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DEVELOPMENT AND PATHOGENETIC SUBSTANTIATION OF THE MODEL OF POST-TRAUMATIC STRESS DISORDER

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Abstract

The spread of post-traumatic stress disorder (PTSD) among the world's population requires the development of new approaches to the treating of this condition. However, actions in this direction are associated with numerous ethical problems; therefore, there is a need to develop experimental models of PTSD in animals. Existing models of PTSD are based on the idea that this pathology is a consequence of only an acute stress reaction. However, modern views on the pathogenesis of PTSD admit the presence of damage in the brain' substrate of psychophysiological processes. The authors set themselves the goal to reconstruct and substantiate a model of PTSD in rats, which would take into account the main pathogenetic mechanisms of this pathology to the greatest extent.

Materials and methods. The PTSD model was recreated in white rats and consisted of several components: mild traumatic brain injury and immobilization- cold stress.

The research results showed that the reproduction of the PTSD model was accompanied by manifestations of fear, suppression of locomotor activity, emotional arousal and embarrassment, convulsive and chaotic nature of the response to external influences. That is, those damages of the psychophysiological sphere which are characteristic of PTSD are defined. Simultaneously, in the cerebral cortex, ganglion cell discharges, edema of the brain matter, perivascular edema of astrocytes, and neuronophagy phenomena are observed. Changes in metabolism indicate a decrease in the energy supply of transmembrane transport and the nitric oxide cycle's intensity. Disturbances in the regulation of metabolic processes were also characterized by an increase in the concentration of uric acid ($p < 0.001$) and a decrease in the content of total protein ($p < 0.05$). On the part of the humoral link of the immune system, signs of the development of inflammatory processes and a weakening of nonspecific defense were established, as evidenced by an increase in the content of circulating immune complexes ($p < 0.05$) and a decrease in the range of heterophilic antibodies ($p < 0.001$).

Conclusions. The authors believe that the systemic disorders that occur during the reproduction of the PTSD model have a pathogenetic significance for the developing of this pathology, and the developed model is pathogenetically substantiated.

Keywords: post-traumatic stress disorder, brain, metabolic disorders, psychophysiological disorders.

Introduction

The current development stage of society is characterized by a significant number of violent conflicts and terrorist acts, natural disasters caused by climate change, the global refugee crisis, the growth of violence in crowded urban areas, the intensification and complication of production processes [1, 2]. This causes a person to stay in extreme long-term situations that provoke exhaustion and disruptions of the organism's adaptive capacity and the development of diseases and disorders of a psychogenic nature [3, 4]. One such disorder is post-traumatic stress disorder (PTSD). Today, PTSD is considered mainly as a manifestation of the consequences of an acute stress reaction - a condition that occurs as a response to a powerful specific physical or/or mental stimulus, manifested by maladjustment, anxiety, amnesia, and behavioral disturbances [5, 6]. For the first time, such mental disorders were observed in the First World War soldiers and were called a mild concussion. Later, these disorders were defined as fatigue syndrome and, today, as PTSD [7, 8]. The need to correct these conditions from the standpoint of pathogenesis faced an ethical problem since it is impossible to conduct in-depth biological research on humans. This led to the active development of models of PTSD in animals.

For example, Alaa Kamnaksh et al. developed a repeated non-explosive brain injury model in rats, with behavioral changes and severe structural brain damage that cannot be investigated in clinical studies in patients with PTSD [9]. Therefore, the authors failed to demonstrate changes in other functional systems of the body. The significance of changes in these systems is associated with damage to synapses and their networks in PTSD, which causes the development of dysregulation pathology, which, through a feedback mechanism, affects the state of reparative processes in the brain [10]. It should be noted that there are many works devoted to models of PTSD, but their feature is the use of psychophysiological stressors. For example, male rats have either experienced direct re-stress due to social damage or have witnessed another male's defeat. Repeated exposure to social stress caused the development of psychosocial disorders, including depression (in the form of anhedonia - decreased interest in activities that were once pleasant, or loss of the ability to enjoy them), and concomitant cardiovascular diseases [11]. Predator stress (situations of death of a partner-neighbor from a predator) is also referred to as social and psychological stressors [12]. Simultaneously, physical trauma to the substrate of higher cognitive functions of the brain substance also reproduces conditions for dysfunction of brain structures, and, accordingly, for changes in psychophysiological processes [13].

Based on the above, this work aimed was to reproduce and substantiate the model of PTSD in rats, which maximally considers the main pathogenetic mechanisms of this pathology.

Methods

Experimental studies were performed on 50 clinically healthy male rats (Wistar line), aged 6-8 months, weighing 180 - 210 in compliance with the humane treatment requirements of experimental animals, regulated by the relevant documents [14]. During the experiment, the animals were in a vivarium at a temperature of 19-240C, the humidity not exceeding 50%, natural light regime "day and night", in plastic cages, on a balanced diet. Animals had free access to water and food. The studies were carried out in an experimental biological clinic (vivarium) State Institution "Ukrainian Research Institute of Medical Rehabilitation Therapy of Ministry of Health of Ukraine", m. Odessa, (Protocol No. 1 dated 20.0.2020. From the Commission on Bioethics). The physiological, morphological, immunological, and biochemical methods used in the study are given in the guidelines and approved by the Ministry of Health of Ukraine [15].

Rats were divided into two groups according to the tasks of the work. The first group - 20 intact animals (were not exposed to any exposure), their data served as a control. The second group - 20 animals in which the PTSD model was reproduced. The obtained indicators were compared with the corresponding indicators of group 1 of intact animals. The PTSD model was performed by applying to the rat, which was under light ether anesthesia, moderate traumatic brain injury (TBI). For this, the rats were placed in a pencil case, where her head was fixed, and on top, from a height of 65 cm on the tube was like in a free fall semicircular

cylinder weighing 85 g. After the application of TBI in rats, immobilization-cold stress was reproduced. For this purpose, the animal was placed in a pencil case 15 cm long and 5 cm in diameter and placed in a refrigerator at 4 - 6 ° C for 4 hr. Seven days after the reproduction of PTSD in animals, a set of studies was carried out, which began with behavior and emotional state. For a comprehensive assessment of experimental animals ' the motor and behavioral activity and their reactions to being under conditions of chronic emotional stress, exposure to damaging or toxic factors, studies of psychophysiological parameters in the open field test are critical and indicative [16]. Features of rats' behavior in the "open field" device is a prognostic criterion for the state of the central nervous system. Placing animals in a new environment leads to the emergence of research motivation, accompanied passive-defensive behavior. A characteristic manifestation of this condition is considered to be the vegetative reaction of animals in the form of defecation (bolus) and urination, as well as a change in the level of motor activity. Grooming is an essential characteristic of the behavior of rats in the open field device. Rats devote a significant part of their time to caring for their fur and skin, and in some cases, this process is dominated by physical activity in terms of duration. Grooming is traditionally classified as a comfortable behavior or a restful state. But when rats perform short-term grooming and its amount is increased, and the duration is reduced, this indicates that the animals are in a state of anxiety [17].

Therefore, to study the functional state of the central nervous system and rats' vigorous activity, we used the generally accepted method "open field" [18]. Changes in behavior and locomotor activity assessed functional state of the central nervous system. In the study of locomotor activity, the number of exits to the center of the "open field" device, the number and duration of stops - indicators of motor activity; the number of squares crossed, the number of vertical stance, and the number of caves peeks. The characteristics of the animals' emotional state were determined by the number and duration of grooming, the number of boluses, and urinations. For each individual rat, the duration of the open field test was 6 minutes.

After researching in the "open field" device, the animals were taken out of the experiment by decapitation under ether anesthesia. When performing autopsies on experimental rats, a visual macroscopic assessment of the brain was performed. From rats, two pieces of brain with a volume of 1 cm^3 were removed. The first piece was fixed in 4% paraformaldehyde solution passed through alcohols of increasing concentration, and poured into celloidin. From the obtained blocks, histological sections of 7-9 μm thick were made, which were stained with hematoxilin-eosin, according to Van Giesonon [19].

The obtained preparations were used for microscopic studies of structural changes in the brain. 5 ml of blood was taken from the animals, serum was accepted, and biochemical and immunological studies were performed.

Biochemical studies determined:

- the level of NOx was measured by the method of reduction of nitrates to nitrite by the Gris reaction and spectrophotometric determination of nitrite,
- the content of total protein by the biuret method (the principle of the method is based on the reaction of proteins with copper sulfate in an alkaline medium with the formation of violet compounds)
- the content of uric acid using a phosphorus-tungsten reagent (a method based on the reduction of phosphorus-tungsten reagent into a blue dye with uric acid in an alkaline medium);
- Ca / Mg-ATPase and Na / K-ATPase activity in the liver homogenate was determined by a method based on the reaction of inorganic phosphate with a molybdovanadate reagent, which leads to the formation of a yellow phosphomolybdovanadate complex.

The study of the state of the humoral immunity was carried out by determining circulating immune complexes (CIC) and the content of heterophilic antibodies (GA), that is, natural antibodies of hemolysins and heterophilic agglutinins. The CEC content determination was carried out by the method of precipitation in solutions of polyethylene glycol 3.5% and 7% with a molecular weight of 6000 Da. The content of GA was determined by the reaction of passive hemagglutination using sheep erythrocytes. The presence of GA is an indicator of

the degree of immunological maturity of the organism and the immune system [20].

Statistical processing of the data obtained was carried out using the program for biomedical research Statistica. Significant shifts were considered those that were within the confidence limits according to the Student's tables p <0.05.

Results

The results of studies on the functional state of the central nervous system and emotional status are shown in Table 1. Before proceeding to the summit of the materials in the table, it is necessary to focus on the fact that at the beginning of the experiment, when each of the rats was placed in the center of the "open field" device, they performed largely by the size of urination, that is, the animals experienced intense fear.

When analyzing the data in Table 1, it was found that the reproduction of PTSD in rats leads to a significant decrease in motor activity due to a decrease in exits to the center of the site by 50% (p <0.001), a decrease in the number of crossed squares by 53% (p <0.001) and an increase in the duration of stops - by 137% (p <0.001). That is, the motor activity of rats with the PTSD model is significantly suppressed. The orientation-exploratory behavior of animals also decreases, as evidenced by a decrease in the number of vertical stance by 53% and a decrease in the number of peeking into caves by 39% against the background of a reduction of crossed squares by 53%. So, in animals with the PTSD model, locomotor activity significantly decreases, as evidenced by a significant increase in the duration of stops by - 137%.

As for the emotional status of rats with a PTSD model, in addition to the above signs of fear and anxiety (a significant decrease in the number of defecations by 44% and an increase in the number of urination acts by 36%), according to the data in Table. 1, there is a significant and reliable increase in the number of grooming acts by - 74%, while maintaining the duration of grooming acts at the level of the control group indicator (p > 0.5). It should be noted that the animals were grooming not only short-term (its duration was 1-4 seconds), but convulsive and disorderly [18]. That is, 2/3 of the experiment's time, the animals were carried out within several squares, briefly engaged in grooming, and recently they were randomly moving around the perimeter of the device "open field". So, the animals showed signs of fright, overexcitement, and disorientation.

In general, it can be stated that modeling of PTSD causes disorders of behavior, emotional status, and cognitive functions in rats.

In addition to psychophysiological disorders, changes in metabolism and immune response were found in rats with a PTSD model. The results of these studies are shown in Table 2. Modeling of PTSD in rats leads to an insignificant but significant increase (by 9%) in the blood of the number of endogenous nitrates and nitrites (NO x). Since this indicator reflects the intensity of the nitric oxide (NO) cycle, its increase can be considered evidence of the inactivation of the NO cycle. A decrease in the activity of the NO cycle negatively affects the state of endothelial function, the transport function of the circulatory system changes, as a result of brain damage, oxygen and substrates are insufficiently provided. The uric acid study showed that its content in the blood significantly increased by 23% (p <0.01). Uric acid is the end product of the exchange of nitrogenous compounds and a regulatory molecule of metabolism (lipid and protein metabolism). An increase in uric acid content may be associated with the need to compensate for violations of neurotrophic regulation of metabolism in connection with the development of PTSD. Confirmation of the presence of violations in metabolic processes regulation is a significant decrease in the total protein content by 11% (p <0.05).

Another event that attracts attention is a decrease in the activity of Na / K-ATP-ase and Ca / Mg-ATP-ase by almost 40% (p <0.01). Moreover, the ratio of these enzymes' activity remained close to that in animals of group 1. That is, the provision of transmembrane transport decreased but remained balanced. Thus, these data on changes in metabolic parameters indicate the development of systemic changes in the body during the development of PTSD and are confirmed by other authors [21].

Changes were also found among the parameters of the immune system - the content of circulating immune complexes (CIC) increased by 12% (which is a leading marker of the development of pathological processes in the body), and the

content of heterophilic antibodies (GA) decreased by 54%. The consequence of such changes may be an increase in the possibility of developing inflammatory processes and a violation of the order of inclusion of amino acids in proteins [22]. The authors point out that in immunodeficiency and different pathological conditions of the body, the titers of GA and other antibodies may be sharply reduced or even not manifested [23].

Histological studies determined the presence of ganglion-clit discharge in the cerebral cortex, mainly in the zone III and IV of the layers of the cortex, swelling of the brain substance, perivascular swelling and edema of astrocytic processes; irregularity of vascular blood filling (along with spasmodic vessels, the presence of vessels with increased blood filling was determined). There were single neurons with a homogeneous cytoplasm and a pyknotic nucleus, surrounded by microgliocytes (Grave hills).

Thus, research results have shown that the PTSD model causes changes in the psycho-physiological sphere in animals, similar to those that have been established and proven in other models [24]. That is, there is a violation of behavior, cognitive functions, emotional status. In general, these changes are similar to those described in a person with PTSD [25].

Discussion

The developed model of PTSD in rats is complex and corresponds to the pathogenesis in humans. A feature of our model is the presence of dysregulation and metabolic disorders that affect the substance of the brain, and which can be considered as a pathogenetic mechanism of PTSD.

Prospects for further research. Animal models are not only an essential tool for improving our understanding of the pathogenesis of PTSD but also serve as an effective basis for the development of drugs, drugs based on products of natural origin, physical healing factors (physiotherapy), and natural healing resources (NHR) such as mineral water, peloids/mud, clay, etc.NHR and other natural origin agents are among the most important factors used for therapeutic and prophylactic purposes in many diseases [26-31]. In connection with the above, our proposed experimental model of PTSD will be used in further studies to determine the curative properties of mineral waters of various chemical composition, to justify further clinical trials in patients with PTSD.

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Table 1. Indicators of the functional state of the central nervous system and emotional activity of rats with a PTSD model

Indicators	1 group control (intact rats)	Group 2 with the PTSD model
	($M_1 \pm m_1$)	($M_2 \pm m_2$)
Number of exits to the center, n	1,67 ± 0,26	0,86 ± 0,12*
Stops, n	1,44 ± 0,21	2,17 ± 0,15*
Stops, c	38,00 ± 8,96	90,14 ± 12,6*
Number of crossed squares, n	63,14 ± 6,98	33,29 ± 4,80*
Number of vertical stance, n	12,78 ± 1,51	6,00 ± 1,23*
The number of peeks in the caves, n	6,89 ± 0,74	4,19 ± 0,93*
Grooming, n	1,44 ± 0,27	2,50 ± 0,05*
Grooming, c	21,67 ± 1,23	23,44 ± 1,29
Number of acts of defecation (bolus),n	2,21 ± 0,15	1,24 ± 0,09*
Number of acts of urination, n	3,78 ± 0,22	5,14 ± 0,12*

Note 1. (M1 ± m1) and (M2 ± m2) are arithmetic means with errors; * - significant changes (p <0.05) were calculated in comparison between the control group and the group with pathology

Table 2. Biochemical and immunological parameters of rats with a model of PTSD

Indicators	1 group control (intact rats)	Group 2 with the PTSD model
	($M_1 \pm m_1$)	($M_2 \pm m_2$)
NO_x, mmol/l	37,90 ± 0,65	41,40 ± 0,87*
Uric acid,µmol/l	120,46 ± 2,96	148,13 ± 5,16*
Total protein, g/l	68,70 ± 1,74	61,08 ± 0,35*
Ca/Mg-ATPasa, mg P/g tissue	9,11 ± 0,93	5,47 ± 0,41*
Na/K-ATPasa, mg P/g tissue	6,40 ± 0,62	3,84 ± 0,34*
CIC, mg/ml	5,70 ± 0,20	6,39 ± 0,17*
GA, cu	6,0 ± 0,8	2,8 ± 0,49*

Note 1. (M1 ± m1) and (M2 ± m2) are arithmetic means with errors; * - significant changes (p <0.05) were calculated in comparison between the control group and the group with pathology