ISSN 1819-7124, Neurochemical Journal, 2017, Vol. 11, No. 2, pp. 168–175. © Pleiades Publishing, Ltd., 2017.
Original Russian Text © A.G. Belyaeva, A.S. Shtemberg, A.M. Nosovskii, O.N. Vasil'eva, Yu.V. Gordeev, V.S. Kudrin, V.B. Narkevich, E.A. Krasavin, G.N. Timoshenko, B.A. Lapin, A.S. Bazyan, 2017, published in Neirokhimiya, 2017, Vol. 34, No. 2, pp. 168–176.

EXPERIMENTAL ARTICLES

The Effects of High-Energy Protons and Carbon Ions (^{12}C) on the Cognitive Function and the Content of Monoamines and Their Metabolites in Peripheral Blood in Monkeys

A. G. Belyaeva[a], A. S. Shtemberg[a, d], A. M. Nosovskii[a], O. N. Vasil'eva[a], Yu. V. Gordeev[a], V. S. Kudrin[a, c], V. B. Narkevich[c], E. A. Krasavin[d], G. N. Timoshenko[d], B. A. Lapin[e], and A. S. Bazyan[a, b, d, 1]

[a] *State Scientific Center of the Russian Federation, Institute for Medical and Scientific Problems, Russian Academy of Sciences, Moscow, Russia*

[b] *Institute for Higher Nervous Function and Neurophysiology, Russian Academy of Sciences, Moscow, Russia*

[c] *Zakusov Institute of Pharmacology, Russian Academy of Sciences, Moscow, Russia*

[d] *Joint Institute for Nuclear Research, Dubna, Moscow Region, Russia*

[e] *Research Institute for Medical Primatology, Russian Academy of Sciences, Sochi, Russia*

Received June 29, 2016; in final form, August 26, 2016

Abstract—Model experiments using monkeys (rhesus macaque monkeys) were performed to investigate the neurobiological effects of two components of galactic cosmic radiation, namely, high-energy protons and carbon (^{12}C) ions. It has been demonstrated that the irradiation of a monkey's head with protons at a dose of 3 Gy did not cause any significant changes in the animal's cognitive function and in the concentrations of monoamines and their metabolites in the peripheral blood. However, exposure to carbon ions at a dose of 1 Gy resulted in a significant cognitive function impairment and a significant decrease in serotonin metabolite concentrations in the blood in the monkeys with the excitable imbalanced type of higher nervous activity.

Keywords: exposure to radiation, learning, monkeys, blood, monoamines

DOI: 10.1134/S1819712417010032

INTRODUCTION

Studies on the effects of cosmic radiation on central nervous system functions are becoming more relevant in the view of the planning and preparation of long-term deep-space missions, in particular, the Mars One Mission. In these missions when a spaceship leaves the Earth's magnetosphere one of the factors that compromise the feasibility of the mission is radiation which, when combined with other stress factors in the space flight, may cause the impairment of CNS functions that determine the astronaut's operator performance. This, in its turn, puts the astronaut's ability to perform flight tasks at risk and poses a real threat to the astronaut's life. Note that, unlike the delayed stochastic radiation effects (that is, probable effects that may or may not manifest themselves some time after the exposure) such as carcinogenesis, cataractogenesis, cytogenetic abnormalities, decreased longevity, etc., ergonomic risks associated with the possible impairment of operator performance may pose a threat to astronaut lives during the space mission. The greatest danger is posed by galactic cosmic radiation, in particular, high-energy protons and heavy ions with energies varying in a broad range up to the ultra-high energies of approximately 10^{20} MeV. It is extremely difficult to protect astronauts from such highly energetic radiation under spaceship conditions.

Therefore, we may speak of a paradigm shift in the area of assessment of radiation hazards since the study of radiation hazards in interplanetary missions is substantially different from that of the hazards in orbital flights. While the latter research is mostly focused on the assessment of delayed stochastic effects, the former research places a premium on assessing the risks of functional impairments in the central nervous system and, consequently, the impairment of operator performance by astronauts.

It should be emphasized that to obtain experimental data that may be used to estimate the probability of impairment of the operator performance of astronauts during an interplanetary mission, as well as to extrapolate the obtained results to a human individual and to estimate the possible threat that these impairments would pose to accomplishment of flight tasks, it is necessary to model certain types of operational activi-

[1] Corresponding author; address: ul. Butlerova 5a, Moscow, 117485 Russia; phone: +7(495)334-7789; e-mail: bazyan@mail.ru.

ties and to study the neural and neurochemical mechanisms that underlie the observed dysfunctions. From this perspective, the experiments with monkeys, which imitate certain operator activities, and analysis of the neurochemical mechanisms governing the processes of interest seems to be the most informative approach. However, we failed to find any data of this kind in the available literature.

In view of all the above issues, the aim of the current work was to study the neurobiological effects of two types of radiation that are typical of open space, namely, high-energy protons and ^{12}C ions, in primate experiments.

MATERIALS AND METHODS

The study involved six 4-year-old male rhesus macaque monkeys (*Macaca mulatta*) weighing from 5–7 kg: three experimental animals (monkeys nos. 257, 311, and 347) and three control animals (monkeys nos. 275, 343, and 344). The animal handling procedures and the experimental outline were approved by the Biomedical Ethics Committee of the Institute for Medical and Biological Problems of the Russian Academy of Sciences

The animals were trained for 12 months according to a psychological testing system that simulates certain elements of operational activity, in particular, monitoring; we modified the system, which was originally developed by American scientists [1, 2]. The experimental unit for the study of the primate higher nervous activity consisted of a computer mainframe, monitor, programmable feeding station for food reinforcement, and a joystick. The joystick design allowed the animals to easily learn different motion types to complete both simple and sophisticated computer tasks. The extremely high motivation of monkeys for game-like testing programs is a substantial argument in favor of using computer-based approaches. The game stimulates the general activity of the animals. During a single experimental day, they can make from 100 to over 500 attempts (instrumental movements) in order to earn a food reward, which is a dosed banana-fruit tablet. Importantly, successful task accomplishment by the monkey does not require any assistance by the researcher, or even their physical presence. The tests are arranged in an order in the software that, first of all, allows monkeys to learn the general approach to earning a food reward. The essential condition is that the cursor should point to a so-called target on the monitor screen. The direction of the cursor movements is determined by the movements of the joystick, which is easily accessible by the monkey by hand when seated in a primate chair or placed in a home cage. The tasks are offered in the following order: first, a blue spot appears on the screen and the cursor must be hovered over it; this area then shrinks to the size of a small rectangle ("target"), which begins to move in a random direction; finally, a circle appears, which embodies a visible "target" that is, however, inaccessible for the cursor. The task is to touch the "target" with the cursor. The initially chaotic manipulations rather quickly (approximately 1–2 days for almost all animals) transform into target-oriented movements as the monkey comes to the understanding, while watching the screen, which is placed close to the cage, that there is an association between the moment of the cursor's touching the target and food-reward delivery, which is accompanied by an acoustic signal. Thus, in such a manner the established eye-hand coordination develops into an instrumental motor-conditioned reflex.

The circle testing regimen was chosen for the experiments. The monitor screen displays a circle-shaped figure whose size may be set by the researcher. The cursor is able to contact the "target" only when it is outside the circle. In the course of the test, the "target" is in constant movement entering the circle and is inaccessible to the cursor when it moves across it.

In the course of training, the total number of motor responses (the number of attempts to accomplish the task) and the percentage of successful attempts were recorded. The monkeys were trained at the Institute of Medical and Biological Problems in Moscow.

The heads of the Monkeys were irradiated with protons in a Fasotron irradiation unit in the Joint Institute for Nuclear Research, Dubna. The delivered irradiation dose was 3 Gy, the proton energy was 170 MeV. The animals from both the control and experimental groups were transported seated in primate chairs in a specially equipped vehicle.

For 40 days after the irradiation, the ability of the animals to redevelop their skills and to proceed further with training was studied. Blood samples were taken from the median cubital vein of ketonal-anesthetized monkeys 1 day after and 1 month after the irradiation for the neurochemical analysis.

At 40 days after the first irradiation, the heads of the monkeys were irradiated with ^{12}C ions in the Nuclotron unit in the Joint Institute for Nuclear Research, Dubna. The delivered irradiation dose was 1 Gy, with the radiation energy of 160 MeV and a linear energy transfer of 0.53 KeV/μm. The control animals were treated essentially in the same way as the experimental animals except for the radiation. Blood samples were taken 8 days and 1 month after the second irradiation.

Venous blood was sampled with a specially designed syringe. Blood in the volume of 5 mL was transferred into plastic tubes and centrifuged at 2500–3000 rpm (150–200 *g*) for 20 min at 4°C. Blood plasma with a volume of 1 mL was frozen in liquid nitrogen for further analysis.

L-DOPA, the dopamine precursor, adrenaline (A), noradrenaline (NA), and 3,4-dihydroxyphenylacetic acid (DOPAC, a metabolite of dopamine) were determined after concentrating them on aluminum oxide. In specially designed tubes (Bioanalytical Systems,

Inc., United States) 500 μL of blood plasma was mixed with 50 μL of 0.1 M $HClO_4$ containing 500 pmol/mL of 3,4-dihydroxybenzylamine (DHBA), catecholamine, which is not encountered in the native tissue, as an internal standard, and 50 mg of aluminum oxide. Then, 1 mL of 1.5 M Tris/EDTA buffer (pH 8.6) was added and the mixture was agitated for 5 min. After sedimentation, supernatant was collected over the aluminum oxide precipitate and the latter was washed twice with 2 mL of deionized water (18 mg Ohm). The aluminum oxide was transferred to a centrifuge tube, 100 μL of 0.1 M $HClO_4$ was added, and the tube was shaken for 2 min. The samples were centrifuged at 1000 *g* for 10 min at 4°C. Supernatant was collected and 20 μL were placed in the chromatograph.

Serotonin (5-hydroxytriptamine, 5-HT), 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, and homovanillic acid (HVA), a dopamine metabolite, were determined after the precipitation with 1.0 M $HClO_4$ (100 μL of blood plasma was mixed with 50 μL of 1.0 M $HClO_4$ containing 500 picomol/mL of DHBA, the internal standard and centrifuged at 12000 rpm (15000–20000 *g*) for 10 min at 4°C). The concentrations of monoamines and their metabolites were determined as described in detail in [3, 4].

To identify significant differences between the parameters in the group of control animals and the group of irradiated animals, one-factor dispersion analysis was performed. Post hoc comparison of the means was carried out using the Fisher's least significant difference (LSD) method (ANOVA). To determine the general direction of the shift in the concentrations of the analyzed compounds after irradiation the nonparametric Z-test was utilized. A difference with the significance level of $p < 0.05$ was considered as significant. *P* values between 0.05 and 0.1 indicated a clearly defined tendency. The final results of the measurements are presented as the mean value ± the standard error of mean.

RESULTS AND DISCUSSION

It should be noted, first of all, that monkeys have very strong differences in their individual behavioral characteristics. For this reason, an essential condition for all primate neurobiological experiments is recognition of the typological behavioral characteristics. In other cases, the individual data variation obtained in monkey experiments may exceed the variation associated with the studied factors. In addition, a monkey is not an abundant experimental animal; therefore, it is usually almost impossible to select animals with similar types of behavioral characteristics. For the same reason, it is not advisable to average the data obtained for different animals. The most appropriate control in such experiments may be an animal's behavior indices recorded before the exposure to experimental factors. We used the control-animal behavior indices only to balance the possible effects of concomitant factors, which are mainly associated with transportation and not related to ionizing radiation.

The typological behavioral characteristics of the monkeys included the strength, balance, and agility of nervous processes during different dynamic activities, such as in cognitive responses to external stimuli and in learning to accomplish various cognitive tasks. In particular, we assessed the level of motion activity, the excitability level (type of response to an unexpected irritant), feeding-motivation level, food preferences, anxiety level, the relationship between the components of anxiety, fear, passive defensive behavior, and exploratory activity in the response to a new environment, unknown objects, etc., and the level of aggression towards the researcher. The animals that were quickly accommodated to contact with a researcher showed a high level of exploratory activity, a quick decrease of the aggression response, and low anxiety level, which are related to the strong and balanced higher-nervous-activity type. The animals that demonstrated a high level and inert mode of excitability and aggression, a low decrease in the rate for these processes, and irritability during training were considered to possess the strong and imbalanced higher-nervous-activity type.

The strong balanced quick nervous-activity type, which corresponds to a sanguineous temperament, was the most evident in monkey no. 257. Monkeys nos. 311 and 347 were characterized by the strong imbalanced type of nervous activity, which corresponds to a choleric temperament. This type of nervous activity is characterized by strong excitation process and delayed inhibitory process; individuals of this type are liable to impairments in higher nervous activity in difficult situations. Monkeys nos. 343, 344, and 275 were also considered as having this type of nervous activity. Their typological characteristics included insufficient agility, or plasticity, of nervous processes, which led to certain problems when the animals were exposed to external stressing factors. As a result, they had an unstable performance during the tests and usually performed only a small number of tests during a day. A comparison of the results of training monkeys as a part of the preparation for the experiment showed that the animals demonstrated different levels of motivation to perform the tests. The monkeys made from 50 to 300 attempts at accomplishing the tests on average.

The test results proved to be heterogeneous. Two control monkeys (275 and 347) may be considered to be incompletely effective. Monkey no. 311 showed the best results. Its active interaction with the computer (the number of attempts and percentage of successful attempts) resulted in a high number of accomplished tests during a single day (from 100 to 300 attempts per day on average). The percentage of successfully performed tasks was stably high and almost always not

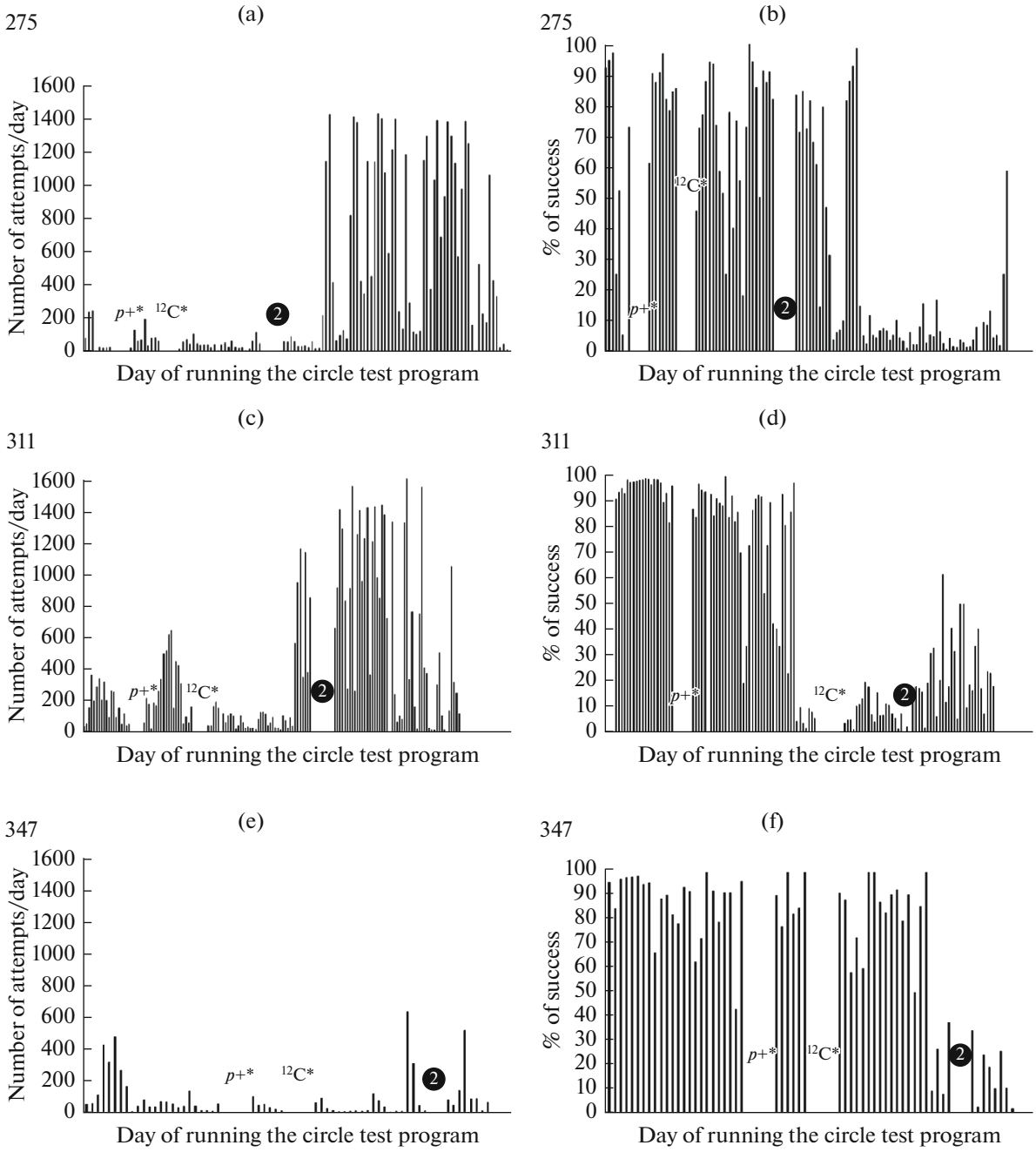

Fig. 1. The cognitive-function indices in the control-group monkeys. p^{+*}, Proton irradiation; ^{12}C, irradiation with carbon ions; 2, the switch to the new complexity level of the game program.

lower than 80–98%. It should be clarified at this point that success in performing a task does not mean the number of accurately completed tasks, but rather the general level of game activity, that is, the total number of attempts to complete the task. All animals from the control group demonstrated a rather low level of activity in general, as can be judged by the total number of performed tasks, while at the same time they showed a relatively high number of proper responses (Fig. 1). The experimental factors (transportation and false irradiation) had no significant effect on these indices.

During training, monkeys from the experimental group showed different background numbers of performed tests. This index value varied from 50 to 100 tests per day. The lower motivation background, as observed in the animals as decreased game activity, did not affect the quality of task accomplishment, with the task success rate being 60–70% on average (Fig. 2).

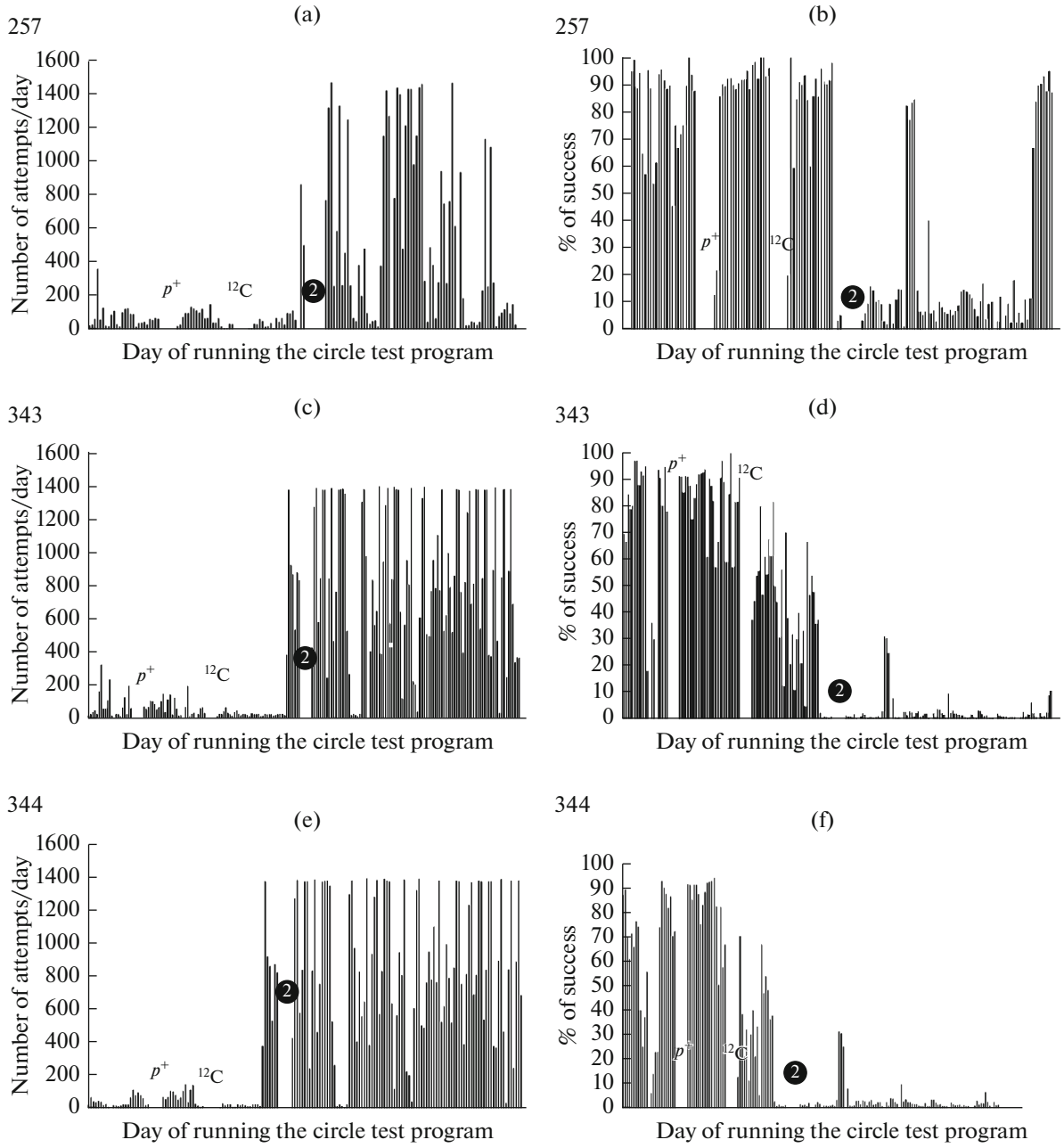

Fig. 2. The cognitive function indices in the experimental group monkeys. Designations are the same as in Fig. 1.

After irradiation with both protons and carbon ions, the monkeys showed the same low level of activity. However, after proton irradiation, the percentage of success was relatively high and showed no significant difference from the background level (Fig. 2). However, after the irradiation with ^{12}C ions, the percentage of success decreased dramatically for monkey no. 343 and especially for monkey no. 344, and remained high only for monkey no. 257 (Fig. 2). As mentioned above, this monkey was considered to be characterized by the strong balanced type of higher nervous activity, which has been demonstrated in many works [5–9] to be highly resistant to radiation.

At 1 month after the irradiation we resumed training the animals according to the circle training regimen, but at the next complexity level. This new complexity level differed from the preceding one in that the "target" turned invisible when it was within the circle; the monkey thus had to extrapolate target movements inside the circle. This new task caused an increase in game activity in monkeys nos. 275 and 311 from the control group followed by a gradual increase in the

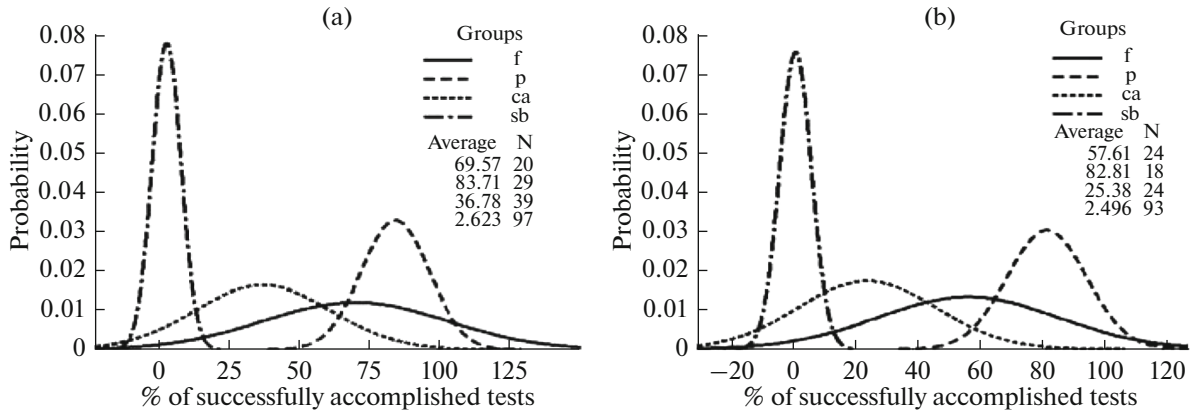

Fig. 3. The distribution function for the cognitive function indices for monkey no. 343 (a) and monkey no. 344 (b). f, Training prior to experimental exposure; p, proton irradiation; ca, irradiation with carbon ions; sb, training according to the circle regimen at the new complexity level.

percentage of success (especially in monkey no. 275). As mentioned above, monkey no. 347 retained the same low level of activity.

At the same time in the experimental group, despite a sharp increase in game activity, monkeys nos. 343 and 344 showed the same low percentage of proper responses. Only monkey no. 257, which was characterized by the strong balanced type of higher nervous activity, revealed a wave-like increase in the number of successful responses (Fig. 2).

We used the Kolmogorov–Smirnov [10] test to process the data statistically. The empirical density of the distribution was calculated for each experiment. Specially designed statistical computer software was utilized to perform this test. We used the professional statistical Minitab v.17 software package designed by Minitab® Statistical Software.

The null hypothesis (H_0) of the equality of the empirical distribution functions was rejected when the Kolmogorov–Smirnov statistic (D_n) value is significant ($p < 0.05$). The plots present the Kolmogorov–Smirnov empirical density of distributions (Fig. 3).

In this manner, in the experimental group, the percentage of successfully accomplished tests increased for all monkeys after proton irradiation compared to the pre-irradiation background ($p < 0.05$). The improvement of conditioned activity upon irradiation was first observed many years ago, with this effect being described both in rats [11] and in monkeys [12, 13]. In particular, X-ray irradiated rhesus macaque monkeys significantly outperformed the control animals in a number of discrimination tests [13]. The causes underlying this effect are still somewhat elusive. The hypothesis exists that, on the one hand, this observation may be explained by so-called attention tunneling when actuator mechanisms of orientation reactions that proceed in the reticular formation and thalamus are suppressed, leading to the animal being less distracted by external stimuli. On the other hand, we may observe the activation of compensatory processes in the central nervous system triggered by irradiation as the manifestation of the first stage of the radioreaction.

However, the irradiation with carbon ions, as well as the switch to the higher complexity level, resulted in a significant decrease in this parameter in monkeys nos. 343 and 344 ($p < 0.05$) (Fig. 3). As mentioned, these parameters for monkey no. 257 remained at the same level or even increased.

To summarize, the analysis of dynamics of cognitive processes in monkeys upon the exposure to two types of ionizing radiation revealed that proton irradiation did not cause any observable impairment of cognitive function, while the irradiation with carbon ions leads to reduced cognitive function in animals with poor plasticity of nervous processes. At the same time, the monkey characterized by the strong balanced type of higher nervous system activity proved to be resistant to both types of radiation, which complies well with the numerous literature data.

The study of the neurochemical characteristics of the experimental animals produced the following results. At the second day after the proton irradiation we observed no major changes in the concentrations of monoamines and their metabolites in the blood plasma (Table 1); however, 1 month after the irradiation we observed a tendency towards an increase in the concentration of L-DOPA, which is a dopamine precursor (Table 2).

At 8 days after the irradiation with ^{12}C ions the concentrations of all the compounds under analysis decreased, with significant results being obtained only for HVA, a dopamine metabolite. We observed a clear tendency towards a decrease in the concentrations of NA, DOPAC, another dopamine metabolite, and 5-HIAA, a 5-HT metabolite (Table 3).

Table 1. The concentrations of monoamines and their metabolites in the blood plasma of monkeys (picomol/mL of plasma) before irradiation and the following day after the proton irradiation

Group	L-DOPA	NA	A	DOPAC	HVA	DOPA	5-HIAA	5-HT
Before irradiation	6.03 ± 0.5	2.95 ± 0.9	0.68 ± 0.2	21.54 ± 4.0	77.35 ± 15.5	0.32 ± 0.1	187.09 ± 18.6	24.15 ± 9.5
After irradiation	5.01 ± 0.5	2.24 ± 0.1	0.64 ± 0.1	29.72 ± 10.3	79.67 ± 18.9	0.22 ± 0.04	143.63 ±21.3	38.81 ± 5.0
p =	0.276	0.556	0.867	0.474	0.929	0.401	0.212	0.320

Table 2. The concentrations of monoamines and their metabolites in the blood plasma of monkeys (picomol/mL of plasma) before irradiation and 1 month after the proton irradiation

Group	L-DOPA	NA	A	DOPAC	HVA	DOPA	5-HIAA	5-HT
Before irradiation	3.95 ± 0.23	1.33 ± 0.16	0.62 ± 0.14	18.50 ± 3.86	50.19 ± 6.97	0.38 ± 0.1	21.69 ± 3.6	53.95 ± 7.5
After irradiation	5.76 ± 0.99	2.08 ± 0.44	0.51 ± 0.27	24.35 ± 4.39	43.48 ± 12.48	0.47 ± 0.2	29.15 ± 8.71	47.98 ± 6.6
p =	***0.097***	0.134	0.687	0.403	0.664	0.679	0.448	0.225

A clear tendency to change, $0.05 < p < 0.1$, is indicated by boldface italics.

Table 3. The concentrations of monoamines and their metabolites in the blood plasma of monkeys (picomol/mL of plasma) before irradiation and 8 days after the irradiation with ^{12}C ions

Group	L-DOPA	NA	A	DOPAC	HVA	DOPA	5-HIAA	5-HT
Before irradiation	6.23 ± 0.56	2.28 ± 0.24	0.50 ± 0.09	19.06 ± 2.16	29.65 ± 1.43	0.44 ± 0.1	72.46 ± 5.76	29.15 ± 11.3
After irradiation	5.58 ± 0.11	1.21 ± 0.22	0.48 ± 0.18	12.53 ± 0.97	18.56 ± 1.49	0.35 ± 0.01	55.05 ± 0.95	21.86 ± 5.0
p =	0.401	***0.054***	0.935	***0.088***	**0.012**	0.450	***0.072***	0.655

Significant changes in bold are underlined. A clear tendency to change, $0.05 < p < 0.1$, is indicated by boldface italics.

At 1 month after the irradiation with ^{12}C ions, we also observed a decrease in the concentrations of all the analyzed compounds (Table 4). Significant changes were observed in the concentrations of NA and DOPAC, the DOPA metabolite. In the cases of L-DOPA, the DOPA precursor, HVA, the DOPA metabolite, and 5-HIAA, the 5-HT metabolite, we observed a clear tendency towards a decrease.

To summarize, 8 days after the irradiation with carbon ions (^{12}C) we observed a marked decrease in the concentrations of the indicated compounds in blood plasma. It is well known that NA, DOPA, and 5-HT are not able to pass the blood–brain barrier, while their metabolites are able to do so. For this reason, an immediate analysis of the effect of the changes in brain monoamine concentrations on monkey behavior is impossible. However, our results may be compared with the results that were previously obtained in a similar experiment involving rat exposure to carbon ions (^{12}C) [3]. In this experiment, we observed a strong decrease in the concentrations of monoamines and their metabolites in the prefrontal cortex, nucleus accumbens, and hippocampus of the rat brain with the significant changes in the prefrontal cortex and nucleus accumbens. Interestingly, we observed the same unidirectional nonspecific effect of irradiation, i.e., a decrease in the concentrations of monoamines and their metabolites, irrespective of whether we analyzed monkey blood liquor, or rat brain structures. In another experimental series, on the 30th day after irradiation with ^{12}C ions, the highest decrease in the concentrations of monoamines and their metabolites was detected in the nucleus accumbens, while the least profound decrease occurred in the hippocampus and striatum [4].

Table 4. The concentrations of monoamines and their metabolites in the blood plasma of monkeys (picomol/mL of plasma) before irradiation and 1 month after the irradiation with ^{12}C ions

Group	L-DOPA	NA	A	DOPAC	HVA	DOPA	5- HIAA	5- HT
Before irradiation	7.23 ± 0.23	3.62 ± 0.12	0.63 ± 0.09	18.56 ± 1.56	28.98 ± 1.16	0.38 ± 0.06	79.13 ± 4.29	32.49 ± 14.0
After irradiation	6.18 ± 0.26	2.2 ± 0.34	0.59 ± 0.09	11.53 ± 0.59	24.56 ± 0.84	0.27 ± 0.04	61.72 ± 3.45	25.19 ± 3.64
p =	***0.068***	**0.042**	0.818	**0.033**	***0.65***	0.297	***0.061***	0.701

Significant changes in bold are underlined. A clear tendency to change, $0.05 < p < 0.1$, is indicated by boldface italics.

In addition, dopaminergic system malfunction and the disturbance of the behavior associated with the functioning of the dopaminergic system, such as motor behavior, the amphetamine-mediated aversive learning test, and operant conditioning were found 3 months after irradiation with ^{56}Fe ions [14]. Irradiation of the 2-month-old rats and testing of operant conditioning 7, 11, and 15 months after irradiation revealed a relationship between the effect of ^{56}Fe ions and ageing. As more time passes from the moment of exposure, the dopamine-dependent behavior deficiency is stronger [15]. ^{56}Fe ion exposure caused a decrease in the amount of ^{3}H-glutamate released from hippocampal synaptosomes 3 and 6 months after irradiation and a concomitant suppression of the NR1, NR2A, and NR2B NMDA receptor subunit expression. At 180 days later, the amount of NR2A subunit still remained low, while the amounts of both NR2B and NR1 subunits regained their normal values and even exceeded them [16]. At 3 months after the irradiation, the animals were tested in the Barnes Maze. The study demonstrated that irradiation with ^{56}Fe ions at even a very low dose (0.5 Gy) impairs spatial memory, which is associated with the functioning of the hippocampus [17]. Spatial-memory impairments revealed in the Morris water maze after irradiation with ^{56}Fe ions at a dose of 1 Gy have been reported previously [18].

Therefore, on the basis of the experimental results obtained in the discussed works we may draw the conclusion that the impairments caused by heavy metal ions delivered even at a very low dose develop and deepen with time. However, we demonstrated that even irradiation with the ions with a much lower relative biological effectiveness than ^{56}Fe causes significant changes in the metabolism of monoamines in both the brain and in the blood liquor and at an earlier time after the exposure. We may speculate here that the radiation causes an intensive effect and affects so many processes in the brain that the decrease in the monoamine metabolite concentrations in the blood liquor of monkeys may correspond to a decrease in the concentrations of monoamines and their metabolites in the monkey brain.

These results are in agreement with our data on the changes in cognitive processes observed in experimental animals. As mentioned above, the most significant decrease in the percentage of correct responses was observed after the irradiation with carbon ions, which suggests that the decrease in the concentration of metabolites in the blood plasma may indirectly reflect the corresponding decrease in the neuromediator concentrations in the key brain structures.

ACKNOWLEDGMENTS

This study was performed within the frameworks of the Russian Academy of Sciences project no. 01201372803 and was supported by the Russian Foundation for Basic Research, project nos. 15-04-003569-a and 15-04-02005-a.

REFERENCES

1. Vasil'eva, O.N., Kapustina, E.A., and Korol'kov, V.I., *Kosm. Biol. Aviakosm. Med.,* 2003, vol. 37, no. 6, pp. 55–63.
2. Washburn, D.A., Rumbaugh, D.M., Richardson, W.K., Gulledge, J.P., Shlyk, G.G., and Vasilieva, O.N., *J. Gravit. Physiol.*, 2000, vol. 7, no. 1, pp. 89–93.
3. Matveeva, M.I., Shtemberg, A.S., Timoshenko, G.N., Krasavin, E.A., Narkevich, V.B., Klodt, P.M., Kudrin, V.S., and Bazyan, A.S., *Neirokhimiya,* 2013, vol. 30, no. 4, pp. 343–348.
4. Belokopytova, K.V., Belov, O.V., Kudrin, V.S., Narkevich, V.B., Klodt, P.M., Bazyan, A.S., Krasavin, E.A., and Timoshenko, G.N., *Neirokhimiya,* 2015, vol. 32, no. 3, pp. 243–251.
5. Lebedinskii, A.V. and Nakhil'nitskaya, Z.N., *Vliyanie ioniziruyushchikh izluchenii na nervnuyu sistemu (Effects of Ionizing Radiation on Nervous System)*, Moscow: Atomizdat. 1960.
6. Livshits, N.N., *Vliyanie ioniziruyushchikh izluchenii na funktsii tsentral'noi nervnoi sistemy (Effects of Ionizing Radiation on Central Nervous System Functions)*, Moscow: Akad. Nauk SSSR, 1961.
7. Livanov, M.N., *Nekotorye problemy deistviya ioniziruyushchei radiatsii na nervnuyu sistemu (Some Questions on the Ionizing Radiation Impact on Central Nervous System)*, Moscow: Medgiz, 1962.
8. Minaev, P.F., *Vliyanie ioniziruyushchikh izluchenii na tsentral'nuyu nervnuyu sistemu (Effects of Ionizing Radiation on Central Nervous System)*, Moscow: Acad. Nauk SSSR, 1962.
9. Shtemberg, A.S., *Izv. Akad. Nauk SSSR, Ser. Biol.,* 1987, no. 4, pp. 547–557.
10. Bol'shev, L.N. and Smirnov, N.V., *Tablitsy matematicheskoi statistiki (Mathematical Statistics Tables)*, Moscow: Nauka, 1983.
11. Blair, W.C., *J. Compar. and Physiol. Psychol.*, 1958, vol. 54, no. 2, pp. 175–178.
12. Harlow, H.F., Scrier, A.M., and Simons, D.G., *J. Compar. and Physiol. Psychol.*, 1956, vol. 49, no. 2, pp. 195–200.
13. Riopelle, A.J., Gronsky, M.A., and Ades, H.W., *J. Compar. and Physiol. Psychol.*, 1956, vol. 49, no. 5, pp. 521–524.
14. Rabin, B.M., Joseph, J.A., and Shukitt-Hale, B., *Adv. Space Res.,* 2004, vol. 33, no. 8, pp. 1330–1333.
15. Rabin, B.M., Joseph, J.A., and Shukitt-Hale, B., *Radiat. Res.,* 2005, vol. 164, no. 4, pt. 2, pp. 552–555.
16. Machida, M., Lonart, G., and Britten, R.A., *Radiat. Res.,* 2010, vol. 174, no. 5, pp. 618–623.
17. Britten, R.A., Davis, L.K., Johnson, A.M., Keeney, S., Siegel, A., Sanford, L.D., Singletary, S.J., and Lonart, G., *Radiat. Res.,* 2012, vol. 177, no. 2, pp. 146–151.
18. Shukitt-Hale, B., Casadesus, G., McEwen, J.J., Rabin, B.M., and Joseph, J.A., *Radiat. Res.,* 2000, vol. 154, no. 1, pp. 28–33.