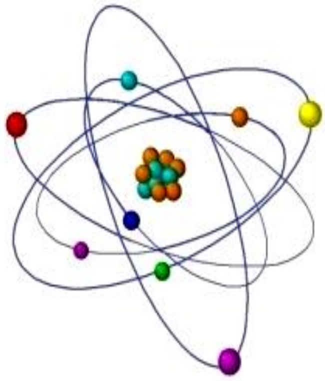
SEX DIFFERENCES IN COGNITIVE PARAMETERS OF LABORATORY MICE DURING ACUTE RADIATION EXPOSURE



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ABSTRACT: *Radiosensitivity, the susceptibility of living organisms to the damaging effects of ionizing radiation, is a critical parameter in various fields of science and medicine. From the development of cancer radiation therapy methods to assessing the risks of radiation exposure in humans, understanding the factors influencing radiosensitivity is of paramount importance. One such factor, often overlooked but potentially significant, is sex differences, particularly in the development of posttraumatic cognitive changes. This study evaluated sex-related differences in laboratory white mice exposed to a 4 Gy dose of gamma radiation. Specifically, cognitive and behavioral parameters were assessed over a three-week period using the Morris water maze and the Open field test. The obtained results of Morris water maze demonstrated that impairments in spatial learning and hippocampus-dependent memory under irradiation conditions were more pronounced in female mice, whereas male mice retained comparatively better spatial memory performance. In the open field test, female mice exhibited higher locomotor and exploratory activity, greater movement speed, and increased time spent in the central zone, indicating relatively lower anxiety-like reactivity compared to males. Nevertheless, both sexes demonstrated enhanced anxiety-associated behavior relative to the control group, confirming the presence of radiation-induced stress-related effects following gamma irradiation. Accordingly, sex-based analysis of the experimental data indicates that radiation-induced cognitive and behavioral alterations do not manifest uniformly and differ substantially between males and females. The findings suggest that sex-related differences play a significant role in post-irradiation cognitive and behavioral responses, emphasizing the importance of considering sex as a biological variable in radiobiological research and medical practice.*

KeywordFs: Sex differences; Irradiation; Cognitive Parameters; White mice

INTRODUCTION

Sex is a critical biological variable that significantly influences physiological and pathophysiological responses; nevertheless, it has historically been underrepresented in experimental and translational radiation research. For decades, male models were predominantly regarded as the experimental standard in biomedical studies, resulting in a substantial gap in knowledge regarding female-specific biological responses and limiting the translational applicability of preclinical findings [1,2]. Emerging evidence indicates that sexual dimorphism substantially modulates both systemic and organ-specific responses to ionizing radiation, including within the central nervous system, underscoring the necessity of incorporating sex as a fundamental variable in radiation biology research [3,4,26].

Radiation-induced neural injury is a multifactorial process involving impaired neurogenesis, increased oxidative stress, neuroinflammation, vascular damage, and disruption of synaptic architecture, ultimately leading to cognitive dysfunction [26,5,6]. The hippocampus is particularly susceptible to radiation-induced damage due to its critical role in spatial memory and learning, as well as its high degree of neuroplasticity [26,7,8]. Importantly, sex-dependent differences in hippocampal neurogenesis and spatial learning strategies have been well documented, even under non-pathological conditions, suggesting that baseline biological differences may substantially influence radiation-induced cognitive outcomes [25].

Following radiation exposure, microglial activation and elevated expression of pro-inflammatory cytokines contribute to the inhibition of neurogenesis, disruption of synaptic remodeling, and dysregulation of neural network integration [9,10].

Recent evidence demonstrates that microglial reactivity and neuroinflammatory cascades differ significantly between males and females, which may explain sex-specific vulnerability to cognitive impairment after brain injury and radiation exposure [24].

Accumulating experimental evidence suggests that sex differences in radiation sensitivity may be mediated by divergent profiles of hormonal regulation, antioxidant defense, immune responses, and neuroinflammatory processes [11,12]. In particular, estrogen has been proposed to exert neuroprotective and radioprotective effects due to its antioxidant and anti-inflammatory properties [27]; however, findings remain inconsistent across experimental models, and in some contexts female animals have demonstrated greater radiosensitivity [26].

Despite these observations, a substantial proportion of radiation neuroscience research continues to rely on animals from only one sex or fails to perform sex-stratified analyses, thereby limiting identification of sex-associated biological differences and hindering comprehensive interpretation of radiation-induced neurobehavioral alterations. This limitation is particularly problematic in behavioral paradigms such as the Morris Water Maze, where males and females inherently differ in exploratory behavior, stress reactivity, locomotion, and spatial learning strategies [26].

Accordingly, the aim of the present study was to evaluate sex-associated differences in cognitive and behavioral responses to ionizing gamma radiation in adult laboratory white mice.

METHODOLOGY

Laboratory white mice (*Mus musculus*) were used in the present study. Due to their genetic homogeneity and well-characterized physiology, laboratory mice represent an important experimental model for investigating numerous biological processes, including those associated with radiobiology [13]. Adult male and female mice aged three months were selected as experimental subjects. The selection of this age group was based on age-related characteristics of radioresistance. It is well established that during early developmental stages, radioresistance in animals varies considerably due to active growth, intensive cellular proliferation, and ongoing tissue differentiation processes. By the age of three months, these processes become stabilized, resulting in relatively uniform radioresistance among animals. To ensure experimental accuracy and the reliability of the obtained results, it was essential to minimize differences in radioresistance between the experimental groups [14,15,18].

The study included four experimental groups: female control group (n=5), male control group (n=5), irradiated female group (n=10), and irradiated male group (n=10). Acute irradiation was performed using a gamma irradiation unit with the radioisotope ^{137}Cs as the radiation source (dose rate: 1.1 Gy/min). According to the experimental design, animals were divided into groups and exposed to radiation doses ranging from 2 to 5 Gy [16,17,29].

Cognitive abilities and behavioral parameters of the experimental animals were assessed using the Morris Water Maze and the Open Field test, while data acquisition and analysis were performed using the EthoVision XT computerized tracking system [18]. In the Morris water maze, the evaluated parameters included total distance traveled, movement speed, and latency to locate the hidden platform [19]. In the open field test, total distance traveled and movement speed were measured. Additionally, the time spent in the central zone of the open field was assessed as an indicator of anxiety-related behavior, whereas activity in the peripheral zone was evaluated as a measure of locomotor activity [20,21].

RESULTS AND DISCUSSION

The obtained findings demonstrated that a dose of 5Gy represents a threshold level for white laboratory mice, beyond which the compensatory mechanisms of the organism are no longer capable of maintaining an adequate physiological response, leading to the development of degenerative alterations in biological systems and increased lethality (Hall & Giaccia, 2018; Ainsworth, 1986). Consequently, detailed analysis of cognitive and behavioral parameters was conducted in animals exposed to a 4Gy dose of gamma radiation.

Behavioral assessment was performed using two validated experimental paradigms: the Morris Water Maze for the evaluation of spatial learning and hippocampus-dependent memory, and the Open Field test for the assessment of locomotor activity and anxiety-related behavior. Both tests were conducted over a three-week period with repeated assessments, allowing dynamic evaluation of post-irradiation cognitive and behavioral alterations (D'Hooge & De Deyn, 2001).

In the Morris Water Maze, the analyzed parameters included total distance traveled, movement speed, and the latency required to locate the hidden platform, all of which represent important indicators of spatial navigation efficiency and learning performance. Each animal was placed in the water maze for 60 seconds; If the platform was not located within this period, the trial was classified as unsuccessful. Additionally, during the probe trial phase, following removal of the platform, the preferentially explored quadrant and swimming trajectories were evaluated, allowing assessment of memory retention and navigational strategies.

The obtained results revealed sex-associated differences in spatial task performance (Figure 1). Specifically, female mice consistently traveled greater distances throughout the experiment compared both with their corresponding control group and with male mice, which may indicate less efficient spatial navigation and less optimized trajectories toward the target platform. In contrast, male mice exhibited shorter swimming distances relative to both females and controls, a pattern that remained stable throughout the entire three-week observation period. These findings suggest that male animals employed more efficient navigational strategies during spatial task performance and reached the platform using shorter trajectories.

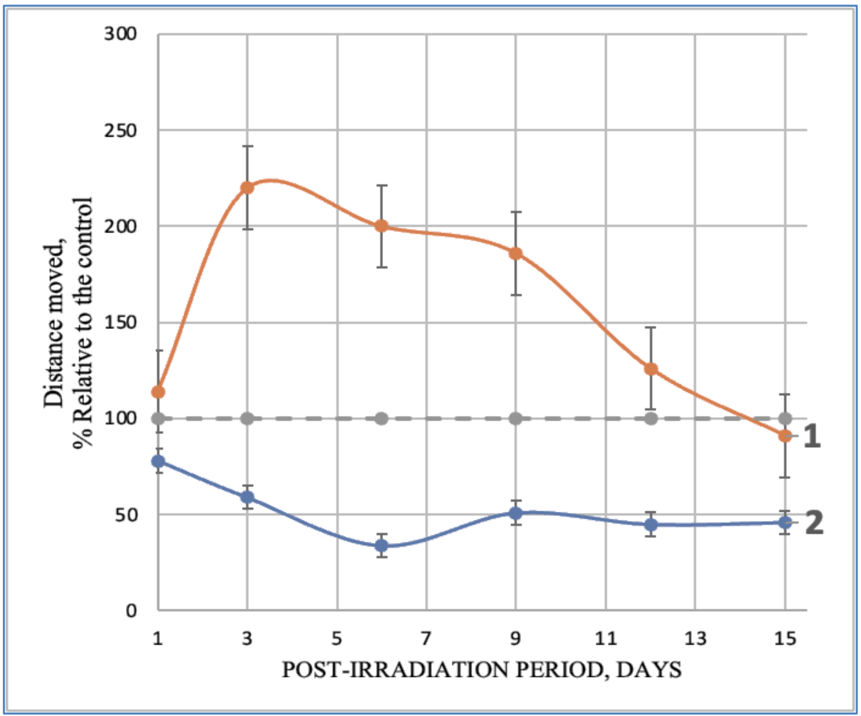

Fig. 1. Changes in the distance traveled by irradiated mice during testing in the Morris water maze.

1 — female; 2 — male; the control group is indicated by the dashed line.

Analysis of movement speed (Figure 2) demonstrated that female mice exhibited higher locomotor activity during the first experimental week compared with both males and controls. However, their movement speed gradually declined over time and, beginning from the second week, approached the values observed in the other groups. This dynamic pattern may reflect increased initial motor activity or heightened stress reactivity in females, which became partially attenuated over time.

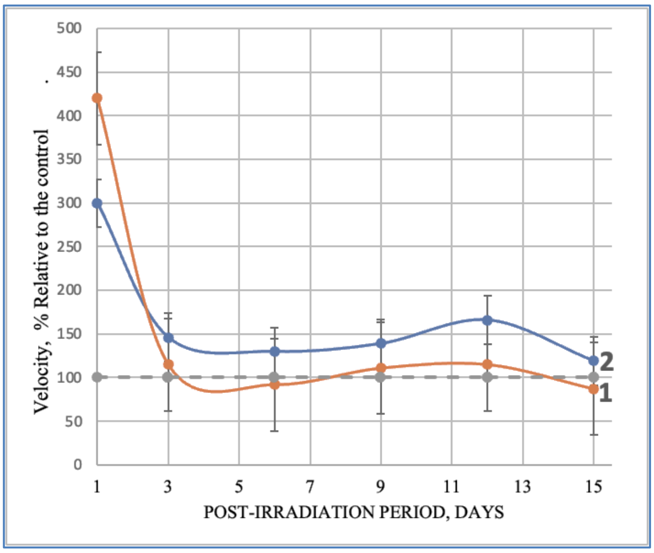

Fig.2. Changes in movement velocity parameters of irradiated mice during testing in the Morris water maze

1 — female; 2 — male; the control group is indicated by the dashed line

Nevertheless, evaluation of latency to locate the hidden platform (Figure 3) showed that female mice required longer periods to identify the platform throughout the experiment. Although their performance partially approached control values by the end of the three-week testing period, differences relative to male mice remained evident. Conversely, male mice consistently demonstrated the shortest latency periods during the entire observation interval, indicating better preservation of spatial learning and hippocampus-dependent memory functions.

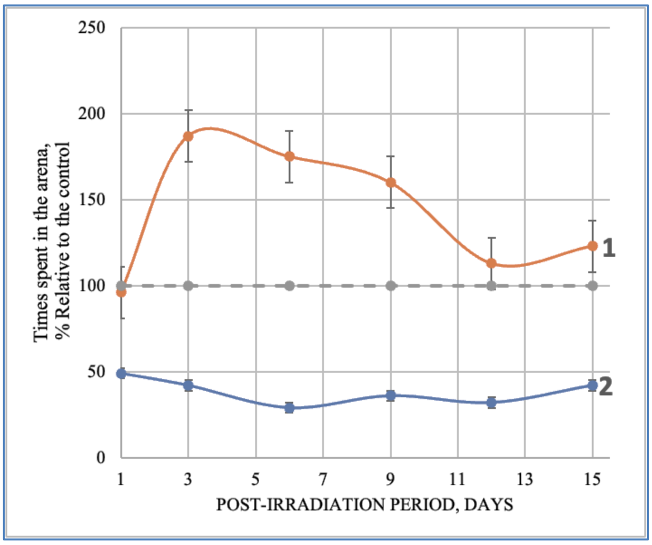

Fig. 3. Changes in the time spent in the arena by irradiated

mice during testing in the Morris water maze

1 - female; 2 - male; the control group is indicated by the dashed line.

The swimming trajectories recorded in the Morris water maze (Figure 4) further demonstrated sex-associated differences in spatial navigation and memory. Male mice displayed more directed and organized movement patterns, rapidly orienting toward the platform and utilizing more efficient swimming strategies. In contrast, female mice exhibited more chaotic and less organized swimming behavior, characterized by frequent directional changes and extensive exploration of the pool area, suggesting difficulties in the formation of effective spatial strategies. During the probe trial phase (3 - male, 4 - female), when the platform had been removed, male mice spent more time in the quadrant where the platform had previously been located, indicating better spatial memory retention and preservation of platform localization. Female mice continued to display non-directed swimming throughout the pool and did not demonstrate a clear preference for the target quadrant, reflecting impaired retention of spatial information and reduced memory consolidation.

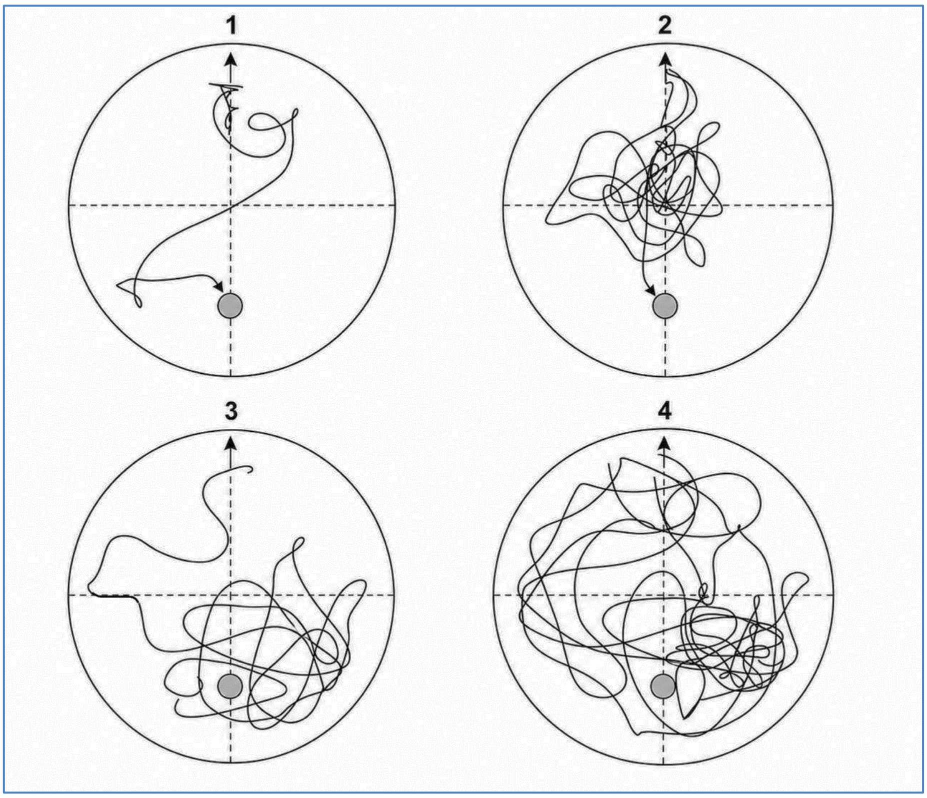

Fig. 4. Tracking visualization (MWM)

1 - Male; 2 - Female; 3 - Male, Probe test; 4 - Female, Probe test

The presented trajectories indicate that impairments in spatial learning and memory under irradiation conditions were more pronounced in female mice than in males, which may reflect sex-related differences in susceptibility of hippocampus-dependent cognitive processes to radiation-induced damage.

In the Open Field test, the analyzed parameters included total distance traveled, movement speed, time spent in the central zone of the arena, and activity within the peripheral zone, all of which represent important indicators of locomotor activity and anxiety-related behavior. Each animal remained in the open field arena for 5 minutes.

The obtained results revealed sex-associated differences in both locomotor and emotional-behavioral parameters. Specifically, female mice traveled greater distances throughout the experiment compared with males (Figure 5), indicating relatively higher spontaneous locomotor activity and increased motor engagement with the environment.

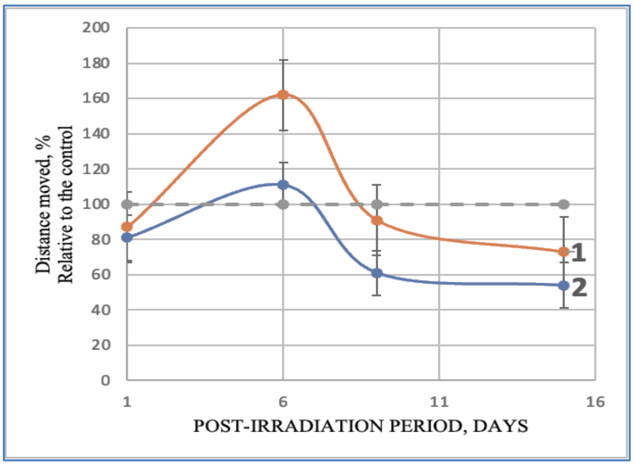

Fig. 5. Changes in the distance traveled by irradiated mice during testing in the Open field test

1 — female; 2 — male; the control group is indicated by the dashed line.

These findings were consistent with movement speed analysis (Figure 6), according to which female mice moved at higher speeds during the entire experimental period. The concordant changes in traveled distance and movement speed confirm that females exhibited more intensive locomotor and exploratory activity in the open field environment.

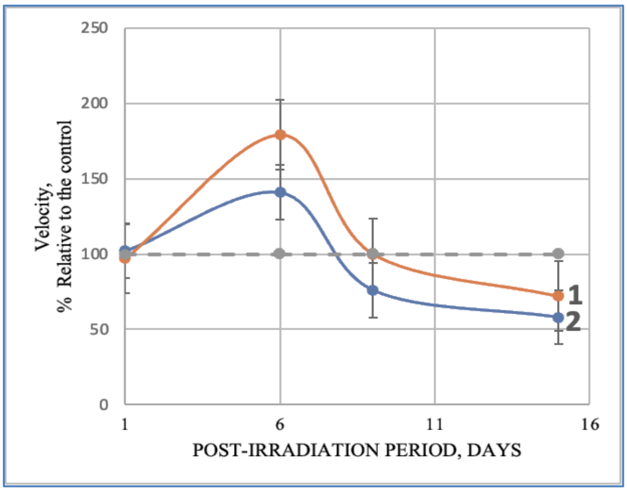

Fig.6. Changes in movement velocity parameters of irradiated mice during testing in the Open field test

1 — female; 2 — male; the control group is indicated by the dashed line.

Particular attention was given to the time spent in the central zone of the arena (Figure 7), as this parameter is widely recognized as an indicator of emotional reactivity and anxiety-related behavior. The obtained data demonstrated that female mice spent more time in the center compared with males, which may indicate relatively lower anxiety-like responses and greater emotional resilience under experimental conditions. However, both female and male experimental groups demonstrated lower values compared with their respective control groups, indicating that gamma irradiation induced enhanced anxiety- and stress-associated behavior in both sexes relative to healthy controls.

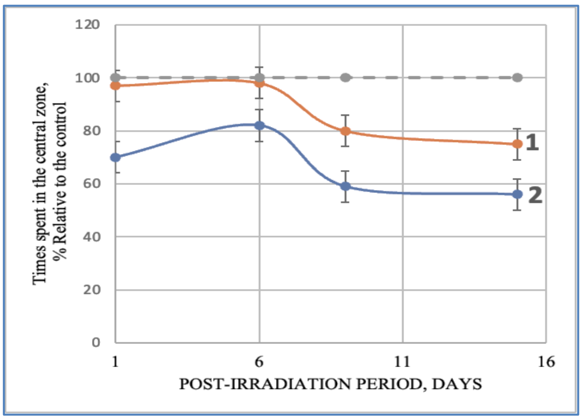

Fig.7. Changes in the time spent in the central zone by irradiated mice during testing in the Open field test

1 — female; 2 — male; the control group is indicated by the dashed line.

Analysis of activity associated with the peripheral zone and arena walls (Figure 8) further confirmed sex-related differences in exploratory behavior. During the final two weeks of the experiment, female mice exhibited higher values for this parameter compared with males, indicating greater environmental interest and increased motor activity. This tendency may reflect sex-dependent differences both in behavioral adaptation and in the neurobiological mechanisms underlying stress responsiveness.

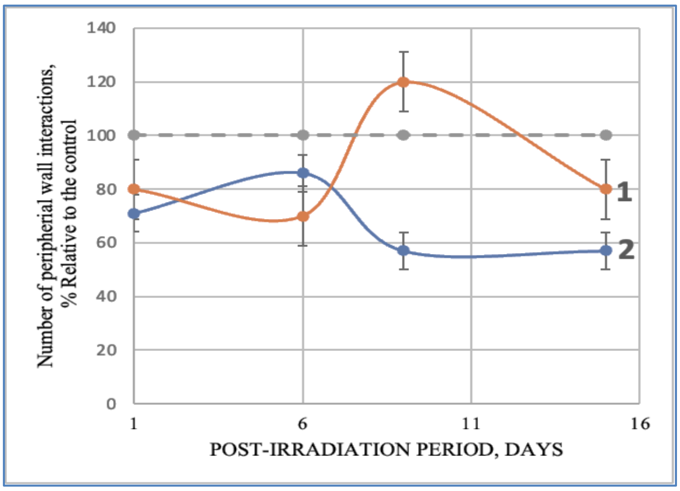

Fig.8. Changes in the number of peripheral wall interactions in irradiated mice during testing in the Open field test

1 — female; 2 — male; the control group is indicated by the dashed line.

CONCLUSION

The obtained results clearly demonstrated sex-associated differences in post-irradiation cognitive and behavioral responses. The observed alterations indicate that ionizing radiation exerts differential effects on hippocampus-dependent cognitive processes and emotional-behavioral regulation in male and female organisms. The data shows that female mice exhibited a more pronounced impairment in spatial navigation and memory performance following irradiation, suggesting a higher degree of functional vulnerability of the hippocampus. At the same time, behavioral parameters indicate that neurobiological mechanisms underlying stress responsiveness and adaptation to novel environments may proceed differently in females. In contrast, the results observed in male mice suggest relatively better preservation of spatial learning and memory, which may reflect greater functional stability of hippocampus-dependent neuronal networks under irradiation conditions. Overall, the findings confirm that sexual dimorphism has a significant impact on radiation-induced neurobehavioral alterations. Consideration of this factor is essential in neuroscience research, both for the interpretation of results and for the design of experimental studies

Sex-based comparisons of male and female animals revealed substantial differences, indicating that post-irradiation cognitive and behavioral changes are not uniform or universally expressed processes, but rather reflect a complex, multifactorial dynamic in which biological sex acts as an important modulator.

Sex-related differences in radioresistance in laboratory mice represent an important, yet often overlooked factor in radiobiology. Systematic evaluation of these differences can substantially improve the accuracy and reliability of experimental findings. Moreover, such data provide a basis for the development of more targeted approaches in radiotherapy, radioprotection, and health risk assessment. Future studies should focus on elucidating the underlying molecular and cellular mechanisms responsible for these sex-dependent differences. Such knowledge may open new perspectives in radiation medicine and contribute to the development of more effective and personalized therapeutic strategies.

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