Research report

Differential organization of open field behavior in mice following acute or chronic simulated GCR exposure


Keywords: Open field, Dead reckoning, Space radiation, Self-movement, Movement segmentation, Allothetic, Idiothetic

Abstract

Astronauts undertaking deep space travel will receive chronic exposure to the mixed spectrum of particles that comprise Galactic Cosmic Radiation (GCR). Exposure to the different charged particles of varied fluence and energy that characterize GCR may impact neural systems that support performance on mission critical tasks. Indeed, growing evidence derived from years of terrestrial-based simulations of the space radiation environment using rodents has indicated that a variety of exposure scenarios can result in significant and long-lasting decrements to CNS functionality. Many of the behavioral tasks used to quantify radiation effects on the CNS depend on neural systems that support maintaining spatial orientation and organization of rodent open field behavior. The current study examined the effects of acute or chronic exposure to simulated GCR on the organization of open field behavior under conditions with varied access to environmental cues in male and female C57BL/6 J mice. In general, groups exhibited similar organization of open field behavior under dark and light conditions. Two exceptions were noted: the acute exposure group exhibited significantly slower and more circuitous homeward progressions relative to the chronic group under light conditions. These results demonstrate the potential of open field behavior organization to discriminate between the effects of select GCR exposure paradigms.

1. Introduction

Astronauts will experience chronic exposure to Galactic Cosmic Rays (GCR) during deep space missions. Understanding the potential effects of GCR on mission critical tasks will depend on using parameters that more closely approximate the astronaut experience in deep space. Ground-based models of space radiation have been observed to disrupt performance on a range of behavioral tasks and influence function across networks of brain structures. For example, proton or HZE particle exposure has been reported to influence hippocampal and cortical structures [1–5], physiology [6,7], and associated behaviors [2,7–10]. Most of this work has focused on low dose acute exposures; however, a more translational approach will need to include chronic mixed field exposures at similar space relevant doses delivered over longer time frames. Such investigations are critical to properly define the potential CNS risks to astronauts translated from rodent behaviors that model information processing deficits related to mission critical tasks.

The organization of rodent open field behavior has been used to investigate the neural basis of spatial orientation. This behavior is spontaneously organized into sequences of stops and progressions around a focal point in the environment termed the home base [11–13]. This organization has been observed to dissociate damage localized to neural systems involved in maintaining spatial orientation. For example, disruptions in this organization restricted to dark testing are commonly associated with damage to the hippocampal formation [14–16]. In contrast, damage focused on the medial frontal cortex spares organization under dark conditions and disrupts organization under light conditions [17]. Additionally, acquired vestibular pathology has been observed to disrupt more selective components of open field organization (between progression change in heading) under both dark and light conditions that persist for at least two months [18]. These dissociations provide a novel behavioral approach to investigate the effects of varying

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aspects of simulated GCR exposure on the neural systems related to maintaining spatial orientation.

In general, varying levels of sexually dimorphic performance has been observed on tasks that assess spatial orientation. While previous work on models of space radiation exposure has typically used male rodents, more recent work has started to include female rodents [19,20]. Recently, a growing body of literature has supported that susceptibility to the effects of space radiation is sex-dependent. For example, female mice exhibit resistance to the neuroinflammatory response that is observed in males exposed to an acute three-particle GCR simulation [19] or low dose helium ions [20]. In addition, male performance compared to females was disrupted to a larger extent in a range of behavioral tasks [20–23]; however, other studies observed male performance to be spared [24,25] following irradiation. Therefore, it is critical to design studies that permit assessment of sexual dimorphisms in response to space radiation exposure [26,27].

The current study implemented the most sophisticated terrestrial simulation of GCR. Male and female mice were exposed either acutely or chronically to similar low doses of GCR. A series of open field sessions were used to assess disruptions in spatial orientation associated with the potential impact of these realistic deep space exposure scenarios. The specificity of disruptions in open field behavior will provide guidance on the behaviors implicated in mission critical tasks following exposure to simulated GCR.

2. Materials and methods

2.1. Animals

Thirty-five female and thirty-six male C57BL/6 J mice were transported from the Jackson Laboratory in Bar Harbor, Maine to Brookhaven National Laboratory (BNL) in Upton, New York. Mice were aged to 6 months prior to irradiation at BNL. All protocols were approved by the University of California Irvine, Northern Illinois University (NIU), and BNL Institutional Animal Care and Use Committees.

2.2. GCR Simulation

Male and female mice were each divided into three experimental groups: sham irradiated controls, acutely irradiated, or chronically irradiated mice using the NASA developed 33-beam GCR simulation (GCR Sim) protocol. The 33 charged particle species were delivered sequentially to simulate the spectrum of radiation experienced during a deep space mission [27,28]. The 33 exposures were delivered in order, energy, and dose as previously described [29]. The NASA Space Radiation Laboratory physics staff performed all radiation dosimetry and confirmed spatial beam uniformity. Chronically irradiated animals received a GCR Sim dose of 2.08 eGy/day, six days a week for four weeks totaling 24 irradiation days with a total accumulated dose of ~50 eGy. Acutely irradiated animals received a one-day total GCR Sim dose of ~40 eGy on the same day the chronically irradiated mice received their final exposure. During whole-body irradiations, two to three cage mate mice were placed together in well ventilated Lucite containers lined with absorbent padding. The number of mice per container was based on their home caging, and each day the same exact mice were grouped together and placed back into the same exact container. Any day that the chronically irradiated mice were restrained and irradiated, all other mice were also similarly restrained in their designated boxes within the animal holding room. For each irradiation or sham exposure, the time between when the mice were loaded into their boxes, irradiated, and then subsequently unloaded back into their respective home cage generally averaged no more than two hours. Mice were shipped to NIU within five days following the completion of GCR Sim exposure.

2.3. Apparatus

The open field apparatus consisted of a circular white wooden table (122 cm in diameter, 34.5 cm in height) located in a rectangular room. The experimental room contained other various distal cues including a sink and cabinets. During dark testing, the ceiling florescent lights were turned off and the room was illuminated by four infrared emitters. During light testing, the room was illuminated by ceiling florescent lights. An infrared bullet camera located on the ceiling recorded the open field sessions for offline analysis.

2.4. Procedure

Behavioral testing began at 3.5–4.5 months post-irradiation with open field behavior recorded under dark conditions followed by light conditions one consecutive day later. The dark open field session was conducted first to reduce potential carry over effects of learned visual environmental cues during the light session. During the open field sessions, mice were transported in a random circuitous path from the colony room to the experimental room in a clear Plexiglass container covered by a towel to prevent learning the location of the experimental room relative to the colony. Experimenters entered the room and placed the mouse on the center of the table. Mice were left to individually explore the table for thirty minutes. If the mouse fell off the table, an experimenter entered the room and placed the mouse back on the center of the table and the fall was recorded. Under dark conditions, night vision binoculars were used to enter the room and place the mouse back on the center of the table without turning the lights on. After completion of the open field session, mice were placed back in the covered transport cage and transported in a random circuitous path back to the colony room. Tables were cleaned with an ammonia-based solution between mice.

2.5. Behavioral analysis

For each session, a 20-minute segment without falls was selected to characterize mouse behavior in the open field. If a mouse fell more than five times, it was excluded from analysis. The segment began two minutes after the mouse was placed on the table. This provided an opportunity for mice to establish a home base prior to analysis of open field behavior [11,30–33]. The position of the home base is associated with many behaviors: frequent stops that increase in duration across time, multiple bouts of rearing and turning behaviors, and faster movements toward versus away from the home base [11,12,30,34]. Clustering of stops was used to define the position and stability of the home base during the session (analysis described in the next section). Noldus Ethovision XT 13 motion tracking software was used to capture the position of the mouse during the 20-minutes at a rate of five samples/second. The tracked x- and y-coordinates from the selected 20-minutes was divided into four five-minute samples (S1 through S4 in Figs. 1–4). The average speed of the mouse was calculated for each 20-minute session and used to segment behavior into stops and progressions. Progressions were two frames or greater in which the speed of movement was either equal to or greater than the average speed of the 20-minute session, whereas stops were two frames or greater in which speeds were less than the average speed of the 20-minutes session. General locomotion measures including total distance traveled and total stop time were calculated across the 20-minute exploratory session in both light and dark conditions.

2.5.1. Stopping organization

To assess stop clustering, the x- and y-coordinates of each stop was converted into a polar coordinate system (r, theta) and analyzed with circular statistics [35]. Stop clustering indicates the concentration of stops within a single direction ranging from one (strong clustering of stops) to zero (weak stop clustering). The first order parameter of
concentration was calculated to represent how concentrated the stops were in one heading direction within a sample. The average heading of each stop within each sample was used to calculate the within-sample estimated home base heading. The second order parameter of concentration was calculated to determine the average heading of stops across all four samples. Additionally, the average heading between the four sample was used to define the between-sample estimated home base heading. Previous literature indicates rats [11,36] and mice [31] prefer to establish home bases near the edge of the open field. Therefore, the distance of each stop from the center during a sample was used to calculate percentage of stops on the edge. The edge of the table was defined as stops within 10 cm of the table, approximately one body length. Change in heading was calculated as the supplementary angle of the angle subtended by the following three x- and y-coordinate points: the previous progression peak speed location, the average stop location, and subsequent progression peak speed location. Change in heading ranged from zero-degrees, consistent with the mouse continuing straight, to 180-degrees, indicating that the mouse completely reversed the direction of their path.

2.5.2. Progression organization

Rodents organize open field behavior throughout the environment into outward and homeward excursions [11,12]. The between-sample estimated home base headings were expanded 45-degrees in each direction to assess homeward bound progressions. The remaining 270-degrees was used to analyze outward progressions. Homeward bound progressions started within the 270-degree sector and ended in the 90-degree home base sector. Outward bound progressions started within the 90-degree home base sector and ended in the 270-degree sector. Once progressions were classified, average path circuity, average peak speed and average peak error was calculated. Path circuity was derived from the ratio between the actual path taken by the mouse and the Euclidean distance [14,16]. This measure ranges from zero (highly circuitous) to one (very direct). Typically, rodent peak speeds occur at the center of progressions. Average peak error reflects the variability in peak speed with the midpoint of the progression representing 0.0 and peak speeds occurring at the start or end of the progression yielding peak error values of 0.5. [16].

2.6. Statistical analysis

Separate mixed designs Analysis of Variance (ANOVA) for each general locomotion, first order parameter of concentration, percent on edge, and disorientation measures were used to test the between-subjects effects of radiation group and sex, the within-subjects effect of sample, and the corresponding interactions, with an alpha cut off at 0.05. Separate two-factor ANOVAs for each directional measure and second order parameter of concentration was used to test the main
effects of radiation group and sex and the corresponding sex by group interaction with an alpha cut off at 0.05. Effect sizes were represented by partial eta squared ($\eta^2_p$). Post-hoc testing was conducted using Tukey HSD and trend analysis. JASP 13.1 and SPSS 26 were used to calculate all statistical results.

3. Results

3.1. General behavior and locomotion

Throughout both dark and light open field sessions, all mice exhibited very few average numbers of falls (Table 1). There were no mice in any of the groups that fell more than five times; therefore, no mice were excluded from analysis.

Mice organized their open field behavior into stops and progressions under dark (Fig. 1A) and light conditions (Fig. 1B). Regarding total distance traveled under dark conditions (Fig. 1C), there were no significant main effects or respective interactions except for a main effect of Sample (Table 2). Polynomial contrasts revealed a significant quadratic trend of Sample [$F(1, 65) = 6.755, p = 0.012$]. Under light conditions (Fig. 1D), there were no observed differences associated with the Group, Sex, or Group by Sex interaction (Table 2), except for a main effect of Sample. Similar to the dark sample results, polynomial contrasts revealed a significant quadratic trend of Sample [$F(1, 65) = 22.524, p < 0.001$] under light conditions. In general, total distance traveled decreased across the four samples under both environmental conditions.

Total stop time was also characterized as a measure of general behavior. No differences were observed under dark (Fig. 1E) or light conditions (Fig. 1F), except for the main effect of Sample (Table 2). Polynomial contrasts of Sample revealed a significant linear trend under dark conditions [$F(1, 65) = 127.224, p < 0.001$]. In general, total stop time increased across the four samples. Conversely under light conditions, trend analysis of Sample revealed a nonsignificant linear trend [$F(1, 65) = 1.698, p = 0.197$] and quadratic trend [$F(1, 65) = 0.076, p = 0.783$]. Total stop time increased across the four samples under both environmental conditions.

3.2. Stopping organization

Circular statistics [35] were used to characterize the concentration of mouse stopping behavior under dark (Fig. 2A) and light conditions (Fig. 2B). The concentration of the stops in each of the four five-minute samples is represented by the first order parameter of concentration for each radiation group across sample. The nested graph represents the second order parameter of concentration for each group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
significant linear trend under dark \([F(1, 65) = 12.988, p = 0.001]\) and light conditions \([F(1, 65) = 13.746, p < 0.001]\). Parameter of concentration increased across samples under both testing conditions, consistent with stopping behavior becoming more focused toward one direction. The concentration of the four sample headings was calculated as the second order parameter of concentration under dark (Fig. 2C & E).
and light conditions (Fig. 2D & F). No differences were found in the Group, Sex, or Group by Sex interaction (Table 3).

The distance from the center of the table was calculated for all stops across samples (Fig. 3A & B). These values were used to calculate percentage of stopping on the periphery. Under dark conditions (Fig. 3C), there was a significant effect of Sample, Group, and Sample by Group interaction (Table 3). Polynomial contrast revealed a significant linear trend [F(1, 65) = 42.331, p < 0.001] with mice decreasing their percentage of stops on the periphery across samples. In general, mice in the acute group had a greater percentage of stops on the periphery than the control group [p = 0.033] and this was significant on the fourth sample [p = 0.016]. There were no observed significant effects under light conditions (Fig. 3D & Table 3).

Finally, change in heading was calculated for all stops under each condition (Fig. 4A & B). There were no significant differences (Table 3) observed in the main effects of Group, Sex, Sample, or corresponding interactions under dark (Fig. 4C) or light conditions (Fig. 4D).

### 3.3. Progression Organization

Several measures were used to quantify progressions relative to the direction of the home base under dark (Fig. 5A) and light conditions (Fig. 5B). Under dark conditions, there were no observed differences between Sex, Group, or Sex by Group interactions for progressions directed away (Fig. 5C) or toward (Fig. 5D) the home base (Table 3). Similar results persisted under light conditions; no differences were associated with progressions directed away from the home base (Fig. 5E). However, progressions directed toward the home base (Fig. 5F) revealed a significant main effect of radiation (Table 4) with the acute group displaying more circuitous paths than the chronic group (p = 0.011).

Although progressions vary in length and direction of movement, the moment-to-moment speeds tended to follow a consistent pattern (see Fig. 6A-D). Specifically, speeds monotonically increased to a peak, then monotonically decreased until the next stop. The peak in speed occurred near the midpoint of the progression. There were no observed differences in peak speed under dark conditions for progressions directed away (Fig. 6E) or toward (Fig. 6F) the home base (Table 4). Comparably
under light conditions, no differences persisted with progressions directed away from the home base (Fig. 6G). However, within progressions directed toward the home base under light conditions (Fig. 6H) revealed that the acute group displayed slower peak speeds than the chronic group \( p = 0.017 \). Under dark conditions, there were no observed differences for peak error associated with Sex, Group, or Sex by Group interactions (Table 4). Similarly, under light conditions there were no observed differences in peak error between Sex, Group, or Sex by Group interactions (Table 4).

### 4. Discussion

The current study assessed the organization of mouse open field behavior under dark and light conditions to compare the impact of acute versus chronic low dose simulated GCR on spatial orientation. This is the first study to assess the impact of full-spectrum GCR on potential sexual dimorphisms and how they might influence the specific organization of mouse open field behavior. All mice displayed highly organized open field behavior centered around one home base. In general, progressions were non-circuitous independent of whether they were directed away or toward the home base. However, there was an observed difference in this organization for the acute group compared to the chronically exposed mice under light conditions. Specifically, with access to environmental cues, mice within the acute group displayed more circuitous progressions and slower average peak speeds with progressions made toward (D) the home base.
Animals segment their movements into progressions and stops focused around one location in the environment called the home base. Typically, animals will make more direct and faster progressions toward their home base and slow more circuitous paths away from their home base [11,30,32,37-40]. These previous studies observed group differences under dark conditions; however, the current study found no differences on these measures under dark conditions. Therefore, neither acute nor chronic exposure to simulated GCR Sim was sufficient to impair self-movement cue processing involved in organizing open field behavior.

Rodents learn to use visual [44], tactile [45], and olfactory [46,47] cues to guide ambulatory movement; however, the spontaneous organization of open field behavior has been shown to be differentially influenced by environmental cues. For example, visual and tactile cues have been observed to anchor home base behavior [39,48,49]. Further, access to visual cues has been observed to attenuate the effects of vestibular pathology on the organization of open field behavior [18,32]. In contrast, removal of odor cues [50] or chemical bulbectomy [36] have not been observed to influence home base behavior. Therefore, visual and tactile cues significantly contribute to the organization of open field behavior, while olfactory cues have a limited role. In the current study, returns to the home base were slower and more circuitous in the acute group relative to the chronic group. These differences are consistent with acute simulated GCR exposure eliciting thigmotaxic behavior in the open field under light conditions. This behavioral pattern will be further discussed in the next section in the context of cortical function.

4.2. Effects of simulated GCR exposure on brain and behavior

Multiple sources of information contribute to organizing open field behavior. Processing each source depends on communication among different brain structures. Previous work has identified several structures that have specific roles in organizing open field behavior. First, the hippocampal formation has been associated with the organization of open field behavior. For example, under dark conditions rats [15,16,50-53] and mice [54] with damage to the hippocampal formation exhibit more circuitous returns to the home base. In contrast, these effects are largely attenuated when provided access to visual cues [15,16]. Similarly, in the current study, the acutely exposed mice displayed more circuitous paths toward the home base compared to the chronically exposed group. However, disruptions linked to hippocampal pathology in previous studies were associated with impaired self-movement cue processing under dark conditions. This is opposite to the current findings where progressions were disorganized solely under light conditions. Therefore, it is possible that acute and chronic exposure to simulated GCR spared sufficient hippocampal function to support movement organization under dark conditions.

The medial frontal cortex has been implicated in processes related to maintaining spatial orientation [50,55] and organizing open field behavior [17]. Under dark conditions, rats with medial frontal lesions do not display differences in behaviors sensitive to spatial orientation. Conversely, under light conditions, rats with medial frontal lesions were
observed to have disruptions within their progression organization by traveling in more circumsit paths and at slower average peak speeds directed toward the heading of the home base [17]. This pattern of behavior is parallel to what was observed in the current study, where the acute group displayed more circumsit and slower paths directed toward the home base under light conditions. In contrast, mice in the acute group exhibited more stops on the periphery of the table relative to the control group under dark conditions, which was not observed with medial frontal lesions [17]. Therefore, future work is needed to evaluate the neuropathology of these potentially affected areas to confirm (or not) whether similar radiation-injury signatures occur, as reported previously in the medial frontal cortex [7].

4.3. Sexual dimorphisms and the effects of space radiation

Sexually dimorphic performance is commonly observed on spatial tasks that depend on access to environmental cues; however, the basis for these differences continues to be debated. For example, during acquisition male rats typically locate the hidden platform within the Morris water task faster and more efficiently compared to females [56–58]. Sexual dimorphisms in stress responses [59] have been posited as a factor contributing to the differences in performance. Sexual dimorphisms in Morris water task performance are enhanced when animals are exposed to unpredictable stress [60] and attenuated when the stress response is blocked [61]. Representation of the environmental cues has also been suggested as basis of the sexual dimorphic performance on spatial tasks. Specifically, within the radial arm maze, both male and female rats use several types of cues (distal, proximal, extra-room), but with repeated training females transitioned to only utilizing distal cues [62]. In the current study, no sex differences were observed in the organization of open field behavior under light conditions. Therefore, it is possible that the open field elicited a similar stress response or similar environmental cues were used to encode the position of the home base.

In general, sex differences have not been observed in processing self-movement cues [58,63–67]; however, one study has reported sex differences [68]. Interestingly, sexual dimorphisms within vestibular system anatomy has been posited as the basis for the higher prevalence of vestibular pathology reported in females [69]. Although the current study did not observe sexual dimorphisms in mouse self-movement cue processing, further work is needed to evaluate development change in susceptibility to vestibular pathology and possible contributions to spatial orientation.

Space radiation has been reported to differentially influence central nervous system function in females and males. Recent research has observed males have greater cognitive deficits and neuroinflammation when irradiated with low doses of helium [20]. This inflammatory response has also been implicated in behavioral dimorphisms. Specifically, male rodents exhibit an increase in general locomotion and greater anxiety-like behaviors compared to females after GCR irradiation [19,25,70]. Although more simplified space radiation exposures were necessary to provide further mechanistic insight regarding sexual dimorphisms.

4.4. Conclusion

In conclusion, this study evaluated the organization of open field behavior under dark and light conditions with acute low dose and chronic low dose whole-body exposure to GCR Sim in male and female mice. In general, males and females organize open field behavior similarly following acute or chronic GCR Sim. Under dark conditions, we did not observe any disruptions in open field organization. However, under light conditions, we observed differences within the acutely exposed group relative to chronic group specific to homeward progressions. Both dosages seemed to spare organization of open field behavior as there were no observed differences compared to controls. Further work is needed to characterize the mechanism(s) for these group differences. Previous work has demonstrated that irradiation can influence neural systems that support spatial orientation [71–75]. The organization of rodent open field behavior provides a novel approach to investigate the effects of space radiation on performance of mission critical tasks.

CRediT authorship contribution statement


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