



Skilled movement and posture deficits in rat string-pulling behavior following low dose space radiation (^{28}Si) exposure

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ABSTRACT

Deep space flight missions beyond the Van Allen belt have the potential to expose astronauts to space radiation which may damage the central nervous system and impair function. The proposed mission to Mars will be the longest mission-to-date and identifying mission critical tasks that are sensitive to space radiation is important for developing and evaluating the efficacy of counter measures. Fine motor control has been assessed in humans, rats, and many other species using string-pulling behavior. For example, focal cortical damage has been previously shown to disrupt the topographic (*i.e.*, path circuitry) and kinematic (*i.e.*, moment-to-moment speed) organization of rat string-pulling behavior count to compromise task accuracy. In the current study, rats were exposed to a ground-based model of simulated space radiation (5 cGy $^{28}\text{Silicon}$), and string-pulling behavior was used to assess fine motor control. Irradiated rats initially took longer to pull an unweighted string into a cage, exhibited impaired accuracy in grasping the string, and displayed postural deficits. Once rats were switched to a weighted string, some deficits lessened but postural instability remained. These results demonstrate that a single exposure to a low dose of space radiation disrupts skilled hand movements and posture, suggestive of neural impairment. This work establishes a foundation for future studies to investigate the neural structures and circuits involved in fine motor control and to examine the effectiveness of counter measures to attenuate the effects of space radiation on fine motor control.

1. Introduction

Deep space flight missions will expose astronauts to galactic cosmic radiation (GCR). Current estimates suggest that astronauts will be exposed to ~ 13 cGy of GCR during each year of a mission to Mars [1], the majority of which will be incurred while in transit. The structure of the spacecraft will offer a degree of shielding to the astronauts, reducing the dose and altering the GCR ion spectrum from that seen in free space. The “Local-Field” spectrum (the radiation spectrum that the internal organs of astronauts will receive within the spacecraft) predicts that the majority of the physical and dose-equivalent space radiation (SR) dose will arise from $Z < 15$ particles [2].

The impact that SR exposure may have on many aspects of astronaut

health has been increasingly investigated. Ground-based rodent studies have shown that SR exposure induces significant changes in many aspects of neurotransmission within the hippocampus [3–9] and other parts of the brain [10–13]. There is increasing evidence that SR exposure impairs performance in several cognitive tasks, including those requiring cognitive flexibility [10–20]. Marked inter-individual differences exist in cognitive flexibility performance of the irradiated subjects, with some rats having performance metrics comparable to shams, while others completely fail to reach criterion in cognitive tasks [10–12,14,16,21]. Our previous studies indicate that $\sim 33\%$ of irradiated rats exhibit cognitive performance metrics that fall below the 5th percentile of the performance metrics of the sham cohorts [15,16,20]. Additional and/or latent performance decrements may be present that are not apparent in

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the irradiated rodents with apparently normal cognitive performance. We have recently shown that rats exposed to 5 cGy 600 MeV/n ^{28}Si had no detectable constrained cognitive flexibility (ATSET task) performance issues but exhibited significant impairments in unconstrained cognitive flexibility tasks [11]. Moreover, there are latent ATSET deficits in rats exposed to Si [22] or neutrons [15] that had apparently perfect ATSET performance under normal rested conditions, which become manifested after mild sleep fragmentation. While there are some individuals that exhibit widespread performance decrements, in general, there does not appear to be a consistent performance impairment in tasks regulated by different brain regions, or that investigate different cognitive domains [10,11,15]. Thus, risk estimates based upon the impact of SR on a single process are likely to severely underestimate the negative impact of SR on astronaut performance.

Astronauts not only have to be cognitively proficient, but they also must be able to physically conduct tasks on the mission. During parabolic flight, astronauts that had been on prior space missions exhibited decreased knot tying ability in a surgical task [23]. The combined effects of space flight stressors, including GCR and microgravity, may act synergistically to influence the central nervous system (CNS) and impair function. However, it is not yet known whether GCR exclusively influences skilled motor control. Ground-based rodent studies have demonstrated that SR exposure results in gross motor deficits [24–27]. However, the impact that SR exposure has on fine motor control which supports many daily activities, such as tool and mission control manipulation, has not been established yet. Given the high level of coordination between multiple processes and brain regions, and the reported issues in neurotransmission and inter-connectivity between brain regions after SR exposure [13], it is conceivable that fine motor skills may be more sensitive to SR exposure than gross motor functions.

The present study examined the performance of rat string-pulling behavior in which animal's balance on their hindlimbs while making hand-over-hand movements to retrieve a food reward attached to the end of the string. Not only does the task involve skilled hand use for performance, but the motor behavior both rats and humans elicit on the task is similar [28–30]. Efficient task performance is dependent upon motor cortex (M1) control, as lesions of this cortical area impair the topographic and kinematic organization of rat string-pulling behavior and compromise task accuracy [31]. Accordingly, the damage produced by SR ions may also impair the structures or systems involved in string-pulling behavior. Therefore, the current study evaluated the effects of a low dose exposure to 5 cGy of 600 MeV/n ^{28}Si ions on the organization of string-pulling behavior in rats.

2. Materials and methods

2.1. Animals

This study utilized a subset of the male (proven breeder) Wistar rats (Hla®(WI)CVF®; Hilltop Lab animals, Inc., Scottsdale, PA, USA) that were used in our previous published study [11]. After arrival at Eastern Virginia Medical School (EVMS), the rats were pair-housed, maintained on a reversed 12:12 light/dark cycle, and given *ad libitum* access to Teklad 2014 chow and water. After one acclimation week, the rats were weighed and implanted with ID-100us RFID transponders (Troxan Ltd, United Kingdom) to facilitate identification of individual animals. The average age of the rats upon arrival at EVMS was 6 months with an average weight of 547 g. This study was conducted in accordance with the National Research Council's "Guide for the Care and Use of Laboratory Animals (8th Edition)" at the animal care facilities of EVMS, Brookhaven National Laboratory (BNL), and Northern Illinois University (NIU), all of which are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International. All procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of EVMS, BNL and NIU.

2.2. Exercise and food regimen

In an attempt to use a model system that resembled astronauts as much as possible, three weeks following arrival at EVMS, rats were placed on a treadmill exercise regimen (Day 1: 30 min at 20 m/min, Day 2–14: 30 min. at 25 m/min), 3 times a week for 2 weeks. Thereafter, rats were exercised for 30 min at 25 m/min, twice a week, for the entire duration at EVMS to correspond to a mild aerobic exercise regimen [32]. Rats were not exercised while housed at BNL or NIU. The exercise regimen acts not only as an "enrichment" activity (minimizing stress associated with being placed in new environments and being handled by humans) but also serves to ensure that the rats did not become obese or develop gross motor deficits.

Two weeks into the exercise regime, the rats were single-housed and switched from *ad libitum* rat chow to a restricted diet. The rats received a daily allowance of ~6 g/day of Cheerios™ (General Mills, Minneapolis, MN); the exact amount was varied daily to maintain an individual rat's weight at ~85 % of its pre-food restriction weight. After 10 days of food restriction, the rats started pre-screening in ATSET as previously described [11,14,15]. After the completion of all behavioral testing the rats were given *ad libitum* access to Teklad 2014 chow. Only those rats that passed all six stages of the prescreening protocol were considered for further study, moreover any rat that took two attempts to pass two or more stages were also excluded from further study. Rats that satisfied these inclusion criteria were paired-housed and then sent to BNL to be irradiated with 600 MeV/n ^{28}Si ions.

2.3. Procedures

2.3.1. Ground-based irradiation

After arrival at BNL, the pre-screened rats were pair-housed, maintained on a reversed 12:12 light/dark cycle, and given *ad libitum* access to Teklad 2014 chow and water. After at least one week of acclimation, the rats were randomly assigned to sham-irradiated or irradiated (whole-body irradiation with 5 cGy 600 MeV/n ^{28}Si ions at the NASA Space Radiation Laboratory (NSRL) cohorts.

The rats were placed in well-ventilated custom-made irradiation jigs that were constructed of ¼ in thick black polyacrylic plastic and exposed to the ion beam at a rate of 2–5 cGy/min. Dose calibration was performed as previously described [33]. Control rats were placed in identical irradiation jigs that remained in the preparation room, while their counterparts were taken into the radiation vault at NSRL. One week after irradiation, the rats remained pair-housed and were transported back to EVMS, where they were maintained on a reversed 12:12 light/dark cycle and given *ad libitum* access to autoclaved Teklad 2014 chow and water. At 12 ± 2 weeks post-irradiation, the performance of the rats in the full ATSET test was established [11]. A cohort of 9 (4 shams and 5 Si-exposed) high ATSET performing rats (passed every stage of the ATSET test, in each instance, in less attempts than the sham cohort average for that stage) were sent to NIU to assess fine motor control in a string-pulling task. Rats remained pair-housed throughout all shipping.

2.3.2. String-pulling

After arrival at NIU, rats were kept on a 12:12 h light/dark cycle in a room maintained at ~72 degrees Fahrenheit and were provided food and water *ad libitum*. Pair-housed rats combined weights were greater than 1000 g; therefore, rats were single-housed in clear standard housing cages. Following one week of acclimation, rats were gently handled, and weights were taken and averaged across three baseline days for control (771.6 g) and irradiated (839.5 g) rats. Rats were then food restricted to 85 % of their free-feeding body weight to increase motivation to engage in the string-pulling task. It took about one week for rats to reach 85 % of their free feeding body weight. Once rats reached their goal weights, habituation to strings (0.2 cm diameter, 100 % cotton) and Cheerios began around 14 months of age. Rats were placed in an empty clear standard-housing cage with strings of varying lengths (30

cm–200 cm), half baited with one half of a Cheerio piece tied to the end. The rats began testing in the string-pulling apparatus the subsequent day. The string-pulling apparatus was a clear Plexiglas box (26.67cm × 25.4cm; see Fig. 1A) placed on a Table (1.5 m above the floor) in a small room. A string-pulling session consisted of 10 trials to pull in a 150 cm string to retrieve half of a Cheerio. A weight was attached to the end of the string in the cage to prevent the string from falling outside of the cage. Rats remained in the apparatus for all 10 trials, and the apparatus was thoroughly cleaned between rats. The first two days of testing were conducted with an unweighted string, followed by one day of string-pulling with a 4 g weight attached to the end of the string after the Cheerio half (see Fig. 1B). The entire task lasted 4 days, including 3 days of testing. A Canon HD video camera (model #: XA30) set at 1:1000 shutter speed and 30 frames per second was positioned perpendicular to the wall of the apparatus to record string-pulling behavior for offline analysis.

2.4. Analyses

2.4.1. Motivation analyses

Once placed in the string-pulling apparatus, rats explored the environment, contacted the weight anchoring the string into the cage, and eventually engaged in string pulling behavior. Motivation to engage in the string-pulling task has been assessed by the approach and pull time. Approach time is the time it takes for rats to initiate string-pulling behavior after being placed in the apparatus. The time it takes rats to pull in the string constitutes pull time. Approach and pull time were evaluated across testing with the unweighted string on days 1 and 2 and the weighted string on day 3 of testing.

2.4.2. Accuracy analyses

Rats typically stand upright on their hindlimbs and use their hands to pull in a string. This upright position allows for the assessment of postural stability. A 3-point scale (0, 1, or 2) was developed to characterize rats' posture in the string-pulling task. A score of "2" indicates an upright stance, symmetrical feet beneath the body, and no leaning on the wall. A score of "1" means that any 2 of the 3 behaviors are present, and a score of "0" represents only one or none of the 3 behaviors present. Rats engage in hand-over-hand movements involving multiple contacts to pull in a string. Occasionally, the mouth is also used to pull in the string. Percent of contacts with the hands and mouth were calculated by first summing all hand and mouth (total) contacts. Then, total contacts

with each body part (left-hand, right-hand, mouth) were calculated as a percent of total contacts (*i.e.*, total left-hand contacts/total contacts). When pulling in the string, rats may miss, or fail to contact the string. Percent of misses with the hands and mouth were calculated by comparing total misses for each body part to total misses (*i.e.*, total left-hand misses/total misses).

2.4.3. Kinematic analyses

String-pulling behavior is organized into multiple reach and withdraw movements. Three trials were selected for kinematic analyses based on previous work showing this provides a robust assessment of the organization of string-pulling behavior. The open-source program, Tracker (<https://physlets.org/tracker/>), was used to manually digitize 30 Hz videos of string-pulling trials frame-by-frame. The digitized x- and y-coordinates were used to calculate moment-to-moment speeds and change in distance on the y-axis. Change in distance on the y-axis was used to divide string-pulling behavior into reach and withdraw movements. Then, several measures of performance were used to quantify reach and withdraw components of movement. First, gross measures of performance, including the time to approach the string and the time to pull the string in to retrieve a food reward, assessed motivation. Second, rats engage in contacts with the hands and mouth to pull in the string; however, rats may miss or fail to contact the string. Third, kinematic and topographic characteristics of movement were investigated across testing. Peak speed is a kinematic feature while measures of topography include distance traveled, path circuitry, concentration and heading direction of reach and withdraw endpoints. Path circuitry was calculated as the ratio of the Euclidean distance relative to the length of the reach and withdraw path. Paths may be very direct (values close to 1) or highly circuitous (values close to 0). Circular statistics was used to transform the start and end coordinates from an x-y cartesian coordinate system, such that the start of the path is the origin (0,0), and the angle of the end coordinate of the path is calculated relative to a polar coordinate system (0: right; 90: up; 180: left; 270: down) to investigate concentration (1st order) and heading of movement (2nd order; [34]). Concentration of movement examined how clustered reach and withdraw endpoints were with more clustered endpoints yielding values closer to 1 and more dispersed endpoints resulting in values closer to 0. The heading direction of reach (toward 90) and withdraw (near 270) endpoints were evaluated across testing.

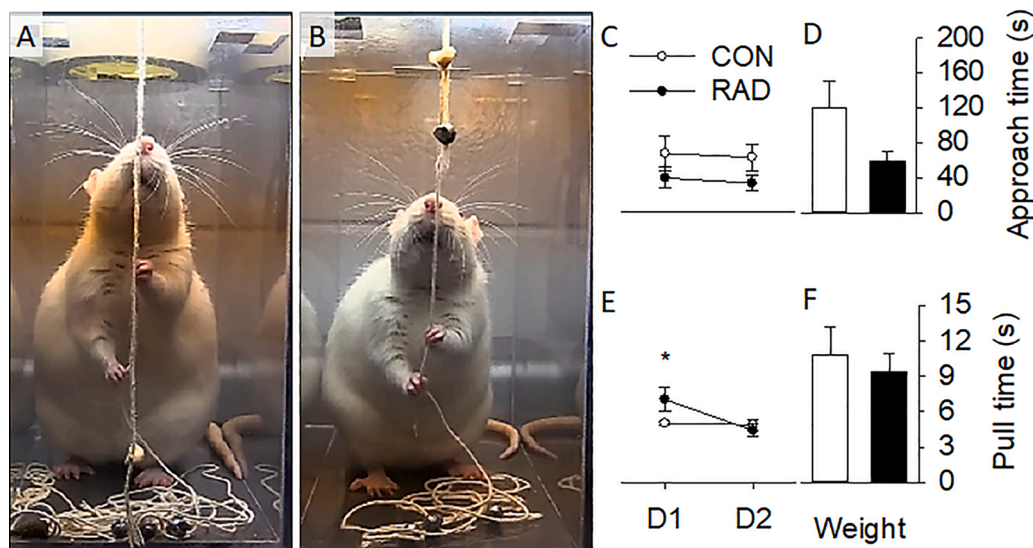


Fig. 1. A rat is shown in the string-pulling apparatus with an unweighted (A) and weighted string (B). Approach (C, D) and pull (E, F) time are plotted for the unweighted (D1-2) and weighted strings. Posture stability scores (G, H) are graphed across testing as well. ($p < 0.05^*$).

2.4.4. Statistical analyses

Repeated measures ANOVAs were used to evaluate the main effects of group, day, and Group by Day interactions with an unweighted string. Independent samples t-tests were used to investigate group differences in pulling when a weight was added to the end of the string. Alpha was set at 0.05, and the effect sizes were examined using partial eta squared (η^2_p) and Cohen's *d* (*d*). Linear trend analysis and Tukey's Honest Significant Difference (HSD) post-hoc tests were used to evaluate significant main effects and interactions. SPSS 25 (IBM, USA) was used to calculate statistical results.

3. Results

3.1. Motivation analyses

Time to approach the string and time to pull in the string were evaluated across testing (see Table 1). Irradiated rats took longer to pull in the unweighted string on day 1 of testing compared to control rats (see Fig. 1E). However, on day 2 of testing, irradiated rats decreased pull time to the level of control rats. Repeated measures ANOVA conducted on pull time revealed a significant Group by Day interaction and a significant effect of day yet failed to reveal a significant effect of group. Analyses of approach time with the unweighted string failed to reveal any differences (see Fig. 1C). Independent samples t-tests failed to reveal any differences in approach (see Fig. 1D) or pull (see Fig. 1F) time on day 3 of testing with the weighted string.

Table 1
Motivational analysis and endpoint measures with an unweighted string.

	f	df	p	η^2_p
Approach time				
Day	0.775	1, 7	0.408	0.100
Day X Group	0.015	1, 7	0.904	0.002
Group	1.826	1, 7	0.219	0.207
Pull time				
Day	6.952	1, 7	0.034	0.498
Day X Group	5.781	1, 7	0.047	0.452
Group	1.446	1, 7	0.268	0.171
Left-hand contacts				
Day	6.872	1, 7	0.034	0.495
Day X Group	2.036	1, 7	0.197	0.225
Group	0.438	1, 7	0.059	0.438
Right-hand contacts				
Day	1.398	1, 7	0.276	0.166
Day X Group	1.813	1, 7	0.220	0.206
Group	0.977	1, 7	0.356	0.122
Mouth contacts				
Day	5.269	1, 7	0.055	0.429
Day X Group	10.787	1, 7	0.013	0.606
Group	0.956	1, 7	0.361	0.120
Left-hand misses				
Day	0.007	1, 7	0.936	0.001
Day X Group	1.188	1, 7	0.312	0.145
Group	0.636	1, 7	0.451	0.083
Right hand misses				
Day	1.050	1, 7	0.340	0.130
Day X Group	1.598	1, 7	0.247	0.186
Group	0.266	1, 7	0.622	0.037
Mouth misses				
Day	0.607	1, 7	0.461	0.080
Day X Group	0.115	1, 7	0.745	0.016
Group	10.239	1, 7	0.015	0.594

3.2. Accuracy analyses

Posture of rats was evaluated across testing during string-pulling behavior using a 3-point scale (see Table 1). A repeated measures ANOVA conducted on average posture score revealed that irradiated rats exhibited less postural stability relative to control rats (see Fig. 1G). Following irradiation, rats were less likely to engage in an upright stance and exhibit symmetrical feet beneath the body, or more likely to lean on the wall relative to control rats. Further, when a weight was added to the end of the string on day 3 of testing, irradiated rats still displayed postural instability (see Fig. 1A vs B). An Independent samples t-test revealed a significant difference between groups on average posture score (see Fig. 1H).

Contacts with the mouth and the left- and right-hands were calculated as a percent of total contacts (see Table 1). A representative mouth contact is shown for a rat (see Fig. 2A). Irradiated rats engaged in a greater percentage of mouth contacts relative to control rats on day 2 of testing, while no differences were observed on day 1 (see Fig. 2B). A repeated measures ANOVA conducted on the percent of mouth contacts revealed a significant Group by Day interaction yet failed to reveal a significant effect of group or day. Analyses conducted on percent of left- (see Fig. 2E) and right-hand (see Fig. 2G) contacts with the unweighted string failed to reveal any significant differences by day, group, or Group by Day interaction. When a weight was added to the end of the string on day 3 of testing, Independent samples t-tests revealed a significant difference in the percent of right-hand contacts (see Fig. 2H) yet failed to reveal any differences in the percent of left-hand (see Fig. 2F) or mouth contacts (see Fig. 2C).

Misses with the mouth and the left- and right-hands were calculated as a percent of each body part's total contacts (see Table 1). A representative mouth miss is pictured for an irradiated rat (see Fig. 3A). A greater percentage of mouth misses were observed by irradiated rats compared to control rats across days 1 and 2 of testing with an unweighted string (see Fig. 3B). Repeated measures ANOVA conducted on percent of mouth misses revealed a significant effect of group. A representative miss with both hands is shown by an irradiated rat (see Fig. 3D). Separate repeated measures ANOVA analyses conducted on percent of left- (see Fig. 3E) and right-hand (see Fig. 3G) misses failed to reveal any differences by day, group, or Group by Day interaction. On day 3 of testing with the weighted string, Independent samples t-tests failed to reveal any differences between groups in mouth misses or misses with the left- (see Fig. 3E) and right-hands (see Fig. 3G).

3.3. Kinematic analyses

3.3.1. Reach component analysis

Rats engaged in upward reaching movements to grasp the string. Representative string-pulling reach trajectories that have been transformed to have the same starting origin (0,0) are plotted for a control (see Fig. 4A) and an irradiated rat (see Fig. 4B). Kinematic and topographic characteristics of the reach component of movement were evaluated across testing (see Table 2). Measures of distance and direction estimation were impaired in irradiated rats during left-hand reaches. First, irradiated rats traveled longer distances with the left-hand compared to control rats on days 1 and 2 of testing with the unweighted string (see Fig. 4C). Repeated measures ANOVA conducted on distance traveled with the left-hand revealed a significant effect of group while no significant effect of day or group by day interaction was observed. Second, irradiated rats exhibited less concentrated left-hand reach end points with an unweighted string (see Fig. 5C). Repeated measures ANOVA conducted on left-hand concentration revealed a significant effect of group but no significant effect of day or group by day interaction. Since left-hand reach concentration (1st order) was significant, left-reach heading (2nd order) was not evaluated for statistical significance. Analyses of peak speed and path circuitry with left-hand reaches failed to reveal any significant effects of group, day, or Group by Day interactions

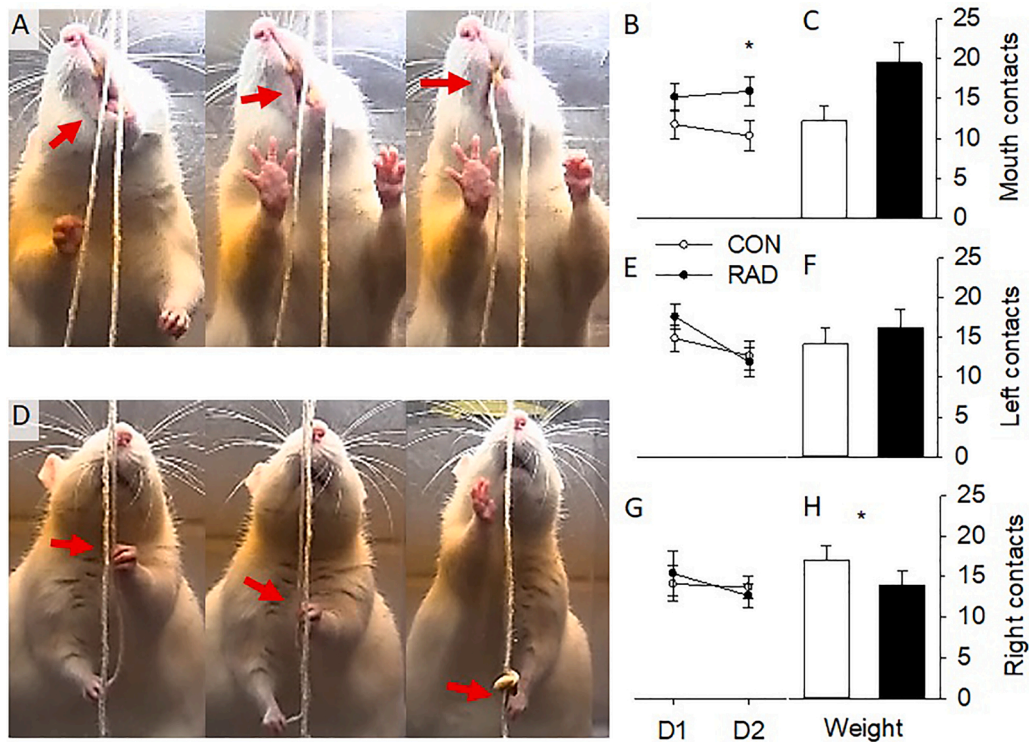


Fig. 2. A representative mouth (A) and left-hand (D) contact is pictured for a rat. The percent of contacts made by the mouth (B, C), the left-hand (E, F), and the right-hand (G, H) are graphed with unweighted (D1-2) and weighted strings. ($p < 0.05^*$).

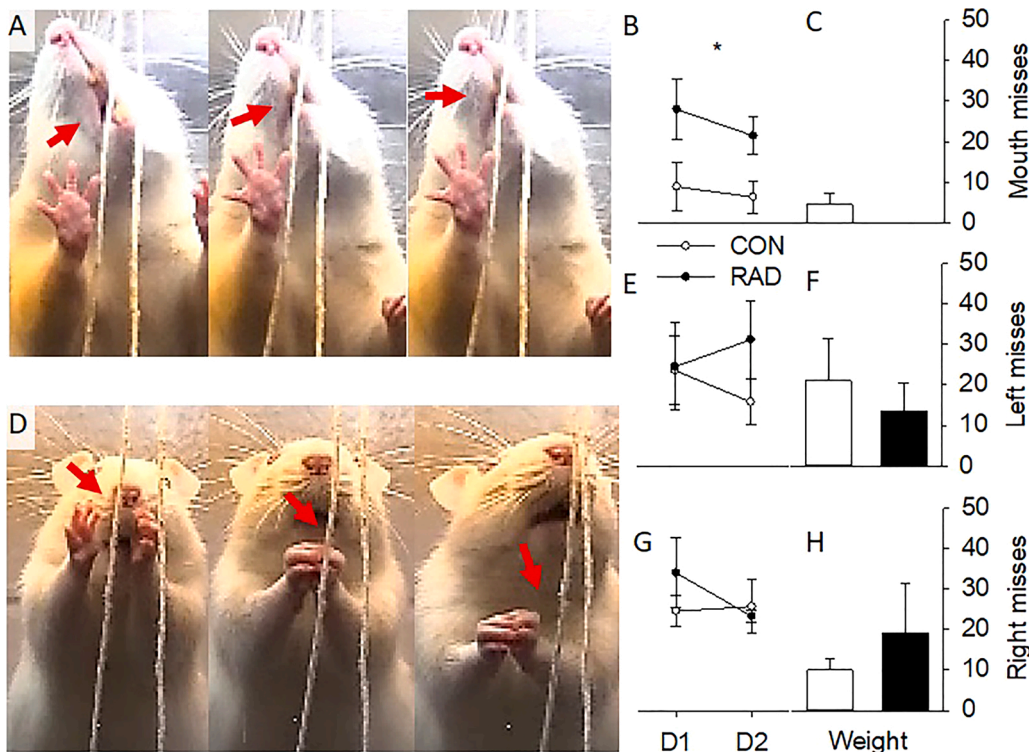


Fig. 3. A representative mouth (A) and hand (D) miss is pictured for a rat. The percent of misses made by the mouth (B, C), the left-hand (E, F), and the right-hand (G, H) with unweighted (D1-2) and weighted strings. ($p < 0.05^*$).

with the unweighted string (see Table 2). No differences were observed in any measures during right-hand reaches with the unweighted string (see Figs. 4–6D). Further, when a weight was added to the end of the string on day 3 of testing, kinematic and topographic measures of left-

(see Figure) and right-hand reaches failed to reveal any differences between groups (see Table 4).

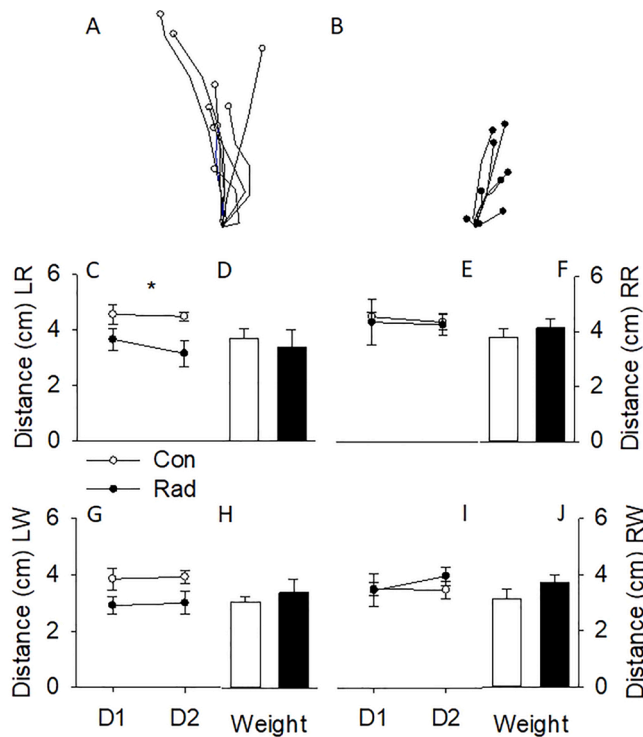


Fig. 4. Representative left reach trajectories that have been transformed to begin at the same origin (0,0) are shown for a control (A) and irradiated (B) rat. Distance traveled is plotted for the left reach (C, D), the right reach (E, F), the left withdraw (G, H), and the right withdraw (I, J) with unweighted and weighted strings. ($p < 0.05^*$).

3.3.2. Withdraw component analysis

Reaches to grasp the string are followed by withdraws to pull the string down. Several aspects of the withdraw component of movement were investigated across testing (see Table 3). Disruptions in distance and direction during left-hand withdraws were observed in rats following radiation exposure. Irradiated rats traveled longer distances during left-hand withdraws compared to control rats across testing with the unweighted string (see Fig. 4C). Repeated measures ANOVA conducted on distance traveled with the left-hand revealed a significant effect of group while no significant effect of day or group by day interaction was observed. Next, irradiated rats pulled the string more closely to the midline of the body during left withdraw movements relative to control rats across testing with an unweighted string (see Fig. 6E). Repeated measures ANOVA conducted on left-hand heading direction revealed a significant effect of group yet failed to reveal a significant effect of day or group by day interaction. Analyses of peak speed, path circuitry, and concentration during left-hand withdraws failed to reveal any significant effects of group, day, or group by day interactions with the unweighted string (see Table 4). No differences were observed in right-hand withdraw measures without a weight on the end of the string (see Figs. 4–6F). Further, irradiated rats organized left- and right-hand withdraw movements during string-pulling behavior similarly to control rats when a weight was added to the end of the string (see Table 4).

4. Discussion

The present study investigated the fine motor skills in rats exposed to 5 cGy ^{28}Si (that had no obvious ATSET performance issues) in a string-pulling task in which rats made hand-over-hand movements to retrieve a piece of Cheerio attached to the end of the string. A variety of alterations in performance were displayed by the irradiated rats, including changes in posture, increased mouth contacts, and decreased pull time with an unweighted string. Accuracy deficits were also observed in distance and

Table 2
Reach kinematics with an unweighted string.

	f	df	p	η^2p
Left-hand distance				
Day	2.676	1, 7	0.176	0.245
Day X Group	1.263	1, 7	0.298	0.153
Group	6.150	1, 7	0.042	0.468
Left-hand peak speed				
Day	0.001	1, 7	0.981	<0.001
Day X Group	3.230	1, 7	0.115	0.316
Group	3.581	1, 7	0.100	0.338
Left-hand path circuitry				
Day	<0.001	1, 7	0.995	<0.001
Day X Group	0.072	1, 7	0.796	0.010
Group	3.170	1, 7	0.118	0.312
Left-hand concentration				
Day	0.325	1, 7	0.587	0.044
Day X Group	0.391	1, 7	0.551	0.053
Group	6.774	1, 7	0.035	0.492
Left-hand heading				
Day	0.324	1, 7	0.587	0.044
Day X Group	0.264	1, 7	0.623	0.036
Group	0.034	1, 7	0.860	0.005
Right-hand distance				
Day	0.338	1, 7	0.579	0.046
Day X Group	0.043	1, 7	0.841	0.006
Group	0.066	1, 7	0.805	0.009
Right-hand peak speed				
Day	0.328	1, 7	0.585	0.045
Day X Group	0.039	1, 7	0.849	0.006
Group	0.313	1, 7	0.593	0.043
Right-hand path circuitry				
Day	0.123	1, 7	0.736	0.017
Day X Group	0.036	1, 7	0.855	0.005
Group	5.533	1, 7	0.051	0.441
Right-hand concentration				
Day	11.321	1, 7	0.012	0.618
Day X Group	0.055	1, 7	0.821	0.008
Group	0.457	1, 7	0.521	0.061
Right-hand heading				
Day	0.009	1, 7	0.926	0.001
Day X Group	1.056	1, 7	0.338	0.131
Group	0.561	1, 7	0.478	0.074

direction of hand reaching and pulling movements after radiation exposure. These results demonstrate that a single exposure to a low dose of ^{28}Si disrupts several aspects of skilled motor function and posture making this the first report that SR exposure produces deficits of this nature. Operationally, SR-induced defects in motor skills may only be evident under light loading conditions. When the string pull assays was conducted with a weight on the end of the string, there was a higher degree of controlled movement by irradiated rats, such that no differences were observed between groups on kinematic or topographic measures. However, the irradiated rats still had postural issues under these conditions.

String-pulling behavior involves the coordination of multiple reach and withdraw movements of the hands and sometimes the mouth. Therefore, the behavior provides a robust way to investigate disruptions in distance and direction estimation related to fine motor control and changes in performance related to compensation or varying task demands [28,29,35]. Left-lateralized deficits in distance and direction estimation were observed in the SR exposed rats. One explanation for the lateralized impairment may be that rats use a lead hand in which one

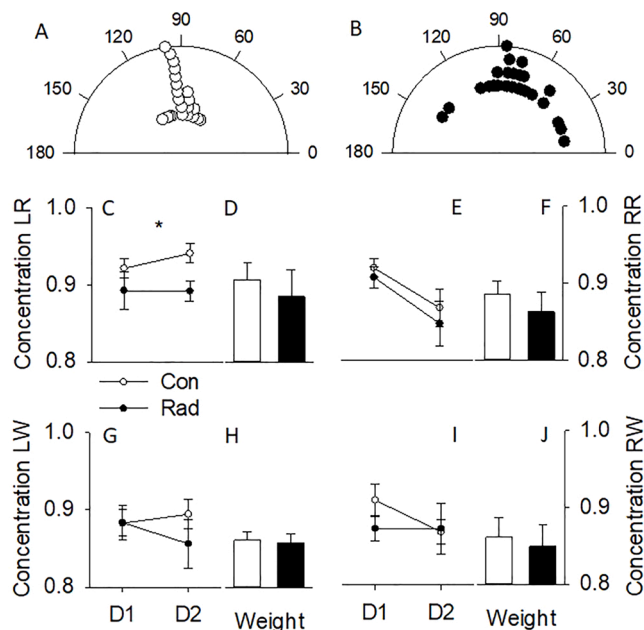


Fig. 5. Representative polar plots of left reach endpoints are displayed for a control (A) and irradiated (B) rat. Concentration of movement endpoints are pictured for the left reach (C, D), the right reach (E, F), the left withdraw (G, H), and the right withdraw (I, J) with unweighted and weighted strings. ($p < 0.05^*$).

hand guides the rhythm of movement [36]. If the rats have a lead, or dominant hand, in the string-pulling task then one hand may be weaker and more susceptible to damage from SR. However, further work is needed to determine the consistency and validity of these lateralized deficits, including assessing baseline pulling behavior prior to radiation exposure. Generally, there is disagreement about whether rodents exhibit handedness [37–41]; regardless, most rats have either a dominant left- or right-hand, and it is possible that a generalized SR-induced impairment in neural function, may affect coordination of bilaterally organized movement. Thus, the integration of multiple neural systems may result in a greater overall load and further contribute to the disruptions observed in movement organization during string-pulling behavior by irradiated rats. The use of both hands, and the mouth, also likely allows for compensation to occur with any potential deficits. For example, irradiated rats exhibited an increase in mouth contacts and a decrease in pull time with the unweighted string. These observations demonstrate the ability of string-pulling behavior to evaluate changes in performance following exposure to SR. In addition, string-pulling behavior may assess similar motor function as the motor praxis task in the Basner battery [42]. The motor praxis task involves moving a cursor to select a box which gradually decreases in size to assess participant sensorimotor function to control a computer interface. Inclusion of a human analogue of the string-pulling task [30] would afford an assessment of bimanually coordinated sensorimotor function and fine motor control. Future work should further examine the neural structures involved in string-pulling behavior and the ability of the task to rehabilitate impairments in fine motor control following damage; nevertheless, some speculation about the neural changes associated with irradiation-induced disruptions in performance described here is warranted.

SR exposure impacts multiple neurotransmitter systems, including the dopaminergic system, that are involved in the posture and skilled motor functions required to effectively retrieve food by pulling in a string [21,25,43]. The SR-induced dopamine decrease in the striatum was associated with impaired performance in a wire hanging task [25, 43]. Irradiated adult rats resembled aged rats (22–24 months old) in dopamine function and performance on motor tasks [44,45]. Dopamine

Table 3
Withdraw kinematics with an unweighted string.

	f	df	p	η^2p
Left- hand distance				
Day	0.217	1, 7	0.656	0.030
Day X Group	0.001	1, 7	0.974	<0.001
Group	4.360	1, 7	0.075	0.384
Left-hand peak speed				
Day	1.073	1, 7	0.335	0.133
Day X Group	2.530	1, 7	0.156	0.265
Group	3.240	1, 7	0.115	0.316
Left-hand path circuitry				
Day	0.166	1, 7	0.696	0.023
Day X Group	<0.001	1, 7	0.986	<0.001
Group	0.683	1, 7	0.436	0.089
Left- hand concentration				
Day	0.102	1, 7	0.758	0.014
Day X Group	0.619	1, 7	0.457	0.081
Group	0.879	1, 7	0.380	0.112
Left-hand heading				
Day	0.033	1, 7	0.861	0.005
Day X Group	1.743	1, 7	0.228	0.199
Group	6.406	1, 7	0.039	0.478
Right-hand distance				
Day	0.483	1, 7	0.510	0.065
Day X Group	0.567	1, 7	0.476	0.075
Group	0.333	1, 7	0.582	0.045
Right-hand peak speed				
Day	1.667	1, 7	0.238	0.192
Day X Group	0.473	1, 7	0.514	0.063
Group	0.160	1, 7	0.701	0.022
Right-hand path circuitry				
Day	0.067	1, 7	0.804	0.009
Day X Group	1.403	1, 7	0.275	0.167
Group	4.803	1, 7	0.065	0.407
Right-hand concentration				
Day	1.694	1, 7	0.234	0.195
Day X Group	1.703	1, 7	0.233	0.196
Group	0.382	1, 7	0.556	0.052
Right-hand heading				
Day	0.198	1, 7	0.670	0.027
Day X Group	0.547	1, 7	0.484	0.072
Group	0.159	1, 7	0.702	0.022

in M1 is necessary for learning a skilled reaching task [46], and damage to this system, such as following unilateral 6-hydroxydopamine nigrostriatal lesions, disrupts bilateral skilled reaching and posture [47,48]. Pharmacological manipulations in rats have shown that posture is decreased by morphine, but movement is not affected, while haloperidol blocks movement and spares, or even releases, posture [49]. The disruptions in skilled movement and posture observed in the string-pulling task may reflect damage to the dopaminergic system or an imbalance between dopaminergic and opioid systems.

Many different neurotransmitter systems that modulate motor control and drive the neuroplasticity underlying motor skill learning are disrupted after SR exposure. For instance, exposure to SR disrupts the readily releasable pools of acetylcholine, GABA, and glutamate in synaptosomes [4,10]. Changes to acetylcholine efflux may have consequences on motor function as an increase in acetylcholine release is observed in the sensorimotor cortex upon stimulation of the forepaw [50]. Further, local depletion of cholinergic afferents to M1 significantly disrupts map plasticity and skilled motor control, and global cholinergic depletion impairs map plasticity similar to M1 depletion while resulting

Table 4
Kinematics with a weighted string.

	t	df	p	d
Reach				
Left-hand distance	0.400	7	0.701	0.262
Left-hand peak speed	0.175	7	0.866	0.117
Left-hand path circuitry	0.072	7	0.945	0.050
Left-hand concentration	0.542	7	0.605	0.059
Left-hand heading	-0.260	7	0.803	0.170
Right-hand distance	-0.763	7	0.470	0.512
Right-hand peak speed	0.030	7	0.977	0.020
Right-hand path circuitry	0.852	7	0.423	0.600
Right-hand concentration	0.774	7	0.464	0.506
Right-hand heading	0.540	7	0.606	0.365
Withdraw				
Left-hand distance	-0.716	7	0.497	0.457
Left-hand peak speed	-1.422	7	0.192	0.905
Left-hand path circuitry	-0.322	7	0.757	0.060
Left-hand concentration	0.193	7	0.852	0.132
Left-hand heading	-1.298	7	0.235	0.920
Right-hand distance	-1.169	7	0.281	0.803
Right-hand peak speed	-1.632	7	0.147	1.069
Right-hand path circuitry	0.817	7	0.441	0.521
Right-hand concentration	0.304	7	0.770	0.205
Right-hand heading	-1.012	7	0.345	0.659

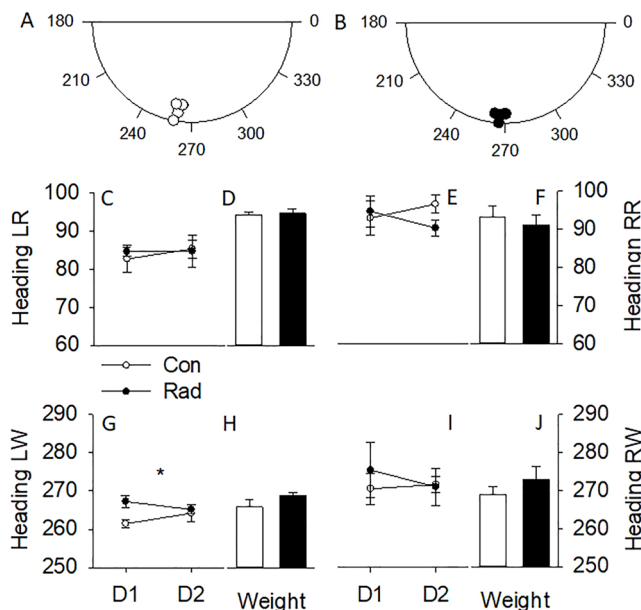


Fig. 6. Average left withdraw headings are displayed for control (A) and irradiated (B) rats on day 1 of testing. Headings are plotted for the left reach (C, D), the right reach (E, F), the left withdraw (G, H), and the right withdraw (I, J) with unweighted and weighted strings. ($p < 0.05^*$).

in significantly greater skilled learning deficits, whereas prefrontal cholinergic depletion fails to produce any changes to motor function [51]. Motor performance is also modulated by a balance of GABA/glutamate levels in motor-related cortical areas [52–54]. For example, following cortical stroke, GABA inhibition, via administration of a benzodiazepine inverse agonist, restored general motor function in a skilled reaching task without significant changes to reaching kinematics [53]. A persistent reduction in glutamatergic function has been reported following low doses of ^{56}Fe [4]. Further, subunits of glutamatergic NMDA receptors are also decreased 90 days after radiation exposure. Dysregulation of monoamines also leads to disruptions in plasticity and motor function (for review see [55]). Following exposure to ^{12}C , irradiated rats displayed a significant modification in normal monoamine

metabolism dynamics in multiple brain regions [56,57]. Damage to these various neuromodulators of motor function may have contributed to the deficits observed in the string-pulling task. However, further work is needed to determine the role of neuromodulators in fine motor control after SR.

SR exposure may also damage cortical and subcortical structures or circuits involved in fine motor control [18,58]. Exposure to 10 cGy ^{28}Si altered synaptic plasticity in the prefrontal cortex (PFC) [11]. The PFC projects to motor areas, [59,60], and interactions between the PFC and M1 may serve as a top-down control signal that inhibits inappropriate responding [61]. Extensive cortical stimulation and lesion work has identified structures critical for the organization of skilled movement. For example, cortical stimulation of M1 regions produces movement in individual digits and the hands [62,63]. Motor learning, including skilled reaching, coincides with M1 plasticity [64,65]. Synaptic transmission of M1 layers II/III is enhanced after rats learn skilled reaching, and the synchrony between M1 individual neuron activity is increased with training [66]. Damage to M1 and related brain regions impairs fine motor control. Unilateral lesions of the sensorimotor cortex or caudate putamen result in similar skilled reaching deficits that improved over time with additional reaching experience, while large cortical lesions abolished effective reaching [67]. M1 activity is also regulated by subcortical loops which contribute to the selection of appropriate motor plans [55]. SR-induced damage may also impact the sensorimotor cortex and lead to disruptions in skilled movement similar to stroke models [31,67]. Focal damage to the forelimb sensorimotor area has been shown to compromise task accuracy and result in persistent deficits in distance and direction estimation in rat string-pulling behavior [30]. Similar impairments in distance and direction estimation were observed in the current study following exposure to a single low dose of SR.

Astronauts may encounter similar disruptions in fine motor control during, or following, extended space flight missions, which could compromise mission critical tasks. String-pulling behavior is organized similarly in rodents [28,29] and humans [30] and may provide a quick and simple behavioral assessment to aid in the identification of SR-induced impairments. The task would be simple to conduct on rodents and astronauts during space flight missions, on the International Space Station, or upon return to Earth. In addition, the application of open-source machine learning algorithms to estimate body position from videos may yield a low cost and efficient analysis of string-pulling behavior [68,69].

Several components were manipulated throughout the study that may have influenced behavior. First, rats were housed in various conditions, including paired- vs. single housing. Second, rats experienced different light/dark cycles during the first and second part of the study with a reversed 12:12 light/dark cycle at EVMS and BNL and a normal 12:12 light/dark cycle at NIU. While these factors have the potential to influence behavior, all rats were housed in similar conditions across irradiated and sham groups. Therefore, deficits in irradiated rat string-pulling performance is likely a result of radiation and not changes in housing conditions. Third, SR characteristics, including dose rate, may impact the severity of deficits observed in the string-pulling task. The dose-rate of radiation exposure in space is orders of magnitude lower than can be reasonably conducted in ground-based studies. CNS effects are not related to cell killing (and are probably independent of DNA damage), so it is unlikely that the classic dose rate effect for cell survival will apply to CNS deficits [70,71]. However, qualitatively and quantitatively, the incidence and severity of cognitive impairments induced by protracted SR exposure (low dose rate: 18 cGy over 6 months) did not differ from that observed with bolus exposure to other SR ions [20,22]. Thus, the use of 2–5 cGy/min as a “standard” dose rate across different SR ions is based upon the readily achievable spill rate of most SR ions in the accelerator, which as a total dose is lowered and becomes more important for dosimetry purposes. This has proved to be an achievable dose rate under most operating conditions. The newer GCRSim beam that is becoming a standard beam for many studies has more

operationally defined dose rates for the various SR beams, which range from 0.2 to 1.5 cGy/min. Future work should investigate the effects of other SR ions, doses, and models, such as GCRSim, on the organization of string-pulling behavior.

In conclusion, the string-pulling task provides a detailed assessment of fine motor control and hand coordination. It is acquired quickly with minimal training, involves bimanually coordinated movements of the hands, and fits within the complex movement organization that astronauts engage in daily, such as manipulating tools and mission controls. The present findings, that string-pulling performance can be altered in several ways following SR exposure, highlights the need for more extensive examination of the effects of SR on motor systems. Future efforts need to determine the longitudinal time course of these impairments, whether rats can develop adaptive responses to circumvent such deficits, and importantly whether there is any sex dependency (with regard to incidence and severity) of SR-induced deficits in fine motor skills.

CRedit authorship contribution statement

Ashley A. Blackwell: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Brandi D. Schell:** Formal analysis, Investigation. **Jenna R. Osterlund Oltmanns:** Investigation, Writing - review & editing. **Ian Q. Whishaw:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **Son T. Ton:** Writing - review & editing. **Natalie S. Adamczyk:** Investigation, Writing - review & editing. **Gwendolyn L. Kartje:** Resources, Writing - review & editing. **Richard A. Britten:** Conceptualization, Methodology, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Douglas G. Wallace:** Conceptualization, Methodology, Software, Validation, Resources, Writing - original draft, Writing - review & editing, Supervision, Project administration.

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