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RADIATION PHYSIOLOGY

The Role of Typological Characteristics of Higher Nervous Activity in Rats in the Neurobiological Effects of Combined Exposure to an Antiorthostatic Suspension, γ-Rays, Protons, and Carbon ^{12}C Ions

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Abstract—The study of the role of typological characteristics of higher nervous activity of rats in the neurobiological effects (rat behavior and its neurochemical mechanisms) of combined exposure to a 10-day antiorthostatic suspension, γ-, and high-energy proton and carbon ion (^{12}C) irradiation demonstrated that the ^{12}C ion irradiation caused more marked distortion of the ratio of orientation and exploratory behavior and passive defensive behavior/anxiety in rats of the excitable anxious emotional type. In the experiment with proton irradiation, the differences between typological groups were more smoothed out, though some parameters of passive defensive behavior in the rats of the experimental groups were also increased. These changes were accompanied by decreased monoamine metabolism in the prefrontal cortex.

Keywords: antiorthostatic suspension, γ-irradiation, high energy protons, carbon ^{12}C ions, typological characteristics of higher nervous activity in rats, rat behavior, monoamine metabolism

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INTRODUCTION

The study of the effects of space radiation on the functions of the central nervous system (CNS) has recently become especially relevant due to planning and preparation for long-duration space missions (particularly the Martian mission). One of the major limiting factors in such missions is radiation. Together with other spaceflight factors, it can lead to impairment of CNS functions underlying the operator activities of cosmonauts. At the same time, the main hazard is galactic cosmic rays, i.e., high-energy protons and heavy ions with energies in a broad range up to ultra-high energies of about 10^{20} MeV [1].

One of the key (though least studied) problems is the combined effect of ionizing radiation and nonradiation space flight factors, the most important of them being hypogravity [2, 3]. The generally accepted model of hypogravity for small laboratory animals is antiorthostatic suspension (AOS) [2, 4]. In our previous works, we have studied various neurobiological effects of combined exposure to AOS and different types of ionizing radiation [5–7].

The important role of individual and typological peculiarities in the functional response of the central nervous system to radiation has been emphasized by most researchers studying the effects of radiation on CNS functions; however, there are comparatively limited systematic data on this issue, and they were obtained long ago [8–11]. The significance of typological peculiarities of higher nervous activity (HNA) for neurobiological effects under combined exposure to radiation and nonradiation factors has not been investigated previously.

Hence, the present work was aimed at studying changes in animals' behavior and the indices of monoamine metabolism in the key cerebral structures of animals with different typological peculiarities of HNA under combined exposure to AOS and three types of ionizing radiation: γ-radiation, high-energy protons, and carbon ^{12}C ions.

MATERIALS AND METHODS

Individual Plexiglas sections (42 × 42 × 40 cm) were arranged into two stacks, 15 sections each, and used for AOS. Animals were suspended by the base of the tail at an angle of 30°–40° to remove the static load from the hind limbs. At the same time, rats were fixed with special clasps fitted to a metal rod, so that they

could move freely within the cage. The resultant antiorthostasis caused redistribution of rat body fluids and removal of the static load from thehind limbs. This technique is the generally accepted ground-based microgravity model for small laboratory animals.

The stacks with suspended animals were put into a radiation chamber: the stack with rats was exposed to both AOS and γ-radiation in the irradiated area at a distance of 3.3 m from the source of radiation, so that all rats would be uniformly irradiated; the stack with rats exposed only to AOS was placed in the same room but beyond the irradiated region. The rats exposed only to radiation were placed on the stack in their home cages.

Two experiments were conducted to investigate the neurobiological effects of synchronous combined exposure to AOS and ionizing radiation, taking into account the typological peculiarities of HNA of the animals. AOS was continued for 10 days. The γ-ray unit GOBO-60 with a ^{137}Cs source was used (72 g-eq. Ra). The radiation intensity was 2.34 sGy/h. There was one 24-h irradiation session three days after suspension. In the first experiment, after termination of γ-irradiation exposure and removal of rats from the suspension apparatus, there was a single exposure of animals' heads to carbon ^{12}C ion irradiation (energy, 420 MeV; dose, 1 Gy) in a U-70 accelerator at the Institute for High Energy Physics (Protvino). In the second experiment, there was a single exposure of animals' heads with proton irradiation (energy, 170 MeV; absorbed dose, 1 Gy) in a proton accelerator at the Tsyb Medical Radiological Research Center (Obninsk).

The irradiation mode was based on the following: a total dose of 3 Gy for rats (with due consideration of their species-specific radiation sensitivity) approximately corresponds to the calculated dose that can be received by cosmonauts during an interplanetary flight.

The following generally accepted techniques were used: neurochemical methods (high-performance liquid chromatography, HPLC) and behavioral methods ("open field," elevated plus maze, Morris water maze test, the conditioned reflex of active avoidance).

The animals were typologized by conditioning the reflex of avoidance of a closed (dark) space ("emotional resonance") according to the modified technique of P.V. Simonov) [12]. The experimental chamber consisted of open (light) and closed (dark) parts. Each experiment was carried out with two rats: an experimental rat and a "victim" rat. Throughout 4–6 experiments, each experimental rat was placed into the open (light) part of the chamber. It was a stress situation for rats, and they moved to the dark compartment of the chamber (the hole exploratory behavior). The stay of experimental rat in the dark compartment was accompanied by a painful electrical stimulation of the "victim" rat causing its anxious vocalizing and motor responses. The total time of the experimental rat staying in the closed compartment and the frequency of going out were recorded during 5-min observation. It has been shown that the differences in animals' behavior in this situation represent their individual typological peculiarities of higher nervous activity and provide the possibility of using this technique for the initial grouping of animals according to this feature [11, 13, 14].

The "open field" technique was used in behavioral studies to find out the levels of locomotor, orientation, and exploratory activities and the ratio of active vs. passive defensive reactions under moderate stress conditions, as well as the efficiency of extinction of these reactions. The open field (OF) was a circle of 100 cm in diameter, divided into sectors with a hole in the center of each sector. The rat was placed into the OF center, and the following parameters were recorded during 4 min of testing (separately for the first and last 2 min): horizontal motor activity (by the number of crossed lines), vertical (orientation) activity (by the number of standing positions), exploratory behavior (by the number of burrow visits), the number of goings out into the OF center and the latent period of orientation and explorative reaction (the time of leaving the center of the ground), and the number of grooming acts and emotional reactivity (by the number of fecal boluses). The tests were carried out under bright artificial light.

The conditioned active avoidance reflex (AAR) was developed in a shuttle chamber (9 × 60 cm, 30 cm in height) divided by a partition into two equal compartments with an independent electric grid floor. The partition had a rectangular hole of 8 cm in width and 6 cm in height with an externally controlled door moving along the plane of the opening. The removable lid of the chamber had two independent built-in lamps located (with the lid closed) in the center of the compartment. An animal was placed into one of the chambers with the compartment door closed and the lights turned off. The chamber was closed with the lid; then the door between the compartments was opened. The animal was taken out in reverse order. The test was started with turning on the light in the compartment with the rat. After 6 s, the electric current (0.5 mA, 100 Hz, meander) was applied to the electrode floor until the rat moved into the other compartment with the light turned on and the absence of current. After the rat had moved, the light was turned off, with the maintenance of a 10-s pause. Then the same sequence of operations was repeated for the number of times specified (a single series of combinations). The number of avoidance reactions (the runs on the conditioned signal) and escape reactions (the runs under the exposure to electric current) was recorded. The learning index was calculated: the ratio of the number of avoidance reactions to the total number of combinations.

The hippocampus-dependent behavior of rats was studied in the Morris water maze, which is a common

test for assessing spatial memory and orientation. The Morris water maze was a light blue pool (150 cm in diameter) with walls of 40 cm in height and a water depth of 21 cm. A round transparent Plexiglas platform of 10 cm in diameter was located at the geometric center of one of the four sectors of the pool; the platform was hidden beneath the surface of water at a depth of 1 cm and thus was invisible for animals against the light blue background; the water was not colored. The platform remained in the same place throughout the experiment. A rat was put into water in a random place of the pool and let swim freely for 60 s. In one single training event, all tested rats were put into the pool at the same place. If a rat found the platform within fewer than 60 s, the finding time was recorded and the animal was left on the platform for 20 s for orientation. Then the rat was taken off the platform and put again into the pool in another random place; thus, two training events comprised one session. There were two sessions per day with a 4-h interval. If a rat could not find the platform, the finding time was recorded as 60 s. The test was carried out for six days. On day 7, a single-stage test without the platform was performed for 60 s, with recording the time the rats stayed in the sector where the platform had previously been located.

The anxiety of experimental animals was assessed in an elevated plus maze with compartments of 50 cm in length and an alley of 10 cm in width; the walls of enclosed compartments were 40 cm high. The light intensity in the center of the enclosed and not enclosed compartments was 12 and 60 lx, respectively. A rat was put into the corner of the enclosed compartment of the maze (the same for all tested animals), and the following parameters were recorded: the latent period of going out into the unenclosed compartment, the number of such goings out, and the time of stay in the unenclosed compartment of the maze.

The results were averaged for each group with respect to the tested parameter, followed by statistical data analysis. A statistical criterion was One-Way Analysis of Variance (one-way ANOVA) and a post-hoc Duncan Multiple Range Test in case of differences in dispersion. The level of significance in all tests was taken to be $p < 0.05$.

The data obtained in the Morris water maze test were analyzed taking the average time of reaching the platform per day; the data were also assessed by the one-way ANOVA and by the post-hoc Duncan test in case of differences in dispersion.

Neurochemical studies. For neurochemical studies, the control and experimental rats were decapitated in ten days and the following brain structures were extracted: the prefrontal cortex, the nucleus accumbens, the hypothalamus, the hippocampus, and the striatum. Brain structures were frozen in liquid nitrogen and weighed. The extracted structures were homogenized at 4°C in a glass homogenizer with a Teflon pestle (0.2 mm) at 3000 rpm. The homogenization and isolation medium was 0.1 N $HClO_4$ with DOBA (3,4-dioxybenzylamine), where no catecholamine substance was found in native tissues, at a concentration of 0.5 nmol/mL as an internal standard. Nucleus accumbens and other brain structures were homogenized in 40 and 20 volumes of the isolation medium, respectively. The samples were centrifuged at 4°C, 10000 *g*, for 15 min. The supernatant was further used to detect monoamines and their metabolites.

The concentrations of monoamines and their metabolites were determined by high-performance liquid chromatography (ion-pair chromatography) with electrochemical detection in LC-304T (BAS, West Lafayette, United States) with a Rheodyne 7125 injector and a 20-μL loop for sample application. Substances were separated in a ReproSil-Pur reversed-phase column, ODS-3, 4 × 100 mm, 3 μm (Dr. Majsch GMBH, Elsiko, Moscow). The pump was PM-80 (BAS, United States); the mobile phase velocity was 1.0 mL/min at 200 atm. Mobile phase: 0.1 mol/L citrate phosphate buffer containing 1.1 mmol/L octanesulfonic acid, 0.1 mmol/L EDTA, and 9% acetonitrile (pH 3.0). The flow rate was 1 mL/min. Measurements were made with a LC-4B electrochemical detector (BAS, United States) on a glass-carbon electrode (0.85 V) vs. the Ag/AgCl reference electrode. The samples were recorded with the MULTICHROME 1.5 (AMPERSEND) hardware and software complex. All reagents used for the analysis were of high purity: ultrapure, chemically pure, or analytical grade. Mixtures of the working standards of the tested substances (500 pmol/mL) were used to calibrate the chromatograph. Monoamine concentrations in experimental samples were calculated by the method of internal standards based on the ratios of peak areas in the standard mixture and in the sample. The levels of noradrenalin (NA), dopamine (DA), and its metabolites 3,4-dioxyphenylacetic acid (DOPAA) and homovanillic acid (HVA), 3-methoxytyramine (3-MT), serotonin (5-oxytryptamine, 5-OT), and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined in five brain structures: the prefrontal cortex, the nucleus accumbens, the hypothalamus, the hippocampus, and the striatum.

The results of neurochemical studies were expressed as the mean ± standard error of the mean. The results were analyzed by one-way ANOVA and the post-hoc Duncan test. The statistically significant result was taken to be $p <$ or $= 0.05$. A p value of 0.05 to 0.099 was considered as the trend of change.

RESULTS AND DISCUSSION

Figure 1 shows the results of the tests with rats in Simonov's chamber: the results of developing the conditioned reflex of avoidance of a closed (dark) space by the squeal of an affected animal. Based on the results

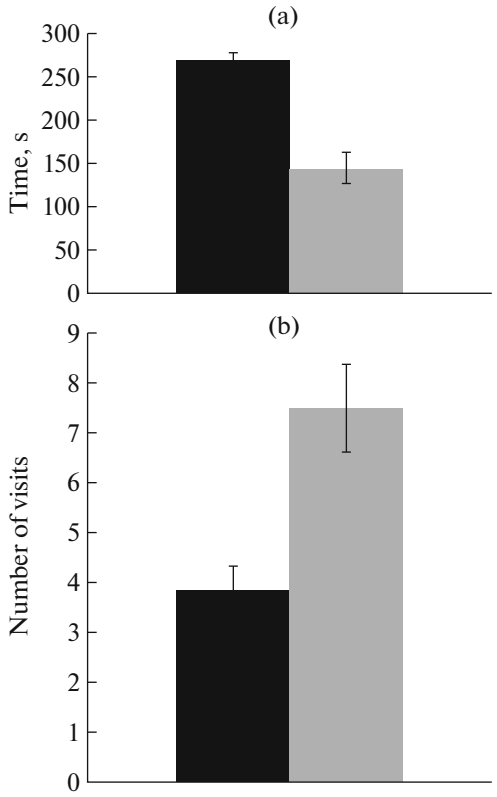

Fig. 1. The results of animal testing in Simonov's chamber. Group 1 (black columns); group 2 (gray columns). (a) The time of rats' stay in the closed (dark) space; (b) the number of visits to the dark space.

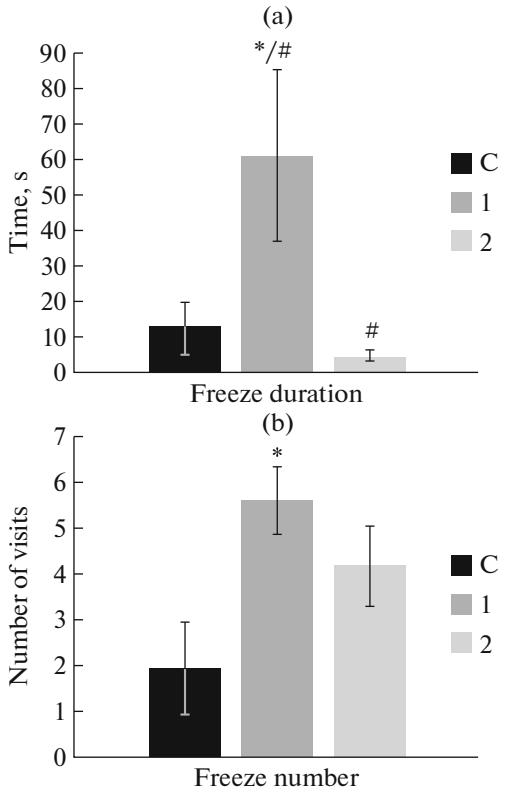

Fig. 2. The values of freeze responses in the "open field" in rats from experimental groups. (A) The average time of freezing; (b) the number of freeze responses.

of these tests, the typological groups of rats can be characterized as follows:

(1) With the predominance of excitation, emotional instability, high anxiety;

(2) With the predominance of inhibition, reduced emotionality, low anxiety.

One can see that the rats of group 1 are characterized by high values for the time of stay in the closed (dark) compartment of the chamber (Fig. 1a) and a lower number of goings out (Fig. 1b).

In the experiment with the exposure to AOS, γ-irradiation, and carbon ^{12}C ion irradiation, the study of rat behavior in the "open field" showed a considerable, statistically significant increase in the indices of passive defensive behavior for rats with the an initially increased excitability and anxiety, as is characterized by high values of the duration and number of freeze responses in these animals (Fig. 2). The indices of active behavior in the "open field" did not change reliably.

These data are generally confirmed by the results of investigation of animals' behavior in the elevated plus maze, a commonly used anxiety test. The rats of group 1 were characterized by the lowest values of stay in the open compartment of the maze (Fig. 3b) and a large number of runs between the compartments (Fig. 3a).

The Morris water maze test for spatial orientation and memory showed a reliable increase in the rate of learning in rats from the experimental groups, more marked in animals with predominant excitation (Fig. 4).

In the experiment with exposure to AOS, γ-irradiation, and high-energy proton irradiation, investigation of rat behavior in the "open field" showed a significant increase in the indices of passive defensive behavior of rats from the experimental group but did not reveal any reliable differences between the typological groups with respect to the duration of freeze responses (Fig. 5a). There was a reliable increase in the number of urination acts in excitable (emotional, highly anxious) rats, which is evidence of stimulation of emotionality and anxiety in animals exposed to the factors under study (Fig. 5b). The indices of active behavior of rats from the experimental groups in the "open field" did not change significantly after the exposure.

The investigation of animals' anxiety in the elevated plus maze test showed a sharp increase in the latent period of going out into the open compartment of the maze in excitable rats after experimental exposures and, at the same time, a sharp increase in the number of visits to this compartment and in the time of staying there (Fig. 6).

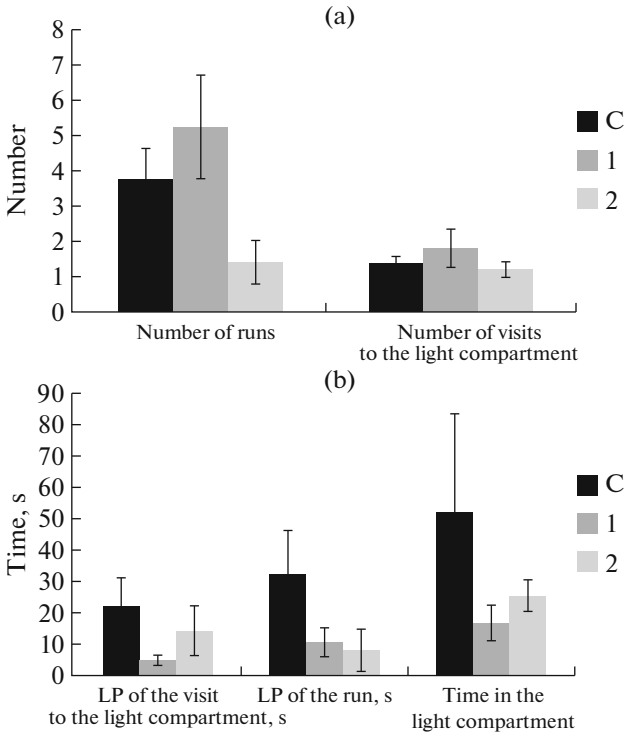

Fig. 3. The behavioral indices of rats from experimental groups in the elevated plus maze. (a) Number of responses; (b) time, s.

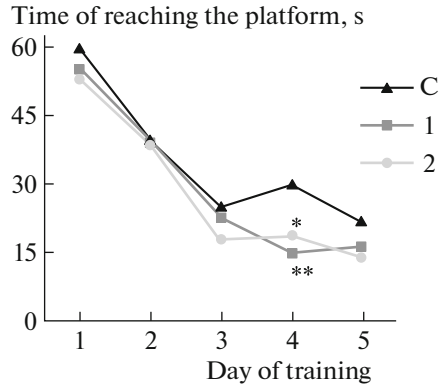

Fig. 4. The behavioral indices of rats from experimental groups in the Morris water maze. C, control; SR_A, group 1; SR_R, group 2.

The Morris water maze test with rats from the experimental groups showed no significant difference in the rate of learning (Fig. 7).

For assessing the cognitive functions of experimental animals, their learning rate was studied by the method of active avoidance conditioning in a "shuttle chamber." The results of this study are shown in Fig. 8. One can see a significant decrease in the learning rate of rats of the inhibitory type (with low anxiety) after experimental exposures compared to rats of the excitable type and to both control groups.

Thus, the comparison of the two experiments aimed at studying the combined effect of AOS and ionizing radiation different in only one factor (with carbon ^{12}C ion and high-energy proton irradiations in the first and second case, respectively) on the behavior of experimental animals showed the following results.

The contribution of carbon ^{12}C ion irradiation leads to more marked distortions of the ratio between orientation/exploratory behavior and passive defensive behavior/anxiety under moderate stress conditions in the "open field" and elevated plus maze tests compared to proton irradiation, which is more marked in excitable, anxious, emotional rats. In this experiment, the typological differences in animal behavior were most pronounced.

Training improvement in the group of irradiated rats demonstrated by the Morris water maze test has been known for a long time and shown for rats [15] and monkeys [16, 17]. The causes of this phenomenon have not been fully elucidated up to now. It is probably due to the so-called attentional narrowing, i.e., the suppression of actuating mechanisms of the orienting response in the reticular formation and in the thalamus (as a result, animals pay less attention to extraneous stimuli); alternatively, there could be the activation of compensatory processes in the CNS caused by irradiation at the first stage of radioreaction.

In the proton irradiation experiment, the differences between the typological groups proved to be more smoothed; however, with respect to some parameters, the passive defensive components of behavior and anxiety in the rats of the experimental groups were also higher. It seems that the training of inhibitory-type rats in the AAR test slows down due to the development of protective inhibition under the influence of defensive reinforcement.

With respect to the results of neurochemical studies, the most marked changes are observed in the prefrontal cortex. The rats of group 1 demonstrate a reliable decrease in the DOPAA, HVA, and 3-MT concentrations. The rats of group 2 show a reliable decrease in the DOPAA and 3-MT concentrations. In addition, there is a reliable decrease in the 5-HIAA concentration in the rats of group 2 relative to the animals of group 1 (Table 1).

Considerable changes in the concentrations of monoamines and their metabolites in the prefrontal cortex of animals decapitated at the early stages of research (in this case, 24 h after the exposure) have been shown many times, in particular, 24 h after the carbon ^{12}C ion irradiation of rats [18], but not after 30 and 90 days [19]. Analogous changes were observed in WAG/Raj rats at early stages of the formation of absence epilepsy, as well as in response to pharmacological effects (introduction of Madopar), 24 h after high-energy proton irradiation, both in the drift [20] and in the Bragg peak position [21].

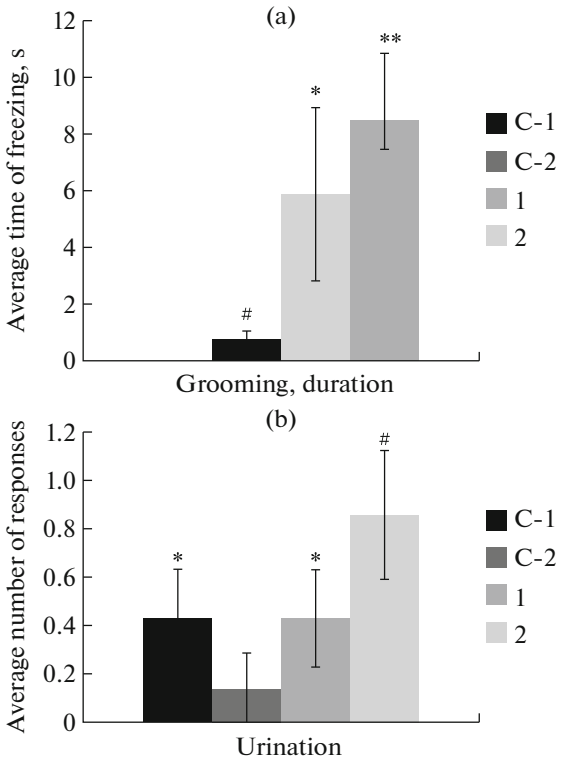

Fig. 5. The indices of passive defensive behavior, anxiety, and emotionality of rats from the experimental groups in the open field test after experimental exposure. (a) The time of freeze responses; (b) the number of urination acts. C-R, the control to group 1; C-A, the control to group 2. Other designations are as in Fig. 2.

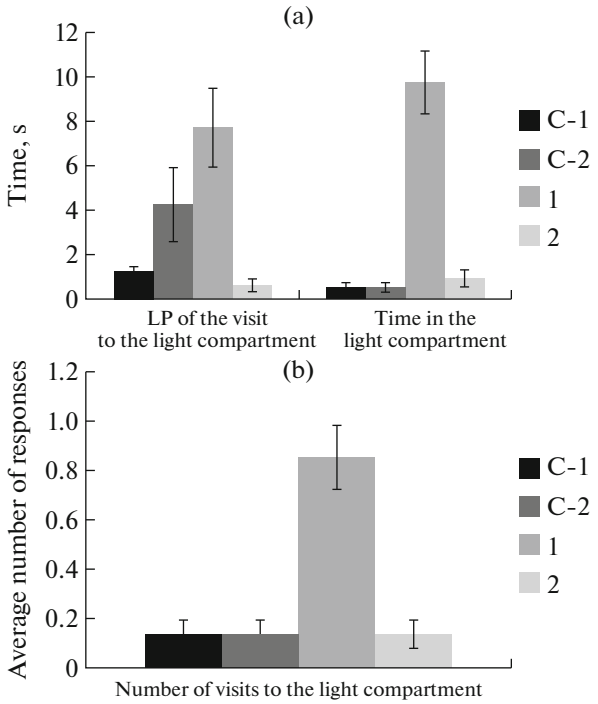

Fig. 6. The behavioral indices of rats from experimental groups in the elevated plus maze. (a) The latent time of rats going out into the open space (left) and the time of rats staying in the open space (right); (b) the number of crossings between the compartments. Other designations are as in Fig. 5.

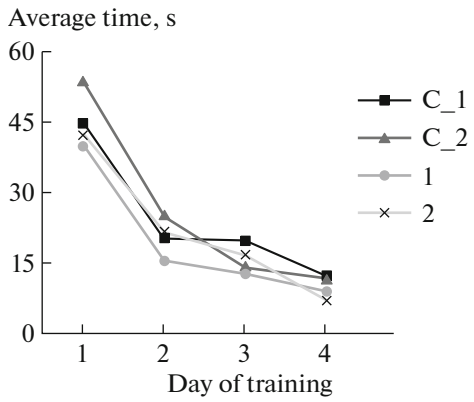

Fig. 7. The behavioral indices of rats from experimental groups in the Morris water maze test. Designations are as in Figs. 5 and 6.

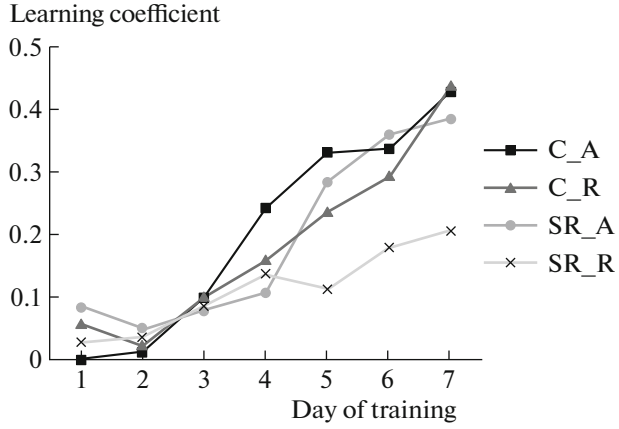

Fig. 8. The indices of training the rats from experimental groups in the shuttle box (the conditioned reflexes of active avoidance). Designations are as in Fig. 5.

The high sensitivity and reactivity of the prefrontal cortex relative to other tested brain structures is a fundamental property of functioning of this structure. Some works show increased stress reactivity of the rat prefrontal cortex, particularly [22]. The medial prefrontal cortex of rats is one of the most important units of the rapid learning system that retrieves recent and remote memories [23]. There was rapid enhancement of the functional coupling between the lateral prefrontal cortex, the basal ganglia (the caudate nucleus and the neostriatum, the putamen), and the orbitofrontal cortex, as well as between the lateral prefrontal cortex and the premotor cortex [24]. The superior dorsolateral prefrontal cortex showed a rapid response to the

Table 1. The concentrations of monoamines and their metabolites (nmol/g tissue) in the prefrontal cortex of rats

Group	NA	DOPAA	DA	HVA	3-MT	5-HIAA	5-OT
Control	6.09 ± 1.15	1.18 ± 0.07	1.51 ± 0.24	0.70 ± 0.13	0.47 ± 0.07	4.31 ± 0.37	6.73 ± 0.49
Group 1	5.79 ± 0.49	0.87 ± 0.05	1.04 ± 0.04	0.28 ± 0.07	0.23 ± 0.06	3.64 ± 0.14	6.65 ± 0.36
p = 1 vs. 2	0.833	**0.010**	0.123	**0.033**	**0.032**	0.173	0.913
Group 2	5.36 ± 0.43	0.82 ± 0.06	1.09 ± 0.03	0.45 ± 0.07	0.18 ± 0.07	3.07 ± 0.11	7.15 ± 0.24
p = 1 vs. 3	0.607	**0.007**	0.166	0.171	**0.039**	**0.021**	0.511
p = 2 vs. 3	0.571	0.595	0.326	0.163	0.723	**0.022**	0.332

Hereinafter: Control, the control group; group 1, experimental group 1; group 2, experimental group 2. Statistically significant values $p <$ or = 0.05 are in bold; p from 0.05 to 0.099 was considered the trend of change (bold italic).

Table 2. The concentrations of monoamines and their metabolites (nmol/g tissue) in the nucleus accumbens of rats

Group	NA	DOPAA	DA	HVA	3-MT	5-HIAA	5-OT
Control	2.71 ± 0.65	5.13 ± 0.52	36.73 ± 5.92	3.69 ± 0.33	0.50 ± 0.09	1.45 ± 0.27	1.84 ± 0.28
Group 1	2.47 ± 0.45	4.81 ± 0.42	39.87 ± 4.51	2.88 ± 0.1	0.72 ± 0.15	1.97 ± 0.27	2.01 ± 0.28
p = 1 vs. 2	0.790	0.678	0.716	**0.067**	0.290	0.262	0.706
Group 2	3.16 ± 0.34	3.03 ± 0.78	43.65 ± 6.51	4.19 ± 0.64	0.60 ± 0.09	1.72 ± 0.42	2.24 ± 0.43
p = 1 vs. 3	0.601	0.922	0.502	0.551	0.509	0.652	0.505
p = 2 vs. 3	0.305	0.831	0.680	0.110	0.537	0.665	0.703

Table 3. The concentrations of monoamines and their metabolites (nmol/g tissue) in the hippocampus of rats

Group	NA	DOPAA	DA	HVA	3-MT	5-HIAA	5-OT
Control	7.79 ± 1.13	0.82 ± 0.27	0.68 ± 0.24	0.49 ± 0.11	0.35 ± 0.08	13.03 ± 1.44	7.88 ± 0.62
Group 1	7.40 ± 0.80	0.41 ± 0.07	0.54 ± 0.18	0.35 ± 0.05	0.32 ± 0.06	14.07 ± 1.05	8.38 ± 0.95
p = 1 vs. 2	0.907	0.218	0.679	0.337	0.795	0.618	0.702
Group 2	7.99 ± 0.77	0.55 ± 0.18	0.88 ± 0.22	0.30 ± 0.05	0.24 ± 0.03	13.52 ± 1.44	9.39 ± 0.64
p = 1 vs. 3	0.801	0.472	0.595	0.198	0.306	0.837	0.165
p = 2 vs. 3	0.644	0.523	0.315	0.523	0.335	0.789	0.449

Table 4. The concentrations of monoamines and their metabolites (nmol/g tissue) in the striatum of rats

Group	NA	DOPAA	DA	HVA	3-MT	5-HIAA	5-OT
Control	0.52 ± 0.12	4.75 ± 0.36	44.16 ± 3.08	4.07 ± 0.30	0.8 ± 0.09	3.87 ± 0.33	1.450.13
Group 1	0.98 ± 0.3	5.67 ± 1.51	59.29 ± 10.03	4.27 ± 1.15	1.23 ± 0.39	4.34 ± 2.00	2.00 ± 0.72
p = 1 vs. 2	0.234	0.631	0.505	0.880	0.463	0.534	0.570
Group 2	0.68 ± 0.11	4.05 ± 0.32	45.54 ± 2.93	2.64 ± 0.19	0.84 ± 0.1	3.14 ± 0.40	1.74 ± 0.08
p = 1 vs. 3	0.405	0.227	0.828	**0.022**	0.752	0.652	0.171
p = 2 vs. 3	0.428	0.403	0.535	0.338	0.407	0.613	0.774

encoding of new stimuli in tasks requiring high-level working memory [25].

In addition, the cellular mechanisms contributing to rapid responses of neurons of the prefrontal cortex have been revealed. The interaction between inhibitory fast-spiking interneurons and excitatory pyramidal neurons in rats facilitates the implementation of fundamental properties of cortical networks. The key functions of fast-spiking interneurons are the rapid inhibition in local networks of the sensory and motor cortex and the processing of information received by the cortex from the thalamus. These results demonstrate the presence of different preferential subnets or local networks between fast-spiking interneurons and pyramidal cells of the prefrontal cortex in rats, which can be specific for this cortical area [26]. Rapid DAer-

Table 5. The concentrations of monoamines and their metabolites (nmol/g tissue) in the hypothalamus of rats

Group	NA	DOPAA	DA	HVA	3-MT	5-HIAA	5-OT
Control	6.70 ± 0.16	0.33 ± 0.03	2.43 ± 0.25	0.31 ± 0.04	0.10 ± 0.01	3.34 ± 0.23	4.24 ± 0.13
Group 1	6.96 ± 0.28	0.31 ± 0.05	1.95 ± 0.16	0.33 ± 0.07	0.15 ± 0.08	3.60 ± 0.19	4.06 ± 0.12
$p = 1$ vs. 2	0.498	0.741	0.184	0.803	0.528	0.464	0.392
Group 2	7.27 ± 0.64	0.22 ± 0.03	1.69 ± 0.18	0.24 ± 0.04	0.10 ± 0.02	3.04 ± 0.43	3.77 ± 0.05
$p = 1$ vs. 3	0.459	**0.023**	**0.063**	0.307	0.915	0.597	0.436
$p = 2$ vs. 3	0.697	0.189	0.355	0.333	0.552	0.321	0.621

Table 6. The concentrations of monoamines and their metabolites (nmol/g tissue) in the amygdala of rats

Group	NA	DOPAA	DA	HVA	3-MT	5-HIAA	5-OT
Control	1.93 ± 0.41	0.87 ± 0.23	9.24 ± 3.13	1.25 ± 0.45	1.01 ± 0.26	4.75 ± 0.09	3.93 ± 0.67
Group 1	0.81 ± 0.08	1.42 ± 0.31	15.03 ± 3.66	1.68 ± 0.32	0.63 ± 0.12	5.22 ± 0.45	3.02 ± 0.38
$p = 1$ vs. 2	**0.046**	0.237	0.249	0.508	0.273	0.688	0.318
Group 2	1.76 ± 0.19	1.52 ± 0.34	15.75 ± 3.73	1.89 ± 0.45	1.46 ± 0.39	5.41 ± 0.08	3.72 ± 0.66
$p = 1$ vs. 3	0.755	0.201	0.266	0.397	0.411	0.641	0.845
$p = 2$ vs. 3	**0.003**	0.857	0.976	0.862	0.741	0.104	0.435

gic modulation of calcium and fast potentials in the dendrites of pyramidal neurons of the rat prefrontal cortex has been revealed [27]. In the prefrontal cortex, DAergic modulation is implemented in less than 0.5 s, whereas in other structures it takes several seconds.

The prefrontal cortex not only forms and controls emotional and motivational states [28, 29] but also plays a key role in cognitive processes [30]. Human cognitive processes can be defined as the acquisition of knowledge and experience and their further applications, while in animals it is the acquisition of experience and its further application. One more basic function of the prefrontal cortex in animal behavior is decision making and selection of action [31] also associated with cognitive activity.

There were no considerable changes in the nucleus accumbens and in the hippocampus (Tables 2, 3).

In the striatum (Table 4), the HVA concentration decreased in the rats of group 2 relative to the control.

In the hypothalamus (Table 5), there was a decrease in the DOPAA concentration in the rats of group 2 relative to the control. The NA concentration in the amygdala (Table 6) decreased in the rats of group 1 relative to the control and to the rats of group 2.

Thus, the decreased activity of the DA system can be considered the major response of monoaminergic systems to the experimental exposures used in the present work.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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