe, Cu/Zn, Zn/Fe were calculated based on the mass fractions of each element in percent and the data between the three groups were compared.

The numerical results were performed using Statistica 6.1 software (StatSoft Inc., serial No. AGAR909E-415822FA). Means and SD values were calculated. Intergroup differences were estimated using the Student’s t-test and considered statistically significant with \( p \leq 0.01 \) or \( p \leq 0.05 \). To establish correlations between the study parameters Spearman’s correlation coefficient was used. Strength of correlation was calculated (r), and \( p \) value \( p \leq 0.01 \) was considered statistically significant.

**Results**

With the help of an elevated plus maze, we investigated changes in motor activity based on indicators of the length of stay in the closed and open arms of the maze, vertical motor activity and grooming, which is a complex act of purposeful movements that provides natural self-care. It is known that the cerebellum participates in the implementation of these processes [11].

The analysis conducted between three groups to separate the effect of halothane on the behavior of rats in the elevated plus maze showed that at this observation period no significant differences were established between the Sham and Intact groups, so the main calculations were carried out between the indicators of the Exp and Intact groups.

The duration of presence in the open arms (Fig. 1) in the Exp rats was 64 % shorter (\( p \leq 0.01 \)) on the 14th day, and 56 % longer (\( p \leq 0.01 \)) on the 21st day.

The time spent in the closed arms (Fig. 2) significantly (\( p \leq 0.01 \)) decreased by 6 % in the Exp rats in the 21st day.

The analysis of vertical motor activity (Fig. 3) showed an increase in Exp rats on the 14th day by 63 % (\( p \leq 0.01 \)), on the 21st day by 67 % (\( p \leq 0.01 \)) and on the 28th day by 72 % (\( p \leq 0.01 \)).

Grooming time (Fig. 4) in the Exp group decreased by 45 % on the 14th day (\( p \leq 0.01 \)). On the 21st day, it was significantly (\( p \leq 0.01 \)) higher by 30 %.
During the analysis of indicators of the biometals ratio in the cerebellum, significant (p ≤ 0.01) changes in Cu/Fe (Fig. 5) were found in the Exp rats: decreased in the 14th day by 8%, in the 21st day by 8% and an increase in the 28th day 32%.

The analysis of the CuZn ratio (Fig. 6) also showed significant (p ≤ 0.01, p ≤ 0.05) changes: by 44% decreased on the 14th day, by 8% increased on the 21st day, and by 20% increased on the 28th day.

And the Zn/Fe ratio (Fig. 7) was significantly (p ≤ 0.01) higher on the 14th day by 39%, on the 28th day by 17%.

Using correlation analysis, a functional relationship (r = 1, p ≤ 0.01) was established between CuZn in the cerebellum and the time spent in closed arms for 21st days. A strong negative relationship was established on the 21st day between Cu/Fe and the time of stay in closed arms (r = -0.8, p ≤ 0.01), as well as on the 28th day between Cu/Zn and the time of presence in open arms (r = -0.8, p ≤ 0.01). A medium positive relationship was established on the 28th day between Zn/Fe and duration in open arms (r = 0.7, p ≤ 0.01), between Cu/Zn and grooming (r = 0.6, p ≤ 0.01). A negative average relationship was determined on the 28th day between Zn/Fe and the duration of grooming (r = -0.7, p ≤ 0.01), as well as with vertical motor activity (r = -0.6, p ≤ 0.01).

**Discussion**

The early period of bTBI is accompanied by significant functional disorders of the brain, the manifestations of which are anxiety, disorientation in space, etc., and the use of additional imaging methods (MRI, CT) does not give a complete picture of the lesion [12]. Violations of the blood-brain barrier as a result of the primary influence of the blast wave established by previous studies [13] and the presence of oxidative stress, which is one of the secondary neurons damage factors [14] during the simulation of bTBI, provide the basis for an in-depth study of new links in the pathogenesis of secondary damage of certain brain structures at different times in bTBI posttraumatic period.

In our opinion, biometals – Fe, Cu, Zn, which are involved in the antioxidant system, the formation and transmission of nerve impulses and various metabolic processes that are important for the normal functioning of the brain, are subject to thorough research [15,16,17].

Significant changes of cognitive activity in experimental group indicate functional disorders of the cerebellum in the form of maladaptation in space with subsequent inhibition of motor centers. This is evidenced by a reduction in the time spent in open arms by 14th day – the presence of a fear to being in open space, and an extension by 21st day, while the period of stay in closed arms was reduced, which indicates suppression of the hiding reflex. During the entire period of the study, an increase in vertical motor activity was observed, which indicates the search for a way out of the maze against the background of being in an unfamiliar place fear.

Similar changes were observed in the duration of grooming – a reduction in time by 14th day and an extension by 21st day. A change in the quality of grooming was also observed. In the Exp animals, this act was inconsistent, intermittent, with signs of anxiety (peri-
Conclusions

1. In the early period of blast-induced brain injury, cerebellar dysfunction was observed in the rats of the experimental group in the form of maladaptation in space with subsequent inhibition of motor centers. This is evidenced by a behavior changes in the elevated plus maze namely changes of time spent in open and close arms, also in grooming duration and in vertical motor activity.

2. The Cu/Fe and Cu/Zn ratios were reduced on 14th and 21st days and raised on 28th day, and Zn/Fe ratio was raised on 14th and 28th days in the cerebellum of experimental compare with sham and intact rats.

3. The correlation analysis showed the presence of different strength and direction connections between Cu/Fe, Cu/Zn and Zn/Fe ratios in cerebellum and behavioral indicators in elevated plus maze (duration in open and closed arms, grooming and vertical motor activity) of experimental rats.

Prospects for further scientific research to establish the role of trace elements in the brain with blast-induced traumatic injury with impairment of various types of memory.

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References


