JOINT INSTITUTE FOR NUCLEAR RESEARCH
Laboratory of Radiation Biology

FINAL REPORT ON THE START PROGRAMME

"The impact of ionizing radiation on brain structure and functions of rats"

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1. Abstract

Radiation's influence on body organs and brain functions can be considered a considerable issue as it usually has damaging results that are histopathological, physiological, and purpose-related issues. Every entity in our surroundings is radioactive to a certain degree from food to human beings. Radiation is necessary for several views of life like in medicine, nuclear medicine therapy, and fundamental research. Cosmic radiation may have threatening consequences for astronauts and is somehow strange till now. During our study, we have considered the impact of high energy proton radiation on Sprague rats by analyzing histopathological samples from rat brains, evaluating the learning and memory abilities of rats using Open Field test, and performing statistical testing to explain the difference between control and test groups. Results illustrated that rats that undergo radiation suffer from significant brain function changes.

2. Introduction

Radiation is a form of energy, either waves or particles, that travels at the speed of light and can penetrate materials and tissues as well (CDC). Radiation can be in the form of waves like Electromagnetic waves like X-rays, Gamma λ rays, and UV. Moreover, it can comprise particles like protons, neutrons, photons, electrons, and heavy nuclei (Jones and Karouia, 2008). It can affect certain people through their work like radiologists, those who work in nuclear settings, miners from radioactive elements, and even airplane crew which can be affected by cosmic rays (Liu et al., 2022). Moreover, humans can be affected by radiation naturally. About 82% of the radiation-absorbed population is affected by background radiation. Background radiation arises from our environment like from soil, oceans, water, and certainly cosmic rays (Shahbazi- Gahrouei et al., 2013, Liu et al., 2022).

Cosmic rays are natural radiations that come from space with high penetration power. The variability in space radiation constitution may be due to Solar Particle Events phenomenon in addition to its interaction with electromagnetic field surrounding the Earth. Solar Particle Events can be a source of Galactic Cosmic Rays GCR which exert an extremely detrimental impact on astronauts on deep space lunar and interplanetary expeditions as they are there outside the protection of earth electromagnetic field. Moreover, Low Earth Orbits LEO can be also exposed to galactic cosmic radiation. Despite the protection of the geomagnetic field against galactic cosmic rays, a negative impact on LEO missions can be noticed due to the entrapment of protons and electrons in Van Allen Belts (Jones and Karouia, 2008). Protons are found to be the most abundant element in Galactic Cosmic Rays and Solar Particle Events as they comprise about 86-91% of galactic cosmic rays as shown in the Figure below (Kiffer et al., 2019, Jones and Karouia, 2008).

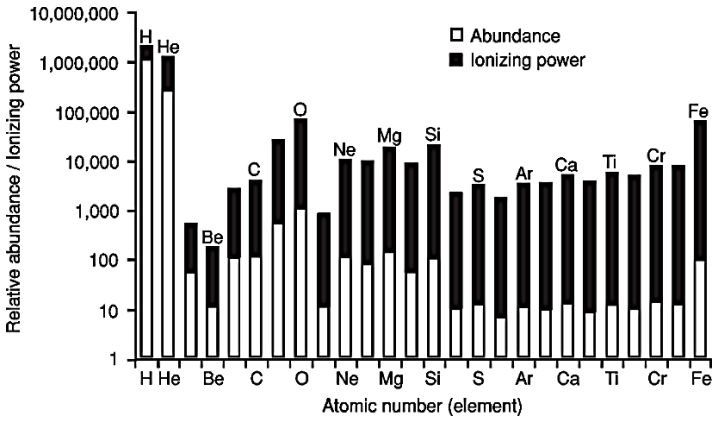


Figure: The abundance and ionizing power of different elements in galactic cosmic rays (Jones and Karouia, 2008).

Protons can be used in cancer therapy with selectivity for tumor tissues rather than normal ones (Mohan and Grosshans, 2017). Moreover, it showed greater effectiveness than photon therapy (Mohan, 2022). However, proton radiation has serious harmful effects on humans and the environment. It can affect people in several settings and can affect approximately all body tissues (Jones and Karouia, 2008). A publication by (Suckert et al., 2021) showed that proton radiation caused mice brain function decline and behavioral changes, skin lesions, and hair loss in addition to recorded histological sample problems like tissue necrosis, blood vessel dilatation, cell edema, and deposited granules in hippocampus with sclerosis. Furthermore, a publication by (Kiffer et al., 2019) has also shown serious data about the effect of ^{1}H radiation on brain function as variating doses can cause alteration in striatum-dependent memory, MWM pattern as animals remain longer in other quadrants than the targeted one, spatial memory, hippocampus-dependent memory and open-field activity, disruption of psychomotor vigilance, lowered motor performance on rotarod in addition to serious structural and functional changes in the Central Nervous System CNS. Therefore, it is very important to study the effect of proton radiation, which is the most abundant element in galactic cosmic rays, to illustrate the detrimental impact of this radiation on astronauts, who are extremely subjected to those rays.

- **Project goals**

In this study, our goal is to understand what are the effects that can be produced in body tissues and brain functions, behavior, and cognition upon exposure to cosmic radiation and the effect of certain drugs with radiation dose produced in the body tissues in addition to the functions, behavior, and cognition upon injection.

Results have shown that proton radiation has a serious detrimental effect on tissues and brain functions.

3. Materials and Methods

a. Animals and study design

A single-blinded study in which 45 female Sprague rats were used and only a few publications studied the effect of radiation on female rats (Kiffer et al., 2019). They were obtained from the "Pushchino" nursery, and they had free access to water and food. Rats were divided into control and test groups. Behavioral and brain function tests were performed after irradiation and then animals were decapitated, and

Histopathological and blood samples were collected and tested. All procedures were performed following the bioethical rules and regulations of (Protocol No. 19-3 Scientific-technical Council of Laboratory of Radiation Biology).

b. Radiation procedures

Two (proton) groups, 9 Sprague rats, were irradiated with a dose of 4 Gy, and 8 Gy of 170 MeV proton radiation as a previous study by (Severyukhin et al., 2019) has shown that it has a significant deleterious effect on visual activity on the 90th day of 170 MeV proton irradiation in addition to amyloid formation and edema in histopathological samples from rat hippocampus (Severyukhin et al., 2023). In addition, two other groups, 16 Sprague rats, for neuroprotection experiments were used to detect the effect of certain drugs with proton radiation on the functional behavior of the mice for the irradiation process.

Rats were placed in cylindrical tubes made from polymethyl methacrylate. The radiation direction was cranio-caudal, starting from rat heads and then down the body. The process took place at the Medical and Technical Complex at JINR.

Figure: Irradiation procedures in medical technical complex, SARRP system using Radiation-specific cylindrical tube JINR.

A. Brain functions and behavioral tests

To test rat brain functions and behavior we have used:

1- Open Field Test OFT

OFT is a main method designed to analyze rat behavior and general activities. It was in the form of a circular area with an enclosure around to prevent the animal from escaping with the ground divided into three main circles that have the same center with holes allocated roughly all over the areas. Several rat actions and activities were noted and set out, quantitatively and qualitatively which means that certain actions have been tested with the exact number of times that these actions have been performed (Gould et al., 2009, Seibenhener and Wooten, 2015). Many actions can be recorded. Moreover, each action has a justification as when they enter the holes in the ground area, this action means an exploratory action. The center entry indicates lower anxiety, grooming means relaxation, rearing, standing up using only two legs

with upper legs free or relying on the wall, and freezing action, where rats have no answer as they become frightened, and finally urination and defecation which indicate exaggerated stress. There were two groups, control and proton groups, with 4 rats for control, 4 for the 8Gy dose of radiation, and 5 for the 4Gy dose of radiation, and each rat was left in the area one time on the day of radiation and 1 day after radiation. Then, their movements, actions, and activities were recorded in 6:02 minute long videos by the camera, noted down by human eyes, and analyzed manually by eye and by Ethovision XT for statistics.

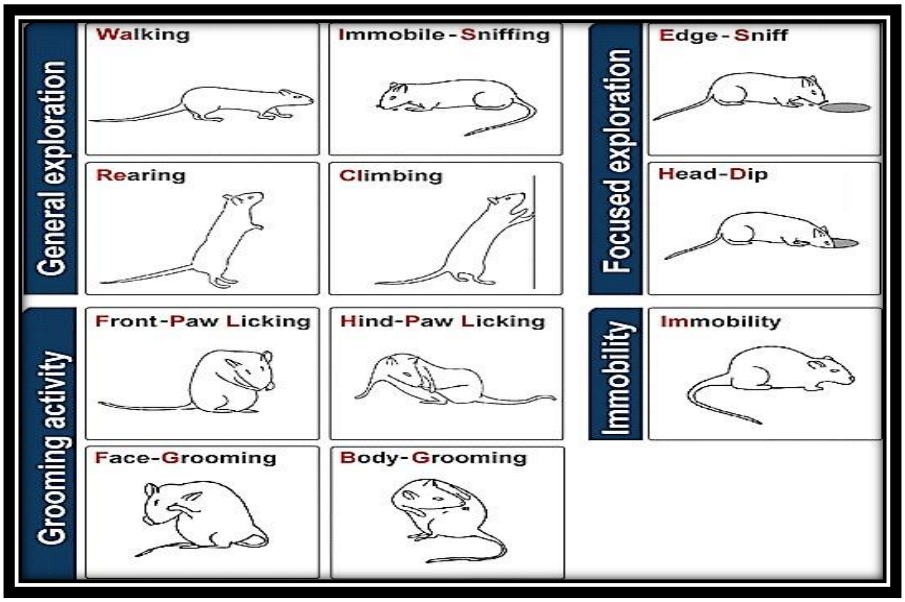


Figure: Some rat actions in Open Field Test OFT

B. Histopathological sample preparation and staining

Three rats were decapitated after 14 days, one from the control group and the other two from the proton groups. Organs were separated, and different processes were performed to preserve and stain tissue.

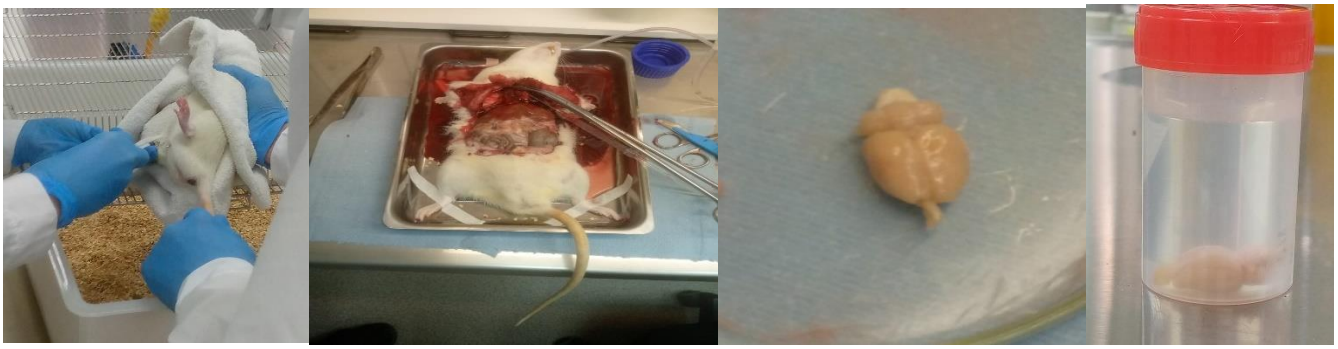


Figure: Process of scarification of the rat and fixation of the brain in 4% formalin

- **Fixation (Irreversible process)**

In formalin 4%, we soaked the fixed organs for 3 days. Washing with tap water is followed to avoid tissue artifacts caused by formalin. Fixation is the first step in staining to keep animal tissue from autolysis and putrefaction (Singh et al., 2019).

- **Pre-embedding, embedding and blocking**

The second process is tissue embedding, which was performed to protect and reinforce the separated tissue and to prevent tissue damage upon slicing (Dey, 2018).

Before paraffin embedding, several stages were followed. Firstly, samples were dehydrated from water using an ascending concentration of ethanol (50% for 1h., 70% for 1h., 95% for 1h., and 100% for 2h for 3 times), This was followed by clearing samples for 1h, 3 times, using xylene to remove the alcohol. Finally, infiltration followed. Paraffin wax with a melting point (60°C) mixed with xylene for 1h for 1 time followed by 2 times without xylene. A low melting point was used to prevent tissue damage by higher temperatures.

Tissues were then transferred from cassettes using worm forceps to a previously wax-filled mold. Then, cassettes were transferred above wax molds followed by additional wax filling. Finally, mold was transferred to chilled water on a cold plate for 30 minutes to form a block in a process called blocking.

Pre-embedding procedures (Reversible process):

Formalin Buffered 4%	3 days	Room temperature
Top water	1.5 h	Room temperature
Ethanol 50%	1h	Room temperature
Ethanol 70%	1h	Room temperature
Ethanol 95%	1h	Room temperature
Ethanol 100%	2h	Room temperature
Ethanol 100%	2h	Room temperature
Ethanol 100%	2h	Room temperature
Xylene	1h	Room temperature
Xylene	1h	Room temperature
Paraffin mixed with xylene	1h	Room temperature
Paraffin	1h	60 deg.
Paraffin	1h	60 deg.
Paraffin	1h(overnight)	60 deg.

- **Sectioning**

Tissue sectioning is a determined and major stage as with good sectioning, specimens will be more apparent under a microscope to detect any damage or artifacts.

Sectioning was done by rotary microtome shown in the figure below and 5 micrometer specimens were produced. Then, thin specimens were floating in a 45°C water bath to flatten. Glass slides were put under tissue slices to adhere to them and samples were left to dry first at approximately 50°C and then left for about 24 hours to dry at room temperature for best sample fixation to slides. Furthermore, the depth of trimming was also recorded to detect which part we had reached, so we could assess if there were hitches related to the process of fixation like autolysis, a problem with the cutting knife, a problem with embedding and pre-embedding processes like dehydration, a concern regarding the temperature of water bath or even drying time. I made slides from irradiated rat brains (4Gy and 8Gy), and control rat brains. Brain slices were taken at several depth points on the tissue starting from zero to 820 micrometers in the case of control blocks, 4Gy irradiated brain slices were taken at zero up to 1445 micrometers, and 8Gy irradiated brains began from zero to 490. I have learned some hacks and tricks like using our breath temperature to

facilitate the process of slicing. Furthermore, the best way to make a section is to be gentle in handling and cutting samples.

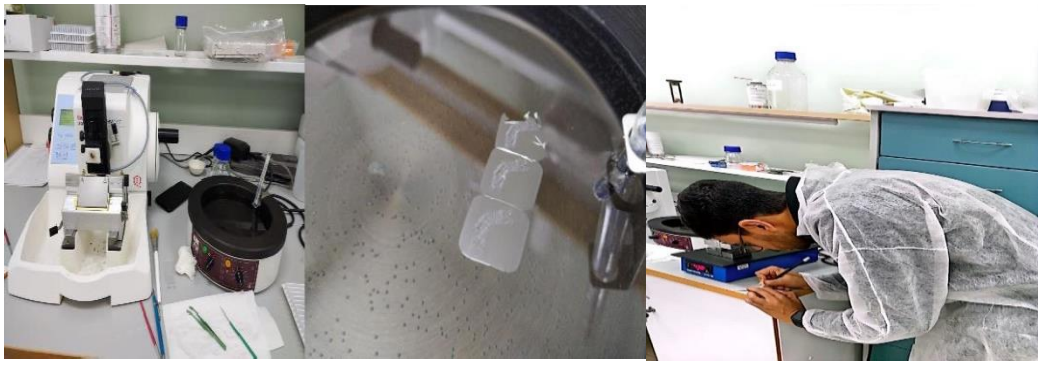


Figure: Sectioning process

- **Staining**

Following the cut of paraffin sections (5 microliters). The slides were completely dry at 37C overnight. Luxol Fast Blue stain (LFB stain) is a commonly used stain to examine myelin under light microscopy. LFB is commonly used to detect demyelination in the central nervous system (CNS) but cannot detect myelination in the peripheral nervous system (*Shin J. Oh (2002*). Luxol fast blue is a copper-phthalocyanine dye that is soluble in alcohol and is attracted to bases found in the lipoproteins of the myelin sheath (*Jocelyn (2006) (John D. Pfeifer2008).* Under the stain, myelin fibers appear blue, neuropil appears pink, and nerve cells appear purple. Tissue sections are treated over an extended period (usually overnight) and then differentiated with a lithium carbonate solution *(John D. Pfeifer2008).*

Staining procedures:

1	Deparaffinization	Xylene	3 changes, 6 minutes each
2	Removal of xylene	100% ethanol	3 changes, 5 minutes each
3	Hydration	95% ethanol	5 minutes, one time
4	Staining	0.1% Luxol fast blue solution	Overnight, 60℃/ or 2h.
5	Rinsing	95% ethanol	1 minute
6	Rinsing	Distilled water	1 minute
7	Differentiation	0.05% lithium carbonate	5 seconds to 20 seconds
8	Differentiation	70% ethanol	3 changes. 20 seconds each
9	Microscopic check, Repeating steps 5-7		Until gray matter becomes white.
10	Rinsing	Eosin	1 minute
11	Rinsing	Distilled water	
12	Dehydration	100% ethanol	3 changes, 5 minutes each
13	Clearing, then coverslip	Xylene	3 changes, 10 minutes each

C. Statistical analysis

We have used Jamovi 2.3.28 for our statistical analysis. In the Open Field Test (OFT), we used the number of certain actions to perform the ANOVA test between the main three groups, control, 4Gy, and 8Gy. Additionally, mean distance and velocities moved were calculated for locomotion change assessment. In the histological part, we differentiate between different myelination sections and calculate the area percentage myelinated using ImageJ in control tissue and 8Gy dose radiated tissues using 3 different tissue slices for each.

4. Results and Discussion

1- Open Field Test OFT

All events in the figure below have been recorded by the camera and tabulated with the exact number of times they happened.

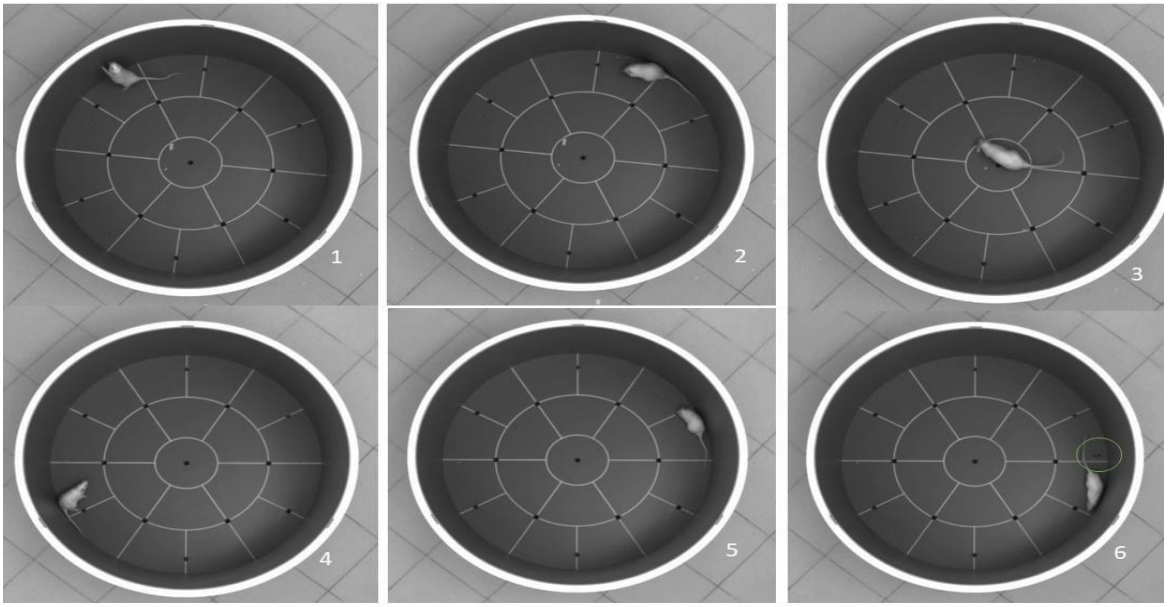


Figure: Actions of rats in Open Field Test (OFT):
1- rearing, 2- grooming, 3- center entry, 4- freezing, 5- hole entry, 6- defecation.

Regarding statistical analysis of OFT findings, Concerning the SARRP test, we have performed an ANOVA test. On day 1, there is a significant difference (p-value 0.04) in center entry as it is significantly lower in the proton group than in the control group as an exploratory action. Followed by grooming action (p-value 0.23) which is supposed to be a way that animals use to remove dirt and objects from their bodies, but it is also found to be related, but not limited, to decreasing anxiety or stress, decreasing pain, and social communication (Rojas-Carvajal et al., 2018). A publication by (Aureli and Yates, 2010) has shown that grooming has a role in distress prevention and anxiety reduction. Moreover, social bonding and relationships are higher after grooming between animals (Schino and Aureli, 2009). Therefore, a significant decrease in grooming after proton irradiation may be due to the reduction of rats' abilities to relax themselves or to communicate and build relationships as an impact of radiation on brain functions

and behavior. Moreover, radiation may have caused depression in certain areas of the brain, so rats are no longer able to mitigate their stress. However, a study by (Molesti and Majolo, 2013) has already reported an increased level of anxiety after grooming and so rats may have also decreased grooming to avoid an extra increase in stress and anxiety that may result from proton radiation.

After 14 days of radiation. We can find that the number of hole entries varies significantly (p-value 0.04) with a higher number of hole entries in the proton group than in the control group. The same result was obtained from the neuroprotection experiments in which the number of hole entries was very significant (p- value 0-13) reflecting the exploratory action. However, the number of hole entries in the case of radiated rats with the injected drug was less than the holes entered in the case of the control group (p-value 0.13). The usual thought about hole entry is that it is related to exploration activity, but a publication by (Birke and Sadler, 1986) has already shed light on this action that animals will not usually have the stimulus of new sites exploration when they are enclosed in a limited area, but their action may be escaping, and entering or even sniffing the holes may be driven by their extreme fear.

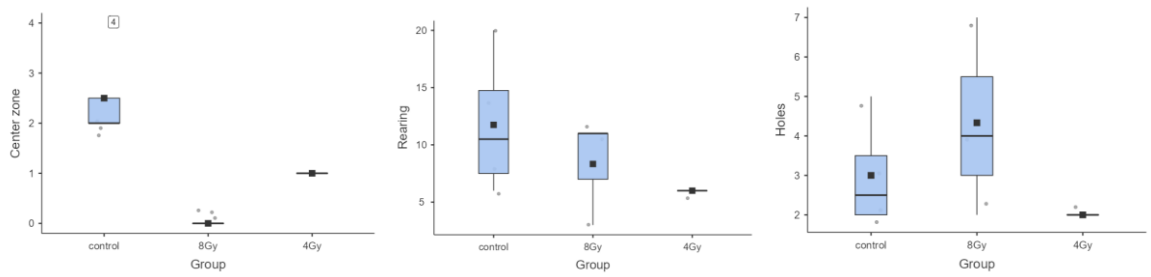
The lower level of significance in the rearing difference between the proton and control group after 14 days from radiation. Rearing is strongly related to animal hyperactivity and exploration (Valvassori et al., 2017). Therefore, the lower rearing in the proton group may be due to the damaging effect of proton radiation after 14 days, which can be irreversible, in the brains of experimental rats and so decreasing the exploratory actions.

- **SARRP experiment**

One-Way ANOVA (Non-parametric)

Kruskal-Wallis

	χ^2	df	p
Center zone	6.447	2	0.040
Rearing	1.334	2	0.513
Holes	1.372	2	0.504
Grooming (long)	2.704	2	0.259
Groomong (short)	0.565	2	0.754
Freezing	2.217	2	0.330



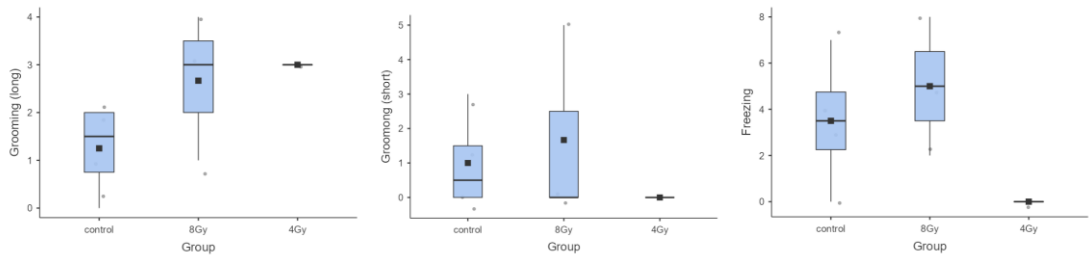


Figure: OFT statistical analysis after one day of radiation

- **Neuroprotection experiment**

Kruskal-Wallis

	χ^2	df	p
Center zone	4.04	3	0.257
Rearing	2.34	3	0.505
Holes	5.65	3	0.130
Grooming(long)	2.33	3	0.507
Grooming (short)	3.30	3	0.348

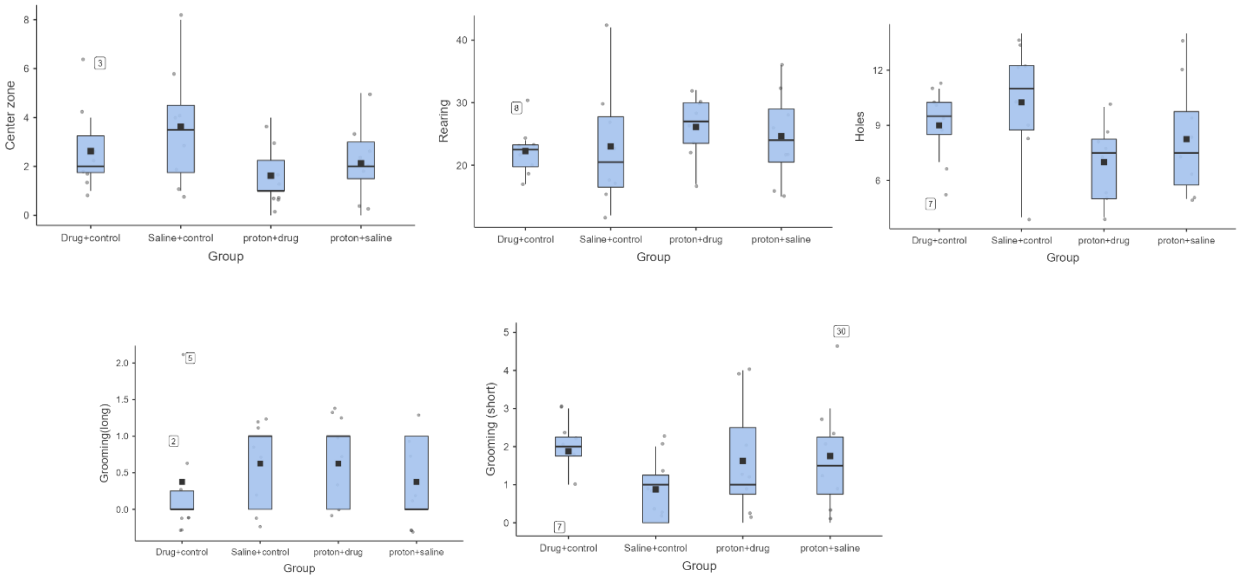


Figure: OFT statistical analysis for neuroprotection experiment

- **EthoVision XT software for OFT**

Videos were uploaded on Ethovision XT for rat tracking and data analysis. The analysis showed heatmaps resulting from rat movement. We can check the difference in movement status, yellow-colored areas show the periods of rat immobility.

One-Way ANOVA (Non-parametric)

Kruskal-Wallis

	χ^2	df	p
Distance moved	**6.17**	**2**	**0.046**
velocity	**6.17**	**2**	**0.046**

Dwass-Steel-Critchlow-Fligner pairwise comparisons

Pairwise comparisons - Distance moved

		W	p
control	**8Gy**	**-3.27**	**0.055**
control	**4Gy**	**-1.00**	**0.759**
8Gy	**4Gy**	**2.00**	**0.334**

Pairwise comparisons – velocity

		W	p
control	**8Gy**	**-3.27**	**0.055**
control	**4Gy**	**-1.00**	**0.759**
8Gy	**4Gy**	**2.00**	**0.334**

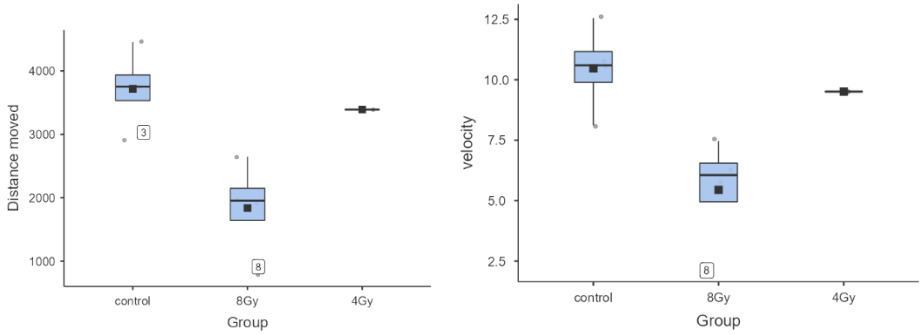


Figure: 4Gy, 8Gy proton and control groups vs velocity and distance

Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p
Distance moved	**Student's t**	**3.78**	**6.00**	**0.009**
	Mann-Whitney U	**0.00**		**0.029**

Independent Samples T-Test

		Statistic	df	p
velocity	**Student's t**	**3.46**	**6.00**	**0.013**
	Mann-Whitney U	**0.00**		**0.029**

Note. $H_a\ \mu_{control} \neq \mu_{8Gy}$

Assumptions

Normality Test (Shapiro-Wilk)

	W	p
Distance moved	**0.898**	**0.279**
velocity	**0.892**	**0.242**

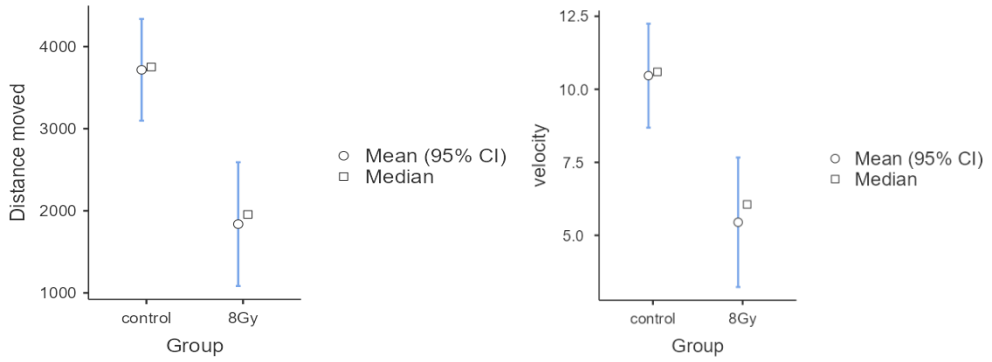


Figure: 8Gy proton and control groups vs velocity and distance

The locomotion of rats has also been assessed. Normality and Student's T-tests were performed showing a significant difference between lower velocity and distance in rats in the proton group than those of the control group as an indicator of the acute damaging effect of radiation on brain functions and behavior.

1- Histological sample examination

We detected overall changes in tissues. Pictures of the brain of the control and irradiation rat groups were taken using ToupView with light microscopy to compare the rate area of myelination between the control group and radiation group.

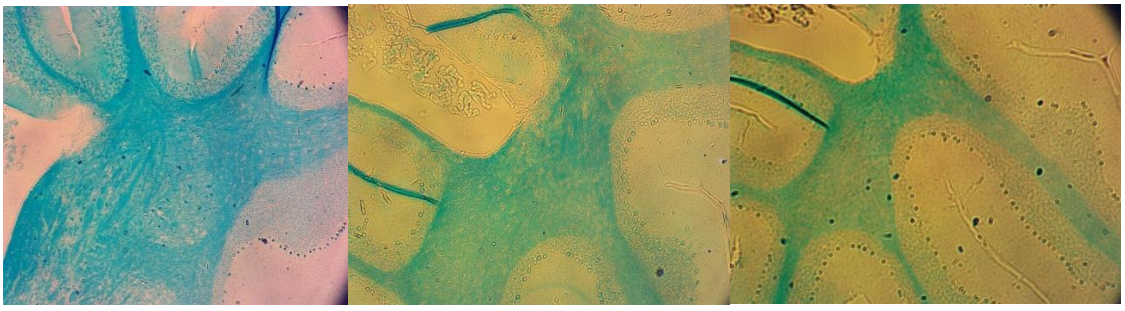


Figure: Control without myelination

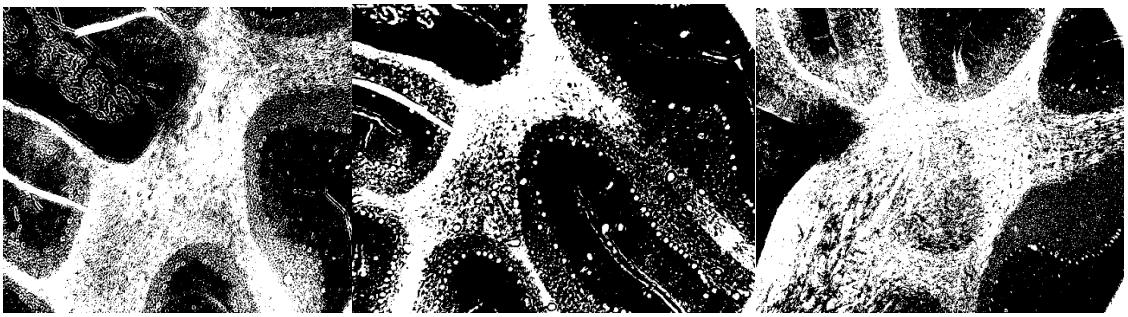


Figure: Control with myelination

Figure: Radiated group without myelination

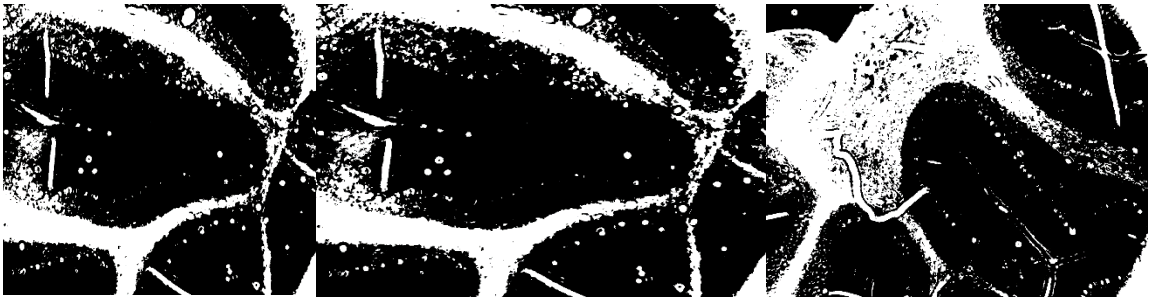


Figure: Radiated group with myelination

5. Conclusion

Our results have shown some behavioral, brain function, and histopathological changes upon rat exposure to 4 Gy and 8 Gy doses of proton radiation. There is a significant difference in hippocampus function between the control and radiation groups. Moreover, some changes in actions like grooming, rearing, and hole entries have been noticed. Histopathological changes were also noticed. This all proves the dangerous effect of proton radiation which is the most abundant part of cosmic rays on the body and brain functions of all living organisms. Astronauts may suffer from the highest damage as they are always exposed to the highest amount of this radiation.

Research in this area must be expanded to understand the long-term effects of cosmic radiation on body tissues and brain functions and their mechanisms. As future research suggests, simulation can be done to save time and cost and to predict the effect of radiation on behavior and body tissue. Moreover, merging research methods of biochemistry, genetics, MD simulation, and computational neuroscience with the techniques used in this work could be very beneficial to fully understand the whole impact of radiation on our bodies.

6. Acknowledgments

It was an honor to attend the STudent Advanced Research Training program START, therefore I would like to thank and express my gratitude to the JINR for giving me this great experience. My sincere thanks to Dr. Iurii Severiukhin for allowing me to attend during this period, allowing me to work and discuss freely, and providing me with great theoretical and practical experience. Moreover, I would like to thank the whole Radiation Biology Laboratory LRB team for being helpful and friendly with me.

7. References

The jamovi project (2022). *jamovi*. (Version 2.3) [Computer Software]. Retrieved from https://www.jamovi.org.

R Core Team (2021). *R: A Language and environment for statistical computing*. (Version 4.1) [Computer software]. Retrieved from https://cran.r-project.org. (R packages retrieved from MRAN snapshot 2022-01-01).

Lenth, R. (2020). emmeans: Estimated Marginal Means, aka Least-Squares Means. [R package]. Retrieved from https://cran.r-project.org/package=emmeans.

ingmann, H. (2018). afex: Analysis of Factorial Experiments. [R package]. Retrieved from https://cran.rproject.org/package=afex.

AURELI, F. & YATES, K. 2010. Distress prevention by grooming others in crested black macaques. *Biol Lett,* 6, 27-9.

BIRKE, L. I. A. & SADLER, D. 1986. Patterns of exploratory behavior in the spiny mouse, Acomys cahirinus. *Behavioral and Neural Biology,* 45, 88-106.

CDC https://www.cdc.gov/nceh/radiation/what_is.html.

CURDT, N., SCHMITT, F. W., BOUTER, C., ISENI, T., WEILE, H. C., ALTUNOK, B., BEINDORFF, N., BAYER, T. A., COOKE, M. B. & BOUTER, Y. 2022. Search strategy analysis of Tg4-42 Alzheimer Mice in the Morris Water Maze reveals early spatial navigation deficits. *Scientific Reports,* 12, 5451.

DAENEN, E. W. P. M., VAN DER HEYDEN, J. A., KRUSE, C. G., WOLTERINK, G. & VAN REE, J. M. 2001. Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus. *Brain Research,* 918, 153-165.

DEY, P. 2018. Embedding of Tissue in Histopathology. *In:* DEY, P. (ed.) *Basic and Advanced Laboratory Techniques in Histopathology and Cytology.* Singapore: Springer Singapore.

ELMORE, S. 2007. Apoptosis: a review of programmed cell death. *Toxicol Pathol,* 35, 495-516.

GOULD, T. D., DAO, D. T. & KOVACSICS, C. E. 2009. The Open Field Test. *In:* GOULD, T. D. (ed.) *Mood and Anxiety Related Phenotypes in Mice: Characterization Using Behavioral Tests.* Totowa, NJ: Humana Press.

JONES, J. A. & KAROUIA, F. J. P. O. C. M. F. S. F. 2008. Radiation disorders. 475-519.

KIANG, J. G. & OLABISI, A. O. 2019. Radiation: a poly-traumatic hit leading to multi-organ injury. *Cell Biosci,* 9, 25.

KIFFER, F., BOERMA, M. & ALLEN, A. 2019. Behavioral effects of space radiation: A comprehensive review of animal studies. *Life Sciences in Space Research,* 21, 1-21.

LIU, C., BENOTTO, M., UNGAR, K. & CHEN, J. 2022. Environmental monitoring and external exposure to natural radiation in Canada. *Journal of Environmental Radioactivity,* 243, 106811.

MACNAUGHTON, W. K. 2000. Review article: new insights into the pathogenesis of radiation-induced intestinal dysfunction. *Aliment Pharmacol Ther,* 14, 523-8.

MOHAN, R. 2022. A review of proton therapy – Current status and future directions. 6, 164-176.

MOHAN, R. & GROSSHANS, D. 2017. Proton therapy – Present and future. *Advanced Drug Delivery Reviews,* 109, 26-44.

MOLESTI, S. & MAJOLO, B. 2013. Grooming increases self-directed behaviour in wild Barbary macaques, Macaca sylvanus. *Animal Behaviour,* 86, 169-175.

POON, I. K., LUCAS, C. D., ROSSI, A. G. & RAVICHANDRAN, K. S. 2014. Apoptotic cell clearance: basic biology and therapeutic potential. *Nat Rev Immunol,* 14, 166-80.

REED, J. C. 2000. Mechanisms of apoptosis. *Am J Pathol,* 157, 1415-30.

ROJAS-CARVAJAL, M., FORNAGUERA, J., MORA, A. & BRENES, J. C. 2018. *Testing experience and environmental enrichment potentiated open-field habituation and grooming behaviour in rats*.

SCHINO, G. & AURELI, F. 2009. Chapter 2 Reciprocal Altruism in Primates: Partner Choice, Cognition, and Emotions. *Advances in the Study of Behavior.* Academic Press.

SEIBENHENER, M. L. & WOOTEN, M. C. 2015. Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *J Vis Exp,* e52434.

SEVERYUKHIN, Y. S., FELDMAN, T. B., OSTROVSKY, M. A. & MOLOKANOV, A. G. 2019. Effects of Cranial Exposure to 170-MeV Proton Radiation at a Dose of 5 Gy on the Visual Behavior and Optomotor Response of Adults Rats. *Biology Bulletin,* 46, 1605-1610.

SEVERYUKHIN, Y. S., LALKOVIČOVÁ, M., UTINA, D. M., LYAKHOVA, K. N., KOLESNIKOVA, I. A., ERMOLAEVA, M. E., MOLOKANOV, A. G., GAEVSKY, V. N., KOMAROV, D. A. & KRASAVIN, E. A. 2023. Comparative Analysis of Behavioral Reactions and Morphological Changes in the Rat Brain After Exposure to Ionizing Radiation with Different Physical Characteristics. *Cellular and Molecular Neurobiology,* 43, 339-353.

SHAHBAZI-GAHROUEI, D., GHOLAMI, M. & SETAYANDEH, S. 2013. A review on natural background radiation. 2, 65-65.

SINGH, H., BISHEN, K., GARG, D., SUKHIJA, H., SHARMA, D. & TOMAR, U. 2019. Fixation and Fixatives: Roles and Functions—A Short Review. *Dental Journal of Advance Studies,* 07.

SLAOUI, M. & FIETTE, L. 2011. Histopathology procedures: from tissue sampling to histopathological evaluation. *Methods Mol Biol,* 691, 69-82.

SUCKERT, T., BEYREUTHER, E., MÜLLER, J., AZADEGAN, B., MEINHARDT, M., RASCHKE, F., BODENSTEIN, E., VON NEUBECK, C., LÜHR, A., KRAUSE, M. & DIETRICH, A. 2021. Late Side Effects in Normal Mouse Brain Tissue After Proton Irradiation. 10.

VALVASSORI, S. S., VARELA, R. B. & QUEVEDO, J. 2017. Chapter 38 - Animal Models of Mood Disorders: Focus on Bipolar Disorder and Depression. *In:* CONN, P. M. (ed.) *Animal Models for the Study of Human Disease (Second Edition).* Academic Press.

VORHEES, C. V. & WILLIAMS, M. T. 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc,* 1, 848-58.

Shin J. Oh (2002). Color Atlas of Nerve Biopsy Pathology. CRC Press. pp. 191–. ISBN 978-0-8493-1676-0. Retrieved 1 January 2013.

Bruce-Gregorios, Jocelyn (2006). Histopathologic Techniques. Goodwill Trading Co., Inc. pp. 241–. ISBN 978-971-12-0270-5. Retrieved 1 January 2013.

Peter A. Humphrey; Louis P. Dehner; John D. Pfeifer (2008). The Washington Manual of Surgical Pathology: Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, Missouri. Lippincott Williams & Wilkins. pp. 681–. ISBN 978-0-7817-6527-5. Retrieved 1 January 2013.