JOINT INSTITUTE FOR NUCLEAR RESEARCH
Laboratory of Radiation Biology

FINAL REPORT ON THE START PROGRAMME

"The effect of cosmic radiation on brain functions, behavior and body tissues of Sprague rats: A Comparative Analysis"

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1. Abstract

Radiation impact on body organs and brain functions is a serious topic as it usually has histopathological, physiological and function related detrimental effects. Every object in our environment is radioactive with a certain degree from food to human itself. Radiation is needed in several aspects of life like in medicine for cancer therapy for and in research. Cosmic radiation which comes from space, may have hazardous outcomes on astronauts and are somehow mysterious till now. In this study, we have investigated the effects of high energy proton radiation on Sprague rats by analyzing histopathological samples from rat brain, intestine and liver organs, assessing learning and memory abilities of rats using Open Field test and Morris Water Maze test and performing statistical testing to elucidate the difference between control and test groups. Results illustrated that rats which undergo radiation suffered from significant brain function changes and some tissue apoptosis.

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 of 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, human can be affected by radiation naturally. About 82% radiation-absorbed population are 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. They are variable in composition and usually are formed of protons and nuclei of elements (Ginzburg and Syrovatskii, 1964). 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 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 Event as they comprise about 86-91% of galactic cosmic rays as shown in figure (1) (Kiffer et al., 2019, Jones and Karouia, 2008).

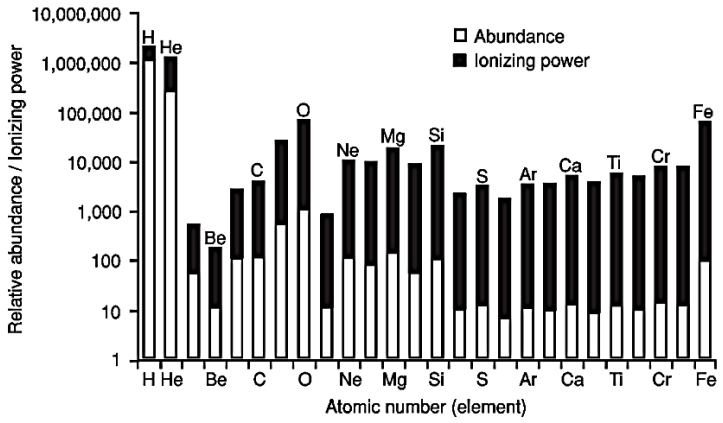


Fig (1): The abundance and ionizing power of different elements in galactic cosmic rays (Jones and Karouia, 2008).

Protons can be used in cancer therapy with selectivity for tumor tissues rather than normal ones (Mohan and Grosshans, 2017). Moreover, it showed a greater effectiveness over photon therapy (Mohan, 2022). However, proton radiation has serious harmful effects on human and the environment. It can affect people in several settings and can affect approximately all body tissues (Jones and Karouia, 2008). As 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 vessels dilatation, cell edema and deposited granules in hippocampus with sclerosis. Furthermore, a publication by (Kiffer et al., 2019) have 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, which 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.

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

3. Materials and Methods

a. Animals and study design

A single blinded study in which 14 female Sprague rats were used as only few publications studied the effect of radiation on female rats (Kiffer et al., 2019). They were obtained from “Pushchino” nursery, and they had a free access to water and food. Rats were divided into control and test groups. Behavioral and brain function tests were performed before and 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 Scientifc-technical council of Laboratory of Radiation Biology).

b. Radiation procedures

Test (proton) group was irradiated with a dose of 3 Gy of 170 MeV proton radiation as a previous study by (Severyukhin et al., 2019) has shown that it has a significant deleterious effect of 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). For irradiation process, rats were placed in cylindrical tubes made from polymethyl methacrylate. Radiation direction was cranio-caudal, starting from rat heads and then down the body. The process took place at Medical and Technical Complex at JINR.

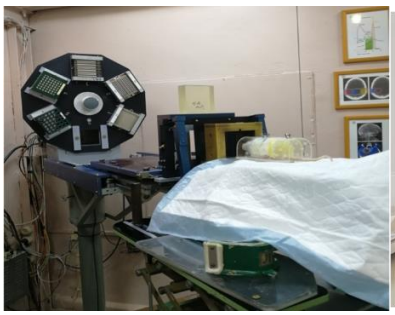


Fig (2): Irradiation procedures in medical and technical complex, JINR.

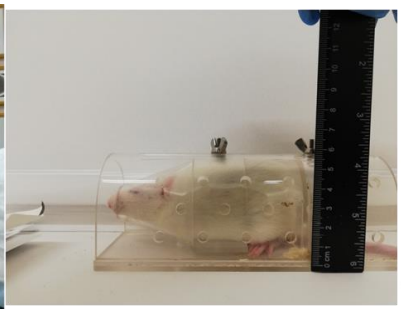


Fig (3): Radiation specific cylindrical tube.

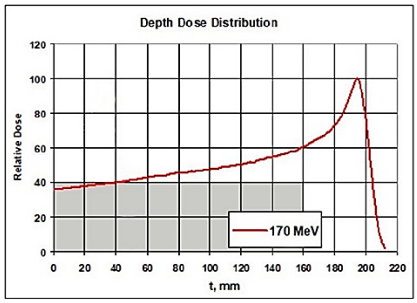


Fig (4): The chart shows the relative dose used with the direction starting from rat head to tail.

c. Brain functions and behavioral tests

To assess rat brain functions and behavior we have used:

1- Morris Water Maze (MWM) test

Morris Water Maze (MWM) is used in our study to assess rat learning functions after irradiation. Moreover, it is also used to evaluate hippocampus functions as MWM depend on NMDA receptor function and Long-Term Potentiation (LTP). It consists of a circular sink, theoretically divided into four equal quadrants, filled with approximately 50% of its volume water with a platform in the center of one of the four quadrants. The platform is partially covered by water so that it is hidden from the rat. MWM is not a typical maze, but the animal has to find its way to the platform. According to the directions and the way by which rats reach the platform, movement pattern can be divided into hippocampus dependent and non-hippocampus dependent movement, so we can assess if there is an actual damage in the hippocampus as a result of proton radiation or not. Regarding Hippocampus dependent search strategies, it includes Direct Path DP, in which animals directly reach the platform without deviation, Direct Search DS, it includes very little deviation, Focal Search FS, which involves searching in very limited space and finally Indirect Search IS which is searching with a major deviation. Non-hippocampus dependent strategies are Chaining

C, in which animal model swims in a circular manner with a fixed distance from the walls of the sink, Thigmotaxis T, in which all rat movement becomes close to the wall of the pool, Scanning S which depends on random search but with wall avoided and is relatively near the platform and Random Searching RS with totally random searching pattern (Vorhees and Williams, 2006, Curdt et al., 2022). All of these strategies were recorded and shown in fig (9). Each animal in both control and proton group was introduced to the sink 3 times at zero point (before radiation), on the 30th day after radiation and on the 90th day. Their searching strategies were recorded with a camera and were analyzed to assess the difference.

2- Open Field Test OFT

OFT is a test designed to examine rat behavior and exploratory and general activities. It can be in the form of rectangular or circular area, but we have used a circular area with a fence around to prevent animal escape with the ground divided into three circles that share the same center and with holes distributed all over these areas as shown in fig (10). Several rat actions and activities were recorded and tabulated, quantitatively and qualitatively which means that certain actions have been recorded with the exact number of times that these actions have performed (Gould et al., 2009, Seibenhener and Wooten, 2015). Several actions can be recorded as shown in fig (5). Moreover, each action that has an explanation like for example when they enter the holes on the ground of arena, this action indicates an exploratory action. Furthermore, grooming, for relaxation, rearing, which is standing up using only two legs with upper legs free or relying on the wall, center entry, which indicates lower anxiety, freezing action, by which rats give no response as they became terrified and urination and defecation which indicate exaggerated stress. There were two groups, control and proton groups, 7 rats in each, and each rat was left in the area one time on the day of radiation and 27 days and 90 days after radiation. Then, their movements, actions and activities were recorded in 6:02 minute long videos by camera and were noted down by human eyes and were analyzed manually by eye and by Ethovision XT for statistics as well.

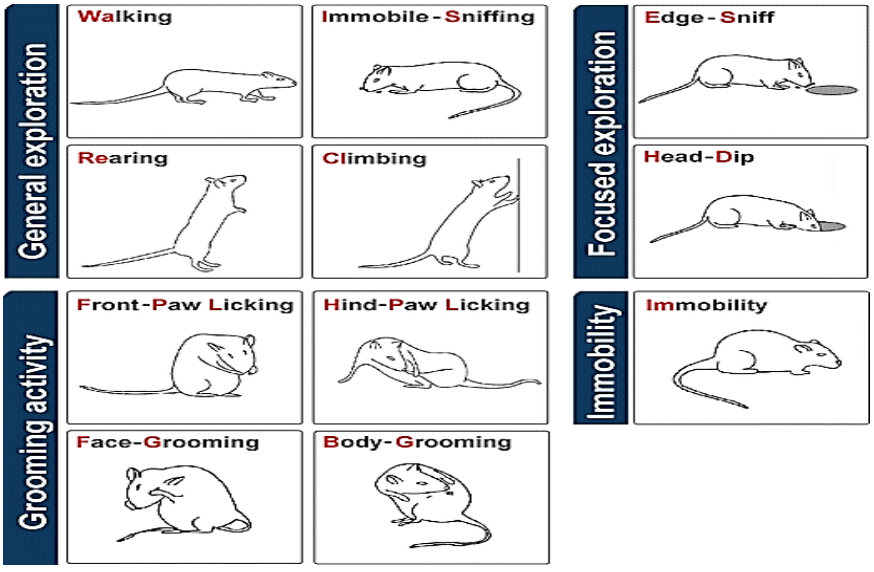


Fig (5): Some rat actions in Open Field Test OFT

d. Histopathological sample preparation and staining

Three rats were decapitated after 30 days, one from control group and the other two from proton groups. Organs were separated, and different processes were performed to preserve and stain tissue.

- Fixation

we worked on already fixed organs in formalin 10% for 24 hours as it causes lower tissue shrinkage and excellent preservation (Vonnie and Thomas, 2018). Washing with tap water is followed to prevent tissue artifacts that may be caused by formalin. Fixation is considered the first step in staining to save 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 buttress 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 ascending concentration of ethanol (50% for 1h., 70% for 2h., 80% for 1h., 96% for 1 night for 2 times and 100% for 30 minutes). This followed by clearing samples for 20 minutes, 3 times, using xylene to remove alcohol. And finally, infiltration followed. Paraffin wax with melting point (56 °C) mixed with xylene for 25 minutes for 1 time followed by 2 times without xylene. Low melting point was used to prevent tissue damage by higher temperatures.

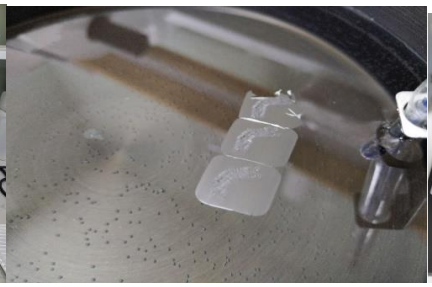
Tissues were then transferred from cassettes using worm forceps to a previously wax filled mold. Then, cassettes were transferred above wax molds followed by additional wax filling. Finally, mold was transferred to chilled water on a cold plate for 30 minutes to form a block in a process called blocking.

- Sectioning

Tissue sectioning is a serious and significant stage as with good sectioning, specimens will be more obvious under microscope to detect any effects or artifacts. Sectioning was done by rotary microtome shown in fig (6) and 6 micrometer specimens were produced. Then, thin specimens were floated in a 45°C water bath to flatten the wax as shown in fig (6,7), glass slides were put under tissue slices to adhere to it and samples were let to dry firstly at approximately 50°C as shown if fig (8) and then left about 24 hours to dry at room temperature for best sample fixation to slides. Moreover, the depth of trimming was also recorded to detect which part we have reached, so we can assess if there are problems 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 problem regarding the temperature of Water Bath or even drying time. I made slides from 24h. irradiated rat brain, 24h. control rat kidney and control and irradiated rat intestines. Brain slices were taken at several depth points on the tissue stating from zero to 168 micrometers, kidney slices were taken at zero up to 144 micrometer, irradiated kidney starting from zero to 108 and control kidney slices were up to 162. I have learnt some hacks and tricks like using our breath temperature to

facilitate the process of slicing. Furthermore, the best way for sectioning is to be gentle handling and cutting samples.

- **Fig (6): microtome and water bath at 45°C.**



- Fig (7): the flattening process of slices over the surface of the warm water.

- Fig (8): the first drying of the slides at approximately 50°C

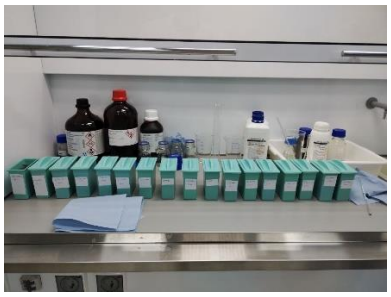
- Staining

Hematoxylin and Eosin (H&E) stain was used with the same order in the table below. Hematoxylin is a basic dye, so it stains nucleus with blue and eosin is an alkaline dye which stain cytoplasm with orange-red color.

Firstly, 800 ml of 95% Isopropanol IPA were prepared by mixing 760 ml of 100% IPA with 40 ml distillated water. Staining followed the time and the order of each stage as in the table and the image below. Wax was removed using Neo-clean instead of xylene due to the higher toxicity of the latter, followed by rehydration with 100% and 95% IPA respectively, as IPA is an economic and safer substitute for ethanol. Then, slides were rinsed with tap water. Slides then were soaked in Hematoxylin for 3 minutes followed by tap water washing. Two minutes of eosin slide soaking for cytoplasm and fibers staining took place before repeated tap water rinsing of slides. Dehydration was done using 95% and 100% IPA respectively and xylene was used to remove excess alcohol and increase the refractive index (we have tried Neo-clean in this stage, but some clouds and fogs appeared on the slide as shown in the photo below, that may be due to an interaction between Neo-clean and the mounting agent). VitroGel®, a mounting agent, was added on the specimen with a cover slip glass above as shown in the photo below. A very gentle-up and down-vertical shaking must be done in all stages especially in dewaxing and dehydration. Slides were dried at room temperature and specimens can be stored at room temperature away from light (Slaoui and Fiette, 2011). Moreover, at first staining were so intense, so we have modified the time for hematoxylin and eosin and the final protocol is mentioned in the table below.

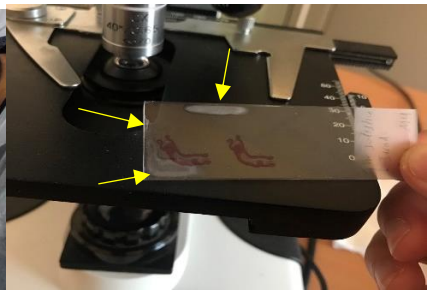
Neo-Clear	**5 min**
Neo-Clear	**5 min**
IPA 100% (Isopropyl Alcohol)	**5 min**
IPA 100% (Isopropyl Alcohol)	**5 min**
IPA 95% (Isopropyl Alcohol)	**5 min**
IPA 95% (Isopropyl Alcohol)	**5 min**
Tap water	**3 min**
Hematoxylin	**3 min**
Tap water	**3 min**
Eosin	**2 min**
Tap water	**1 min**

IPA 95% (Isopropyl Alcohol)	**2 min**
IPA 95% (Isopropyl Alcohol)	**2 min**
IPA 100% (Isopropyl Alcohol)	**2 min**
IPA 100% (Isopropyl Alcohol)	**2 min**
Xylene	**2 min**
Xylene	**2 min**
Cover	**VitroGel®**



- Stages of H&E staining

- addition of the mounting agent)



- A drawback: Clouds appeared (Solved by using xylene in last 2 stages)

e. Statistical analysis

We have used jamovi 2.3.24 for our statistical analysis. In Morris Water Maze test (MWM), we compared control and proton group research strategies, whether Hippocampus dependent or independent, using x^2 test of association as independent samples. In Open Field Test (OFT), we used the number of times of certain actions to perform normality test, depending on its result, results underwent Student's t test and Mann-Whitney U test. Moreover, mean rat velocities and distance moved were calculated for locomotion change assessment. In the histological part, we have counted the number of apoptotic cells using ImageJ in control tissue and 4h, 24h and 48h after 3Gy dose radiated tissues using 3 different tissue slices for each. We have captured 5 pictures for each tissue slice with a total amount of 60 pictures. We have used small intestine with the main focus on intestinal crypts. Repeated Measures ANOVA test was done.

4. Results and Discussion

1- MWM test

we have recorded the patterns of movement of the rats or their search strategies, and they showed a variety in their search strategies as shown in figure (9).

Data from MWM test were then used for statistical analysis. There is a comparison between control and proton group at zero point (before radiation), after 30 days of radiation and then after 90 days according to the type of search strategy, whether HD or NHD which gives indication if there is an irradiation induced hippocampus damage or not. As shown in table (a), there is no significant difference in HD and NHD search strategies between control and proton groups before irradiation. On the other hand, and as shown in table (b), there is a significant difference (P = 0.005) between the two groups 30 days after irradiation which elucidates the devastating effect of radiation on hippocampus. Contrary, P value (P = 0.057) was higher than that obtained from data after 30 days with a lower NHD strategies as shown in table (c) which

may indicate that there is some hippocampal tissue repair or regeneration as a recovery from radiation during this whole period.

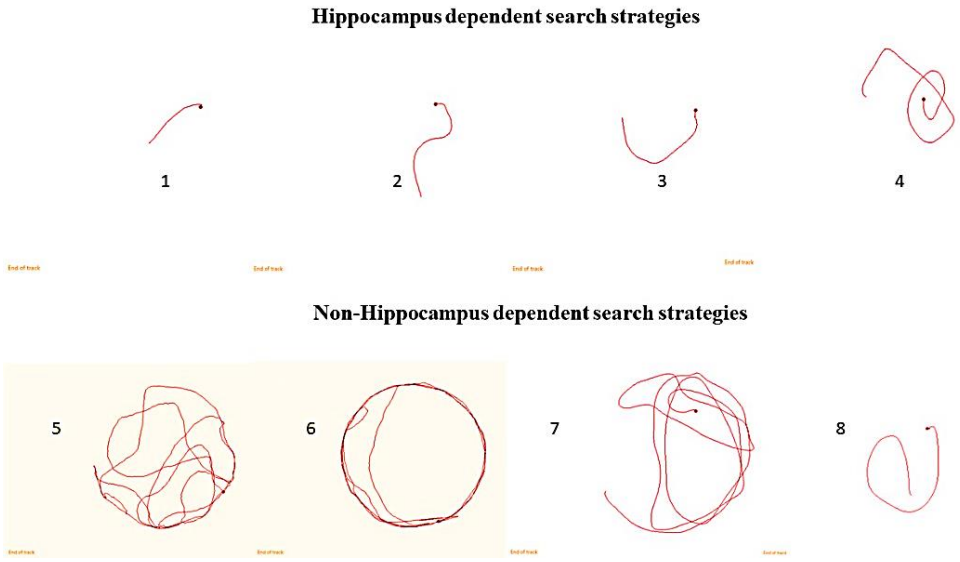


Fig (9): The figure shows different search strategies that rats have already used to reach the platform: (1) Direct Path DP, (2) Direct Search DS, (3) Indirect Search IS, (4) Focal Search FS, (5) Random Search RS, (6) Thigmotaxis T, (7) Scanning S, (8) Chaining C.

Contingency Tables

Contingency Tables

	Before		
Group	**NHD**	**HD**	**Total**
control	62	22	84
protons	54	29	83
Total	116	51	167

χ^2 Tests

	Value	df	p
χ^2	1.51	1	0.220
N	167		

(a)

Contingency Tables

Contingency Tables

	30 days		
Group	**HD**	**NHD**	**Total**
control	72	12	84
protons	56	27	83
Total	128	39	167

χ^2 Tests

	Value	df	p
χ^2	7.76	1	0.005
N	167		

(b)

Contingency Tables

Contingency Tables

	90 days		
Group	**HD**	**NHD**	**Total**
control	65	7	72
protons	47	13	60
Total	112	20	132

χ^2 Tests

	Value	df	p
χ^2	3.63	1	0.057
N	132		

(c)

2- Open Field Test OFT

All events in fig (10) have been recorded by camera and tabulated with exact number of times they happened.

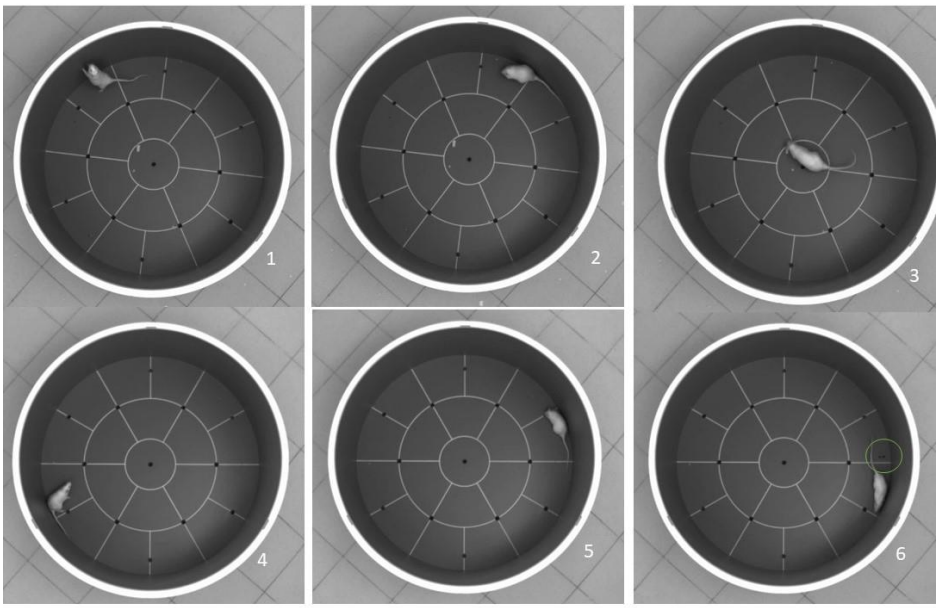


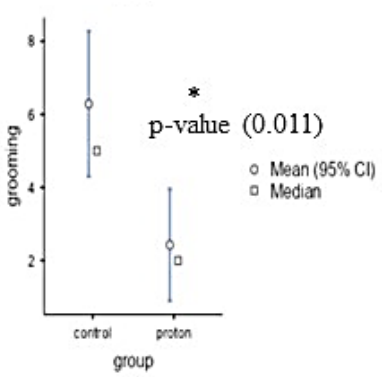
Fig (10): Actions of rats in Open Field Test (OFT): 1- rearing, 2- grooming, 3- center entry, 4- freezing, 5- hole entry, 6- defecation.

Regarding statistical analysis of OFT findings, we have performed a normality test to see which variables should undergo Student's t test or Mann-Whitney test. At day 1, there is a significant difference (p-value 0.011) in grooming as it is significantly lower in proton group than in control group as shown in fig (11). Grooming 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 communications (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 have been found to be higher after grooming between animals (Schino and Aureli, 2009). Therefore, a significant decrease of 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 a depression in certain areas of the brain, so rats are no more 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 be resulted from proton radiation.

After 27 days of radiation and as shown in fig (12), we can find that the number of hole entries varies significantly (p-value 0.003) with higher number of hole entries in proton group than in control group.

The usual thought about hole entry is that it is related with exploration activity, but a publication by (Birke and Sadler, 1986) have 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.

Finally, fig (13) has shown a lower level of significance in rearing difference between proton and control group after 90 days from radiation. Rearing is strongly related with animal hyperactivity and exploration (Valvassori et al., 2017). Therefore, the lower rearing in proton group may be due to the damaging effect of proton radiation after 90 days, which can be irreversible, in the brains of experimental rats and so decreasing the exploratory actions.



Assumptions

Normality Test (Shapiro-Wilk)

	W	p
center entries	0.893	0.089
rearing	0.943	0.456
grooming	0.923	0.246
freezing	0.814	0.007
holes entries	0.959	0.709
defecation	0.510	< .001

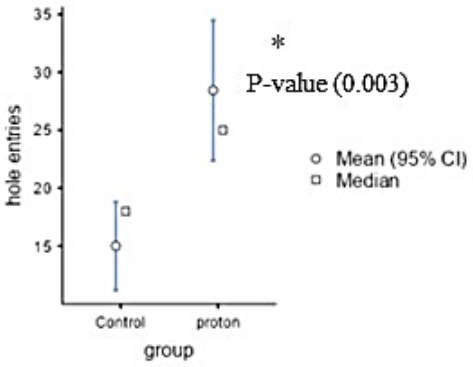
Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p
center entries	Student's t	1.271	12.0	0.228
	Mann-Whitney U	14.00		0.188
rearing	Student's t	1.726	12.0	0.110
	Mann-Whitney U	12.50		0.140
grooming	Student's t	3.006	12.0	0.011 *
	Mann-Whitney U	6.00		0.021
freezing	Student's t	-0.397	12.0	0.698
	Mann-Whitney U	17.50		0.372
holes entries	Student's t	1.356	12.0	0.200
	Mann-Whitney U	16.00		0.302
defecation	Student's t	1.000 *	12.0	0.337
	Mann-Whitney U	21.00		0.391

Fig (11): OFT statistical analysis at the day of radiation.

(*) indicates a significant value



Assumptions

Normality Test (Shapiro-Wilk)

	W	p
center entries	0.929	0.293
rearing	0.977	0.954
grooming	0.893	0.089
freezing	0.668	< .001
hole entries	0.951	0.582

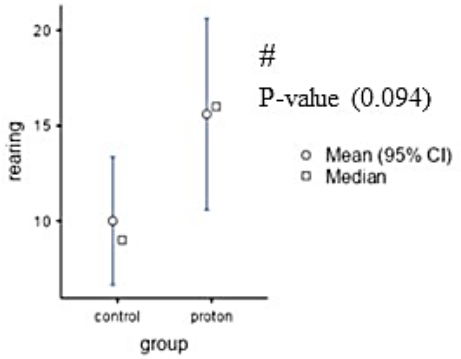
Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p
center entries	Student's t	0.756	12.0	0.464
	Mann-Whitney U	18.500		0.477
rearing	Student's t	1.172	12.0	0.264
	Mann-Whitney U	15.000		0.247
grooming	Student's t	0.885	12.0	0.393
	Mann-Whitney U	19.500		0.554
freezing	Student's t	-0.188	12.0	0.854
	Mann-Whitney U	19.000		0.473
hole entries	Student's t	-3.672	12.0	0.003 *
	Mann-Whitney U	0.500		0.003

Fig (12): OFT statistical analysis after 27 days of radiation.

(*) indicates a significant value



Assumptions

Normality Test (Shapiro-Wilk)

	W	p
center entries	0.934	0.456
rearing	0.950	0.639
grooming	0.882	0.109
freezing	0.880	0.103
holes entries	0.954	0.698

Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p
center entries	Student's t	-1.3401	9.00	0.213
rearing	Student's t	-1.8739	9.00	0.094 #
grooming	Student's t	-0.0323	9.00	0.975
freezing	Student's t	-1.1176	9.00	0.293
holes entries	Student's t	-0.3826	9.00	0.711

Fig (13): OFT statistical analysis after 90 days from radiation

(#) indicates a lower level of significance

- EthoVision XT software for OFT

Videos were uploaded on Ethovision XT for rat tracking and data analysis. Fig (14) shows heatmaps resulted from rat movement. We can check the difference in movement status, yellow-colored areas show the periods of rat immobility. (d) and (f) heatmaps are related to proton groups after 27 and 90 days respectively and how there are longer periods of motionless than (c) and (e) of control groups. This may

be an indicator for certain brain area problems or depression. Conversely, different patterns can be obvious in (a) and (b) and this may indicate extreme fear and anxiety after radiation.

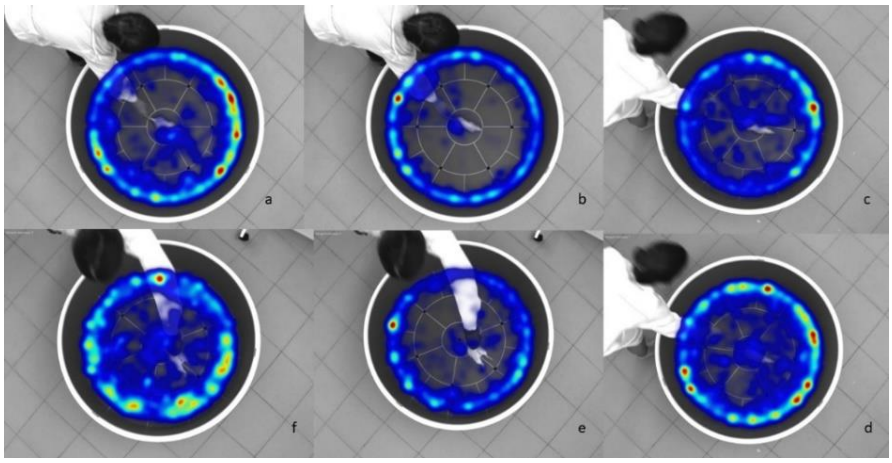


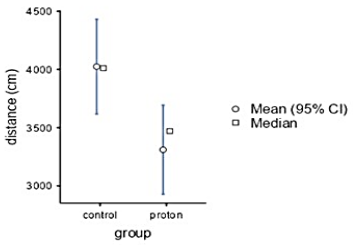
Fig (14): EthoVision XT Heatmaps: Day 1: (a) control, (b) proton – Day 27: (c) control, (d) proton – Day 90: (e) control, (f) control.

Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p	
distance (cm)	Student's t	2.50	12.0	0.028	*
velocity (cm/s)	Student's t	2.48	12.0	0.029	*

Note. H_a $\mu_{control} \neq \mu_{proton}$



Assumptions

Normality Test (Shapiro-Wilk)

	W	p
distance (cm)	0.955	0.635
velocity (cm/s)	0.955	0.642

Note. A low p-value suggests a violation of the assumption of normality

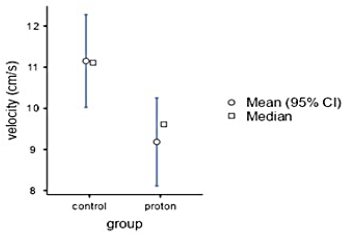


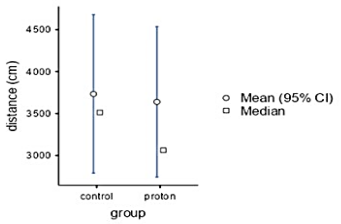
Fig (15): Day 1 proton and control groups vs velocity and distance

Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p
distance (cm)	Student's t	0.142	12.0	0.889
velocity (cm/s)	Student's t	0.139	12.0	0.892

Note. H_a $\mu_{control} \neq \mu_{proton}$



Assumptions

Normality Test (Shapiro-Wilk)

	W	p
distance (cm)	0.934	0.344
velocity (cm/s)	0.934	0.347

Note. A low p-value suggests a violation of the assumption of normality

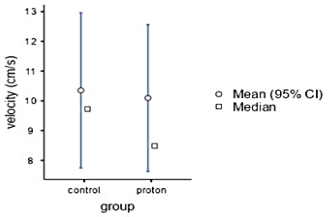


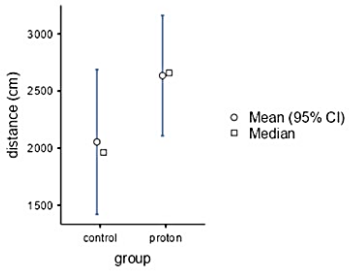
Fig (16): Day 27 proton and control groups vs velocity and distance

Independent Samples T-Test

Independent Samples T-Test

		Statistic	df	p
distance (cm)	Student's t	-1.34	9.00	0.212
velocity (cm/s)	Student's t	-1.35	9.00	0.209

Note. H_a $\mu_{control} \neq \mu_{proton}$



Assumptions

Normality Test (Shapiro-Wilk)

	W	p
distance (cm)	0.959	0.754
velocity (cm/s)	0.955	0.714

Note. A low p-value suggests a violation of the assumption of normality

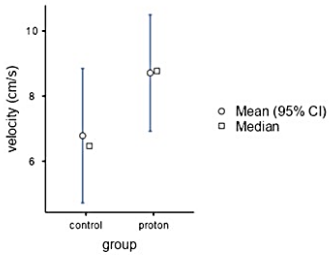
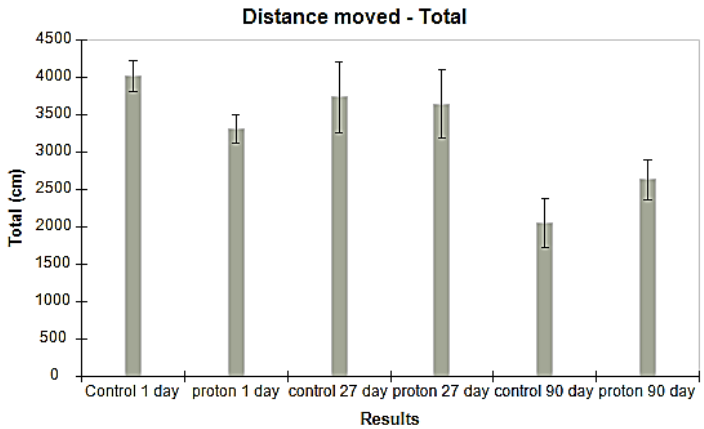


Fig (17): Day 90 proton and control groups vs velocity and distance

Locomotion of rats has been also assessed. Normality and Student's t tests were performed. On Day 1, fig (15) shows a significant difference with lower velocity and distance in rats in proton group than those of the control group as an indicator of the acute damaging effect of radiation on brain functions and behavior. However, on Day 27 and 90, fig (16) and (17), there is no significant difference, which may be evidence on brain tissue repair with this dose of radiation, but brain may not be able to do so with higher continuous radiation doses. Moreover, a lower level of adaptation has been detected after radiation as the difference between the percentage of the mean distance moved by rats in control rats and proton rats after 90 days is lower than the difference between both groups at day 1 with the difference percentages of 20% and 49% respectively. And this means that proton group after 90 days consumed more time to adapt the place with a higher mean distance than day 1 proton group. This decrease in adaptation may suggest the destructive effect of proton radiation on rat amygdala and ventral hippocampus region as previously mentioned in (Daenen et al., 2001) study.



	Control %	Proton %
1 day	100%	100%
90 days	51.03%	79.90%
difference	49%	20%

Fig (18): total distance moved during the experiment for all groups and the percentage difference in total distance mean between control and proton groups at day 1 and 90.

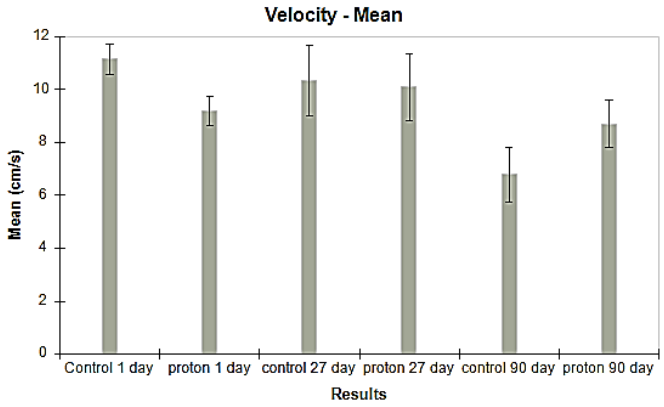


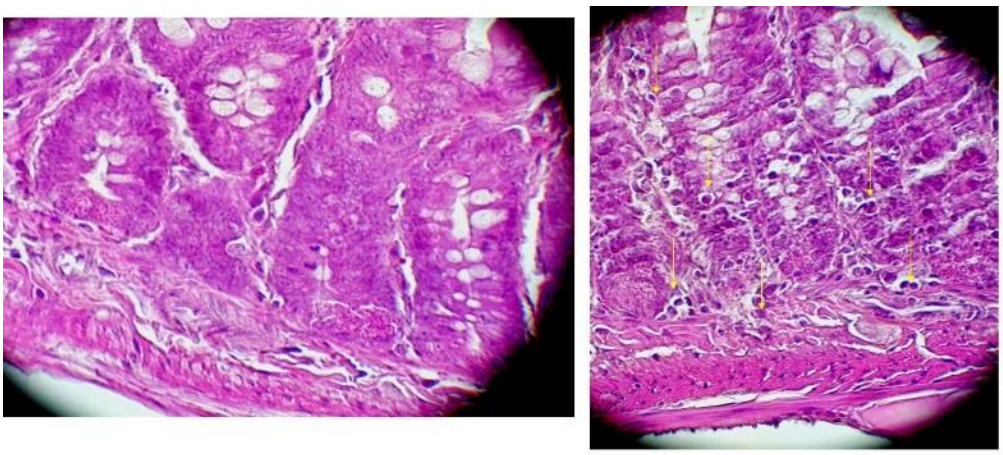
Fig (19): mean velocities for all groups during the whole experiment

3- Histological sample examination

We detected overall changes in tissues and cells. One of these changes was apoptosis. Apoptosis is considered a programmed cell death in which cell may die naturally due to aging, due to certain drugs and chemotherapy or due to occupational or therapeutic radiation to maintain the integrity and health of other cells. It mainly occurs due to DNA damage by one of the mentioned agents which is the stimulus of apoptotic cascade beginning (Elmore, 2007). A publication by (Reed, 2000) has shown apoptosis mechanism and pathways. The damage caused by radiation depends on several factors like radiation duration, dose, organ sensitivity and comorbid conditions. One of the highest sensitive body organs towards radiations is gastrointestinal tract with the acute effects to appear on the intestinal crypts as radiation cause crypt epithelial cell mitosis dysfunction resulting in apoptosis (MacNaughton, 2000, Kiang and Olabisi, 2019).

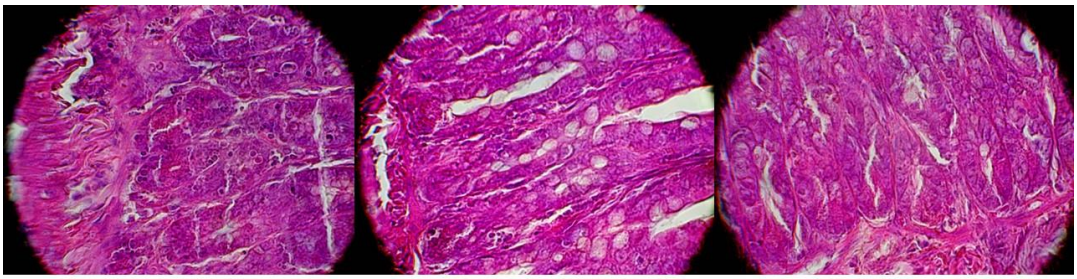
Pictures for intestinal crypts of control and irradiation rat groups were taken using ToupView with light microscopy and apoptotic bodies were detected in crypt bodies of the irradiated rat group as shown in Fig (20).

In Fig (21), four hours after irradiation, crypts showed the highest apoptotic bodies which then decreased in 24 h. sample with the lowest number in the intestine sample taken 48 h. after radiation. This can be due to phagocytosis clearance of apoptotic cells as an important role of an intact immunity which allows keeping the homeostasis of the tissues (Poon et al., 2014).



Control Radiation

Fig (20): intestinal crypts with normal cells in control group and with apoptosis indicated by arrows in the radiation group.



4h. after radiation 24h. after radiation 48h. after radiation

Fig (21): apoptosis dynamics in 4, 24 and 48 hours after radiation samples.

Repeated Measures ANOVA

Within Subjects Effects

	Sum of Squares	df	Mean Square	F	p
RM Factor 1	41679	3	13893	23.4	< .001
Residual	24927	42	594		

Note. Type 3 Sums of Squares

Post Hoc Tests

Post Hoc Comparisons - RM Factor 1

Comparison							
RM Factor 1		RM Factor 1	Mean Difference	SE	df	t	p_{tukey}
control	-	4h	-73.40	6.93	14.0	-10.599	< .001
	-	24h	-25.87	7.63	14.0	-3.392	0.020
	-	48h	-30.13	9.96	14.0	-3.025	0.040
4h	-	24h	47.53	7.64	14.0	6.220	< .001
	-	48h	43.27	11.17	14.0	3.874	0.008
24h	-	48h	-4.27	9.29	14.0	-0.459	0.967

Fig (22): Repeated Measure ANOVA test and Post Hoc test.

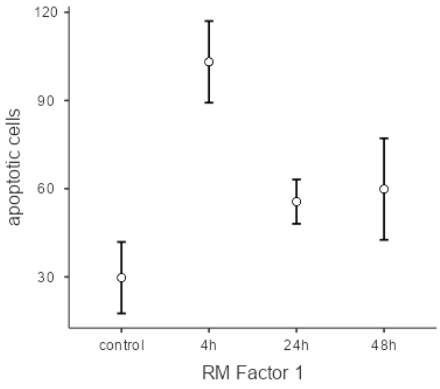


Fig (23): the chart shows the difference in apoptotic cells between all groups.

Repeated Measures ANOVA test was performed. As shown in the tables below, ANOVA test has shown a significant difference in the number of apoptotic bodies between the four groups with p-value < .001 and Post Hoc test has also shown a significant difference between the control group and 4h after radiation group and between 4h and 24h after radiation groups with p-value < .001 for both. Difference between all groups was significant except that between 24h and 48h after radiation groups. Figure (23) also shows apoptotic bodies population difference between all groups with approximately no difference between 24h and 48h ones.

During counting apoptotic bodies, it was obvious that apoptotic bodies were lower in number in 24h and 48h groups than in 4h group, however, there were a higher number of more complex apoptotic bodies or cells in 2nd stage of apoptosis in 24h and 48h groups.

5. Conclusion

Our results have shown some behavioral, brain function and histopathological changes upon rat exposure to 3Gy dose of proton radiation. There is a significant difference in hippocampus function between control and radiation groups. Moreover, some changes in actions like grooming, rearing and hole entries have been noticed. Histopathological changes like apoptosis 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 suggestions, simulation can be done to save time and cost and to expect 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.

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