Cent. Eur. J. Med. • 9(5) • 2014 • 714-721
DOI: 10.2478/s11536-013-0338-3

Central European Journal of **Medicine**

Analysis of ultrastructural synaptic apparatus in parietal neurocortical and midbrain neurons in animals with experimental mild craniocerebral injury prior to and following magneto-laser therapy

Research Article

Olena Zubkova[1], Vira Semenova[1], Ivan Samosiuk[2], Walery Zukow*[3], Viktoria Vaslovich[1], Natalia Samosiuk[2]

1 State Enterprise Institute of Neurosurgery named acad. AP Romodanov Academy Medical Sciences of Ukraine, Kyiv, Ukraine

2 National Medical Academy of Postgraduate Education named PL Shupyk, Kyiv, Ukraine

3 Kazimierz Wielki University, Bydgoszcz, Poland

Received 28 June 2012; Accepted 29 May 2013

Abstract: Background. Magnetic laser therapy is widely used in general medicine in a complex treatment of many diseases. This is due to its unique and versatile biological properties; it which activates intracellular metabolic processes and expedites the repair of damaged tissues. Magnetic laser therapy is an important area of modern theoretical and clinical physiotherapy; It is used in the treatment of dermatological, general surgical, endocrinological, and dental diseases; pain syndromes; and other conditions. Objectives. Our objective is to assess the therapeutic efficacy of magnetic laser therapy in experimental mild traumatic brain injury in rabbits. Material and Methods. We evaluated the therapeutic efficacy of magnetic laser therapy in experimental mild traumatic brain injury in 20 rabbits based on an analysis of ultrastructure of synaptic apparatus of neurons in the parietal and midbrain regions. Magnetic transcranial laser influence exercised by our developed method. Results. The course of magnetic laser therapy led to statistically significant changes in quantitative characteristics of the ultrastructural components in the synaptic apparatus of neurons that express the activation of compensatory processes and restoration of structural and functional integrity of the synaptic apparatus of neurons in experimental mild traumatic brain injury.

Keywords: *Experimental mild craniocerebral injury • Magnetic laser therapy • Synaptic apparatus of neurons • Morphometry*

1. Introduction

Magnetic laser therapy is widely used in general medicine in a complex treatment of many diseases. Its unique and versatile biological properties activate intracellular metabolic processes and expedite the repair of damaged tissues [2,4]. Magnetic laser therapy is an important tool of modern theoretical and clinical physiotherapy: It is used in the treatment dermatological, endocrinological, and dental diseases; in general surgery; to treat pain syndromes, and other conditions. [8,15,19,20].

However, in clinical neurosurgery, particularly in the treatment of mild traumatic brain injury in the acute stage, this method is not yet sufficient verified [11,14,21], which may result from a lack of substantional safety features

* E-mail: w.zukow@wp.pl

and influence of biological factors on the structure of the central nervous system (CNS). In particular, the known reaction of synaptic apparatus of neurons, changes which reflect the nature of adaptive plastic rearrangements play a crucial role in the effectiveness between neuronal transmission of nerve impulses in the brain.

This requires the purposeful study of ultrastructure of synaptic apparatus of neurons in the cortex and midbrain of animals with experimental mild traumatic brain injury after magneto laser impact. The relevance and practical importance of these issues proved that in the pathogenesis of brain injury, regardless of its severity, the most important change is the structural and functional substrate with synaptic apparatus of neurons, leading to disruption of intercellular regulatory relationships [5].

In this study, we present the results of morphometric analysis of ultrastructure of synaptic apparatus of neurons in the parietal cortex area of the brain and midbrain of experimental animals with experimental mild traumatic brain injury after a course of magnetic laser therapy.

2. Materials and Methods

Mild head injury in 20 experimental rabbits sexually mature male-type chinchilla weighing 3.5–4.0 kg was produced using a spring drummer dosed by applying effort to the left parietal area of the brain by the method of A. Kopiov (1984). In experiments performed in the laboratory of the State Institute, the A. P. Romodanov Institute of Neurosurgery pf the National Academy of Medical Sciences of Ukraine, shake-up of the brain, was produced using a special spring drummer by the graduated nonfixed non-draging attack on the head of livestock (by O. V. Kopiev, 1988). We have perfected the technique of experimental light cranio-cerebral trauma for the study of changes in the nervous tissue as the injured and intact areas of the brain. In dosed animals, this blow was applied to the left fronto-parientals area. In 90% of the experimental animals, we observed clinical picture typical of brain shaking – the animal fell on the side with its head thrown and straightened; maximum allotted limbs were in this position for 30 to 90 seconds. Then, after a few uncoordinated movements of the limbs and head, the rabbits rose. Further, for 1 to 3 minutes, there was weakness, their movements were poorly coordinated, rocking was noted, and then the animals returned to normal. No focal neurological symptoms were observed, macro-vand microscopic features of the face of the brain were absent [5].

The experimental animals (n = 20) divided into 4 groups:

- Group 1 (n = 5) – control, healthy individuals;
- Group 2 (n = 5) – rabbits with experimental mild traumatic brain injury;
- Group 3 (n = 5) – rabbits with experimental mild traumatic brain injury and subsequent magnetic laser therapy effects carried out sequentially in 3 zones of the biologically active points (BAP), T20, T16, T14.

Methods of transcranial magnetic laser effects on experimental animals in Group 3:

Magneto laser influence was consistently carried out on the contact areas of the relevant BAP T14, T16, T20. The BAP have been selected with the pathogenesis of light cranio-cerebral trauma and their anatomical-functional specificity:

- when exposed to the projection of BAP T20 (projection of the parasagittal area of the brain, the sine of the phase sagittal, great anastomotic vein) improves the absorption of CSF to the venous system, stimulation of hormone systems and synthesis of serotonin hormones of the epiphysis;
- when exposed on projection BAP T16 (projection of the tank and the shaft of the brain), stimulation of the brain and liquor-dynamic status of the autonomic nervous system;
- projection of BAP T14 – one of the major acupuncture points is the projection of C8-Th3 segments of the spinal cord (the 7th cervical and 1st thoracic vertebrae), providing all the cute vegetative innervation the structure of the brain [15].
- Group 4 (n = 5) – rabbits with experimental mild traumatic brain injury and subsequent magnetic laser therapy performed on an impact area BAP T14.

Immediately after application of the experimental light head injury animals received a course of magnetic laser therapy that we developed [10,15]. We used an infrared laser radiation (λ = 0,89 mm, P imp. = 7.8 W, frequency–500 Hz, magnetic induction–100 mT). Magnetic laser effects on the brain of animals was carried out using the KMILTA apparatus daily transcranial, consistently, to these areas BAP. The dose of radiation during a session in one area was 1–2 J/cm² (total dose per session – 3–4 J/cm²). After 10 sessions of magnetic laser therapy for 14 days, the rabbits were sacrificed by decapitation according to the requirements set by the National Bioethics Committee (NCU). The brain was removed from the cranial cavity, fragments of the parietal cortex area and the midbrain (in the area of substantia nigra) were isolated for electron-microscopic study, carried out by standard methods [9].

Using electrongramms, we performed morphometric analysis of the features, ultrastructure axon-dendritic and axon-spine asymmetric synapses of neurons Type I (by Gray) [18], who studied in digital photomicrographs neuropil obtained by electron microscope EM 400T firm PHILIPS (Netherlands) and РЭМ-100-01 firm SELMI (Ukraine) in the computer image analysis CAI-01ABN using software Kappa opto-electronics GmbH (Germany), with Standard Zoom (X 22000). In the electron density of asymmetric synapses were counted with a clear synaptic cleft by 100 mkm^2 neuropil. In each observation we analyzed 30 randomly selected neuronal synaptic contacts (SC), which were evaluated for common indicators as well, including the ratio of the length of active zone synapse (LAZS) to the length of synaptic contact (LSC) and the percentage of different types of presynaptic terminals on the content of secretory vesicles [7]. Among the presynaptic terminals quantified count: 1–normal terminal building filled with synaptic vesicles to ½–⅔ of their area, 2–terminal swollen with low electron density and depletion of synaptic vesicles sharp, occupying no more than ¼ of their area, and 3–terminal, totally filled synaptic vesicles. Assessed as the percentage of different types of synaptic contacts in the form of internally slit line. As conventional standard used flat punch synapse with active zone as a continuous line [1,3].

Considerable attention was paid also to the structure of mitochondria, which reflects the energy metabolism of cells. Comprehensive assessment of these indicators reflects the morphofunctional condition of the synaptic apparatus of neurons and their changes in different versions of experiments.

For statistical processing of the indicators we used parametric method of variation statistics (Student's t test criteria, two selective t-test with different variances) using the software package MS Excel 2003 [6]. The difference was considered statistically significant at $p < 0,05$.

All experimental studies conducted in compliance with the European Convention for the Protection of Vertebrate Animals and approved by the First National Congress of Bioethics of Ukraine (September, 2001).

3. Results and discussion

We compared the ultrastructural features of the synaptic apparatus of neurons of the 2nd layer of the cortex parietal region and the midbrain in the substantia nigra area of the rabbit brains with experimental mild traumatic brain injury without magnetic laser effect (group 2) and after magnetic laser impact (group 4) revealed significant differences in the state of these structures in both experiments compared with intact animals (group 1). Thus, in the parietal cortex of the brain areas of control (intact) animals most neuronal synapses presynaptic terminals (PST) had normal building containing small spherical transparent vesicles. In the area of the midbrain neuronal axons near the end of synaptic vesicles transparent cholinergic type contained also peptid ergic granules with tightened core and transparent rim. In synaptic terminals, the synaptic vesicles were grouped mainly around good contour synaptic cleft, occupying about 1/3 of the presynaptic ending and did not show signs of destructive changes; the ultrastructure of mitochondria was unchanged.

In contrast to these results, in synaptic ultrastructure system neurons of the same brain areas of rabbits with experimental mild traumatic brain injury (group 2) were observed quite significant structural violations as neurons in the parietal cortex area, and neurons in the midbrain compared with controls (intact) animals. In much of the neuronal synapses of both parts of the brain recorded signs of degeneration: presynaptic ending acquired high osmiophilia unlike postsynaptic who were enlightened and swollen. Such synapses were marked signs of swelling of mitochondria with destruction of mitochondrial crystals, which is the morphological equivalent of hypoxic changes of these organelles as a result of mild traumatic brain injury and represents a violation of both energy and water exchange. Pathologically altered synapses were located in clusters or forms contained between invariably preserved synaptic contacts. A few synaptic vesicles were grouped mainly near the synaptic cleft or in the center. Places were noted as adhesion or destruction of individual synaptic vesicles (Figure 1). In neuropil, we observed signs of edema. It is important to emphasize that only a small part of neuronal synapses in both areas of the brain retained a normal

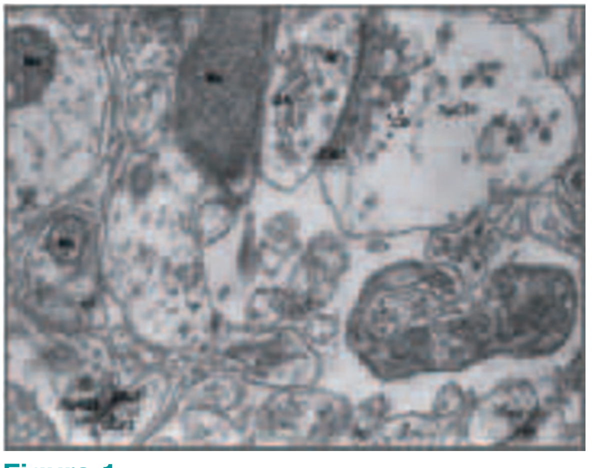

Figure 1. Axon-arrow concave punch synapse layer II of the rabbit brain TCD experimental mild craniocerebral injury. Signs neuropile edema, swelling of pre- and postsynaptic terminals and arrow's apparatus synapses. Reducing the number, adhesion and erratic distribution of synaptic vesicles (SV - arrow) in the presynaptic terminal. Electron micrograph, increase x 22 000.

ultrastructure, comparable to the control samples of the brain of experimental animals.

Of particular interest are the results of ultrastructural study of synaptic apparatus of neurons in both parts of the brain in rabbits with experimental mild traumatic brain injury and following a course of magnetic laser therapy (group 3). In the synaptic contacts of neurons cortex of these animals relative to the comparison group (group 2 - rabbits only experimental light head injury), an increase in the number and length of active zones increase their osmiophilia thickening of postsynaptic membrane and synaptic vesicle accumulation at the presynaptic membrane that may reflect the increasing structural and functional activity of synapses (Figure 2). In addition to those, we identified presynaptic terminal filled with synaptic vesicles, and synaptic terminals appeared hypertrophied mitochondria.

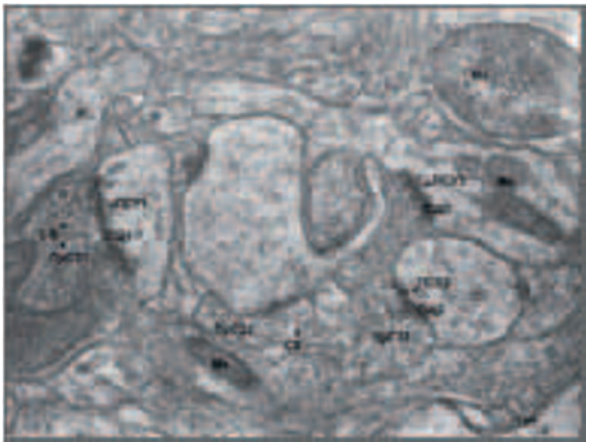

Figure 2. Axon-arrow activated neurons synapse concave punch TCD layer II of rabbit brain with experimental mild craniocerebral injury after magneto-laser therapy. Reduce swelling, swelling of the synaptic apparatus. PS full synaptic vesicles. Hypertrophic MTX in synaptic terminals. Electron micrograph, increase x 22 000.

Along the same in both parts of the rabbit brains with experimental mild traumatic brain injury after magnetic laser therapy appeared synapses with perforated contact zones, considered as compensatory-adaptive reactions in response to functional exhaustion and partial degeneration of synapses and other related one source neosynaptogenesis [13,19]. It is known that these features are crucial in the implementation mechanisms of synaptic plasticity. This is accompanied by changes in individual contacts curvature pre-and postsynaptic membranes, which can serve as an indicator of increased functional status and synapse reflect plastic reorganization interneural bonds [17].

It is also important to emphasize that the ultrastructural study of neuronal synapses in the midbrain fragments of rabbits with experimental mild traumatic brain injury and subsequent magnetic laser therapy in the synaptic apparatus of neurons observed changes similar to those found in the synapses of the cerebral cortex in these same options experiments.

As dendrites of neurons in synaptic areas neuropil, they tend to have normal structure, but focal in some neurons mainly in the parietal cortex area of the brain were observed ultrastructural signs of damage to cytoskeleton structures in the form of random allocation and their partial fragmentation. These features were characteristic of the brains of animals with experimental mild traumatic brain injury (group 2) in contrast to the brains of animals with experimental mild traumatic brain injury, followed by magnetic laser therapy (group 3, 4), in which such changes are manifested in a slight volume. At the lumbar sections of some of the dendrites

Table 1. Morphometric characteristics of interest rate (%) value for different types of synaptic contacts, axo-spine and axo-dendritic synapses parietal cortex area and the midbrain rabbits with different bending plane of contact in rabbits with experimental mild traumatic brain injury before and after magnetic laser therapy (M±m).

Group study	Number of synapses per 100 mkm^2 neuropil	Synaptic contacts concave, %	Synaptic contacts flatconvex, %	Synaptic contacts	Percentage of perforated synaptic contacts of the total, %	LAZS / LSK
Group 1 PCA	15,8±0,76	36,7	40	23,3	13,3	0,81±0,02
Group 1 MB	11,9±0,5	33,37	40	26,7	10	0,79±0,03
Group 2 PCA	9,3±0,47*	26,7	33,3	40	6,7	0,45±0,04*
Group 2 MB	8,2±0,51*	30	33,3	36,7	3,3	0,44±0,04*
Group 3 PCA	14,8±0,79**	50	30	20	16,7	0,65±0,03* **
Group 3 MB	10,8±0,55*	46,7	33,3	20	13,3	0,62±0,03* **
Group 4 PCA	12,2±0,71* **	36,7	36,67	26,7	10	0,63±0,03* **
Group 4 MB	9,8±0,42*	36,7	33,3	30	10	0,59±0,04* **

**–statistically significant compared with controls (P <0.05),*
***–statistically significant between the groups of study (P <0 , 05).*
SC, synaptic contacts; PCA, parietal cortex areas; MB, midbrain; LAZS / LSC, the ratio of the length of active zone synapse (LAZS) to the length of synaptic contact (LSC).

appeared empty against neuropil with numerous intact (intact) structures, but the whole phenomenon of edema-swelling in the brain neuropil animals with experimental mild traumatic brain injury + magnetic laser therapy (group 4) and especially in the brain of animals with experimental mild traumatic brain injury + magnetic laser therapy (group 3) proved significantly less in contrast to animals with experimental mild traumatic brain injury without magnetic laser therapy, in which brain edema-swelling characteristics neuropil. mitochondria dendrites and synaptic terminals in neuronal synapses kept unchanged or building, or showed reactive changes such as swelling, extending crystal, high osmiophilia, with signs of hypertrophy. These facts can be explained by the reaction of these organelles in the development of hypoxia-induced light replaced to experimental brain injury and their activation in response to magneto laser effect due to increased energy metabolism in the synapses.

The results of ultrastructural study obtained by morphometric analysis found a number of important quantitative characteristics of synaptic contacts of neurons parietal cortex area and the midbrain rabbits with experimental mild traumatic brain injury before and after magnetic laser therapy (Table 1). In particular, Table 1 presents the results quantify percentage of different types of synaptic contacts axo-spine and axo-dendritic synapses in neurons of the parietal cortex area and the midbrain rabbits with different bending plane of contact before and after magnetic laser therapy (M ± m) in various forms of research.

According to the results presented in Table 1, is the relationship between the degree of curvature of synaptic contacts, and its length: flat synapses usually the longest and most curved-convex-soon. Also established that presynaptic terminals area concave synapses is greater than the area of convex. Along with the size of the active zone synapse and postsynaptic neurons consolidation correlate with the activity of synaptic function, reduction of which is accompanied by a decrease in these parameters [1].

According to our research, in the parietal cortex neuropil parts of the brain control animals dominated flat functionally mature contacts, the fate of which was 40% of synaptic contacts (Table 1). Thus the lowest density of synaptic contacts found for convex contacts (23% and 26% respectively for the cortex and midbrain areas). In the brain of animals with experimental mild traumatic brain injury fate concave contacts compared with the control decreased 1.4 times in Synaptic apparatus of neurons of the cerebral cortex and only 1.1 times (almost unchanged)–neurons in the midbrain.

In the brain of animals with experimental mild traumatic brain injury after magnetic laser therapy (group 3 and 4) relative to comparison group (group 2) the density of concave contacts increased by 1.4 and 1.2 times respectively for the parietal cortex synapses areas and midbrain of animals for group 4 and in 1.9 and 1.6 times for the relevant departments of the brain for group 3, in the latter case, exceeding targets by 1.4 times. These results indicate the increasing proportion of actively functioning synapses in neurons of these areas of the brain under the influence of magnetic laser therapy. At the same time it is important to emphasize that the synaptic plasticity of neurons in the parietal cortex area of the brain by our data proved more variable than in neurons of the midbrain.

Based on the data, it can be concluded that the percentage of concave in form synaptic contacts varies mainly due to convex contacts and share does not change the fate of relatively flat contacts. Thus, confirmed the assumption that after mild traumatic brain injury observed concave transformation of synaptic contacts of neurons in the convex type, which leads to a sharp decrease of functioning synapses. Intensive contacts in the transition concave convex are probably due to reversible processes of regulation violation conformational state of microfilaments subsynaptic units due to autointoxication and hypoxic brain tissue in mild traumatic brain injury [12].

Restoration of the relative content of concave and convex synaptic contacts in neurons of the cortex and midbrain of animals in experiments of groups 3 and 4 are likely caused by the reverse transition fate convex concave synaptic contacts with stable content of flat contacts and, obviously, can be the result of recovery mechanisms regulating synaptic transmission induced by magnetic laser impact.

Significant results were obtained in the analysis of neuronal density of synaptic contacts in layer II cortex parietal region and midbrain in terms of 100 mm² neuropil. It recorded a sharp decline in this indicator to control light in experimental traumatic brain injury (group 2) by 1.7 and 1.5 times respectively for the parietal cortex and midbrain areas. In the group of animals 4 (after magnetic laser impact on a zone BAP) density of synaptic contacts in neurons statistically significant increased relative to the comparison group by 1.3 and 1.2 times for the respective parts of the brain, but returned to control values. Under study 3 – after magnetic laser impact zone of 3 BAP, the density of synaptic contacts in both areas relative to the comparison group increased to a greater extent – respectively by 1.6 and 1.3. However, this figure in the parietal cortex area decreased slightly relative to control, almost reached control values.

Note that our data ultrastructure of synapses of neurons in the midbrain after experimental mild traumatic brain injury changed to a lesser extent than in the cerebral cortex. But after magnetic laser therapy, this figure did not reach control values. Increasing the density of synaptic contacts in both areas of the brain of animals of groups 3 and 4 after magnetic laser therapy relative to comparison group indirectly points to start the process neosynaptogenesis in response to magnetic laser impact.

Analysis of the percentage of different types of presynaptic terminals shows that in animals with experimental mild traumatic brain injury (group 2) synaptic terminal with a sharp depletion of vesicles were observed in 40%-43% of cases, and a terminal full of synaptic vesicles–only 3% of cases. In research groups synaptic apparatus of neurons in the brain of animals with experimental mild traumatic brain injury and subsequent magnetic laser therapy (groups 3 and 4) the following changes in presynaptic terminals with low electron density and depletion of synaptic vesicles sharp detected in much lesser extent – according to 20% and 30% for both parts of the brain.

Thus, the number of abnormal synaptic terminals of neurons in the "treated" group of animals with experimental mild traumatic brain injury was 1.5-2 times lower than in the group of animals with experimental mild traumatic brain injury without treatment, although not as high control values. In 10-13% have been identified as a terminal filled with vesicles unselected mediator.

It should be noted that the synaptic cleft in a large part of synaptic contacts in the brain of animals with experimental mild traumatic brain injury without magnetic laser therapy (Group 2) seemed narrowed and sometimes reduced, turning in a solid electron dense cord, in addition to 37-40 % neuronal synapses in the middle of the synaptic cleft observed accumulation of electron-dense material, uncharacteristic of intact neurons, which probably serves as a mechanical barrier to the selection of mediators in the postsynaptic end and reduces the effectiveness of their between neuronal transport.

In the "treated" groups of rabbits synapses with neurons reduced synaptic cleft met in study group 4 in 17-23% and in group 3–13% of cases, that is 2-3 times less than in the brain of animals with mild traumatic brain injury without magnetic laser effect, which is an indirect manifestation of the normalization function selection mediator.

Consideration of value LAZS / LSC neurons suggests that this figure statistically significantly decreased in the brain of animals with experimental mild traumatic brain injury (group 2) with respect to control of 1.8 times for both the studied areas of the brain (Table 1).

Also registered statistically significant rate increase LAZS / LSC in the brain of animals with experimental mild traumatic brain injury and subsequent magnetic laser therapy (group 4) relative to Group 2 in 1.4 and 1.3 times respectively for the parietal cortex area and the midbrain reflecting increasing the length of active zones of neuronal synapses in the injured brain after magnetic laser therapy and meets previously registered an increase of fate concave synaptic contacts in comparison with non treated group of animals with experimental mild traumatic brain injury. This reflects the activation of metabolic processes in neuronal synapses after Magneto laser impact. Likely to reduce the length of active zones of synapses may be due to pathological damage of paramembraneous filaments and a violation of their relationship with neuronal cytoskeleton neurofilamentous terminations due to a sharp increase of lipid peroxidation of membrane structures under the influence of experimental mild traumatic brain injury [13]. In contrast, we recorded a statistically significant increase in the length of the core synaptic apparatus of neurons after magnetic laser therapy in both studied areas of the brain (groups 3 and 4) relative to 2 groups probably related to normalization and activation of filaments synaptic contact, laser-induced magnetic influence.

Thus, a comparative morphometric analysis of ultrastructural elements synaptic apparatus of neurons parietal cortex area of the brain and substantia nigra area of the midbrain in rabbits with experimental mild traumatic brain injury before and after the course (10 sessions) of magnetic laser therapy by criteria that reflect elimination of symptoms or tumor between neuronal synapses, destruction or restoration of synaptic vesicles with the characteristic accumulation of indirect-output mediators, state and membrane structures paramembraneous internal slit line synaptic endings. Proved the positive influence of magnetic laser therapy on the course structural and functional changes in animals with experimental mild traumatic brain injury.

4. Conclusions

1. Experiments revealed ultrastructural changes in synaptic apparatus of neurons in the parietal cortex area of the brain and substantia nigra area of the midbrain rabbits with experimental mild traumatic brain injury is a morphological manifestation of violation of their ultrastructure and synthesis of neurotransmitters to decrease the effectiveness of their between neuronal transport.
2. In experiments with experimental mild traumatic brain injury in animals followed by magnetic laser therapy revealed ultrastructural signs of functional activity in

the vast number of neuronal synapses in the cortex as parietal areas of the brain and in the substantia nigra area of the midbrain rabbits. This justifies the conclusion of activating regeneration-compensatory processes that ensure recovery of structure and function of synaptic apparatus of neurons as a result of these combined factors.
3. Morphometric analysis of complex quantitative parameters of synaptic apparatus of neurons in both studied areas of the brain of animals with experimental mild traumatic brain injury before and after a course of magnetic laser therapy showed greater efficacy of these physical factors on the projection of three zones of biologically active points than only one.
4. Comparative analysis of quantitative indicators of synaptic apparatus of neurons in the two studied areas of the brain of animals with experimental mild traumatic brain injury indicates higher vulnerability of neuronal synapses parietal cortex area of the brain compared with those in the area of substantia nigra midbrain rabbits as of traumatic factors, so and to magnetic laser influence.

Conflict of interest statement

Authors state no conflict of interest.

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