

# The Effects of Low-Dose, High-LET Radiation Exposure on Three Models of Behavior in C57BL/6 Mice

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To investigate the behavioral consequences of exposure to whole-body irradiation such as might occur for astronauts during space flight, female C57BL/6 mice were exposed to 0, 0.1, 0.5 or 2 Gy accelerated iron ions (<sup>56</sup>Fe,  $Z = 26$ ,  $\beta = 0.9$ , LET = 148.2 keV/ $\mu$ m) of 1 GeV per nucleon using the Alternating Gradient Synchrotron at the Brookhaven National Laboratory. Animal testing began 2 weeks after exposure and continued for 8 weeks. Under these conditions, there were few significant effects of radiation on open-field, rotorod or acoustic startle activities at any of the times examined. The lack of radiation effects in these behavioral models appears to offer reassurance to NASA mission designers. These results suggest that there may be negligible effects of HZE radiation on many behaviors during a 2–8-week period immediately after radiation. © 2004 by Radiation Research Society

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## INTRODUCTION

Although it has been over three decades since humans first landed on the surface of the moon, there is a surprising lack of consistent data on the effects of the spaceflight environment on astronaut health and behavior. This is particularly true of the low-dose/low-dose-rate radiation aspect of this environment (1). Until recently, radiation effects on behavior were not of particular concern to mission designers since the central nervous system (CNS) was considered to be fairly radioresistant. However, there is a growing body of research that indicates that there are indeed effects of low-dose radiation on CNS function (2–6), and many of these effects may have long-term consequences.

This may be of critical importance to mission risk management for NASA when one considers that astronauts will

undoubtedly experience the impact of a number of unique psychological factors in any long-term spaceflight (general mission-related stress, neurovestibular influences, etc.) (7, 8). For example, microgravity-associated changes have been reported in astronaut motor/spatial behavior as well as in their ability to respond to visual cues in three-dimensional space. Furthermore, depending on the duration of the mission, these changes appear to go through several adaptive stages (9, 10). Should radiation exaggerate or impair these adaptive responses to the microgravity environment, there may be a significant downstream impact on astronaut function and safety (e.g. navigation and landing, performance of medical procedures). When one considers the proposed increases in International Space Station resources, and as plans for future exploration missions develop (e.g. to Mars), the importance of a scientifically based risk assessment system becomes apparent (7, 11).

Earth and low-Earth orbiting spacecraft are protected from much of the interplanetary radiation environment (e.g. Van Allen radiation belts). However, beyond the protective geomagnetic shielding of Earth, astronauts will be exposed to low doses of radiation in the form of solar particle events (SPEs) and galactic cosmic rays (GCR). While most of this radiation is made up of high-energy protons, only about 2% of the fluence in this environment is made up of high-Z, high-energy (HZE) particles. Nevertheless, these HZE particles are predicted to account for many of the biological consequences (7, 11–13).

Given current technology, a mission to Mars will likely last 2 to 3 years, with 8–12 months of that time in transit (14). The unpredictable nature of the relatively high-dose-rate SPEs (when compared to GCRs) makes these events likely threats to astronaut safety. Recent reports of nine coronal mass ejections over a 2-week period in October 2003 further emphasize the reality of this potential danger. Three particularly large solar particle events (November 1956, August 1972 and October 1989) have been used by mission designers to model worst-case scenarios. Depending on shielding conditions, these models indicate that the dose to astronauts over the course of a mission may be as

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high as 3 Gy, with most of the dose occurring during SPEs (11, 15, 16).

While research in this area is extremely limited, there have been reports of long-term neurophysiological and behavioral effects after radiation treatment. Glial degeneration and vascular disruption generally occur within weeks to months after exposure (17, 18). In addition, children who received relatively low-dose cranial radiotherapy for acute lymphoblastic leukemia (19) or tinea capitis (20, 21) generally scored lower on scholastic aptitude, intelligence and other psychological tests, sometimes even 20 years after treatment.

In several animal models, disruptions in behavior have been shown to occur after exposure to X rays (22, 23), electrons (22, 24, 25),  $\gamma$  rays (25, 26), neutrons (22), mixed fission-spectrum neutrons/ $\gamma$  rays (27), protons (2), and iron-particle radiation (3, 6, 28, 29). Results from these studies indicate that the effects of  $\gamma$  radiation cannot be used to extrapolate to other radiation types (3, 30).

NASA's Strategic Program Plan for Space Radiation Health Research suggests that while protons make up 90% of the dose equivalent in SPEs, their contribution drops to 19% in GCRs. The rest of the dose environment is made up of other radiation species including HZE particles such as iron, oxygen, carbon and silicon. In the case of GCRs, ~41% of the total dose equivalent is predicted to be due to HZE particles (31).

In the present study, we used iron-particle radiation as a surrogate for all of these HZE particles to characterize a behavioral dose response. Furthermore, given this predicted dose distribution, the doses used overlap with those predicted for astronaut exposures.

Previous work in this laboratory indicated that low-dose proton irradiation can have an influence on some behavioral models. Changes include deficits in open-field exploratory behavior, acoustic startle habituation, and rotorod performance. Furthermore, the influence of proton radiation tended to be acute and transient. However, the maximum dose in our previous study was 4 Gy (2). Given that the linear energy transfer (LET) of the iron-particle radiation used in the current study (~148 keV/ $\mu$ m) is considerably higher than that of protons (~0.4–0.5 keV/ $\mu$ m), we thought it possible that 2 Gy  $^{56}\text{Fe}^{26}$  particles would be enough to elicit a response similar to (if not greater than) that of 4 Gy protons. Typically, an increase in LET has been associated with altered biological effects, including unique gene expression patterns (32, 33) and impairment of conditioned taste aversion (4, 34). In most behavioral models, when there are radiation-induced decrements in performance, an increase in LET typically results in greater performance deficits (3, 30, 34). However, this does not always appear to be the case (2, 22).

As in our previous study, we felt it prudent to assess several behavioral models simultaneously. To be consistent with behavioral neurotoxicology testing guidelines suggested by the U.S. Environmental Protection Agency, the

National Toxicology Program, and others (35–37), we selected three behavioral models: open-field activity, rotorod, and habituation of acoustic startle. The open-field test was chosen to assess locomotor and spontaneous exploratory activity (38, 39). The rotorod task was chosen to examine the effects of radiation on balance, coordination and motor control (40). Finally, acoustic startle was chosen to assess changes in the function of a brainstem-level reflex (41). Pilot studies using proton irradiation indicated that these tests were adequate to establish the effects of radiation on CNS function (2).

## METHODS

### Animals

All protocols were approved by the Loma Linda University (LLU) and Brookhaven National Laboratory (BNL) Institutional Animal Care and Use Committees (IACUC).

Female C57BL/6 mice ( $n = 14$ –17/group, 62 animals total) were purchased from Charles River Breeding Laboratories (Hollister, CA) at 8 weeks of age and shipped directly to BNL. They were allowed to acclimate and recuperate from shipping stresses for a week under standard vivarium conditions. Within 1 week of irradiation, the animals were sent to the LLU Animal Care Facility via courier service. In both facilities, animals were maintained in large shoebox cages (7–8 mice/cage) on a 12:12-h light/dark cycle (lights on 0700 h) at 30–40% humidity and 65–70°F.

Approximately 1 week prior to irradiation, preprogrammed identification/temperature transponders (BioMedic Data Systems, Inc., Maywood, NJ) were inserted subcutaneously in each mouse and the tails were tattooed (AIMS, Inc., Budd Lake, NJ) with an identification number. The mice were mildly sedated using 100% CO<sub>2</sub> prior to the transponder insertion procedure. After the insertion of the transponders and tattooing, each mouse was assigned randomly to one of the four experimental groups ( $n = 14$ –17/group).

### Whole-Body Irradiation

Animals were irradiated with heavy iron ions ( $^{56}\text{Fe}$ ,  $Z = 26$ , 1087 MeV/nucleon at extraction and 1055 MeV/nucleon after passage through the vacuum line plus 2 m of air, LET = 148.2 keV/ $\mu$ m at target). Total doses delivered were 0.1, 0.5 and 2.0 Gy. Irradiations were performed at the entrance plateau region of the beam; each dose was delivered in a single fraction with  $\pm 10\%$  uniformity. Dose rates ranged depended on the total dose: 0.8–1.3 Gy/min for 0.5 and 2 Gy and 0.1–0.2 Gy/min for 0.1 Gy. Total irradiation times ranged from 0.5–2.5 min. The contribution of secondary particles at the center of the mouse would be similar to what has been described previously for tissue culture flasks with water (42, 43). A maximum of four mice were irradiated per exposure. Animals were shipped to the LLU Animal Handling Facility within 1 week of irradiation. After irradiation, animals were observed daily for signs of toxicity.

### Behavioral Testing

Behavioral testing was performed twice per week, on two consecutive days, during the light period of the cycle (start time 0900). Animals were tested with each behavioral model at 2, 4 and 8 weeks postirradiation. On each day, behavioral tests were performed in order of increasing potential for stress: open-field, rotorod and acoustic startle. At behavioral testing, the experimenters were blind to the radiation exposures of the mice.

### 1. Open field

We have reported the details for each of these tests previously (2). In brief, locomotor activity was measured using the SDI Open Field System (San Diego Instruments, San Diego, CA). The open field was a 20 × 40-cm Lexan™ shoebox cage, and activity was detected using arrays of LED photosensors. The cage and frame system were enclosed on four sides with neutral-colored walls and a translucent, white plastic filter top to block external visual cues. Cages were cleaned thoroughly with quatricide after each mouse was tested.

For each open-field test session, animals were placed in the center of the field, one animal per cage, and activity was monitored for 5 min. After 5 min, a 4-cm-diameter rubber disc with a nubby texture was placed in the front half of the cage, and activity was again monitored for 5 min.

The following parameters were evaluated: beam breaks in the center (BB<sub>C</sub>) and disc (BB<sub>D</sub>) ends of the cage, total and active time spent in the center (T<sub>C</sub> and TA<sub>C</sub>) and disc end (T<sub>D</sub> and TA<sub>D</sub>) of the cage, number of rears (N<sub>R</sub>), and time spent rearing (T<sub>R</sub>). The periphery included a 4-cm-wide strip around the base of the cage. The disc region was defined as a 16 × 16-cm area centered on the disc.

### 2. Rotorod

The rotorod tests were performed using the SDI Rotor-Rod System (San Diego Instruments, San Diego, CA). The test chamber consisted of a rotating 9-cm-diameter textured nylon rod at a height of approximately 45 cm above a foam floor. Each trial began when an animal was placed on the rotating rod and ended when the animal fell. If the animal stayed on the rod for ≥150 s, the trial was terminated and a duration of 150 s was recorded. There were six consecutive trials in each test session. The trials with the longest and shortest durations for each test session for each animal were discarded prior to analysis. The animals were tested at 18 and 26 rpm on the first and second days of each week, respectively.

### 3. Acoustic startle

The acoustic startle tests were performed using the SDI SR-Lab Startle Reflex System (San Diego Instruments, San Diego, CA). A constant white noise background was set at 70 dB. The startle response was elicited with a 50-ms, 120-dB burst of white noise with near-instantaneous onset and offset. Each test session included 50 consecutive startle trials with a 10-s intertrial interval. Parameters examined included startle amplitude, latency to peak, and startle frequency.

### Design and Analysis

The mixed factorial design for these experiments included one between-group factor, radiation dose (0, 0.1, 0.5 and 2 Gy), and the two within-group factors of week (2, 4, and 8 weeks) and day (two sessions per week). In addition, for the open-field data, object (no disc, disc present) was included as a repeated factor. For the acoustic startle test, trials were combined into bins (10 trials/bin for a total of 5 bins) and included as a within-group factor.

Behavioral testing is notoriously sensitive to environmental factors and testing conditions such as temperature, noise, lighting, odors and personnel (44). The variability in results due to these sensitivities is likely compounded when testing occurs over a period of weeks to months. Therefore, to minimize the day-to-day variability, all data were normalized to those for 0-Gy controls tested on the same day. For the open-field test, data were normalized by dividing each data point by the average of all data points taken from the control animals during the “no disc” portion of the test. Similarly, each data point from the rotorod test was divided by the average of the data from 0-Gy controls. Finally, for the startle test, each data point was divided by the average of all data points collected from control animals during the first bin of testing.

In all cases in which an analysis included more than two levels of a repeated measure, Greenhouse-Geisser corrections were applied to guard against violations of the sphericity assumption. Epsilon correction factors

are reported along with uncorrected degrees of freedom and corrected *P* values.

## RESULTS

### Open Field

#### 1. Activity in the open field

There were no main effects of dose, week or day on any of the parameters measured in the disc end of the open field, including BB<sub>D</sub>, T<sub>D</sub>, TA<sub>D</sub> and D<sub>D</sub> (Fig. 1). However, there were the expected significant main effects of placing a novel object in the field on all of these parameters. For example, the mice investigated the disc, spending more time in the vicinity of the novel object [T<sub>D</sub>: F(1,55) = 279, *P* < 0.001; TA<sub>D</sub>: F(1,55) = 249, *P* < 0.001] but breaking fewer beams and traveling less distance [BB<sub>D</sub>: F(1,55) = 37.5, *P* < 0.001; D<sub>D</sub>: F(1,55) = 43.0, *P* < 0.001]. Parallel effects were observed when examining the behavior of mice in the center of the field (data not presented). Importantly, no measure of the response to the object was modified by exposure to radiation. None of the interactions involving dose reached significance.

#### 2. Rearing activity in the open field

Rearing (Fig. 2), which may be considered a general exploratory behavior in the open field, declined after week 2 and with investigation of the novel object [main effect of object: N<sub>R</sub>: F(1,55) = 88.1, *P* < 0.001; T<sub>R</sub>: F(1,55) = 116, *P* < 0.001; week × object interaction: N<sub>R</sub>: F(2,110) = 4.60, *ε* = 0.96, *P* < 0.05; T<sub>R</sub>: F(2,110) = 6.74, *ε* = 0.98, *P* < 0.005]. Rearing increased on the second test day of each week as reflected by a main effect of day on the time spent rearing [T<sub>R</sub>: F(1,55) = 6.77, *P* < 0.05]. Importantly, however, there were no significant main effects or interactions associated with exposure to radiation.

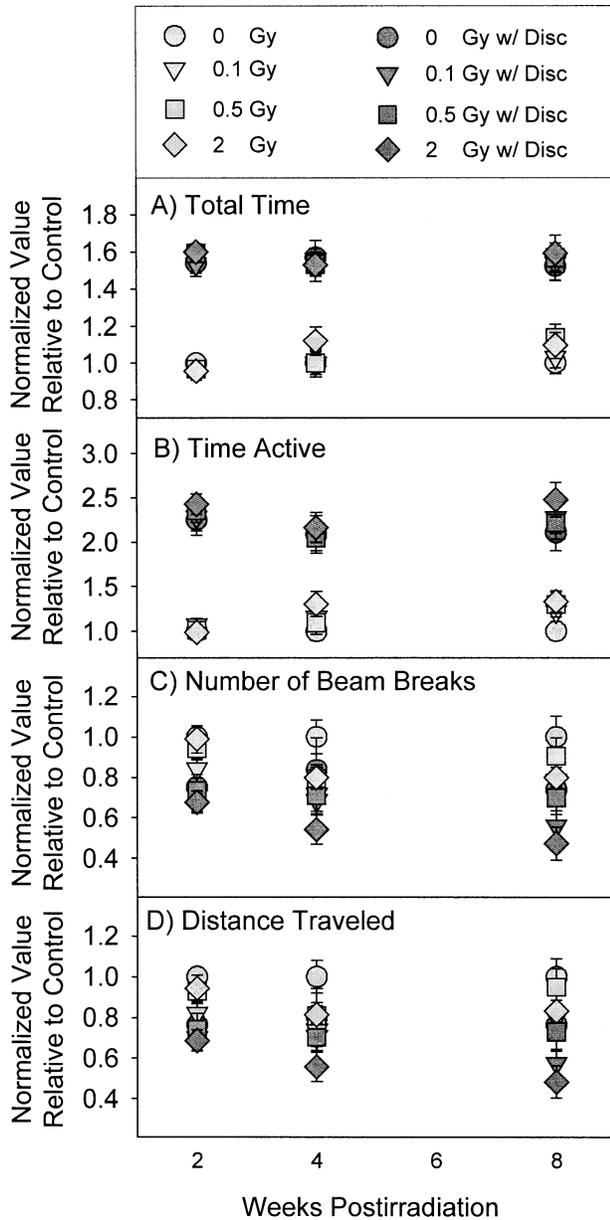
#### Rotorod: 18 and 26 rpm

Prior to normalization to the 0-Gy controls, there was a significant main effect of angular velocity [F(1,112) = 5.28, *P* < 0.05], with time on the rod being inversely proportional to rpm (Fig. 3). When normalized, there were no main effects of dose or week on the time spent on the rotorod at either angular velocity, nor were there any significant week × dose interactions.

### Startle

#### 1. Peak amplitude

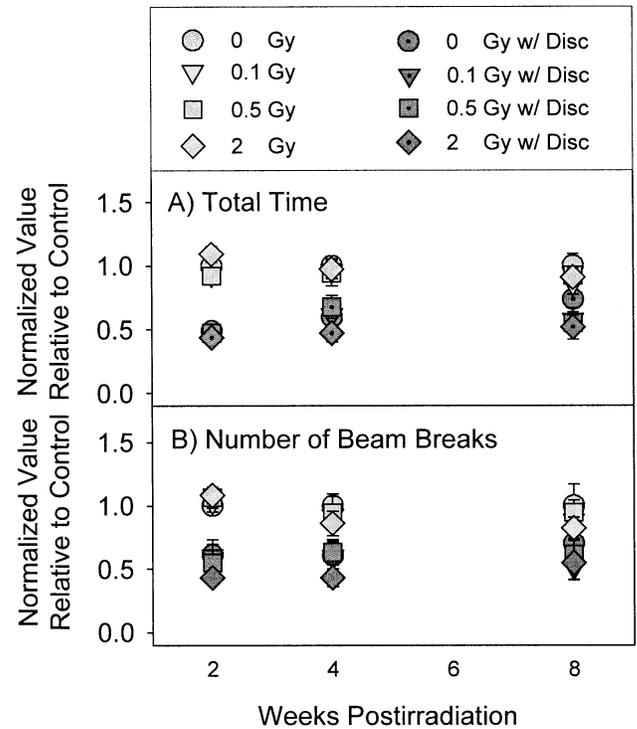
As indicated in Fig. 4, habituation of the startle response was generally observed across trial bins and from week to week, producing main effects of bin [F(4,208) = 7.42, *ε* = 0.88, *P* < 0.001] and week [F(2,104) = 5.14, *ε* = 0.87, *P* < 0.01] and a week × bin interaction [F(8,416) = 2.65, *ε* = 0.80, *P* < 0.05]; however, sensitization in responding was typically observed on the second day of testing within



**FIG. 1.** Effects of radiation on open-field activity near the disc. The disc region was defined as a  $16 \times 16$ -cm area centered on the disc. Prior to normalization, time parameters were measured in seconds and distances were measured in centimeters. All data were normalized to 0-Gy controls on each testing day. Data were normalized by dividing each data point by the average of all data points taken from the control animals during the “no object” portion of the test. Values are means  $\pm$  SEM. There were no significant main effects of radiation on any of the open-field measures.

a week (main effect of day [ $F(1,52) = 2.79, P < 0.01$ ] and week  $\times$  day interaction [ $F(2,104) = 7.56, \epsilon = 0.97, P < 0.001$ ]).

Although there were no significant main effects of dose on the acoustic startle response, there was a significant day  $\times$  bin  $\times$  dose interaction [ $F(12,208) = 2.55, \epsilon = 0.89, P < 0.005$ ]. Posthoc Tukey comparisons indicate that this statistical effect is not likely to be a meaningful behavioral



**FIG. 2.** Effects of radiation on rearing activity in the open field. Prior to normalization, time parameters were measured in seconds. All data were normalized to 0-Gy controls on each testing day. Data were normalized by dividing each data point by the average of all data points taken from the control animals during the “no object” portion of the test. Values are means  $\pm$  SEM. There were no significant effects of radiation on rearing activity.

result. The statistical interaction was apparently a result of response decreases in bins 3 ( $P < 0.01$ ) and 4 ( $P < 0.1$ ) in the 2-Gy group on the first, but not second, day of each week. Furthermore, animals exposed to 0.5 Gy appeared to have a consistently greater response than other groups across bins on both days. However, this difference reached significance only between the 2-Gy and 0.5-Gy groups ( $P < 0.05$ ) for day 1, bin 3.

## 2. Latency to peak

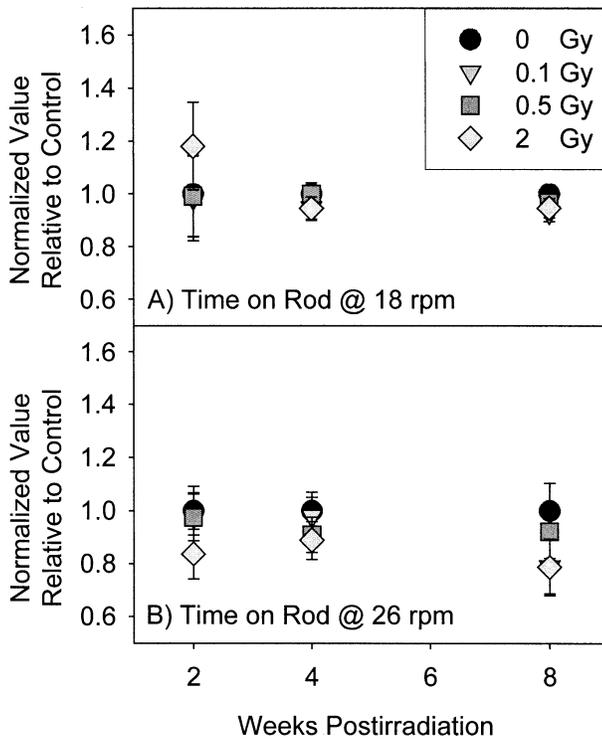
There were no significant main effects of dose, day or bin on latency to peak (data not presented). However, there was an effect of week [ $F(2,104), \epsilon = 0.84, P < 0.001$ ]. Similarly, with the exception of a week  $\times$  bin interaction [ $F(8,416) = 2.35, \epsilon = 0.78, P < 0.05$ ], there were no significant interactions for any variable on latency.

## 3. Startle probability

There were no significant main effects or interactions for any parameter on the probability of a startle response (data not presented).

## DISCUSSION

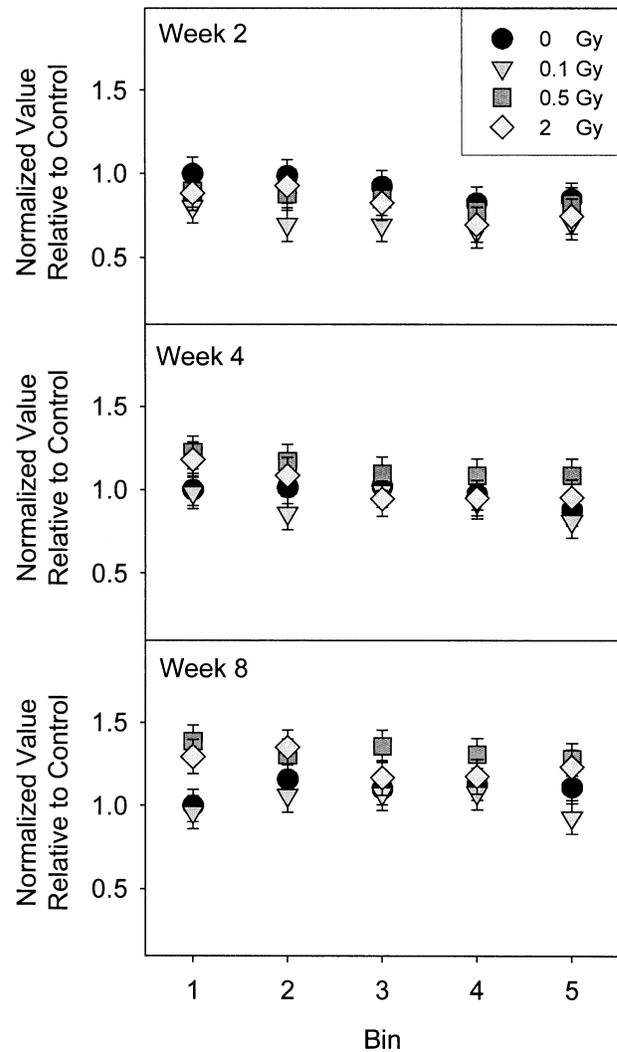
Three behavior paradigms targeting a variety of brain-behavior interactions were chosen to study the effects of



**FIG. 3.** Effects of radiation on rotorod activity. Prior to normalization, time parameters were measured in seconds. All data were normalized to 0-Gy controls on each testing day. Each data point was divided by the average of the data from 0-Gy controls. Values are means  $\pm$  SEM. There were no significant effects of radiation on the time spent on the rotorod at either 18 or 26 rpm. The early variability in the time spent on the rod at 18 rpm was likely due to a lack of animal exposure on the hardware and subsequent habituation.

low-dose whole-body iron-particle irradiation: open-field activity, rotorod and habituation of acoustic startle. These tests require single, relatively short test sessions and can be administered repeatedly to assess the time course of any effects. To minimize any day-to-day variability, all data were normalized to the controls on each day. The primary result of this study is that radiation did not produce any measurable change in performance on any of these behavioral assessments during the 2–8-week window of observation.

There were no significant effects of iron-particle irradiation on spontaneous locomotor or exploratory activities (as modeled by the open field). This is consistent with our previous results with protons. When testing began immediately after doses of up to 4 Gy protons, mice did not habituate to repeated exposures to a novel object in the open field. However, a 2-week delay between irradiation and the initiation of testing was enough to resolve any radiation effects on open-field activity (2). These results are also consistent with the lack of a radiation effect reported for male C57BL/6 mice 30–90 days after similar doses of whole-body iron-particle irradiation (45). However, because we did not initiate testing immediately after irradiation, we



**FIG. 4.** Effects of radiation on startle activity. All data were normalized to 0-Gy controls on each testing day. Each data point was divided by the average of all data points collected from control animals during the first bin of testing. Values are means  $\pm$  SEM. There were no significant main effects of or consistent interactions with radiation on startle activity.

cannot rule out transient acute changes that may have occurred immediately after treatment.

The rotorod behavioral model allows the characterization of radiation-induced changes in on balance, coordination and motor control (40). Unlike previously reported work with protons, where exposure to up to 4 Gy led to a decrease in time spent on the rotorod (2), there does not appear to be any effect of low-dose iron-particle radiation on rotorod activity. After a 2-week delay between treatment and testing, animals performed equally well, regardless of radiation dose, at both angular velocities.

The lack of a response with the higher-LET iron particles would not be inconsistent with the “reverse LET effect” reported previously for this model (2, 22). The lack of an effect may be related to particle fluence. If one assumes that the cell nucleus is the primary target of radiation, and that the distribution of particles is Poissonian, one can cal-

culate the proportion of nuclei will be “hit” (or traversed) by particles of various radiation types. The average diameter of a nucleus in the rat cerebellum is  $5.5 \mu\text{m}$  (46). The lowest dose of radiation reported here was  $0.1 \text{ Gy } ^{56}\text{Fe}^{26}$  particles, which corresponds to a fluence of  $4.22 \times 10^5$  particles/cm<sup>2</sup>. At this fluence, only about 9.5% of the nuclei will be hit by one or more iron particles. On average, each nucleus will be hit by 0.1 particles. However, with an equal dose of protons, there is a fluence of  $1.25\text{--}1.56 \times 10^8$  particles/cm<sup>2</sup>, and 100% of the nuclei will be hit by an average of 30–37 protons. In the visual cortex, the average nuclear diameter is  $10 \mu\text{m}$  (47). For iron particles, 28% of the nuclei will be hit by one or more particles with an average of 0.331 hits/nuclei. For protons, 100% of the nuclei will be hit by an average of 122–98 particles.

When one considers that rotorod behavior involves the cerebellum, one of the most tightly packed regions of the brain in terms of neurons, the possibility that particle fluence may play a greater role than total radiation dose or LET becomes apparent. Much of the work in this area has been reported in the rat model. In the rat, there do not appear to be any differences across the various areas of the brain in terms of synaptic density:  $1\text{--}2 \times 10^9$  synapses/mm<sup>3</sup> in the cerebellum (48), visual cortex (47, 49), and somatosensory cortex (49). However, there is considerable disparity in neural density. Cerebellar granule cell densities alone are  $1\text{--}3 \times 10^6$  cells/mm<sup>3</sup> (46, 48, 50). Purkinje cell densities add an additional  $2\text{--}10 \times 10^3$  cells/mm<sup>3</sup> to this figure (48, 50, 51). In sharp contrast, total neuronal densities are  $50 \times 10^3$  cells/mm<sup>3</sup> in the somatosensory cortex (49),  $70\text{--}90 \times 10^3$  cells/mm<sup>3</sup> in the frontal and visual cortex (49, 52), and  $110 \times 10^3$  cells/mm<sup>3</sup> in the occipital lobe (52). Fewer data have been reported for the mouse model. Cerebellar granule cell densities have been reported to be  $\sim 1.5 \times 10^6$  cells/mm<sup>3</sup> (53) compared to total neuronal cell densities of  $7\text{--}12 \times 10^4$  cells/mm<sup>3</sup> in the cerebral cortex (54). These differences of at least an order of magnitude in neuronal densities suggest at least the possibility that fluence may play a role. However, until similar doses or fluences of radiations with differing LETs can be assessed simultaneously, these effects cannot be confirmed.

The high degree of variability seen in week 2 may be due to a lack of animal training. Because these animals were shipped directly to the Brookhaven Animal Care Facility and then returned to Loma Linda for testing, there was no opportunity to “train” the animals prior to irradiation. In subsequent weeks, the animals likely habituated to the testing protocols and equipment, leading to a decrease in variability.

Interestingly, there is at least one report of an effect 30 days after 2 Gy whole-body iron-particle irradiation using an accelerating rotorod model (45), suggesting that radiation may influence adaptive rather than uniformly repetitive motor responses.

The effects of radiation on startle activity were subtle at best. There were no significant main effects of dose on

amplitude, latency or frequency. Furthermore, there was only one dose-related interaction that was unlikely to be behaviorally meaningful. A lack of a response would be consistent with our previous results with protons. In that study, effects on the startle response were limited to the first week after irradiation, where treatment reduced the level of habituation across bins. However, there did not appear to be any influence of radiation on startle behavior when there was a 2-week delay between irradiation and behavioral assessment (2).

The lack of radiation effects in these behavioral models may offer reassurance to NASA mission designers. However, though these results are encouraging, it is important to recognize that the null hypothesis cannot be proven, and this investigation did not have the power to reliably detect small effects. Nevertheless, these results suggest that there may be negligible effects of HZE-particle radiation on many behaviors during a 2–8-week period after irradiation. Though one cannot exclude the possibility that changes may occur after the 8-week testing period reported here, our data are consistent with our previous proton data (2), as well as the reports with other behavioral models, suggesting that the behavioral effects of radiation tend to be acute and transient (22). Effects of proton radiation on open-field exploration, rotorod performance and acoustic startle habituation were observed during the first week after exposure but resolved within 14 days (2). Conditioning in a taste aversion paradigm is disrupted in rats within 3 days of exposure to proton or  $^{56}\text{Fe}$ -particle radiation at doses of less than 3 Gy (4, 34). Within a day of exposure to 5 Gy  $\gamma$  or X radiation, disorientation (55) and impaired learning and memory (56, 57) have been reported. Similarly, within hours after radiotherapy, patient cognitive function is sometimes degraded (58, 59). Following from this evidence of the acute and transient nature of radiation effects on behavior, it may be possible (or even advisable) to avoid mission-critical operations immediately after an SPE.

The potentially transient nature of radiation-induced changes in behavior may reflect a time-dependent response of various cell types. While neurons tend to be fairly radioresistant, radiation likely has an influence on the cell populations surrounding the neurons (e.g. glia and astrocytes). This is particularly pertinent since ionizing radiation has been shown to induce inflammation in the brain, involving the acute activation of glial cells, and significant increases in proinflammatory cytokine mRNA expression (60, 61). After irradiation of the brain with 7–25 Gy photons, the expression of IL1B mRNA in the brain is dependent on time. Levels increase rapidly (i.e. 4 h), return to baseline by 24 h (61), and consistently remain at baseline levels for up to 6 months after irradiation (62). In our own studies, we found significant increases in IL1B using an ELISA 10 days after 3 Gy whole-body  $\gamma$  irradiation. There was only a trend for an increase in IL1B expression 35–36 days after 5 Gy  $^{56}\text{Fe}^{26+}$ -particle head-only irradiation compared to age-matched controls (manuscripts in preparation).

This pro-inflammatory cytokine has been shown to decrease the peak magnitude of GABA-induced currents in whole-cell voltage-clamped hippocampal neurons (63), inhibit long-term potentiation in the hippocampus (64), and reduce inhibitory currents, possibly leading to increased synaptic efficacy. While a link between radiation-induced inflammation in the brain and changes in behavior has not yet been made, the temporal correlations alone suggest possible mechanistic relationships.

Another explanation for the lack of a significant long-term radiation effect on these models might involve the sensitivity of our models. Reports suggest that, when animals are tested on multiple behavioral models, exposure to the first test can influence the results of a second (65). Because all of our animals were tested with all three behavioral models, the effects of radiation may have been masked.

Similarly, radiation effects may have been masked due to the relatively small sample sizes used in this study. One can get around this limitation by examining the "treatment" effects. When the radiation groups were collapsed into two conditions, with and without exposure, there were still no significant main effects of exposure (data not presented). However, there were a limited number of interesting interactions. There were significant week  $\times$  day  $\times$  exposure interactions for both time ( $P < 0.05$ ), and time active ( $P < 0.05$ ), in the disc end of the of the open field. In both cases, there were exposure-associated increases in activity seen on day 2 of week 8. There were also significant day  $\times$  exposure interactions, which did not interact with week, in the time ( $P < 0.05$ ) and time active ( $P < 0.05$ ) in the center of the open field. There was an exposure-associated decrease in activity seen on day 2. There was also a significant week  $\times$  exposure interaction in startle frequency reflecting an exposure-associated increase in startle frequency in week 8. Although the importance of these interactions is unclear, they may reflect a delayed onset of radiation-induced behaviorally relevant effects.

Finally, one must also consider potential synergism with adaptation to the microgravity environment or other stress-related aspects of spaceflight (e.g. isolation, interpersonal stress on extended missions) (8, 66, 67). If there is an effect of radiation on adaptive motor control, adaptation to changes in the inertial environment may also be subject to influence by radiation.

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