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Modifications of the nervous system in rats after proton and gamma irradiation

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Abstract

One of the serious problems of long-distance space flights is the effect of space radiation on the astronaut's body, in particular on the central nervous system and cognitive abilities. And as a result, the ability to carry out a space mission.

An experiment is underway at the LRB, the essence of which is the simulation of cosmic radiation in outer space and the study of behavioral and histochemical changes in the nervous tissue in laboratory rats.

Aim of research to identify the changes that have occurred in the nervous tissue of rats 450 days after irradiation. The purpose of study is to analyze and understand the changes made to nervous system by radiation to prevent or treat astronauts involved in space programs. Our research highlights the effects of radiation affecting neurons in the nervous system and find a correlation between changes the behavior and changes in nervous tissue of exposed rats.

The central nervous system (CNS) can be exposed to ionizing radiation after a nuclear accident or during space fight, atomic weapon trials and medical treatment. Although the adult brain is less vulnerable to radiation than other organs, it is likely that even relatively low doses of radiation can cause some cognitive impairment [1]. It is known that fractionated X-ray and gamma irradiation in the neonatal period of development of rats lead to a defciency in certain behavioral characteristics, degranulation of the dentate gyrus, and reduction in the area of olfactory bulbs and the hippocampus in adulthood [2].

Introduction

One of the challenges of long lasting space missions is the exposure of the astronauts to galactic cosmic rays (GCR) during the mission [3]. The component of cosmic radiation can be expressed as a percentage as follows: 86-91% protons, 8-13% helium nuclei, and 1% heavy energetic nuclei (HZE) [4]. In low-orbit missions astronauts are protected from exposure to charged particles due to the Earth's magnetic field except the areas where we meet the Van Allen belts. The mission to Mars would require between 800-1100 days of which about 500 days are on the Martian surface. These details depending on how mission is designed [5]. The equipment for space missions also provides radiation shield but an amount of the radiation passes the shield. Behind the shielding provided by Mars Science Laboratory and en cruise to Mars, the GCR dose rate was approximately 0.481±0.080 mGy/day [6]. Mission Curiosity on the Martian surface indicated a GCR dose rate of 0.210 ± 0.040 mGy/day [7].The shield used in space missions

provides protection on the martian surface the problem remains the radiation dose received on the way to cosmic space.

The body of literature examining animal behavioral outcomes in response to high-energy charged-particle radiation suggests differential effects in response to different particles and energies. Our study looks at behavioral changes in adult rats 450 days after proton irradiation. To investigate behavioral changes the T-maze method and Open Field Test were used. These tests allow the analysis of different disorders as an example the disease of hippocampus are analyzed using T-maze: rats and mice naturally tand to alternate the chosen arm in the T-maze test. This involves short-term memory or "working memory". Alternation reflects the motivation of the animal to explore its environment and locate the presence of resources as food, water etc. The phenomenon is called spontaneous alternation. Our measurements showed that rats that have been irradiated have difficulty in remembering the arm of the maze or avoid choosing, this is due to the level of anxiety. To quantify the level of anxiety the study analyzes anxiety level with Open Field Test. For a correct and rigorous interpretation of the experimental data for both T-maze and Open Field Test, we statistically processed and analyzed the recorded rats behaviors and the frequency of their occurrence [8].

The destructive effects of ^{1}H radiation on brain cells have been observed since the very early days of particle accelerators [9]. To analyze the effect of radiation at the cellular level we performed histological measurements on the nervous tissue. The microscopy measurements revealed damage caused by radiation at the cellular level.

Measurements and method

In the experiment, 31 rats (females) of the SD line (Sprague Dawley) of the specific pathogen free (SPF) category of about 200-245 g weight were used. At the time of irradiation, the animals were 15 weeks old. The rats were obtained from the nursery of laboratory animals "Pushchino" and underwent preliminary adaptation to the conditions of the vivarium. During the entire maintenance, the animals had unlimited access to water and food. They were divided into groups 10 weeks prior to irradiation by randomization. After that, they were sacrificed by decapitation on the 30th day after irradiation, the section and selection of organs for the study were performed at the same time. The procedures carried out on the animals corresponded to the rules of work approved by the bioethical commission of the Federal State Budgetary Institution of Science of the State Scientific Center of the Russian Federation - the Institute of Biomedical Problems of the Russian Academy of Sciences.

Irradiation procedures

Irradiation with protons was carried out on the proton beam of the Phasotron, DLNP, JINR. The animals were divided into 4 groups. Immediately before irradiation, the rats were placed in transparent irradiation containers made of polymethyl methacrylate (OOO NPK Otkrytaya Nauka), while the head and body of the animals were placed in a cylinder 160 mm long. Three groups were irradiated in the cranio-caudal direction (Fig. 1; 2)

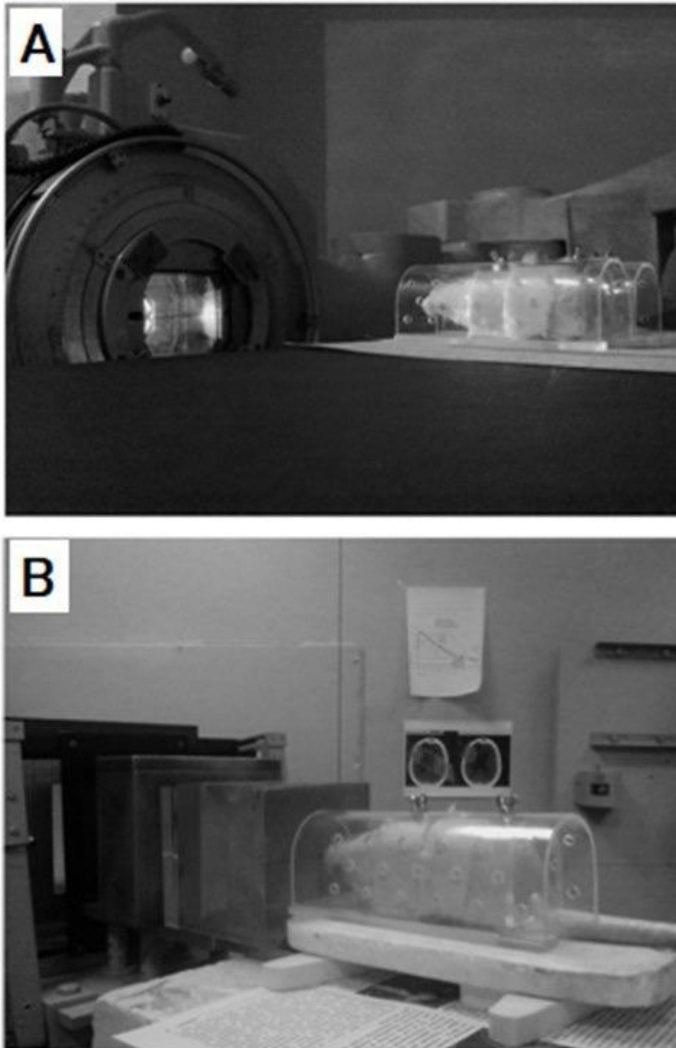


Figure 1. Rats in plastic fixing containers for head irradiation procedure (in process).

Figure 2. Rats in plastic fixing containers for head irradiation procedure.

Average value of LET = 0.49 keV / μm. The dose in both cases was 1 Gy; the second group was irradiated with an additional 120 mm water-equivalent moderator. The particle flux at the exit from the collimator was $1,16*10^9$ particles /$см^2$. Dosimetric calibration was performed with the ionization chamber TM30013 of the PTW UNIDOS-E clinical dosimeter. Animals of the third group were exposed to gamma-irradiation ^{60}Co at a dose of 1 Gy using the ROKUS-M device of the JINR Medical and Technical Complex. The dose rate was 0.869 Gy / min. The SSD value was 75 cm. The animals of the fourth - control group were transported and placed in containers, but were not exposed to ionizing radiation. Anesthesia was not performed.

On the 30th day after irradiation, the animals were sacrificed by decapitation. Blood was taken for hematological examination. The animal's brain was placed in a fixative (Carnoy's solution (60% ethanol, 30% chloroform and 10% acetic acid). After dehydration and defatting, the right lobe of the brain was taken and processed into paraffin blocks, and the left one was archived. Next, brain sections with a thickness of 10 and 6 μm were prepared using a microtome (Thermo Fisher Scientific HM 340E Electronic Rotary Microtome). No differences were found between the average depth of the prepared sections and the brain mass in different groups of animals. (Thermo Fisher Scientific HM 340E Electronic Rotary Microtome).

Histological research

To study the effects of radiation on the nervous system of irradiated rats and to learn about the changes that take place at the cell level the study subjected the histological analysis the nervous tissue of rats from both groups. After guillotining the rats and after extracting the samples were held 24h in Formaline 10% for process of fixation. To integrate organs into paraffin and to be able to perform microscope samples the following protocol was used:

1. Dist. water (2 h)
2. Ethanol 70% (1 h)
3. Ethanol 80% (2 h) (1h for eyes)
4. Ethanol 96% (12 h) (1 h for eyes)
5. Ethanol 100% (1h)
6. Chloroform 1 (30 min)
7. Chloroform 2 (30 min)
8. Chloroform + Paraffin mix (30 min)
9. Paraffin 1 (1h)
10. Paraffin 2 (1 h)

Figure 3 a) the organs fixed in paraffin; **b)** perform microscope samples the following protocol.

For histological analysis of the samples we used two contrast procedures. The first: contrast method used was Hematoxylin and Eosin, one of the principal tissue stains used in histology [10]. The idea is that hematoxylin stains cell nuclei blue and eosin stains the extracellular matrix and cytoplasm pink. The protocol used for this method followed a sequence of steps:

1. Xylen 3 min;
2. Xylen 3 min;
3. 96% ethanol;
4. 80% ethanol;
5. 60% ethanol;;
6. 40% ethanol;
7. d. H_2O 2min;
8. Hematoxylin 2 min;
9. d. H_2O+ NaOH;
10. d. H_2O;
11. Eosin 1 min;
12. d. H_2O 1 min;

13. 40% ethanol;
14. 60% ethanol;
15. 80% ethanol;
16. 90% ethanol;
17. Xylen 2 min;
18. Xylen 2 min;

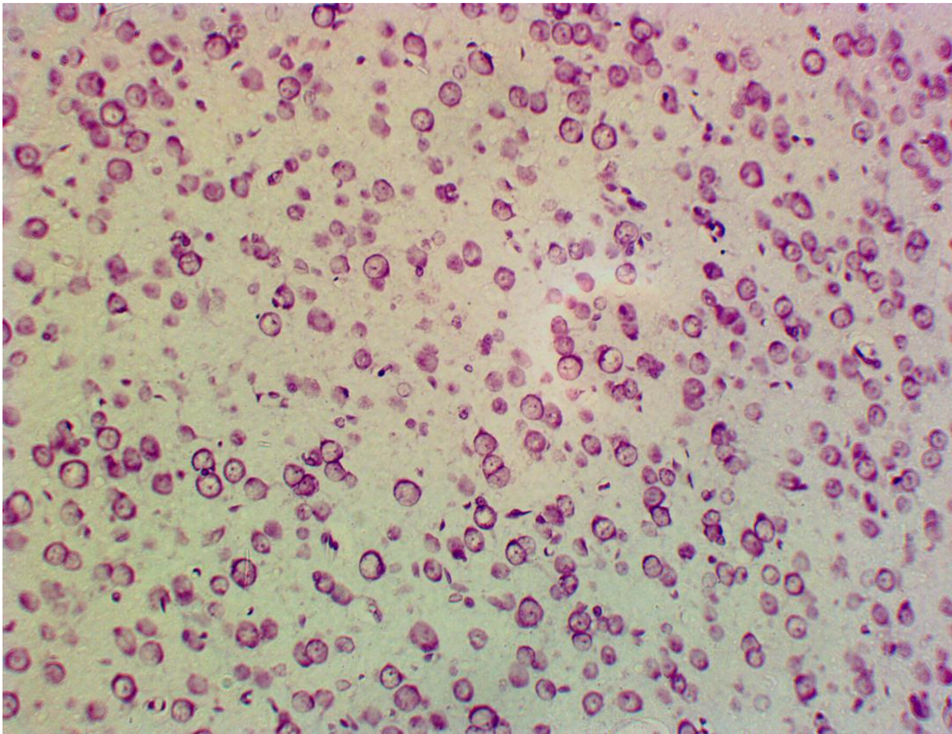
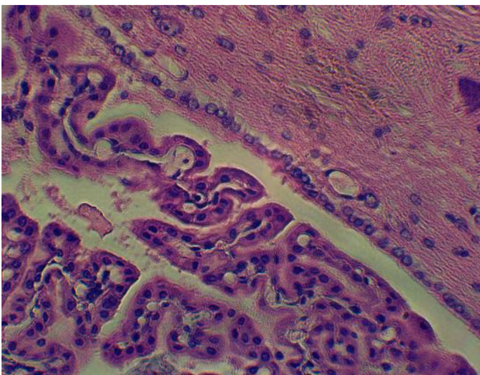


Figure 4. Images of brain tissue and retina were acquired using the microscope.



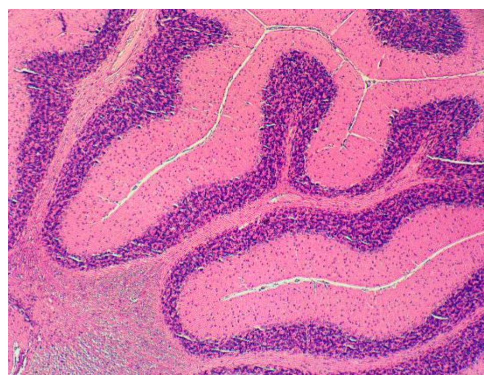


Figure 5 a; b). Microscopic image at different regions of the rat brain; Malignant 40X

a) Ependymal cells

b) Cerebrum cells

The second histological method to analyze the degeneration of neurons due to proton exposure we used Fluoro-Jade B stain [11] on samples taken from control and irradiated rats. The protocol used has the following steps:

1. Xylene 1 (3 min)
2. Xylene 2 (3 min)
3. Xylene 3 (3 min)
4. Ethanol 80% (5min)
5. Ethanol 70% (2min)
6. dist. water (2 min)
7. 0,06% $KMnO_4$ (2min)
8. dist. water (2 min)
9. Fluoro-Jade B (0,01%) 30 min
10. dist. water 3 stage (1 min)
11. towel dry

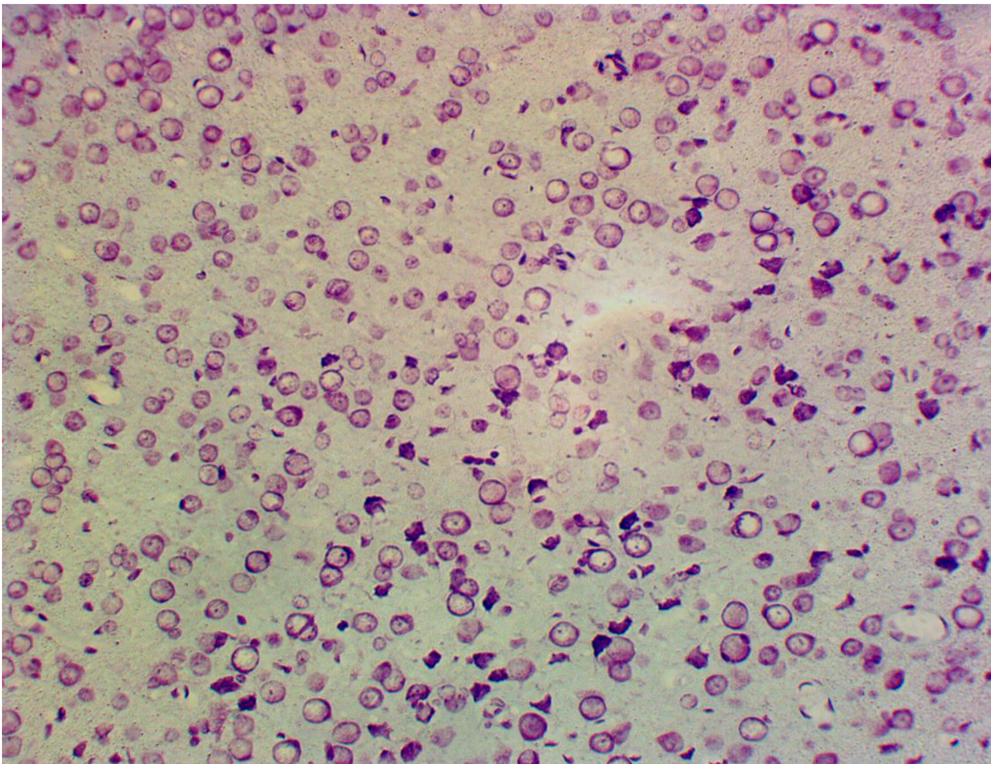


Figure 6. Brain tissue with degenerated neurons; E = 70 ke/V.

Figure shows areas with degenerate neurons. The presence of these areas may be due to proton irradiation but the presence of degenerated neurons in the nervous tissue was found in both rats groups: control and irradiated group. This suggests that a more likely cause is the age of the rats analyzed.

Neurons and glial cells change slightly in size during their life cycle. Dying cells in apoptosis change markedly in size relative to normal cells. cell morphometry was used for numerical evidence. Cell counting and their morphometry are carried out in the program ImageJ. ImageJ is public domain software for processing and analyzing scientific images. The screenshot of this program is shown in figure 7.

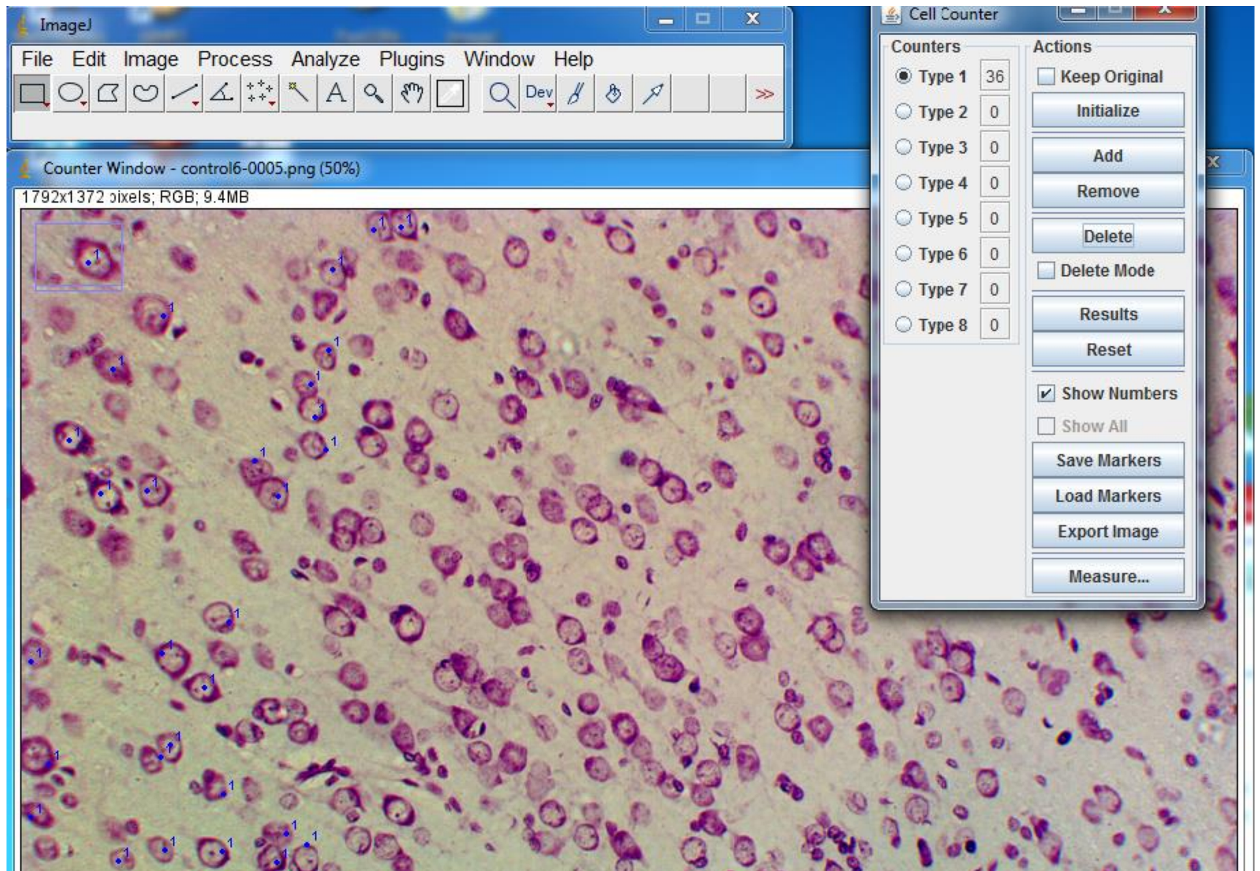


Figure 7. Application working environment of ImageJ.

Conclusions

Analysis of behavioral test results indicated that there are no statistically significant differences between the control rats group and the irradiated group. This can be explained by the development of compensatory mechanisms which in long term repair the effects caused by proton irradiation.

Analysis of nervous tissue in process.

In the future it is important to consider tests with long term training of animals, identifying more complex behavioral functions and establishing correlations with morphological changes in the brain.

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