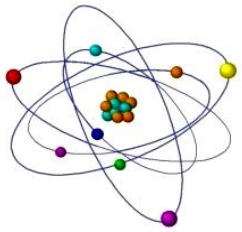
EFFECT OF ACUTE GAMMA IRRADIATION ON THE BEHAVIORAL PARAMETERS IN WHITE MICE



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ABSTRACT: *High doses of ionizing radiation exposure have been shown to induce impairments in the limbic system. As hippocampal abnormalities have been linked to an impairment of behavioral functions, the present work aimed to investigate whether exposure to 5Gy of ionizing radiation can develop behavioral deficits in adult mice. White mice (Mus musculus) were irradiated with ^{137}Cs. Experimental animals were tested in an open field maze over a period of 30 days after irradiation. A study of open-field parameters revealed that gamma irradiation can be considered a radiobiological factor inducing anxiety and emotionality in mice.*

Key words: Gamma irradiation, white mice behavior, open field test

INTRODUCTION

Radiation-induced brain injury is a long-term and dynamic process. Post-radiation brain injury results in neurobehavioral disorders as a consequence of functional and anatomic deficits [1]. Radiation-induced cognitive impairments are related to the hippocampus, a key subcortical structure in the mammalian brain, involved in three primary functions: the formation of new memories, spatial learning, and emotions. Increasing evidence indicates that radiation-induced early cerebral changes may be determining factors of behavioral and emotional impairments [2,3]. The *Cornu Ammonis* (CA) is a seahorse-like structure that describes the different layers of the hippocampus. There are four hippocampal regions CA1, CA2, CA3, and CA4. CA3 receives fibers from the dentate gyrus granule cells. The entorhinal cortex–hippocampal neuron network plays a major role in episodic memory and spatial information about the occurrence of former events [4,5,6]. Pathophysiological responses to radiation-induced brain injury include the following changes increased numbers of apoptotic cells, reduced neurogenesis in the subgranular zone (SGZ), demyelination, or blood-brain barrier (BBB) disruption [7,8,9]. Stem cells in SGZ of the hippocampal Dentata Gyrus and the SVZ along the lateral ventricles have special sensitivity to ionizing radiation [10,11]. There is growing evidence of significant cognitive impairment during the post-irradiation period when there are no expressed histological abnormalities. Elevated zero maze, the Open Field test, the elevated plus maze, the Morris water maze, and the elevated-type multi-way maze are the most commonly used tests to measure behavior in animal models [12]. High doses of gamma irradiation significantly increase impairment in short-term memory, decreases the spatial learning process, and cause radiation-induced aging [13]. Though the mechanisms involved in this process are still the subject of study.

This study aimed to determine the influence of acute gamma irradiation on behavioral parameters using an open-field maze.

MATERIAL AND METHODS

The experimental protocol was by the guidelines for the care and use of laboratory animals as adopted by the Ethics Committee of the Tbilisi State Medical University (TSMU). Animal care and maintenance: 3-month-old male mice (Mus musculus), were obtained from the Vivarium of Tbilisi State Medical University. They were housed in animal cages, with room temperature maintained at 200 -220C and relative humidity of 50-70%. Also, a time-controlled system provided 08:00-20:00 h of light and 20:00-08:00 h dark cycles. All mice were given a standard rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes.

Experimental design.

After acclimatization for a week to laboratory conditions, the mice were divided into two groups. The I control group of 3-month-old not irradiated mice, II experimental group of 3-month-old irradiated mice. Mice whole-body irradiation with ^{137}Cs was performed at a dose rate of 1,1Gy/min for a total dose of 5Gy with the equipment "Gamma-capsule-2".

Anxiety-like behavior and exploratory activity were estimated in the open field. The open field consists of a Plexiglas enclosure (40×40 cm) placed in the center of a normally lit experimental room. Mice were placed in the left corner of the enclosure; head facing an open space, their anxiety-like and exploratory behaviors were recorded for 5 min across the area divided into 16 squares. The parameters measured were the time spent in the center of the arena (four central squares), the total time spent near the walls, the number of rearing, and defecation. Tests were performed on days 2, 4, 6, 8, 10, 12, 14, and 30 after irradiation.

RESULTS AND DISCUSSION

Our study aimed to evaluate radiation-induced behavioral changes using an open-field test. The open field test is often used to assess anxiety and locomotor activity; The main variables recorded during the test period are the time taken to leave the starting square, time spent in the center of the arena rearing, grooming frequency, and several defecation boli [15,16].

Less time spent in the central area of the box, increased rearing, grooming, and defecation frequency demonstrates anxiety-like behavior in rodents. Furthermore, thigmotaxis refers to the specific behavior of animals (staying close to walls in the open field), which is a well-established indicator of animal anxiety and fear [17, 18].

In this study, we assessed four parameters of the Open field test (OFT) to evaluate the anxiety and emotionality of experimental animals. As shown in Fig.1 compared to the control group rearing behavior increases on the 2nd, 4th, 6th, 14^{th,} and 30th days after irradiation (5Gy). On the 8th, 10th, and 12th days after irradiation, the difference from the control group was not statistically significant. Rearing behaviors could be considered as an additional measurement of anxiety in rodents and may be used in the repeated testing process [19,20]. Anxiety-like behavior conditions are thought to be mediated by the hippocampal formation which is a key target of the stress response [21]. Recently, oxidative stress has also been implicated in high anxiety levels. The formation of reactive oxygen species after irradiation could cause hippocampal impairments resulting in behavioral changes [22].

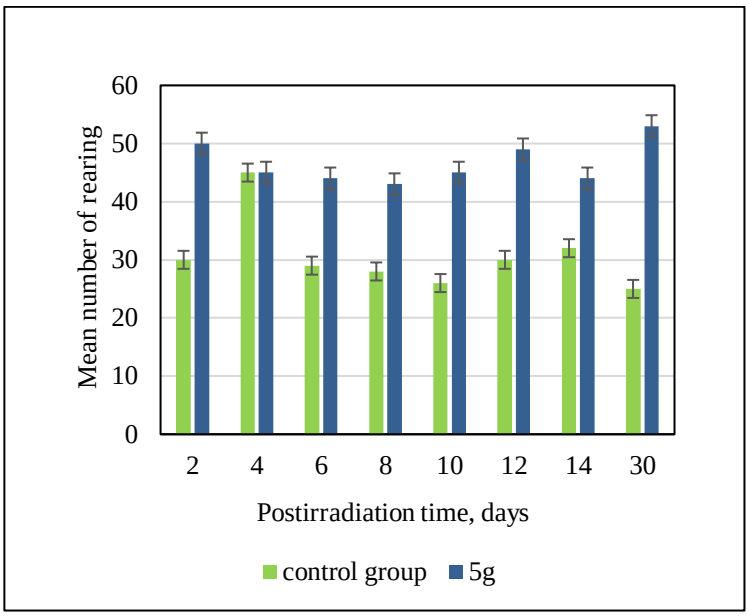

Fig.1. Mean number of rearing in experimental animals during the post-radiation period

Thigmotaxis or wall-hugging behavior is observed in experimental mice and is in relation to anxiety-related behaviors. Increased time spent in the outer zones of the maze is linked to thigmotaxis [23,24]. In our study on the 2nd, 4th, 12th, 14th, and 30th days of irradiation time spent in the center is less than time spent at the walls of the maze (approximately 1 minute). Animals of the experimental group showed increased thigmotaxis compared to the control group (Fig.3,4).

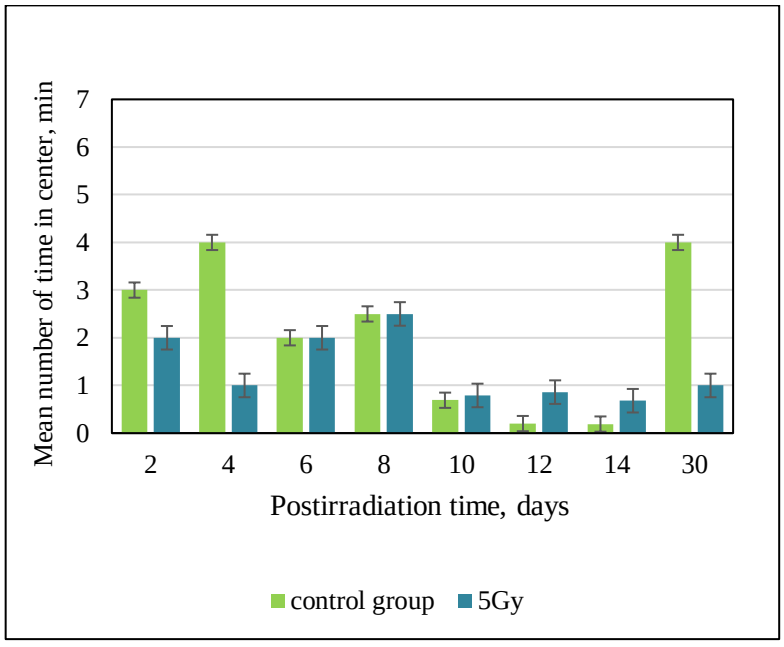

Fig 2. Mean number of time spent in the center of open field in experimental animals during the post-radiation period

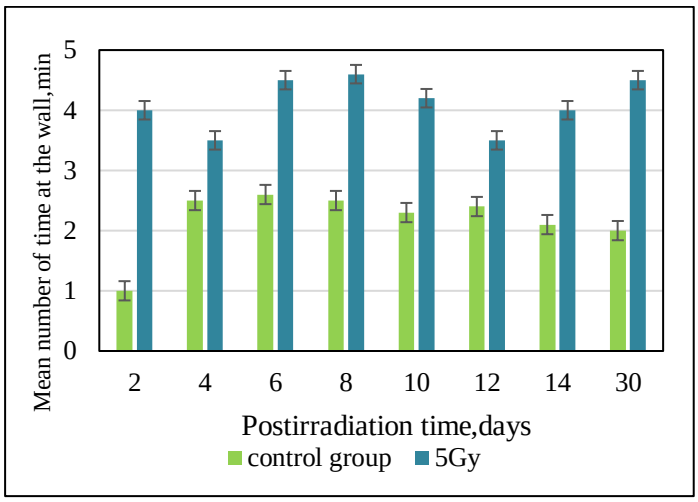

Fig 3. Mean number of time spent at the walls of open field in experimental animals during the post-radiation period

The fourth parameter assessed in the open field was the number of defecations as an increased number of boli can be indicative of anxiety and emotionality in the mice (the amount of the boli was calculated after a testing period) Fig. 4 shows a mean number of the bolus according to post-radiation period. On the 2nd, 4th, 6th, 8th, and 30th days of irradiation amount of bolus increases in comparison with control group animals. On the 10th 12th and 14th days, the number of defecations is approximately the same as in the control group.

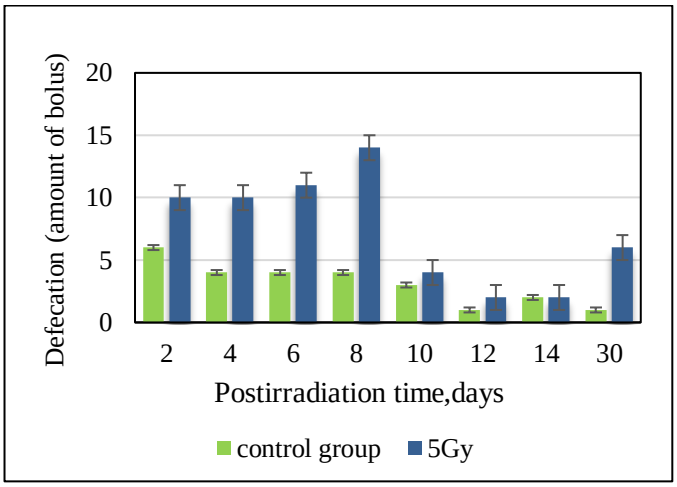

Fig. 4. Defecation: number of fecal boluses during the post-radiation period

CONCLUSION

Using a laboratory white mouse model, we showed that ionizing radiation exposure could lead to increased anxiety-like behavior. A study of open-field parameters revealed that gamma irradiation can be considered a radiobiological factor inducing anxiety and emotionality in mice. Behavior changes can be considered as an integrated radiobiological effect, which includes both injury of the central nervous system, as well as implications for other organ systems in this process.

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