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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

Yu. V. Kozlova*

Dnipro State Medical University, Ukraine

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

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***E-mail:** kozlova_yuv@ukr.net

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 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 study 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.

Ключові слова: травма головного мозку, мікроелементи, піднесений хрестоподібний лабіринт, мозочок.

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Вплив змін мікроелементів у мозочку на поведінку щурів у піднесеному хрестоподібному лабіринті в ранньому періоді легкої вибухо-індукованої травми головного мозку

Ю. В. Козлова

Мета роботи – визначити, чи відбуваються зміни мікроелементів у мозку щурів із вибуховою травмою і чи мають взаємозв'язок ці зміни з порушенням поведінки у піднесеному хрестоподібному лабіринті.

Матеріали і методи. Дослідження здійснили на 126 статевозрілих білих щурах-самцях лінії Вістар. Тварин рандомно поділили на 3 групи: експериментальну, де щури зазнавали впливу вибухової хвилі з надлишковим тиском 26,4 ± 3,6 кПа (n = 42); контрольну (n = 42), де після інгаляційного наркозу галотаном тварин фіксували в горизонтальному положенні; інтактну (n = 42). Поведінку досліджували в піднесеному хрестоподібному лабіринті. У всіх групах протягом 3 хвилин реєстрували тривалість перебування у відкритих і закритих рукавах, кількість стійок, тривалість ґрумінгу. Після цього тварин евтаназували галотаном, видаляли головний мозок. Мозочок повністю відокремлювали для спектрального аналізу за допомогою енергодисперсійного рентгенівського флуоресцентного аналізу (EDRFA) на аналізаторі EXPERT 3 XL.

Результати. Виявили значні зміни когнітивної активності в експериментальній групі, що свідчать про функціональні порушення мозочка, зокрема дезадаптацію у просторі з пригніченням надалі рухових центрів. Співвідношення Cu/Fe знижене на 14 і 21 добу, підвищене на 28 добу. Співвідношення Cu/Zn знижене на 14 добу. Співвідношення Zn/Fe вище на 14 і 28 добу. Встановили кореляційні зв'язки між змінами мікроелементів і порушенням поведінки експериментальних щурів.

Висновки. У ранньому періоді вибухової травми головного мозку в щурів експериментальної групи виявили дисфункцію мозочка – дезадаптацію в просторі з наступним пригніченням рухових центрів. Кореляційний аналіз показав наявність різних за силою і напрямом зв'язків між співвідношенням Cu/Fe, Cu/Zn, Zn/Fe у мозочку та поведінковими показниками у піднесеному хрестоподібному лабіринті (тривалість перебування у відкритих та закритих рукавах, ґрумінг і вертикальна рухова активність) експериментальних щурів.

Applying various explosives in military conflicts around the world and currently in Ukraine leads to an increase in the number of craniocerebral injuries as a result of the blast wave effect – blast-induced traumatic brain injury (bTBI) [1]. This type of brain injury is characterized by diffuse damage, even with a slight impact of the blast wave, which, as the main pathogenic factor of the explosion, leads to damage due to excess pressure and intense energy [2]. However, bTBI itself is often not separated, focusing on injuries caused by additional factors of the blast (thermal 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 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 Dnipro State Medical University. The rats were kept in rectangular plastic cages (floor S = 1500 cm^2) with a wire mesh on top. In each cage were 3 rats, which allowed to adequately divide the rats into groups and did not cause emotional reactions in the animals that remained in the cage after the euthanasia of others. Rats were marked with a permanent marker. Wood shavings with a thickness of 2–3 cm were used as bedding. In the room with the rats, the air temperature was maintained in the range of 20–25 °C and the humidity was in the range of 50–60 %. The daily light: dark cycle was 12:12 hours. Food and water were provided ad libidum. All rats were fed with commercial pellets, corn, and wheat, identical in composition and quantity. Tap water in 500 ml drinking cups was used for drinking.

All studies were conducted in accordance with modern international requirements and standards of humane treatment of animals (Council of Europe Convention of 18.03.1986 (Strasbourg); Helsinki Declaration of 1975, revised and supplemented in 2000, Law of Ukraine of February 21, 2006 No. 3447-IV), as evidenced by the extract from the minutes of the Biomedical Ethics Commission of Dnipro State Medical University meeting No. 3 of November 2, 2021.

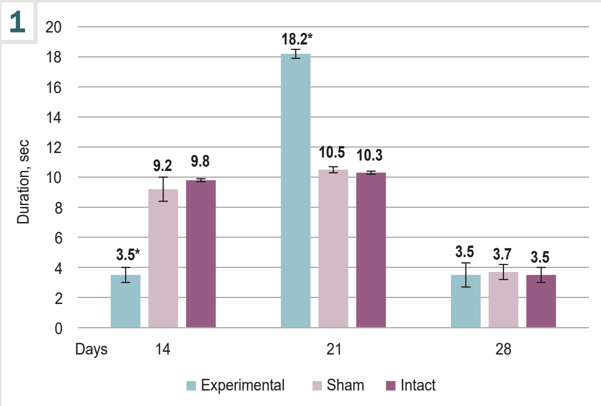
The selected rats were randomly divided into 3 groups: Group I – Experimental (Exp) with bTBI (n = 42); Group II – Sham (n = 42), the animals of which were subjected only to inhalation anesthesia with halothane and fixation in a horizontal position; and Group III – Intact (n = 42). Sham and Intact groups were created to limit the effect of additional pathogenic factors on the behavior and composition of biometals (anesthesia, fixation).

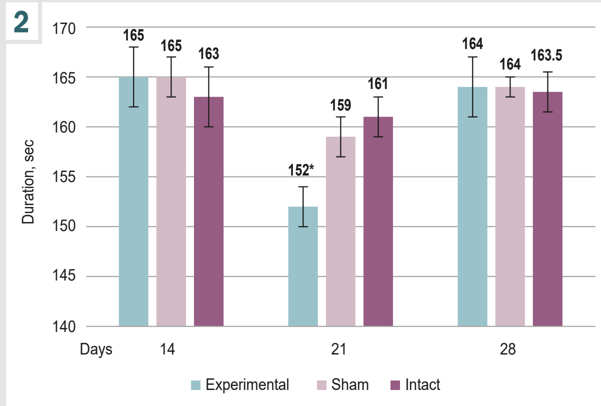
The Exp animals were anesthetized with halothane (Halothan Hoechst AG, Germany), softly fixed in a horizontal position on the abdomen with the rat muzzle at a distance of 5 cm from the opening of the device. The blast wave was created by instant (using an electromagnetic valve) opening of a chamber filled with compressed air (up to 15 atm, i. e., ≈1520 kPa). Under the conditions of our experiments, this generated a baroacoustic wave with an excess pressure of 26–36 kPa, on average [8].

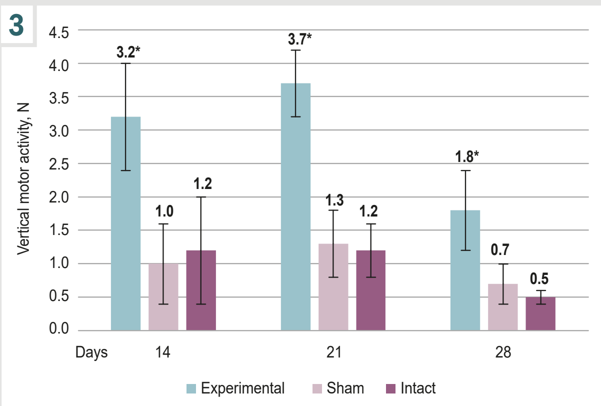
A study of behavior in the elevated plus maze was conducted on the 14st, 21st, and 28st days after bTBI simulation at the same time (11:00 a. m.). Animals were brought to the laboratory 1 hour before the start of the study. After that, behavioral profiles were determined in an elevated plus maze, which is represented by an arena with crossed arms – 2 open arms, 2 closed arms, length 50 cm, width 14 cm, the height of the closed arms walls were 30 cm, which creates sufficient darkness. At the intersection of the arms there was an open area, which is the starting point for the test. The arena is raised 55 cm above the floor level [9]. The duration (in seconds) of presence in the open and closed arms, the number of stands (vertical motor activity), the duration of grooming (in seconds) were recorded in all groups of rats for 3 minutes.

Immediately after the behavior study on the 14st, 21st, and 28st days of the posttraumatic period, 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. This analysis refers to physical non-destructive express method of quantitative determination of the elemental composition of biological material [10]. 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.

Fragments (cerebellum) of the native unfixed brain were introduced into the measuring chamber with exposure to radiation for 10 minutes (600 s). After that, with the help of software (manufacturer: LLC “Scientific and Production Enterprise Institute of Analytical Control Methods (“INAM” LLC), Kyiv, Ukraine) on the basis of the laboratory of the Communal Institution “Dnipropetrovsk Regional Bureau of Forensic Medical Examination” 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 with







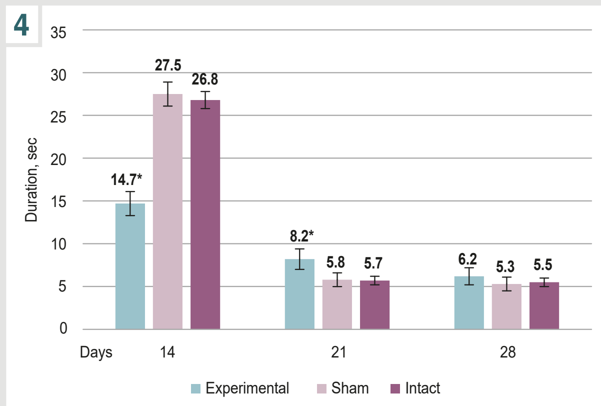


Fig. 1. Dynamics of the duration in open arms shown by rats of different groups in the elevated plus maze. **Vertical scale:** time in seconds. Days of observation (14, 21 and 28) are shown above the diagrams. Means and s. e. m. values are shown. *: cases of significant p ≤ 0.01 differences from the Experimental and Intact groups.

Fig. 2. Dynamics of the duration in closed arms shown by rats of different groups in the elevated plus maze. **Vertical scale:** time in seconds. Days of observation (14, 21 and 28) are shown above the diagrams. Means and s. e. m. values are shown. *: cases of significant p ≤ 0.01 differences from the Experimental and Intact groups.

Fig. 3. Dynamics of the intensity of vertical motor activity shown by rats of different groups. **Vertical scale:** number of full vertical stands on the hindlimbs within the period of observation. Days of observation (14, 21 and 28) are shown above the diagrams. Means and s. e. m. values are shown. *: cases of significant p ≤ 0.01 differences from the Experimental and Intact groups.

Fig. 4. Dynamics of duration of grooming episodes within the observation period. **Vertical scale:** time in seconds. Days of observation (14, 21 and 28) are shown above the diagrams. Means and s. e. m. values are shown. *: cases of significant p ≤ 0.01 differences from the Experimental and Intact groups.

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 ≤ 0.01 or p ≤ 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 ≤ 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].

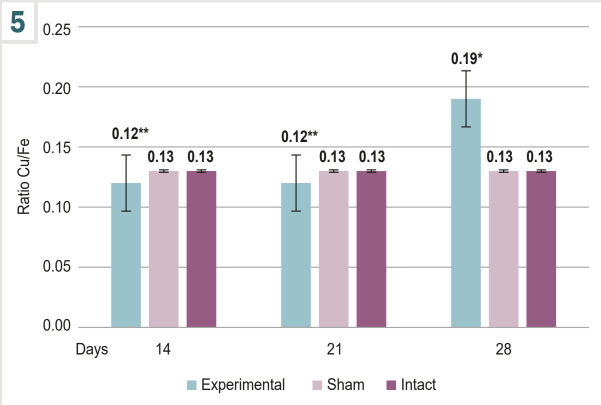
The analysis conducted between three groups to separate the effect of halothane on the behavior of rats in the elevated plus maze showed that at this observation period no significant differences were established between the Sham and Intact groups, so the main calculations were carried out between the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

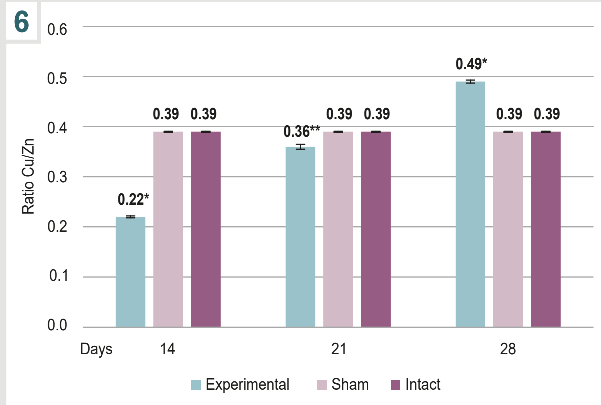
The duration of presence in the open arms *(Fig. 1)* in the Exp rats was 64 % shorter (p ≤ 0.01) on the 14^{th} day, and 56 % longer (p ≤ 0.01) on the 21^{st} day.

The time spent in the closed arms *(Fig. 2)* significantly (p ≤ 0.01) decreased by 6 % in the Exp rats in the 21^{st} day.

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

Grooming time *(Fig. 4)* in the Exp group decreased by 45 % on the 14^{th} day (p ≤ 0.01). On the 21^{st} day, it was significantly (p ≤ 0.01) higher by 30 %.





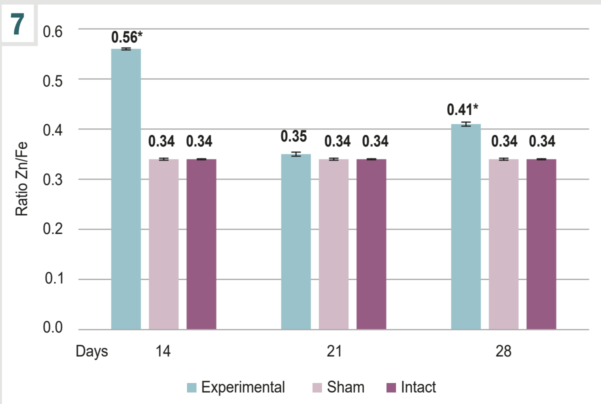


Fig. 5. Dynamics of Cu/Fe ratio in cerebellum shown by rats of different groups. **Vertical scale:** ratio indexes. Means and s. e. m. values are shown.
***:** cases of significant $p \leq 0.01$ differences from the Experimental and Intact groups;
****:** cases of significant $p \leq 0.05$ differences from the Experimental and Intact groups.

Fig. 6. Dynamics of Cu/Zn ratio in cerebellum shown by rats of different groups. **Vertical scale:** ratio indexes. Means and s. e. m. values are shown.
***:** cases of significant $p \leq 0.01$ differences from the Experimental and Intact groups;
****:** cases of significant $p \leq 0.05$ differences from the Experimental and Intact groups.

Fig. 7. Dynamics of Zn/Fe ratio in cerebellum shown by rats of different groups. **Vertical scale:** ratio indexes. Means and s. e. m. values are shown.
***:** cases of significant $p \leq 0.01$ differences from the Experimental and Intact groups;
****:** cases of significant $p \leq 0.05$ differences from the Experimental and Intact groups.

During the analysis of indicators of the biometals ratio in the cerebellum, significant ($p \leq 0.01$) 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 Cu/Zn ratio *(Fig. 6)* also showed significant ($p \leq 0.01$, $p \leq 0.05$) 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 ($p \leq 0.01$) higher on the 14th day by 39 %, on the 28th day by 17 %.

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

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 (periodic, quick, short-term glances in different directions, performed by rats without noise and visual stimuli) [18]. We consider that this indicates a violation of regulation on the cerebellum side, because its function in the implementation of the act of grooming has been confirmed by scientists [19].

Numerous studies of the biometals functions in the metabolic processes of the brain indicate the extremely important role of Fe, Cu and Zn [5,6,7]. Currently, it is being investigated how violations of certain biometals affect the brain and what consequences these changes have [20,21]. It is known that when Fe, Cu and Zn are accumulated separately, they have the same neurotoxic effect, and their deficiency leads to a violation of energy exchange and nerve impulse transmission processes [22,23]. Meanwhile, these biometals individually regulate each other's metabolism, and when one is imbalanced, the metabolism of another biometals is disturbed [24,25]. We found that increasing the Zn/Fe and Cu/Fe ratios on 28ts day significantly increased the risk of neurodegeneration in the remote period of bTBI [26,27].

Also, an increase in Fe concentration is the leading mechanism of disruption of Cu and Zn homeostasis. The role of the blood-brain barrier in the accumulation of Fe is integral, because it is known that this structure strictly regulates the entry of any nutrients and active substances into the brain [27]. In addition, diapedesis hemorrhages due to the increased permeability of blood-brain barrier vessels leads to the release of iron from erythrocytes [13].

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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Information about the author:

Kozlova Yu. V., MD, PhD, Associate Professor of the Department of Pathological Anatomy, Forensic Medicine and Pathological Physiology, Dnipro State Medical University, Ukraine.
ORCID ID: 0000-0002-1364-1910

Відомості про автора:

Козлова Ю. В., канд. мед. наук, доцент каф. патологічної анатомії, судової медицини та патологічної фізіології, Дніпровський державний медичний університет, Україна.

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