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Behavior of Rats in the Open Field within the Early Period after Light-Degree Blast-Induced Neurotrauma

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Blast-induced neurotrauma (BINT) is a specific type of traumatic brain injury (TBI). At present, this type of injury is rather widespread throughout the world due to its occurrence in military conflicts and terrorism acts. Consequences of such traumas form an important social and medical problem. Brain injuries in the case of BINT result mostly from a specific action of the main pathological factor of explosion, the shock wave. Experimental studies of BINT consequences have been concentrated on its different aspects and were carried out using variable techniques of modeling; thus, some links of the BINT pathogenesis have been insufficiently examined. In our study, we modeled BINT in rats using a self-made pneumatic device that produced a baroacoustic wave with the excess pressure of about 25 kPa and examined behavioral activity of the experimental animals subjected to the action of this factor in the open-field test within acute and early segments of the post-traumatic period. After initial increases in the intensities of horizontal and vertical motor activities (in the case of vertical activity, dramatic and more long-lasting), these behavioral phenomena were later on significantly suppressed. Exploration of openings in the arena floor (burrows) and the performance of grooming episodes were suppressed within the entire observation period. A part of grooming events was transformed into an abortive pattern. It should be concluded that high anxiety related to primary pathological effects of the shock wave were obvious within the acute period (days 1–7). Within the early post-traumatic period (days 7–21), the animals experienced fear and demonstrated signs of depression; this was probably related to secondary damage to the brain against the background of neuroinflammation and neuromediatory imbalance.

Keywords: blast-induced neurotrauma (BINT), open field, motor activity, orientational/research activity, emotional state.

INTRODUCTION

At present, blast-induced neurotrauma (BINT) is rather widespread throughout the world. Due to the use of various explosive devices in military conflicts, both servicemen and, frequently, civilians suffer [1]. Other sources of BINTs are terrorism acts, “domestic” events (related to an uncontrolled spreading of blast devices), and, more rarely, industrial explosions [2]. The occurrence of BINT comprises a significant component in the level of disability among relatively young people in both acute and remote post-traumatic periods, and this forms an important social problem [3], which is especially urgent in today Ukraine.

From the other aspect, BINT is a serious medical problem, as its treatment is rather difficult. BINT belongs to a specific type of traumatic brain injury (TBI) [1]. Damage to brain tissue in the case of explosion is based on specific physical and biological features associated with the action of the main damaging factor of an explosion, the shock wave. The latter leads to displacement and concussion of the brain, induces a cavitation phenomenon, and generates hemodynamic and hydraulic shocks. These factors, even when being relatively mild, may lead to significant functional disorders [4]. It was reported that there is a probability for clinical signs of strong behavioral disorders in people subjected to even mild explosive TBI [5]. Within the acute and early periods of BINT, the patients may demonstrate disorientation, agitation, abnormal aggression, and considerable autonomic disorders under conditions where modern research techniques are unable to establish

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noticeable signs of brain damage [6, 7]. This is why such disorders are frequently not diagnosed and treated in time. As a result, “vicious circles” and secondary damage to the brain and other organs occur [8, 9], serious complications appear [10], and the general condition of patients may significantly deteriorate.

Experimental studies of BINT have been concentrated on various aspects of the respective problem, and a variety of methods of modeling BINTs was used in such experiments; thus, only some links of the BINT pathogenesis have been sufficiently elucidated at present. A few nonspecific and specific BINT markers remain disputable. Therefore, all of the above determines the relevance of the chosen topic and the need for further research in this field, which would help to develop pathogenetically-based approaches to the treatment and prevention of complications of BINT. We have developed a technique of producing a baroacoustic wave capable of inducing a light-degree BINT in experimental rats and examined the behavior of the latter in the open-field test.

METHODS

This study was carried out on 20 adult (6–7 months) male Wistar rats (body mass 220–270 g) randomly divided into three groups. Animals of the experimental group Exp (n = 6) were anesthetized with halothane and 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 26.4±3.6 kPa, on average [11]. The device was provided with an electronic manometer, APZ 3420G (Piezus, Russia), and a noise meter [12], Robotron RFT 00024 (Germany). Two other animal groups were formed to estimate the action of additional pathogenetic factors (anesthesia, fixation). Rats of the sham group, Sh (n = 7) were subjected to inhalation halothane anesthesia and fixation, while animals of the intact group, Int (n = 7), were not subjected to any manipulations.

The behavioral activity of the animals was examined using a standard open-field test. The wooden rectangular 100×100-cm test arena with 40-cm-high walls was divided into 25 equal squares (20×20 cm). Holes with a diameter of 1 cm (burrows) were made at intersections of the borders of each square.

The behavioral studies were performed on the 1st, 3rd, 7th, 14th, and 21st days after modeling of BINT. The test period of observation was 3 min long. In each animal, the number of crossed squares (horizontal motor activity, HMA), that of vertical stands performed by the animal (vertical motor activity, VMA), the number of inspected holes (burrows), and the number of grooming episodes were calculated [13]. Among groomings, we differentiated complete (comfort) and abortive ones. The former included systematic washing of the muzzle, nose, and zones behind the ears, and also cleaning of the entire body; when the animal washed itself inconsistently and intermittently or cleaned only one part of the body, the grooming episode was considered abortive.

Statistical processing of the numerical results was performed using STATISTICA 6.1 software (StatSoftInc., serial No. AGAR909E415822FA). Means and s.d. values were calculated. Intergroup differences were estimated using the Mann–Whitney U-test and considered statistically significant with $P < 0.05$ or $P < 0.01$.

RESULTS

We performed paired statistical comparisons of behavioral indices shown in the open-field test by rats of the following above-mentioned groups, experimental (Exp, blast-affected) vs. sham (Sh), Exp vs. intact (Int), and Sh vs. Int. It was found that the differences between all measured indices in the latter two groups (Sh and Int) never reached the level of statistical significance ($P > 0.05$, Figs. 1–4). Therefore, inhalation anesthesia by halothane and fixation of the animal exerted no considerable systematic long-lasting effects on rats of the Sh group; thus, from the statistical aspect, groups Sh and Int were identical to each other. This situation provided us with well-based reasons to focus our attention on a comparison of the two groups, Exp and Sh (believing that the values in group Sh are very close to those in the fully intact group Int).

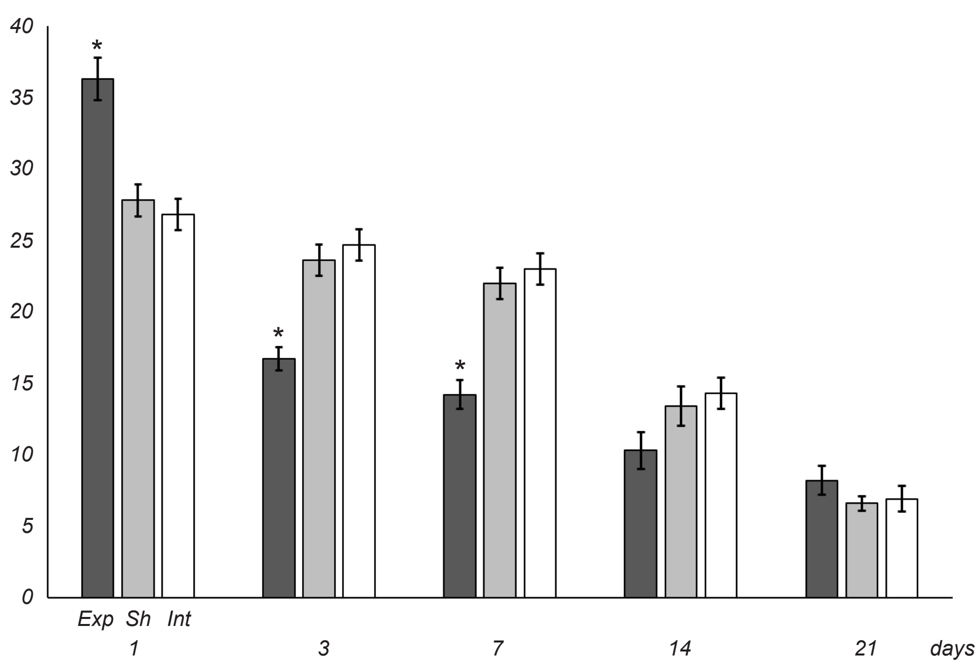
Significant effects of the action of light-degree BINT on behavior indices of the experimental rats were obvious. On the 1st day after simulation of the explosion and induction of BINT, the level of HMA (characterizing mostly the intensity of locomotion of the animal within the arena) in the Exp group was, on average, 31% higher than that in group Sh ($P < 0.05$). Later on (days 3–14), this type of activity was noticeably suppressed in comparison with the control, Sh (by 35–23%; $P < 0.05$). Then, on day 21, this index increased again noticeably (but statistically insignificantly) and exceeded the control value ($P > 0.05$, Fig. 1). In other words, the dynamics of HMA in blast-affected rats within the early post-trauma period demonstrated at least a two-phase (and maybe even a three-phase) pattern.

The number of complete stands on the hindlimbs (VMA) performed by the animal, which is usually interpreted as a manifestation of mostly explorative/orientational activity, in rats with light BINT within days 1, 3, and 7, was dramatically greater ($P < 0.01$) than that in Sh rats. On these time sections, VMA of traumatized rats corresponded, on average, to 340, 220, and 234% of that in control animals. Later on, however, vertical activity of rats with BINT demonstrated significant suppression (by about 70%, $P < 0.05$) in comparison with the respective value in the Sh group.

Inspections of the holes (burrows) in the floor are supposed to be a most "pure" manifestation of research activity in the open-field test. This type of behavioral phenomena in blast-affected rats was dramatically suppressed throughout the 21-day-long period of observation. Within days 1–7, inspection of burrows by rats with BINT was 37–53% less intense than that shown by Sh animals, and within days 14–21, the respective index demonstrated further negative dynamics (on day 21, down to only 14% of that in group Sh; $P < 0.01$).

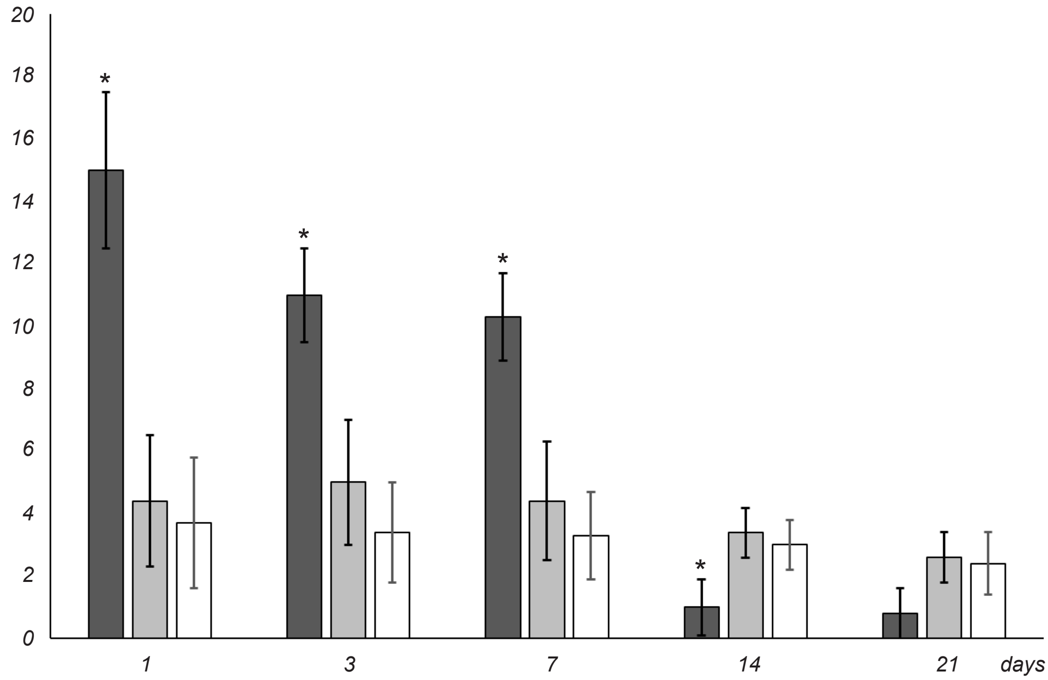
When analyzing grooming behavior, we should distinguish two types of the respective episodes. Complete grooming included "washing" movements directed toward the muzzle, nose, and areas behind the ears and also cleaning of the entire body. In a part of the grooming episodes, the rats cleaned only single parts of the body and head, and such activity demonstrated an interrupted pattern. Such episodes were qualified as abortive grooming. The latter modifications of grooming behavior were rather rare in animals of groups Sh and Int, but abortive grooming constituted up to about one-third of the grooming episodes in rats subjected to BINT.

Typical (complete) grooming behavior was significantly suppressed in rats with BINT throughout the entire period of observation in comparison with the analogous manifestations in rats of groups Sh and Int ($P < 0.05$ on days 1, 3, 7, and 21; Fig. 4, A). Episodes of abortive grooming were

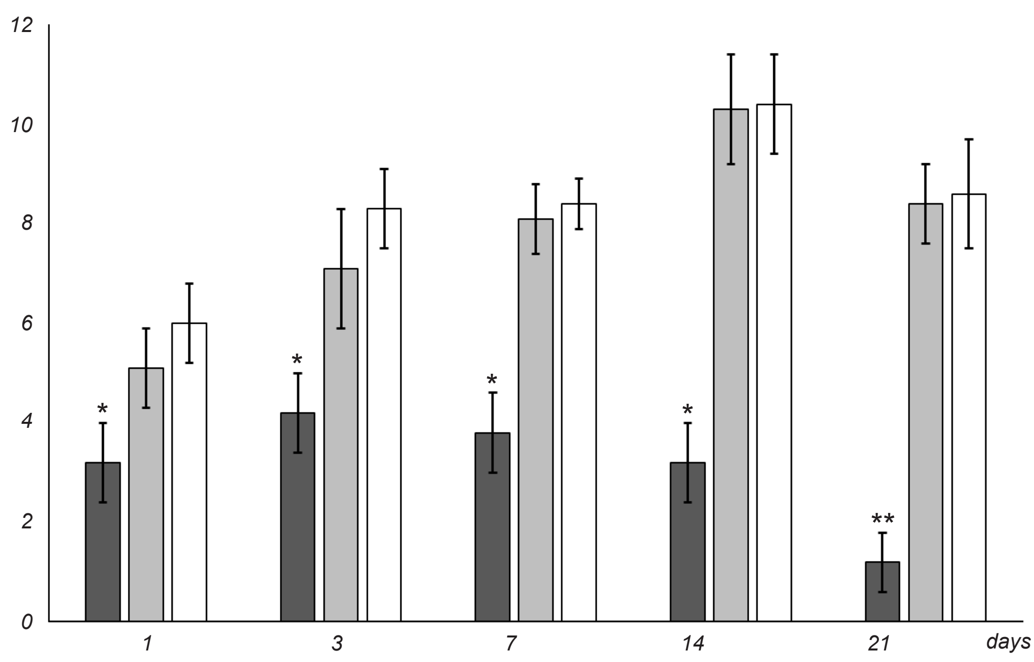


F i g. 1. Dynamics of the intensity of horizontal motor activity (HMA) shown by rats of different groups in the open-field test. Vertical scale) Number of crossed squares within the test period of observation (3 min). Filled, gray, and open columns corresponded to groups of animals, Exp (experimental, with mild BINT), Sh (sham), and Int (intact), are indicated below the columns. Days of observation (1–21) are shown above the diagrams. Means and s.e.m. values are shown. Cases of significant ($P < 0.05$) differences between the Exp and Sh groups are indicated by asterisks.

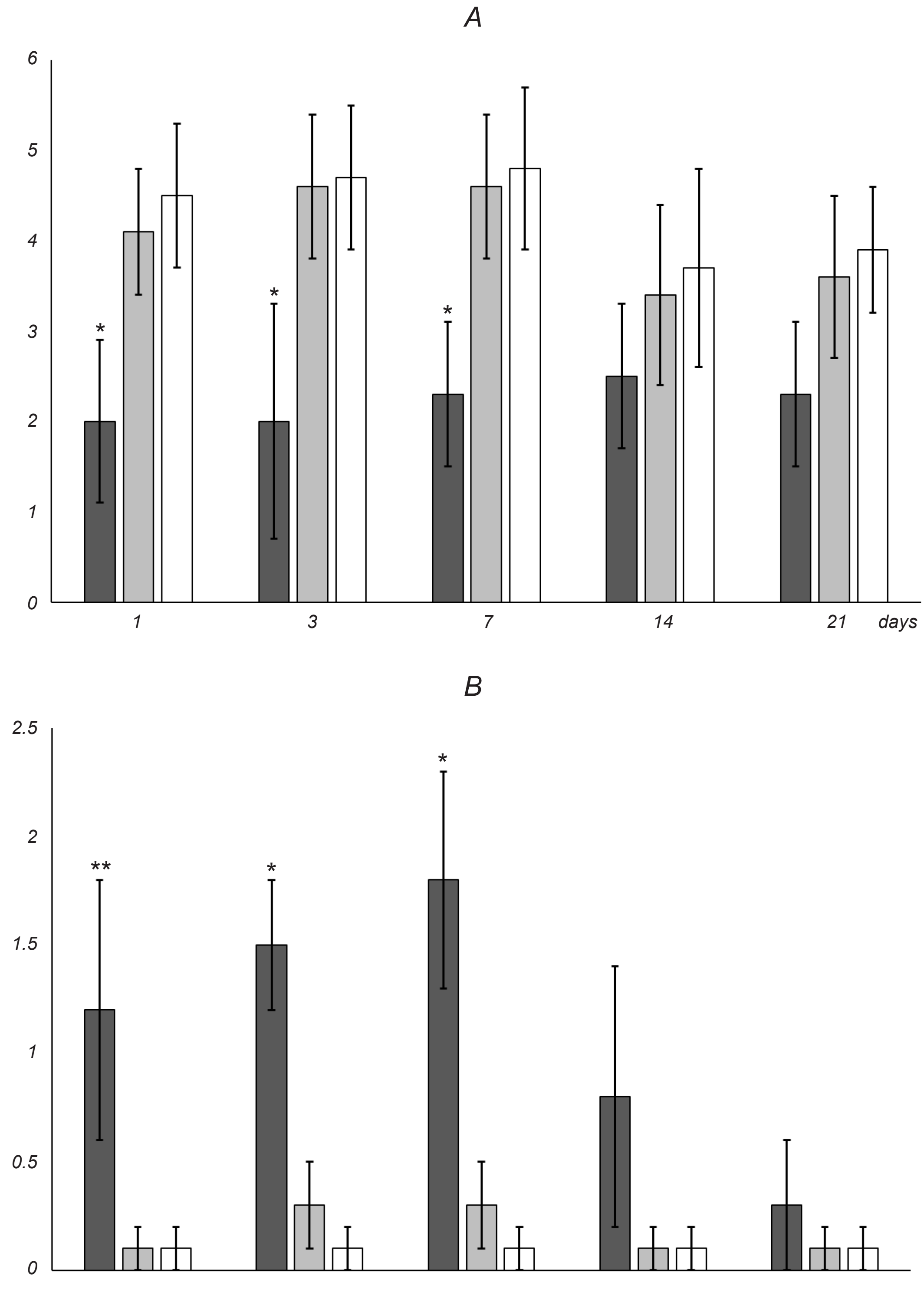
relatively frequent on days 1, 3, and 7 ($P < 0.01$ and $P < 0.05$ in comparison with the respective indices in groups Sh and Int), and their number progressively decreased on days 14 and 21. It should be noted that the occurrence of episodes of abortive grooming in all groups was rather extensively individually variable.



F i g. 2. Dynamics of the intensity of vertical motor activity (VMA) shown by rats of different groups. Vertical scale) Number of full vertical stands on the hindlimbs within the period of observation. Other indications are similar to those in Fig. 1.



F i g. 3. Dynamics of the intensity of "burrow" research activity shown by rats of different groups. Vertical scale) Number of inspections of openings in the arena floor. Other indications are similar to those in Fig. 1



F i g. 4. Dynamics of number of grooming episodes within the observation period; A) that of complete (normal) groomings, B) that of incomplete (abortive) ones. Other indications are similar to those in Fig. 1

DISCUSSION

The use of measuring instruments (an electronic pulse manometer and a noise meter) in our experiments showed that our device allowed us to mimic the main damaging factors of an explosion, namely a shock wave and an intense sound. Rapid opening of a chamber with a high air pressure (up to 15 atm) generated a mechanical oscillation (leap of pressure) with a wavelength of the order of 10–100 nm and a sound of about 130 dB. Thus, we succeeded in mimicking the effects of a small blast without using any explosive substance and a possibility to regulate the power of a "blast" within a wide range. At a distance of the rat's muzzle of about 5 cm from the device mouth, the experimental animals, on the one hand, demonstrated clear changes in their behavior related to the induction of BINT. On the other hand, damage to the traumatized rat induced by the mimicked "explosion" was accompanied by zero mortality. Thus, modeling of the light-degree BINT can be considered adequate.

The shock wave is the most striking and dangerous, according to its consequences, factor of explosion. This dramatic and very rapid change in the air pressure penetrates the defense system of the brain (skull bones and meninges) and destroys the blood–brain barrier (and also the cell structures in general). It should be taken into account that induced changes in the pressure, density, temperature, and rate of movement of the substance of brain tissues spread with a supersonic velocity.

There is another important mechanism responsible for damage of the cerebral structures; this is cavitation [15]. The latter is a physical phenomenon of the formation of vapor-gas vesicles within local zones of the low pressure in the fluid due to the action of the shock wave, with their subsequent destruction after getting to the areas with a high pressure. This generates local hydraulic shocks of superhigh frequency and pressure. Such shocks provide strong destructive mechanical effects (that can ruin even metal objects). Cavitation events readily appear within the zone of action of a shock wave with an initial high pressure and subsequent sharp drop in this parameter.

Within the dynamics of BINT-related phenomena, pathophysiologists distinguish two different pathogenic links connected with primary (mostly mechanical) injury [16] and subsequent secondary changes, mostly of a biochemical nature, which are related to neuroglial responses to the primary damage; both of these points should be subjected to in-depth study.

Modifications of brain functions related to the action of an explosion and modeling of light BINT can be studied by recording the behavioral phenomena demonstrated by the experimental animals. The open-field methodical approach is one of the most widespread techniques in studies of the actions of different factors on the state of the CNS. Registration of motor manifestations, orientational/research activity, and complex behavioral acts (like grooming) allows researchers to formulate well-based conclusions on the specificity of the integrative activity of the CNS related to the actions of the above factors. It should, however, be taken into account that most indices recorded in the open-field studies are not highly specific. In particular, VMA is believed to be a manifestation of orientational/explorative activity and inevitably includes some locomotor component. In turn, HMA also inevitably includes some explorative aspect. Correlation of the occurrence of grooming episodes with the emotional state of the animal is neither direct nor simple. Thus, the above conclusions on the specificities of the CNS activity can be formulated only after taking into account the entire complex of indices recorded in the course of an open-field study. Nonetheless, this test remains an adequate and sufficiently informative method of the respective studies [13] and is extensively used, e.g., in testing the effects of newly developed pharmacological agents.

Usually, animals put in novel unknown conditions feel uncertainty, increased anxiety, and even fear. In this case, rats try to examine, as soon as possible, the unknown environment by increasing both HMA and VMA and manifest stress signs. As a rule, these animals first avoid central zones of the open field and move mostly within outer squares of this field. Healthy animals become rather rapidly adapted to new conditions and begin to visit the central zone. At the same time, animals with some disorders of the CNS functions are unable to rapidly adapt to the unknown circumstances and demonstrate modifications of the above types of activity.

The dynamics of indices of behavioral activity in the open-field test, namely the intensity of HMA and VMA, number of examined burrows, and that of grooming episodes, which were demonstrated by experimental animals with mild BINT, give strong reasons to conclude that these animals, in comparison with sham and intact rats, are characterized by a high anxiety level within the acute period (days 1–7) [13]. Rats with BINT demonstrated uncertainty, preferred to move along the device walls, examined corners of the field, and avoided its center. This was combined with relatively increased locomotor activity on the first post-trauma day. The latter activity (HMA) began to decrease until day 3, while the activity qualified mostly as orientational/explorative one (VMA) was first strongly intensified (within days 1–7) and demonstrated a certain decline only later (after day 7). It should be emphasized that such behavioral phenomena as inspections of burrows, usually qualified as a "pure" research activity related mostly to a "social" aspect of behavior, were significantly suppressed in experimental (blast-subjected) rats within the entire period of observation, from its very beginning (day 1). Thus, there was some disagreement between the dynamics of modifications of different types of explorative activity.

As is supposed, highly intensified VMA is indicative of a high individual nonspecific excitability of an animal and may be related to domination of such individual in the population and "excessive" aggressiveness [13]. Therefore, it may be supposed that the aggressiveness of rats with BINT was temporarily increased within the first days after blast-induced TBI.

According to the existing concepts, grooming is an inherent component of normal innate behavior of rodents and is supposed to be a manifestation of the comfort state of the animal. The grooming pattern is believed to be one of the most informative indices of behavior under open-field conditions and is assumed to reflect the resistance of the animal to stress [13]. The constant significant decrease in the number of grooming episodes shown by rats with BINT (Fig. 4 A) is indicative of considerable disorders in integrative activity of the CNS responsible for the formation of normal behavior. It should be especially emphasized that grooming activity in rats with BINT undergoes not only quantitative but also qualitative modifications. Integral normal episodes of grooming are subjected to dissipation and are transformed into distorted abortive ones. In sham and intact rats, the latter episodes were very rare, while in rats of the Exp group these were relatively frequent (Fig. 4 B). This is one other confirmation of the statement that even light BINT may lead to significant disorders of the integrative cerebral activity. Animals with experimental light BINT are characterized by a low resistance to stress, i.e., their inherent adaptation mechanisms are significantly destroyed.

Therefore, our observations under conditions of the open-field test show that rats with light-degree BINT demonstrate significant disorders of behavior. Within the acute post-traumatic period (days 1–7), these disorders are related mostly to primary pathological effects of the action of blast-induced factors. Such disorders are based, to a significant extent, on high anxiety and accompanied by suppression of some aspects of behavioral activity. Within the early post-traumatic period (days 7–21), the respective modifications of behavior correspond to the development of depression, disorders of the adaptation mechanisms, and general suppression of orientational/research phenomena. Both above behavioral shifts (initial and later) are probably related to significant disorders in the neuromediator regulation and to hypoxic damage to the neurons [17]. Suppression of both types of motor activity (HMA beginning from day 3 and VMA from day 7) may be related to the development of neuroinflammation in the CNS of experimental animals. At the same time, high anxiety and fear within the later period are probably preserved to a significant extent, and the observed modifications are indicative of the organism's attempt to save resources for post-traumatic recovery [18]. These events are probably based on secondary damage to the brain against the background of neuroinflammation and neuromediatory disbalance.

All experimental procedures on animals were carried out in accordance with the European Directive of the Council of Communities of November 24 1986 (86/609 / EEC), as well as in full compliance with the Law of Ukraine of 21.02.06, № 3447-IV "On protection of animals from cruel treatment."

The author, Yu. V. Kozlova, confirms the absence of any conflicts over commercial or financial relations, relations with organizations or individuals that could in any way be related to the study.

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