# *The Effects to Exposure of Simulated Spaceflight Radiation on Behavioral Health of Male and Female Mice*

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## Abstract

Exposure to space radiation is a principal consideration of spaceflight missions as risk is leveraged as time and dose—both expected to increase with future missions to the Moon, Mars, and beyond. Previous mission exposure levels, galactic cosmic radiation (GCR) and solar particle events (SPE), have been characterized as increased compared to those natural to Earth and are predicted to cause robust deficits at higher doses and longer durations. The cognitive health implications of this critical difference are understood as risks to mission and crew operations. We examined potential radiation-induced disruptions on brain health through resting-state in-cage behavior. 23–24-week-old male and female mice were exposed to 0 cGy (Sham), 5 cGy, 15 cGy, and 50 cGy via Five-Ion GCR Simulation (H, Si, He, O, Fe) at the NASA Space Radiation Lab in Brookhaven National Labs. Behavioral and cognitive performance were evaluated via frequency/duration of species-typical behaviors including digging, rearing, and grooming within the 72-hour period immediately following irradiation. Additionally, during this time we evaluated nestlet building using a 5-stage Deacon score, rating shredding and shelter assembly of cotton material from untouched (1) to shredded and formed into a crater shape (5). We have observed differences in Deacon score only among the 15 cGy subset. Further comparative performance analysis will be completed evaluating the differences in irradiation effects between male and female mice. These experimental design aspects that allot for gender-inclusivity is supportive of the diversification of future space travel mission plans. Investigating gender differences is an element under our main objective of determining radiation dose-response curves. In brief, these studies identified a space-relevant radiation dose of 15 cGy that that can be utilized for future standardized ground studies on the nervous system.

## Background and Introduction

- Key space environment stressors of behavioral health studies are microgravity, radiation, and social isolation. Exposure to these stressors are known to result in altered circadian rhythmicity, anxiety and degraded cognitive performance (Euston et al., 2012; Parihar et al., 2015, 2018; Raber et al., 2018).
- On a mission level, stressor effects on the central nervous system and brain may result in adverse changes to astronaut behavior, mood, and performance of critical tasks. Our brain and behavioral health study meet critical neurological risk research needs: HRR Gaps BMed 101-108, SM-104.
- Ionizing radiation: one of the challenging spaceflight stressors, Galactic Cosmic Radiation (GCR) and Solar Particle Events (SPE), and serious research consideration as such exposure is not easily administered in terrestrial human studies.
- Our model study organism is C57BL/6 mice. Ionizing radiation was found to alter neuronal morphology(Parihar et al., 2015). The decrease in neuronal complexity was accompanied by behavioral deficits: decreased cognitive flexibility, elevated anxiety, impaired spatial, episodic and recognition memory sometimes lasting up to 1-year post-irradiation (Euston et al., 2012; Parihar et al., 2015, 2018; Raber et al., 2018).
- We hypothesize that deficits in performance of immune, neural, and cognitive nature will be more robust at greater IR doses.
- We further expect sex differences to be observed by our methods.

## Methods

- This project is the first stage in a multi-year study to investigate the multi-variate effect of spaceflight radiation on behavioral health
- By determining any significant behavioral deficits, we plan to establish a dose-response curve:
    - Inform dose selection that evokes a mid-range response: a mid-range dose that is low enough to reflect spaceflight exposure, yet high enough to elicit a response
    - Determine the dose dependence of acute Five-Ion GCR for immune, brain and behavioral performance responses.
- Analyze for any cognitive changes within resting-state in-cage footage recorded within the 72 hours after irradiation
- Compare among different exposure groups and sexes for behavioral health profiles
- Prepare blind study to quantify mice behavior categories of digging, rearing, and grooming based on duration, frequency, and location
- Evaluate Nestlet development among exposure groups and sexes
- The only material available in the cage for the mice to interact with is cotton material for nestlet building
    - Nestlet development: a WT behavior and construction task that is reflective of cognitive health, is evaluated in stages as determined by the Deacon score. The lowest stage (scored as 1) is depicted by largely untouched cotton material and the highest (scored as 5) is depicted by shredded cotton material formed into a crater shape to serve as the mouse's shelter
- Organized results to measure the frequencies of the activities separated by exposure groups
- Indicate the behavior frequency mean of each exposure group with upper and lower standard deviation (SD) error bars using GraphPad Prism (version 9.2.0) software. The statistical test used for one sex was a one-way ANOVA with Dunnett's multiple comparison post hoc test. When comparing the male and female behavior frequencies, the statistical test used to compare both sexes was two-way ANOVA.

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## Results: Behavior Frequency

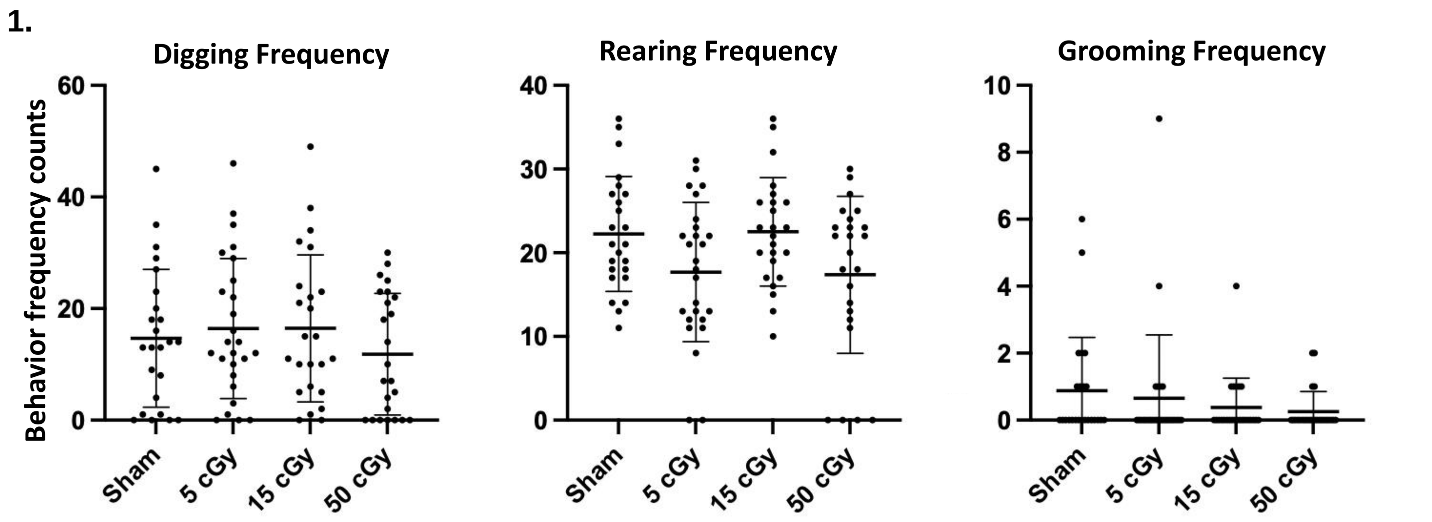


*Figure 1. Male (n≈25 per exposure group) behavior frequencies across all exposure groups (Sham (0 cGy), 5 cGy, 15 cGy, 50 cGy). Error bars indicate mean with upper and lower SD.*

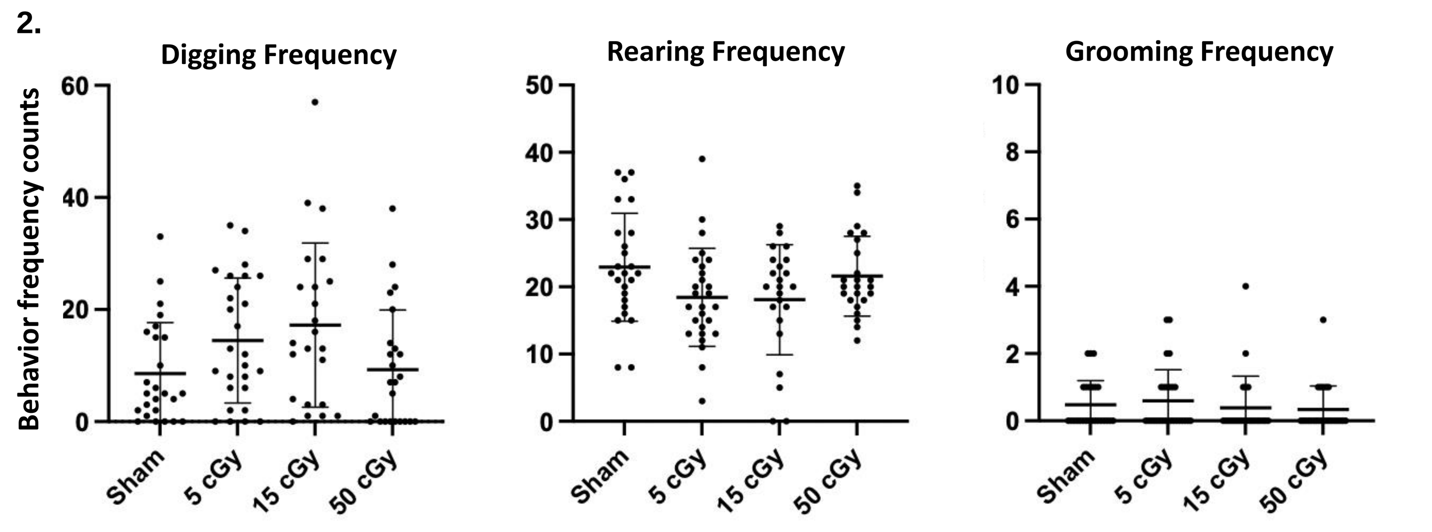


*Figure 2. Female (n≈25 per exposure group) behavior frequencies across all exposure groups (Sham (0 cGy), 5 cGy, 15 cGy, 50 cGy). Error bars indicate mean with upper and lower SD.*

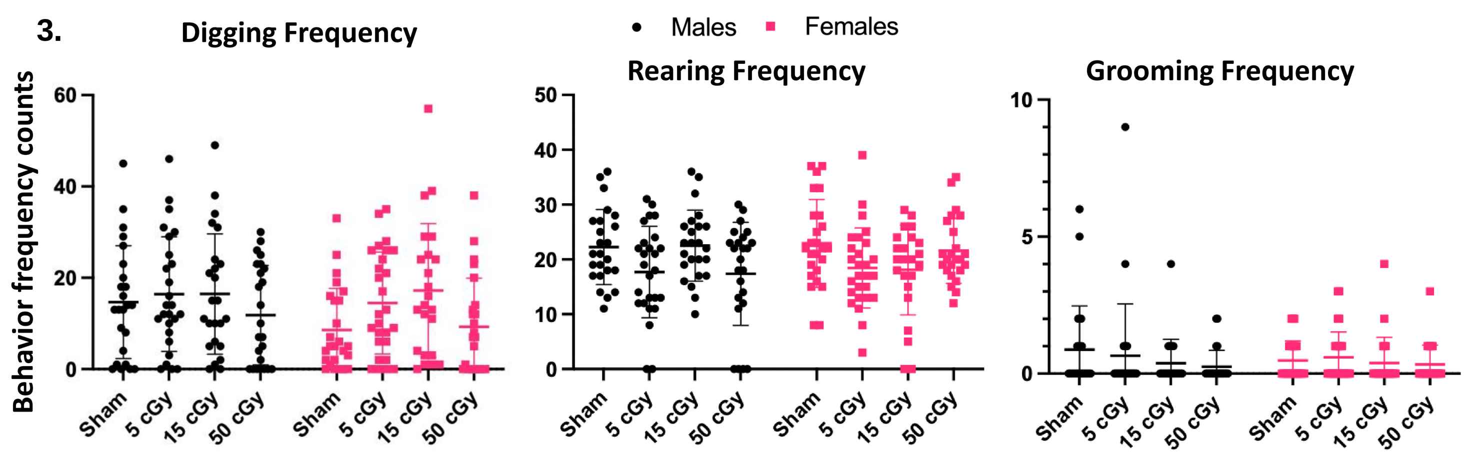


*Figure 3. Comparison of behavior frequencies across sexes and all exposure groups (Sham (0 cGy), 5 cGy, 15 cGy, 50 cGy). Error bars indicate mean with upper and lower SD.*

## Discussion: Behavior Frequency

Our methods found that there was no significant difference of male behavior frequencies among exposure groups (Figure 1). As the data is the same as sham, these frequency rates are normal. Among the females, we saw again that there was no statistically significant difference of behavior frequencies compared with the sham exposure group (Figure 2). Our methods found that there were also no significant sex-specific differences observed among behavior frequencies (Figure 3). A reason for this observation might be that due to such an immediate evaluation window following radiation exposure, there may not be obvious developed adversities in behavior yet. We are continuing to look at the duration and location of the recorded behavior frequencies to further investigate the responses.

## Results: Nestlet Building

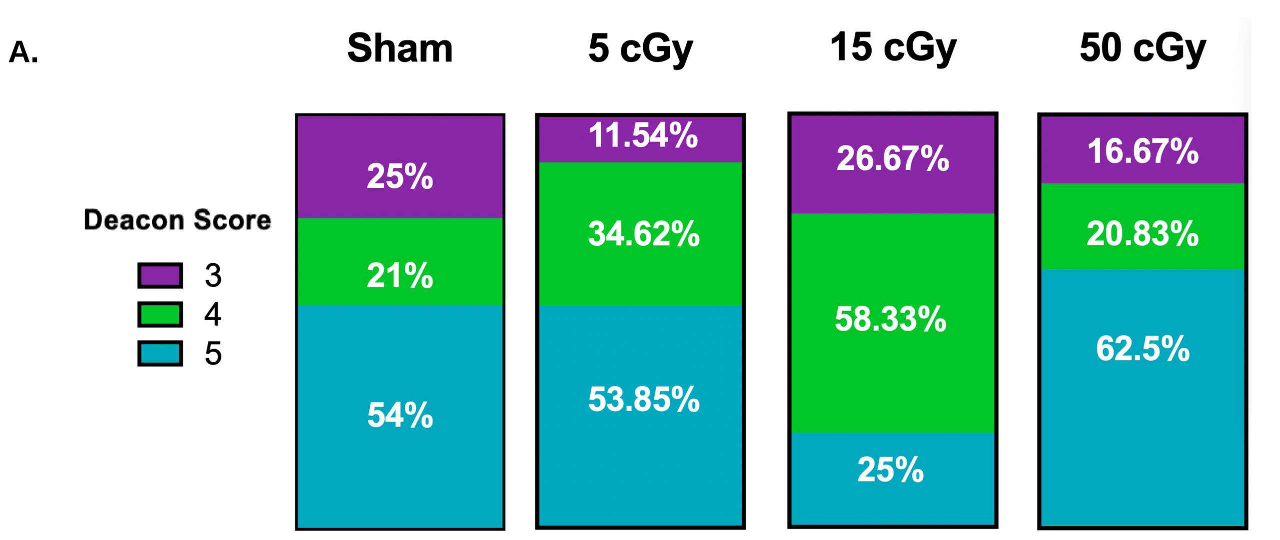


*Figure A. Deacon scores of nestlets built by male mice across all exposure groups (0 cGy, n=24; 5 cGY, n=27; 15 cGy, n=24; 50 cGy, n=24)*

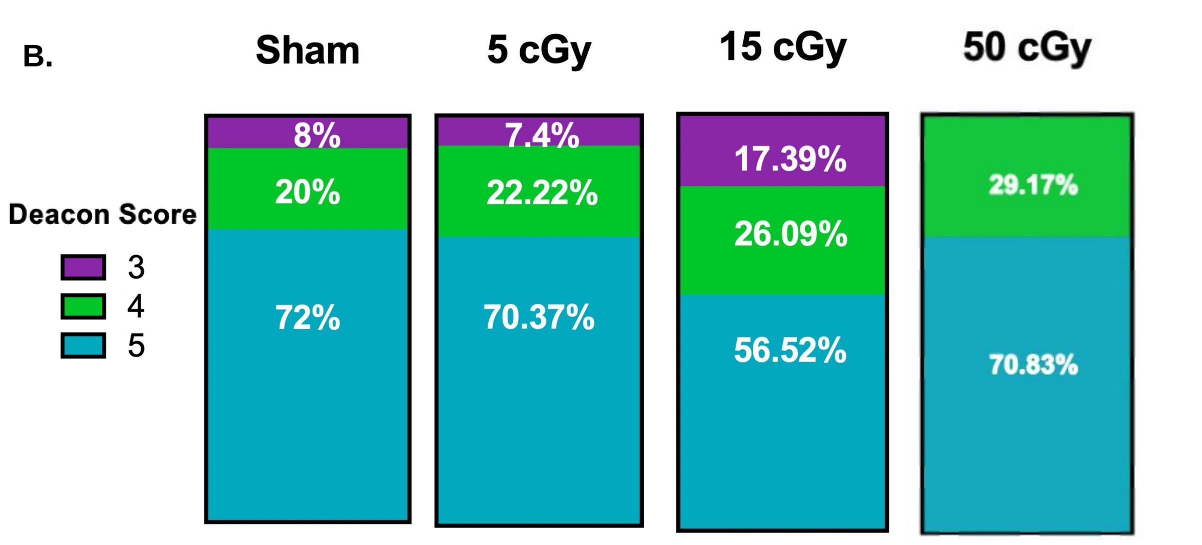


*Figure B. Deacon scores of nestlets built by female mice across all exposure groups (0 cGy, n=25; 5 cGY, n=27; 15 cGy, n=23; 50 cGy, n=24)*

## Discussion: Deacon Score

- Males in the 15 cGy subset had a majority observed percentage of score 4 nestlets, whereas the other male exposure groups have an observed majority of score 5 nestlets (Figure A)
- Of the males, the 50 cGy exposure group had the most score 5 nestlets observed (Figure A)
- Of the males, the 5 cGy exposure group had the least number of low scores of 3 observed (Figure A)
- No score 3 nestlets were observed among the 50 cGy female exposure group (Figure B)
- Overall, score 5 nestlets were observed for majority of the female exposure groups (Figure B).

- Comparing sex and exposure groups: higher overall Deacon scores were observed among the female groups, the majority of scores being a 5 (Figure A, B)
- Both females and males in the 5 cGy exposure groups had the lower Deacon score of 3 observed, omitting 50 cGy females which had no scores of 3 (Figure 4, 5)

- The anatomy and physiology of the behavior are currently under investigation as these same mice are undergoing blood, brain and tissue sampling, and for the rest of the mice, more cognition tests will be conducted to determine any long-term effects. Future statistical tests will determine if the differences we see here with the Deacon score are significant or not.

C.

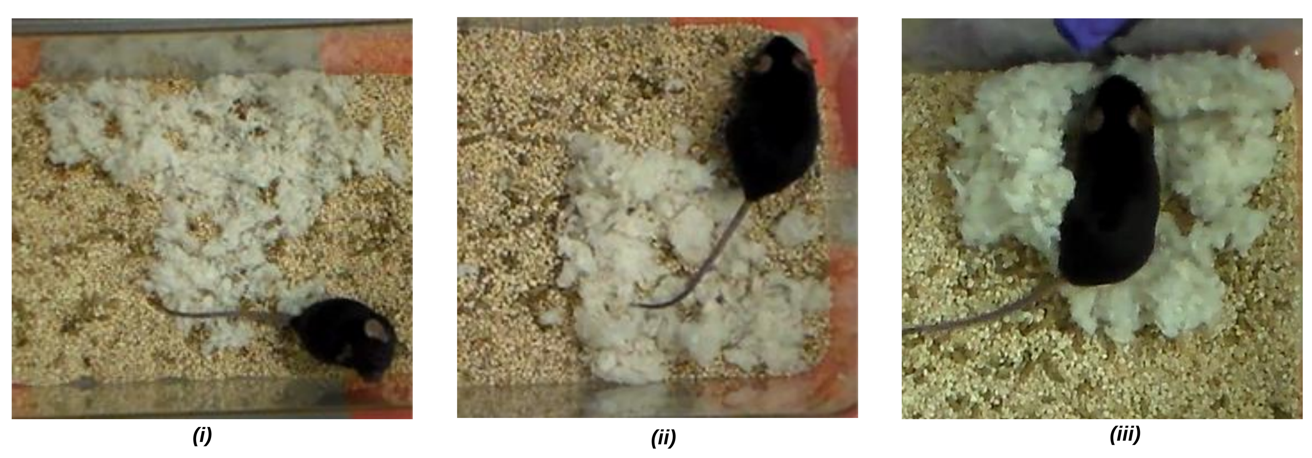


*Figure C. Images of nestlets rated by the following Deacon scores from left to right:*
*(i) Score 3, Cotton material is mostly shredded but no identifiable nest site: less than 50% of the nestlet remains intact, but less than 90% is within a quarter of the cage floor area*
*(ii) Score 4, An identifiable but flat nest: more than 90% of the Nestlet is torn and the material is gathered into a nest within a quarter of the cage floor area*
*(iii) Score 5, More than 90% of the Nestlet is torn and the nest is a crater, with walls higher than mouse body height for more than 50% of its circumference.*

## Conclusions

The summaries for phase 1 tests completed so far of the multi-year plan looking at radiation and behavior as follows: 1) We did not observe any significant differences of behavior frequencies among exposure groups nor sexes. 2) We found that the lowest occurrence of score 5 nestlets were observed among the 15 cGy exposure group males. 3) We also found that the 50 cGy females had no score 3 nestlets built. Only score 4 and 5 nestlets. 4) When comparing the Deacon scores, females had an overall more frequent score 5 nestlets. The lowest counts of score 3 nestlets being observed at the 5 cGy exposure levels for both sexes, excluding the fact that the 50 cGy females had no score 3 nestlet results. The conclusions that we can draw from this study are that males show more pronounced responses to radiation exposure than females, specifically in the cognition skills needed to construct a fully developed nestlet shelter. More research is needed to further contextualize our findings of the male and female behavior frequencies—of which we found no statistically significant differences among sex nor radiation exposure groups.

## Future Directions

My study focused on the 72-hour window immediately after an acute exposure of radiation. Analysis of cognitive performances and behavioral health (i.e. Nestlet scoring) was conducted with a sensitivity to baseline functional assessment—of which astronauts' high operational workload quality and mission success is dependent on. Furthermore, we must study how biology adapts to the long-term presence of space radiation as we plan missions to the Moon and Mars. These biological adaptations occur on a molecular level before manifesting as a behavior and a prevalent factor in radiation-behavior health adversities is oxidative stress. Space radiation countermeasure studies are currently investigating oxidative stress mitigation on the hippocampus of animal models that are genetically engineered to express increased levels of antioxidants (Rubinstein et al Nature Microgravity, 2021). During my time at SLSTP, I also quantified hippocampal microglia CD68 protein expression which represents brain immune response to oxidative stress brought on by radiation (Rubinstein et al in preparation). This neuroimmunology study and the cognition study connect because I'm able to see the effect spaceflight radiation may cause on both the molecular level and the behavior level.

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