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Behavioral consequences of radiation exposure to simulated space radiation in the C57BL/6 mouse: Open field, rotorod, and acoustic startle

MICHAEL J. PECAUT, PAUL HAERICH, CARA N. ZUCCARELLI, ANNA L. SMITH,
ERIC D. ZENDEJAS, and GREGORY A. NELSON
Loma Linda University, Loma Linda, California

Two experiments were carried out to investigate the consequences of exposure to proton radiation, such as might occur for astronauts during space flight. C57BL/6 mice were exposed, either with or without 15-g/cm^2 aluminum shielding, to 0-, 3-, or 4-Gy proton irradiation mimicking features of a solar particle event. Irradiation produced transient direct deficits in open-field exploratory behavior and acoustic startle habituation. Rotorod performance at 18 rpm was impaired by exposure to proton radiation and was impaired at 26 rpm, but only for mice irradiated with shielding and at the 4-Gy dose. Long-term (>2 weeks) indirect deficits in open-field activity appeared as a result of impaired experiential encoding immediately following exposure. A 2-week recovery prior to testing decreased most of the direct effects of exposure, with only rotorod performance at 26 rpm being impaired. These results suggest that the performance deficits may have been mediated by radiation damage to hippocampal, cerebellar, and possibly, forebrain dopaminergic function.

Astronauts and mission designers of future long-term spaceflight missions must include potentially dangerous ionizing radiation in any complete risk assessment. The effects of radiation on central nervous system function and behavior must be considered. This is particularly true when radiation may act synergistically with psychological factors already known to be inherent to long-term spaceflight (i.e., general mission-related stress, neurovestibular influences, etc.; Committee on Space Biology and Medicine, 1998). Astronaut motor/spatial behavior and the response to visual cues in three-dimensional space have proven to be altered by the microgravity environment, going through several adaptive stages, depending on the duration of the mission (Tafforin & Campan, 1994; Tafforin, Thon, Guell, & Campan, 1989). Although many of these modifications in behavior appear to be natural adaptations to the microgravity environment, significant exaggeration or impairment of adaptation during transition phases owing to radiation stress could prove detrimental to astronaut safety, particularly if crewmembers are unable to navigate and land vehicles, communicate threats to supporting ground crews, or perform medical procedures.

To date, there has been little information acquired on the effects of low-dose/low-dose-rate ionizing radiation such as would be expected in space flight (Todd, Pecaut, & Fleshner, 1999). This is especially true for the effects of charged particle radiation on behavior. The construction of the International Space Station and planning for future exploration missions (e.g., to Mars) have served to emphasize the importance of a scientifically based risk assessment system to evaluate the unavoidable exposure to space radiation environments (Committee on Space Biology and Medicine, 1998; Fry et al., 1989; Setlow et al., 1996; Swenberg, Horneck, & Stassinopoulos, 1991). Moreover, the use of charged particle radiation to treat cancers and nonmalignant diseases of the central nervous system represents an additional need for an understanding of the behavioral consequences of radiation exposure (Blakely & Kronenberg, 1998; Tofilon & Fike, 2000).

The Van Allen radiation belts protect the Earth and low earth-orbiting spacecraft, such as the space shuttle, from much of the space radiation environment. However, once outside this protective geomagnetic shielding, astronauts will be exposed to two sources of radiation: solar particle events (SPEs) and galactic cosmic rays (GCRs). Roughly 85% of this radiation is made up of high-energy protons. The remainder consists of mostly helium (~13%) and high Z energy particles (~1%; Committee on Space Biology and Medicine, 1998; Fry et al., 1989; Setlow et al., 1996; Swenberg et al., 1991). Current mission designs for the human exploration of Mars suggest a possible du-

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ration of 2–3 years, 8–12 months of which will be spent in the space environment. Because SPEs are relatively unpredictable and produce a transient elevation in the rate of exposure (when compared with GCRs), these events are likely to pose the greatest radiation-based threat to astronaut safety. Data from three SPEs (November 1956, August 1972, and October 1989) have allowed mission designers to model worst-case scenarios. From such models, it has been estimated that astronauts may be exposed to total doses of 3 Gy or higher, depending on shielding conditions (Fry et al., 1989; Letaw, Silberberg, & Tsao, 1989; Moore, 1992; Parsons & Townsend, 2000; Setlow et al., 1996; Simonsen, Cucinotta, Atwell, & Nealy, 1993; Swenberg et al., 1991; Townsend, Cucinotta, Shinn, & Wilson, 1992; Townsend, Shinn, & Wilson, 1991).

Most radiation-induced changes in behavior appear quickly and are often transient (Bogo, 1984). For example, conditioning in a taste aversion paradigm is disrupted in rats within 3 days of exposure to proton or ^{56}Fe radiation at doses of less than 3 Gy (Rabin, Hunt, & Joseph, 1989; Rabin, Hunt, Joseph, Dalton, & Kandasamy, 1991). Fatigue and weakness occur in humans after doses of X rays as low as 1 Gy (Anno, Baum, Withers, & Young, 1989). Disorientation (Anno et al., 1989), and impaired learning and memory (Fields, 1957; Urmer & Brown, 1960) have been shown to occur within a day of exposure to doses of 5-Gy γ- or X-irradiation. In patients undergoing clinical radiotherapy, cognitive function is sometimes degraded within hours of being treated with 10 Gy or less (Hochberg & Slotnick, 1980; Maire, Coudin, Guerin, & Caudry, 1987; Taphoorn et al., 1992).

Long-term neurophysiological and behavioral effects after radiation treatment have also been reported. For example, there is evidence of long-term glial degeneration and vascular disruption, which appear only after weeks to months following exposure (Tofilon & Fike, 2000; van der Kogel, 1986). Children who underwent the relatively low dose cranial radiotherapy for acute lymphoblastic leukemia consistently scored lower on the Wechsler Intelligence Scale for Children (WISC), when compared with children with solid tumors treated with similar extracranial doses. These differences appeared up to a year after treatments (Ladavas, Missiroli, Rosito, Serra, & Vecchi, 1985). Similarly, mean doses of 1.3 Gy have been shown to produce long-term impairment of the mental function of children who received X-ray treatment for *tinea capitis*, a fungal scalp infection. When evaluated almost 20 years later, these patients scored lower on scholastic aptitude, WISC, and other psychological tests and had an increased risk for mental hospital admissions (Danoff, Cowchock, Marquette, Mulgrew, & Kramer, 1982; Ron, Modan, Floro, Harkedar, & Gurewitz, 1982; Shore, Albert, & Pasternack, 1976).

Most of the literature regarding low-dose radiation effects on behavior has been focused on γ- or X-radiation. Disruptions in behavior have been shown to occur in several animal models after exposure to X- (Bogo, 1984), electron- (Bogo, 1984; Hunt, 1983; Mickley, 1980; Mickley & Teitelbaum, 1973), γ- (Bruner, Bogo, & Jones, 1975; Chaput & Wise, 1970; Hunt, 1983), neutron- (Bogo, 1984), and mixed fission-spectrum neutron/γ-irradiation (Casarett & Comar, 1973; Franz, 1985). However, there has been an almost complete absence of data describing the effects of protons. Furthermore, there is evidence to suggest that results from behavior studies using γ-radiation cannot be used to extrapolate the effects of protons (Rabin et al., 1989; Rabin et al., 1991). Therefore, we conducted an experiment to investigate the effects, both short and long term, of low-dose proton radiation on a set of widely used behavioral tests. Given the scarcity of information from previous work, our primary goal in this effort was to determine whether any behavioral effect associated with proton radiation could be measured with these tests and, if observed, to assess the magnitude of the effect. In addition, we were interested in the time course of the expression of any measurable effects associated with irradiation.

Mindful of the guidelines for behavioral neurotoxicology testing set forth by the U.S. Environmental Protection Agency, the National Toxicology Program, and others (Office of Prevention, 1998a, 1998b; Pryor, Uyeno, Tilson, & Mitchell, 1983; Reiter, 1978; Reiter & MacPhail, 1979; Tilson, 1987, 1990; Tilson & Mitchell, 1984; Tilson & Moser, 1992), three behavior paradigms targeting a variety of brain–behavior interactions were chosen for the pilot studies presented here: open-field activity, rotorod, and habituation of acoustic startle. Unlike the radial arm maze or most conditioning tasks, each of these tests requires a single, relatively short test session to be administered. Each test can be administered repeatedly across a series of weeks in order to assess the time course of any effects.

The open-field test was chosen to assess locomotor and spontaneous exploratory activity (Cabib, Algeri, Perego, & Puglisi-Allegra, 1990; Crawley et al., 1997; Hall, 1934, 1936; Lijam et al., 1997; Walsh & Cummins, 1976; Wilcox & Broadhurst, 1967). The rotorod task was chosen to examine the effects of radiation on balance, coordination, and motor control (Buccafusco, 2000). Finally, acoustic startle was chosen to assess changes in the function of a brainstem-level reflex. Moreover collecting data on the startle response was expected to be of value because this measure and its modification (e.g., prepulse inhibition, habituation) has become one of the most common behavioral measures during the past 2 decades. Together, these tests provide a framework for the initial assessment of the effects of charged particle radiation exposures relevant to clinical and spaceflight environments.

To simulate features of a space radiation exposure on the scale of an SPE, mice were irradiated with relatively low doses of mono-energetic (250 MeV) protons (3 or 4 Gy). Current models for space radiation exposure incorporate aluminum shielding at varying degrees of thickness for the purpose of providing some protection

to the astronauts from the radiation environment (Badhwar, Cucinotta, & O'Neill, 1994; Fry et al., 1989; Letaw et al., 1989; Moore, 1992; Setlow et al., 1996; Simonsen et al., 1993; Swenberg et al., 1991; Townsend et al., 1992; Townsend et al., 1991). To explore the effects of shielding, 15 g/cm^2 aluminum shielding was used to modify the energy spectrum of particles during the irradiation of some animals.

In addition, because many of the reported effects of radiation occur immediately and are transient, two parallel experiments were conducted. In one, Experiment 1, mice were tested immediately following irradiation and, again, after 1, 2, 4, 8, and 12 weeks. In Experiment 2, the mice were allowed to recover and were first tested at 2 weeks following irradiation and then again at 4, 8, and 12 weeks. The rationale for these two experiments was to separate the immediate from the long-term effects of radiation exposure.

METHOD

Animals

Female C57BL/6 mice ($N = 90$) were purchased from Charles River Breeding Laboratories (Hollister, CA) at 8 weeks of age and were allowed to acclimate and recuperate from shipping stresses for 2 weeks. Animals were maintained in large shoebox cages (7–8 mice/cage) on a 12:12-h light:dark cycle (lights on at 0700 h) at 30%–40% humidity and 65°–70°F. Behavior testing was performed during the light period of the cycle (start time, 0900 h).

Approximately 1 week prior to irradiation, preprogrammed identification/temperature transponders (BioMedic Data Systems, Maywood, NJ) were inserted subcutaneously in each mouse, and their tails were tattooed (AIMS, Budd Lake, NJ) with an identification number. The mice were mildly sedated using 100% CO_2 for the transponder insertion procedure. At behavioral testing, the experimenters were blind to the radiation condition of the mice.

Following the insertion of the transponders and the tattooing, each mouse was assigned randomly to one of five groups and to one of the two experiments (Experiment 1, $n = 6$/group; Experiment 2, $n = 12$/group). Behavioral testing was performed twice per week, on two consecutive days. Two to 7 days prior to irradiation, the animals were exposed to the environment of the startle and rotorod tasks. On each of the 2 days immediately preceding irradiation, each animal was placed in the startle chamber for 10 min with 70-dB background noise. Each animal was then placed on the rotorod at an angular velocity of 18 rpm, until they were able to stay on the rotating rod for 5 min. In Experiment 1, the animals were tested on Weeks 0, 1, 2, 4, 8, and 12. The designation *Week 0* is used for the first set of tests, which were performed within 24 h of irradiation. In Experiment 2, the animals were tested on Weeks 2, 4, 8, and 12 postirradiation. All the animals participated in each of the three behavioral tests, which were performed in order of increasing potential for stress: open field, rotorod, and acoustic startle.

Whole-Body Irradiation

The mice were irradiated at 10 weeks ± 3 days of age. In each of the two experiments, there were five groups: controls and four radiation treatment conditions. The control animals were housed and treated identically to the irradiated groups but were not exposed to radiation. The remaining four groups were exposed to either 3 or 4 Gy of total physical dose. One group at each radiation dose was irradiated with an unmodulated proton beam, whereas the other was exposed behind 15-g/cm^2 aluminum shielding to modify the radiation spectrum (without changing the total dose).

All irradiations were conducted at Loma Linda University Medical Center's Proton Treatment Center (Coutrakon et al., 1997). Each mouse was placed into a ventilated rectangular plastic box ($3 \times 3 \times 8.5$ cm). The boxes were arranged in the radiation target area so that eight boxes were irradiated simultaneously with a single dose of protons at a dose rate of 75–95 cGy/min (in some runs, phantoms were included in otherwise empty boxes). The mice were irradiated in the entrance region of the proton beam (250 MeV) by placing the surface of the boxes behind a 400×400 mm^2 polystyrene phantom at a water-equivalent depth of 26.4 mm. The surface of the polystyrene phantom was located at the isocenter of the proton beam. Sham-exposed control animals were placed in exposure boxes and remained at the target area for the same amount of time as exposed animals but were not irradiated. Calibration was performed with a Markus parallel plate ionization chamber, traceable to the National Institute of Standards and Technology, at depths corresponding to the center of the mice.

Open Field

The tests were performed using the SDI Open Field System (San Diego Instruments, San Diego, CA). The open field was a 20×40 cm Lexan shoebox cage placed in a double frame of LED photosensors, one above the other. The lower set, 1 cm above the cage floor, consisted of a 4×8 LED-sensor grid (4-cm interbeam distance) and was used to monitor overall locomotor activity. The upper set, 3 cm above the cage floor, consisted of 4 LED sensor rows (4 cm interbeam distance, arrange along the 20-cm width of the cage) and was used to monitor rearing activity. The cage and frame system was enclosed on four sides with neutral-colored walls to block external visual cues. The cage was covered with a translucent, white, plastic filter top to provide consistent lighting conditions, and the cages were thoroughly cleaned with quatricide after each mouse was tested. Four identical open-field systems were monitored simultaneously by computer.

For each open-field test session, the animals were placed in the center of the field, 1 animal per cage, and activity was monitored for 5 min. After 5 min, a flat, round object (a 4-cm-diameter rubber disk with a nubby texture) was placed in the front half of the cage (by hand, with laboratory gloves to minimize identifying odors), and activity was again monitored for 5 min. A freshly cleaned disk was placed in the same location within the field for each of the test sessions.

The computer monitored beam breaks every 0.1 sec, recording both location and rearing activity simultaneously. The following parameters were evaluated: total number of beam breaks; beam breaks in the periphery, center, and disk ends of the cage; time spent in the periphery and disk ends of the cage; amount of rearing activity; and time spent rearing. The periphery included a 4-cm-wide strip around the base of the cage, as monitored by the outermost columns and rows of the 4×8 LED sensor grid. The disk region was defined as a 16×16 cm area centered on the disk (exactly one half of the 4×8 LED sensor grid).

Rotorod

The rotorod tests were performed using the SDI Rotor-Rod System (San Diego Instruments, San Diego, CA). The system consisted of four rotorod chambers. Each chamber included a 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, breaking a photobeam that automatically recorded the trial duration. If the animal stayed on the rod for ≥150 sec, the trial was terminated, and a duration of 150 was recorded. The test session consisted of six consecutive trials. The trials with the longest and shortest durations for each test session for each animal were discarded, leaving four trials per session. All the animals were given one training session at 18 rpm with the rotorod 1 week prior to irradiation. Following irradiation, the ani-

mals received two test sessions per week, at each rotation speed, on consecutive days. On the 1st test day of the week, the animals were tested at 18 rpm. On the second day, the animals were tested at 26 rpm.

Acoustic Startle

The acoustic startle tests were performed using the SDI SR-Lab Startle Reflex System (San Diego Instruments, San Diego, CA) including four parallel chambers. A constant white noise background was set at 70 dB. The startle response was elicited with a 50-msec, 120-dB burst of white noise with near-instantaneous onset and offset. The startle response, measured via an accelerometer attached to the chamber floor, was measured as the maximum displacement during the 100 msec following onset of the startle pulse. Each test session included 50 consecutive startle trials with a 10-sec intertrial interval. Each animal was placed in a startle chamber for two 10-min no-startle acclimation periods on each of the 2 days preceding irradiation (or sham irradiation for control animals).

Design and Analysis

The general design for these experiments included repeated factors of *week, session* (two sessions per week), and, for the open-field data, *object* (object present or no object). There were two between-group factors: radiation *dose* (0, 3, and 4 Gy) and *shielding* (with or without shielding). For the analyses, the two between-group factors were sometimes combined into a single, five-level factor of *radiation group*. 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

Experiment 1: Testing Begins Immediately With Time Points at Postirradiation Weeks 0, 1, 2, 4, 8, and 12

Open Field

Ambulation. The data for open-field activity are presented in Figure 1. Although inspection of the figure suggests that animals in the 4-Gy exposure group were less active than those in the other groups, the statistical analysis indicated that similar levels of locomotor activity were displayed across the five radiation groups [$F(4,25) = 1.46$, $p > .10$]. The sham-irradiated mice increased their overall ambulation in each of the first 3 weeks from about 111 beam breaks per 5-min observation period to an asymptotic level of about 155 beam breaks, which was maintained throughout the experiment (Figure 1A). During the first 3 weeks' testing, the activity levels of the control mice were maintained following the presentation of the object. However, beginning with Week 4, object presentation produced a decrease in ambulation. The magnitude of this decrease grew from Week 4 through Week 12 (Figure 1B). In exposed mice, locomotor activity was similar to that in sham-exposed control mice for the 5-min period prior to the presentation of the object. However, unlike for the control animals, presentation of the object was associated with a decrease in ambulation beginning at Week 0. Moreover, in contrast to the decline in object-associated activity observed in the control group from Weeks 4 to 12, the locomotor activity of exposed mice did not decrease (the 3-Gy group) or actually increased across weeks (the 4-Gy group). These differences among the groups in week-to-week activity following presentation of the object were confirmed by a significant three-way interaction of radiation group, object, and week [$F(20,125) = 183$, $\varepsilon = 0.83$, $p < .05$]. Analyses of simple effects revealed a significant object × week interaction for the 0- and 4-Gy groups (Fs > 2.57, εs > 0.80, ps < .04; but for the 3-Gy group, $p > .10$). Additional analyses examining the number of beam breaks and the time spent in the center of the field, in the periphery, and in the vicinity of the object revealed a similar pattern of results (data not shown). Object presentation was associated with a decrease in beam breaks and in time spent in the center of the field and with increases for each of the measures in the object area [Fs(1,25) > 60.45, ps < .001]. The three-way interaction was observed in these measures at least at marginally reliable levels (ps < .06).

None of the main effects or interactions involving the shielding factor was significant.

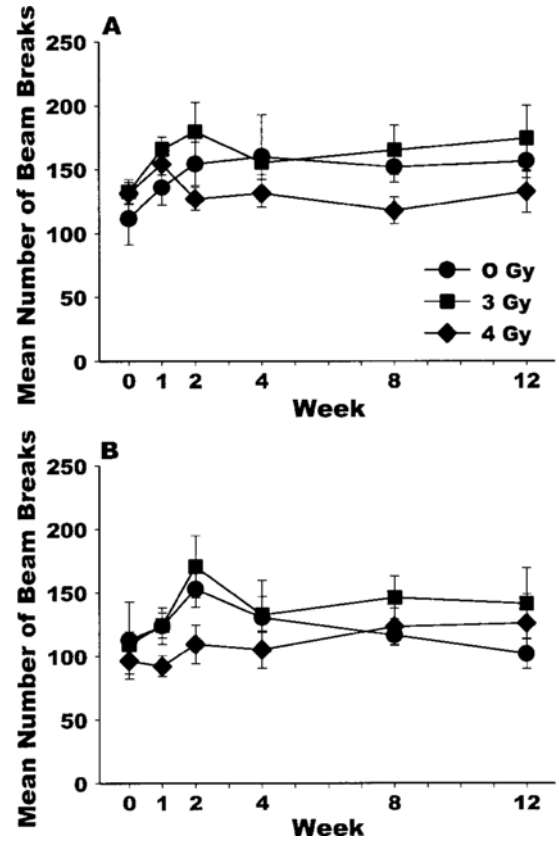

Figure 1. The numbers of beam breaks for each of the three radiation dose levels are depicted across Weeks 0–12 postirradiation (Experiment 1). The error bars represent standard errors of the mean. (A) Ambulatory activity in the first 5-min period prior to the presentation of the object. (B) Ambulatory activity in the second 5-min period after the presentation of the object.

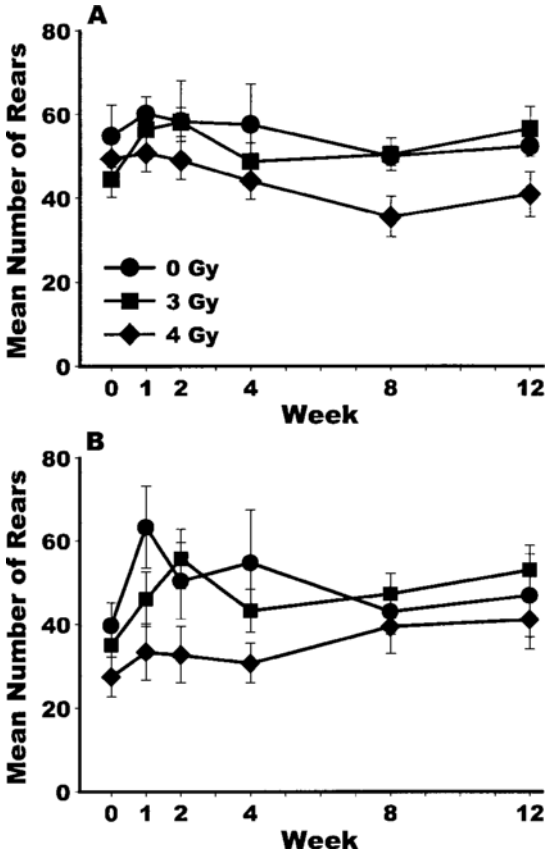

Figure 2. The number of rears for each of the three radiation dose levels are depicted across Weeks 0–12 postirradiation (Experiment 1). The error bars represent standard errors of the mean. (A) Rearing activity in the first 5-min period prior to the presentation of the object. (B) Rearing activity in the second 5-min period after the presentation of the object.

Rearing. The data for rearing activity are presented in Figure 2. Although the 4-Gy animals appear to have displayed less rearing activity than did the other groups, the main effect of radiation group was not significant in the statistical analysis [$F(4,25) = 1.61, p > .10$]. Neither the main effect nor the interactions involving shielding were significant. However, a significant main effect of week was observed [$F(5,125) = 2.84, \varepsilon = 0.84, p < .05$], apparently reflecting the relative increase in rearing frequency at Weeks 1 and 2. As with ambulation, the strongest influence on rearing activity was that of presentation of the object, which resulted in a significant decrease in rearing activity [$F(1,25) = 14.75, p < .001$]. The effect of object presentation was greatest in the initial observation sessions, producing a significant object × week interaction [$F(5,125) = 3.36, p < .01$].

Rotorod

Data for rotorod performance are presented in Figure 3. As would be expected, mice maintained balance on the rotorod for a longer time in 18-rpm trials (Figure 3A), as compared with 26-rpm trials [Figure 3B; $F(1,25) = 120.32$, $p < .001$]. Although the mice performed well at 18 rpm, remaining on the rod for more than 2 min on average, a relative decrease in performance was observed in those mice exposed to proton radiation [$F(2,27) = 3.31, p = .05$]. Inspection of Figure 3A, supported by the lack of a significant effect of week, suggests that rotorod performance was impaired immediately following exposure and never recovered. No significant effect of shielding was found.

At 26 rpm, the mean time spent on the rod decreased across weeks for all the groups of mice [$F(5,125) = 4.18$, $\varepsilon = 0.81, p < .005$]. Moreover, although there was no significant interaction, inspection of Figure 3B suggests that much of the performance decrement across weeks was driven by those mice irradiated without shielding and those mice receiving 4 Gy. In fact, the 4-Gy unshielded group displayed the shortest rotorod balance times. However, statistical analyses supported the protective effect of shielding on rotorod performance only at 26 rpm [$F(2,27) = 3.33, p = .05$]; that is, there was no significant effect of or interaction with dose.

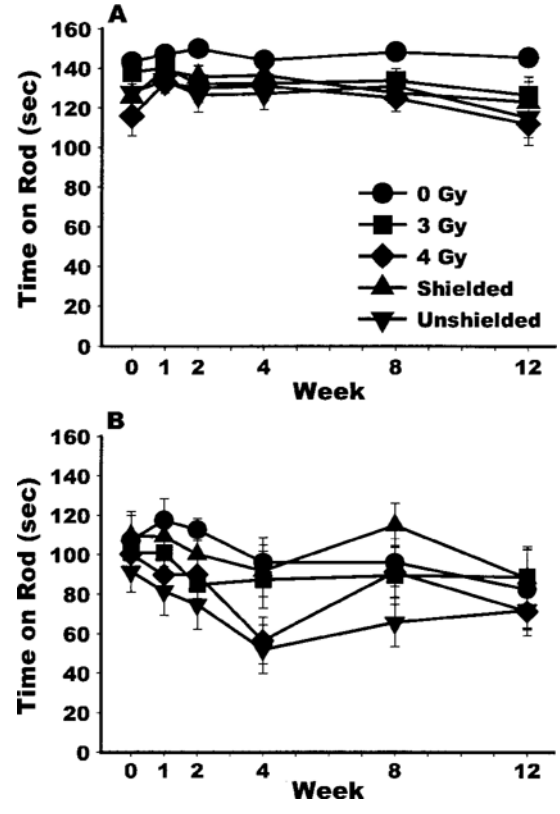

Figure 3. Latency to fall from the rotorod (in seconds) is depicted across Weeks 0–12 postirradiation (Experiment 1). The data depicted for each dose group include all the animals exposed at that dose, both with shielding and without shielding. Similarly, the data depicted for each shielding condition include all the animals from both dose levels. The error bars represent standard errors of the mean. (A) The latency to fall from the rotorod at 18 rpm. (B) The latency to fall from the rotorod at 26 rpm.

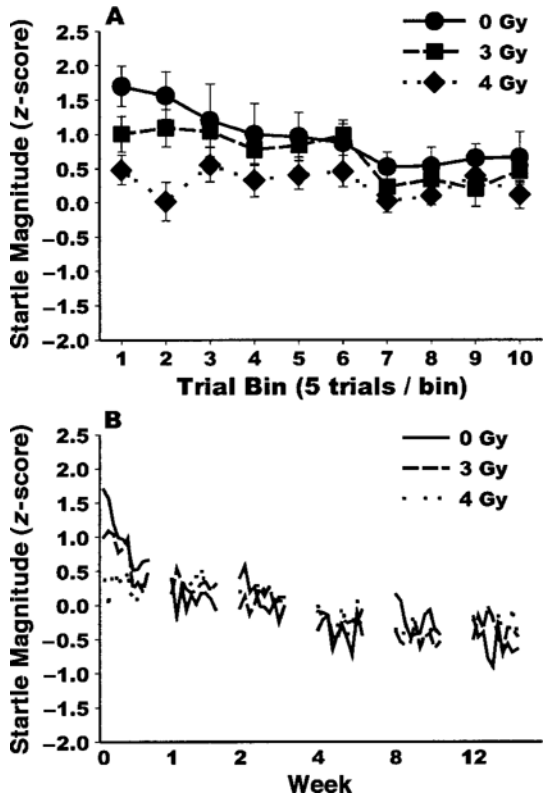

Figure 4. The magnitude of the acoustic startle response (*z*-score transformed) for Experiment 1 is depicted. Acoustic startle response magnitude across 50 trials collapsed into bins of 5 trials each. (A) The startle magnitude for each of the three radiation dose levels is depicted across the 10 trial bins for the Week 0 test session. The error bars represent standard errors of the mean. (B) Startle magnitude for each of the three radiation dose levels across Weeks 0–12. Note that the data represented in panel A are from Week 0 and that the 0-, 3-, and 4-Gy dose groups are represented by solid, dashed, and dotted lines, respectively, in both panels.

Acoustic Startle

The data for habituation of acoustic startle are presented in Figure 4. A dose-related reduction in habituation was observed in testing during Week 0 (Figure 4A). The sham-exposed control mice displayed a robust habituation across trial bins, whereas the mice exposed to 4-Gy proton radiation displayed little habituation. The habituation of the 3-Gy-exposed mice was intermediate between the other two groups. An analysis of variance revealed a main effect of dose [$F(2,27) = 4.66$, $p < .025$] and a marginally reliable interaction of dose and linear trend across trial bins [$F(2,27) = 2.68$, $p = .08$]. Parallel analyses exploring the impact of shielding yielded no effect ($F < 1$).

Figure 4B presents the startle response data for Weeks 0–12. Short-term within-session habituation was observed at each of the five subsequent test sessions [$F(9,225) = 4.06$, $\varepsilon = 0.69$, $p = .001$]. In addition, long-term habituation was observed across weeks [$F(5,125) = 20.42$, $\varepsilon = 0.65$, $p = .03$]. The short- and long-term habituation effects appear to have been similar across dose and shielding groups. For these test sessions, no significant main effects or interactions involving the dose or the shielding factors were observed.

Experiment 2: Testing Beginning at Postirradiation Weeks 2, 4, 8, and 12

Open Field

Ambulation. The data for open-field ambulation in Experiment 2 are presented in Figure 5. As may be seen in Figure 5A, overall locomotor activity in the first 5-min observation declined from Week 2 to Week 4. There was also a decrease in activity each test week associated with the presentation of the object. These were confirmed in the statistical analyses as main effects of week [$F(3,55) = 3.77$, $\varepsilon = 0.621$, $p = .02$] and object [$F(1,55) = 52.79$, $p < .001$]. There were no main effects or interactions involving the radiation group, shielding, or dose factors. Importantly, there was no radiation group

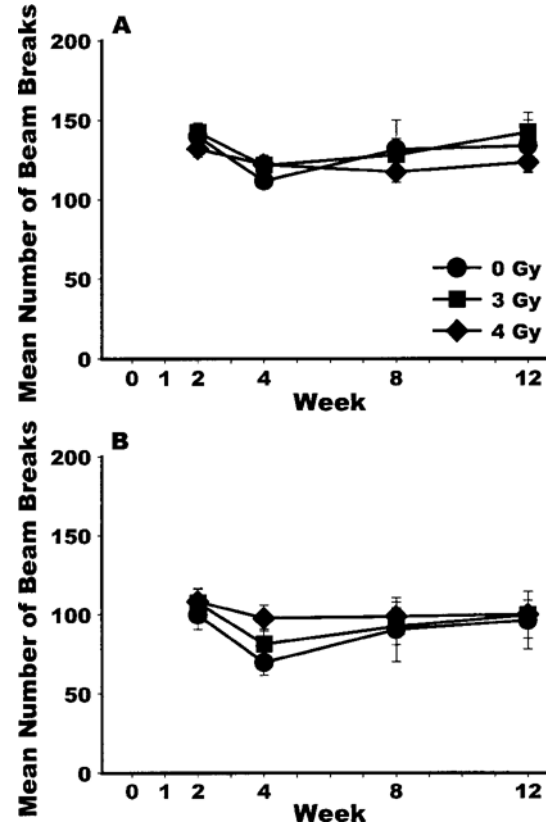

Figure 5. The numbers of beam breaks for each of the three radiation dose levels are depicted across Weeks 2–12 postirradiation (Experiment 2). The error bars represent standard errors of the mean. (A) Ambulatory activity in the first 5-min period prior to the presentation of the object. (B) Ambulatory activity in the second 5-min period after the presentation of the object.

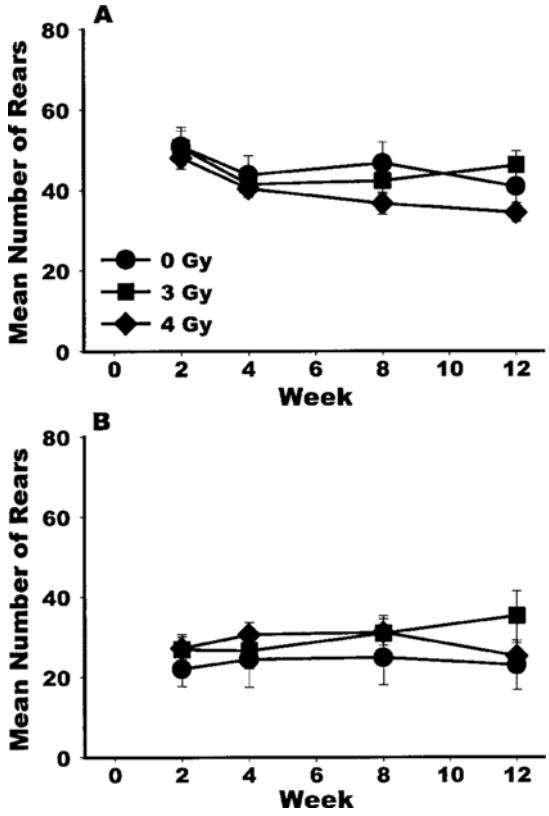

Figure 6. The numbers of rears for each of the three radiation dose levels are depicted across Weeks 2–12 postirradiation (Experiment 2). The error bars represent standard errors of the mean. (A) Rearing activity in the first 5-min period prior to the presentation of the object. (B) Rearing activity in the second 5-min period after the presentation of the object.

× object × week interaction ($F < 1$), suggesting that the critical time point for generating the interaction observed in Experiment 1 involved the first 2 weeks (i.e., Weeks 0 and 1).

Rearing. The data for rearing activity are presented in Figure 6. The number of rears decreased from the first to the second 5-min observation with the presentation of the object. This effect was greatest on the 1st week of testing (Week 2). Statistical analyses revealed a significant effect of object [$F(1,55) = 87.75$, $p < .001$] and an interaction between object and week [$F(3,165) = 11.56$, $\varepsilon = 0.94$, $p < .001$]. There were no main effects or interactions involving the radiation group, shielding, and dose factors.

Rotorod

The data for rotorod performance in Experiment 2 are presented in Figure 7. As in Experiment 1, the mice were able to maintain balance on the rotorod longer at 18 rpm than at 26 rpm [$F(1,55) = 90.28$, $p < .001$]. Analyses revealed no significant main effects or interactions for rotorod performance at 18 rpm. At 26 rpm however, a significant interaction of radiation group and the quadratic trend across weeks [$F(4,55) = 3.23$, $p = .02$], along with a marginally reliable interaction of radiation group and week [$F(12,165) = 1.63$, $\varepsilon = 0.90$, $p = .09$], was observed. Unlike in Experiment 1, we observed no main effect of shielding. Nevertheless, inspection of Figure 7B suggests that the effects of radiation were similar to those observed in Experiment 1, but smaller in magnitude. Exposure to 4-Gy proton radiation and a lack of shielding were associated with a decrease in 26-rpm rotorod performance from Week 2 to Week 4. In fact, the group of mice exposed to 4 Gy without shielding maintained balance on the rotorod for barely over 1 min, on average, at the second test point (Week 4, mean = 69 sec).

Acoustic Startle

As was suggested in Experiment 1, there were no apparent long-term effects of irradiation on acoustic startle. Neither the main effect nor either of the interactions involving radiation group reached significance (ps > .13). Both long-term and short-term habituation continued to

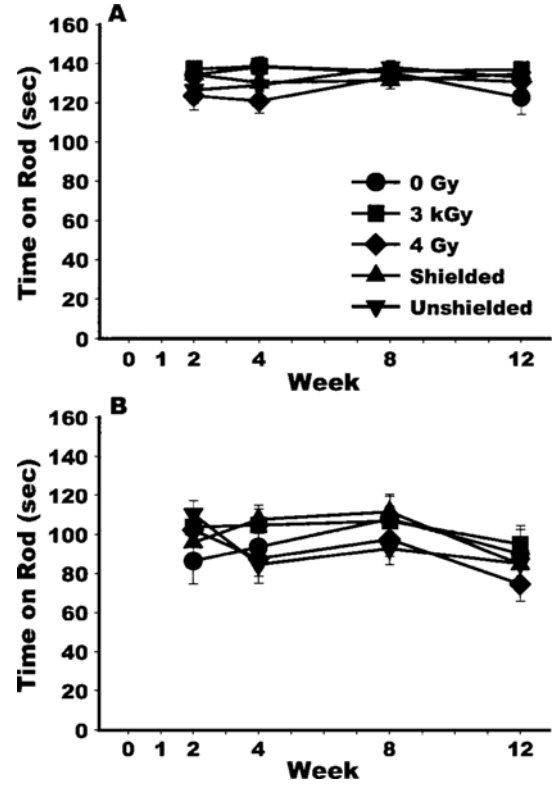

Figure 7. Latency to fall from the rotorod (in seconds) is depicted across Weeks 2–12 postirradiation (Experiment 2). The data depicted for each dose group include all the animals exposed at that dose, both with shielding and without shielding. Similarly, the data depicted for each shielding condition include all the animals from both dose levels. The error bars represent standard errors of the mean. (A) The latency to fall from the rotorod at 18 rpm. (B) The latency to fall from the rotorod at 26 rpm.

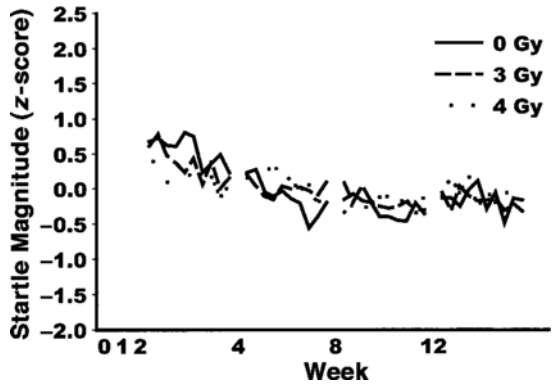

Figure 8. The magnitude of the acoustic startle response (*z*-score transformed) at each of the three radiation dose levels for Experiment 2 is depicted.

be present and were observed as significant main effects of week [$F(3,165) = 17.44$, $\varepsilon = 0.83$, $p < .001$] and trial bin [$F(9,495) = 6.68$, $\varepsilon = 0.80$, $p < .001$], respectively (see Figure 8).

DISCUSSION

A primary goal motivating these experiments was to determine whether proton irradiation produces measurable changes in behavioral performance in open-field activity, rotorod, and habituation of the acoustic startle response. Our results indicate that proton radiation does impair performance on each of these three tasks. In the open field, mice exposed to 3- or 4-Gy proton radiation failed to exhibit the habituation displayed by the sham-exposed controls to the repeated presentation of a nubby-textured disk. Rotorod performance at 18 rpm was impaired in exposed mice; at 26 rpm, mice receiving unshielded radiation showed the greatest impairment across the 12 weeks of testing. Finally, short-term habituation exhibited a dose-related reduction in magnitude, which was observable during the 1st week of startle testing.

The second goal for this study was to document the time course for the appearance of radiation-related effects. The most important result to notice regarding the time course is that after delaying 2 weeks before beginning testing (in Experiment 2), the effects of radiation were negligible. That is, it appears that the direct effects of radiation in these performance measures seem to occur during the day(s) immediately following exposure and that 2 weeks seemed to be sufficient for recovery.

Radiation may produce transient neurophysiological damage that appears and resolves quickly, on the order of days. If a particular task requires the operation of that population of impaired neurons, impaired behavioral performance will be observed. This may be called a direct effect of radiation exposure. In addition, the activity of this impaired population may modify the activity of an unimpaired neuron population. Such a modification of activity might then persist following recovery and appear as a persistent impairment. This impairment may be classified as an indirect effect of radiation exposure. Radiation may also result in chronic or developing membrane damage, inflammation, and necrosis. The direct and indirect effects of such impairment may be expected to intensify over time, resulting in an increasing behavioral impairment. The three tasks used in this study may provide examples of each of these types of effects of proton radiation.

The habituation of acoustic startle appears to be an example of direct, rapidly recovering impairment. Habituation was impaired only when testing occurred within 24–48 h immediately following exposure. There were no persistent effects, since both short- and long-term habituation appeared similar across radiation groups for all the test sessions following Week 0 in Experiment 1 and throughout Experiment 2.

In the open-field task, the response to the presentation of the object (appearing as the three-way interaction of radiation group, object, and week) provides an example of the indirect effects of radiation. A relatively persistent effect results from animals' being tested prior to their recovery from the direct effects of exposure. For the sham-exposed mice in Experiment 1, the presentation of the object was associated with an increase in object-related exploration, seen as a shift in activity to the area of the object without a decline in ambulation. However, across time, the animals habituated to the repeated presentation of the object, and a decrease in ambulation was observed in the second 5-min period, as compared with the first (see Figure 1). In contrast, exploration of the object failed to habituate in the animals exposed to proton radiation. Moreover, all of the groups of the animals in Experiment 2 displayed a decrease in activity associated with presentation of the object. Taken together, these findings suggest that the failure to habituate observed in Experiment 1 resulted from the experience in the open field during the first 2 weeks following exposure. In particular, a putative radiation-induced impairment in the encoding or storage of the prior experience with the field or the object may have contributed to the impaired habituation observed in later weeks.

Rotorod performance also appeared to reflect a direct impairment with both short- and long-term aspects. At 18 rpm, impaired performance was observed in the exposed mice throughout the 12 weeks of Experiment 1. This impairment was not observed in Experiment 2, suggesting that it may have been, at least in part, an indirect effect. In contrast, impairment at 26 rpm was observed in both experiments, becoming particularly apparent around 4 weeks postexposure in mice receiving the 4-Gy dose and in those exposed without shielding.

Half of the mice receiving radiation were exposed behind 15-g/cm^2 aluminum shielding. However, for two of the three tests, acoustic startle habituation and the open field, no significant effects of shield were observed. Because performance was impaired following radiation exposure in each of these tests, it appears as though the

shielding failed to provide measurable protection. Nevertheless, the shielding did have a protective effect in rotorod performance, since the significant impairment at 26 rpm was observed primarily in those mice exposed without shielding.

This study was designed as an initial exploration of the potential effects of low-dose proton radiation on performance. It is, therefore, premature to draw any but speculative conclusions. However, these results suggest that astronauts may be vulnerable to various performance impairments following radiation exposures, such as those associated with an SPE. Diminished startle habituation which represents impaired perceptual learning and response, might appear as difficulty responding to and evaluating warning signals occurring during critical operations. Rotorod and open-field performance deficits might reflect negatively impacted motor control and memory for recent events. One might say that the bad news is that the results of this study suggest the possibility of performance deficits following exposure to proton radiation. The results also suggest that shielding, such as the type used in this study, is not completely effective in providing protection from proton radiation. However, the good news is that the results also suggest that, for the most part, the deficits are acute and transitory. Performance deficits resolve within 14 days, with the possible exception of some effects indexed by the rotorod task.

The specific nature of any performance deficits would depend on the precise brain regions and neural structures affected.

The hippocampus is among the structures known to be sensitive to the damaging effects of radiation. For example, granule cell agenesis and hypoplasia in the neonatal rat hippocampus are induced by exposure to X- and γ-radiation (Jensh & Brent, 1988; Mickley & Ferguson, 1989; Mintz, Yovel, Gigi, & Myslobodsky, 1998) at doses of 13 Gy and above. In the adult guinea pig, γ- or X-irradiation in the 5- to 10-Gy range can significantly impair the ability of hippocampal (CA1) neurons to generate and maintain the membrane potentials required for axonal spikes and synaptic function, with the duration of the impairment being 5–7 days (Pellmar & Lepinski, 1993; Tolliver & Pellmar, 1987). Disorientation in humans (Anno et al., 1989) and impaired learning and memory in rats (Fields, 1957; Urmer & Brown, 1960) occur within a day of exposure to doses of 5-Gy γ- or X-irradiation. Transient damage of this sort to the hippocampus would be a possible source of the open-field performance deficits. Cellular impairment over the course of 14 days might affect performance and learning in the open field during the first two or three test sessions, producing long-term performance deficits at later test points.

Although a point for parsimony could be scored if damage to a hippocampal mechanism could explain the diminished habituation immediately following proton exposure and an early report suggested that hippocampal lesions might impair long-term habituation (Groves, Wilson, & Boyle, 1974), evidence suggests that short-term habituation is unaffected by lesions of the hippocampus (Groves et al., 1974; Leaton, 1981; Sobotka et al., 1996). Therefore, it is unlikely that radiation-induced functional impairment of the hippocampus diminished habituation in irradiated mice at Week 0.

There have been a number of reports that indicate that dopaminergic function is impaired following exposure to ionizing radiation. Exposure of rats to 4- to 14-Gy X or γ rays produced small changes in measures of dopamine activity, metabolism, or K^+-stimulated dopamine release in various brain regions, including the hypothalamus (Pausescu, Chirvasie, Teodosiu, Lugojan, & Muntiu, 1973), the striatum (Chen & Kandasamy, 1996), and the pineal gland (Kassayova, Ahlersova, Pastorova, & Ahlers, 1995). At higher energy and doses, X or γ rays caused decreases in measures of dopamine metabolism in the caudate nucleus (Hunt, Dalton, Joseph, & Rabin, 1990). These effects were transient, occurring on the order of 1–2 h. In contrast, ^{56}Fe-irradiation may produce longer-lasting effects on dopamine systems. For example, dopamine metabolite concentrations were decreased in the caudate nucleus of rats during a 3- to 14-day window following exposure to 5-Gy (but not 0.5- or 1-Gy), whole-body ^{56}Fe-irradiation (Hunt et al., 1990). Rats exposed to 0.1- to 3-Gy whole-body ^{56}Fe-irradiation showed a decrease in the K^+-induced enhancement of DA release in the striatum during the same postirradiation period (Joseph, Hunt, Rabin, & Dalton, 1988, 1992). These impairments in dopaminergic function in the striatum were accompanied by parallel decrements in wire-hang performance (Joseph et al., 1988, 1992). Therefore, the time course of the striatal dopaminergic effects of ^{56}Fe radiation is consistent with the open-field performance in both Experiments 1 and 2. However, although a transient reduction in dopaminergic function might provide an explanation for the increase in ambulation across Weeks 0–2 in Experiment 1, this would not explain why the sham-exposed mice exhibited the same increase. This transient change in forebrain dopamine might modulate short-term habituation of the acoustic startle response. Dopamine infused into the rat nucleus accumbens inhibits both short-term habituation and the prepulse inhibition of startle (Schwarzkopf, Mitra, & Bruno, 1992; Swerdlow, Braff, & Geyer, 1990). Modulating the accumbens' output to the striatum has similar results (al-Amin & Schwarzkopf, 1996; Swerdlow & Geyer, 1999).

Finally, although mice with focal cerebellar lesions can still function on the rotorod, performance is considerably reduced in comparison with nonlesioned controls (Barlow et al., 1996; DeFries, Gervais, & Thomas, 1978; Glickstein, 1992; Lalonde, Bensoula, & Filali, 1995). It has been reported that the radiation-induced changes in granule cell development in the neonatal cerebellum resemble those of focal cerebellar lesions, in that both produce decrements in motor behavior—specifically, ataxic gait (Guelman, Zorilla Zubilete, Rios, Dopico, & Zieher, 2000) and rotorod performance (Le Marec, Dahhaoui, et al., 1997; Le Marec,

Stelz, Delhaye-Bouchaud, Mariani, & Caston, 1997). Exposing adult mice to 50–100 Gy γ- or X-irradiation reduced the time animals spent on a shock-motivated form of accelerod (Bogo, 1984; Bogo, Zeman, & Dooley, 1989; Cockerham, Bogo, & Gosset-Hagerman, 1984).

Because it is a cerebellum-dependent behavior, the architecture of the cerebellum might suggest a potential mechanism for the protective effects of shielding on rotorod performance. The high firing rates common to Pukinje neurons require a significant amount of metabolic fuel and are correspondingly rich in mitochondria. Not only are mitochondria susceptible to radiation-induced membrane damage (Boloor, Kamat, & Devasagayam, 2000; Kamat & Devasagayam, 2000; Kim & Shin, 1994; Shen, Ye, & Wu, 1989; Somosy, 2000), the abundance of mitochondria could promote a pro-oxidant status, making neuronal tissue in this area even more susceptible to radiation-induced damage (Lewen, Matz, & Chan, 2000). Because cerebellar cells are more densely packed, than any other part of the brain, particle tracks are likely to cause damage to functional groups of neurons, rather than being distributed to individual cells. This suggests the cerebellum may be more strongly affected by variations in particle fluence than by linear energy transfer (LET). This is further suggested by the *reverse LET* effect oft-noted in this behavior.

When a three-layer version of the BRYNTRN (Baryon Transport) model is used, the spectrum of particles to which the animals are exposed, regardless of shielding condition, is complex (Mark Shavers, personal communication, January 2002). As the primary (or incident) beam moves through the upstream beam-shaping materials (i.e., lead, polycarbonate, or Rexolite), secondary particles, including neutrons, alpha particles, and additional lower energy protons, are generated through a series of atomic collisions and interactions. Placing 15-gm/cm^2 aluminum shielding between the animal and the beam causes a shift in the number, energy, and species identity of the particles within this distribution. The end result is a shift in the track-averaged LET, or the most probable average LET for particles that interact to cause cellular or genetic damage in the mouse. The shift is from 0.54 keV/μm in the unshielded condition to 0.61 keV/μm in the shielded condition. Therefore, because we kept the dose constant, regardless of shielding condition, one would expect that the 13% increase in the track-averaged LET that was due to shielding would have some effect on behavior.

Typically, an increase in LET has been associated with altered biological effects, including unique gene expression patterns (Green et al., 2001; Woloshak, 1997) and impairment of conditioned taste aversion (Rabin et al., 1989; Rabin et al., 1991). In most models, an increase in LET results in a decrease in behavioral performance. However, despite the vastly different doses used in previous studies, a reverse LET effect has been found previously with rotorod behavior (Bogo, 1984; Bogo et al., 1989). This would be consistent with the main effect of shielding found here.

While the shielding used in the present study increased the average LET of the particles that interacted with tissues, there may have also been a decrease in particle fluence. A decrease in particle fluence owing to shielding could lead to a decrease in tissue damage, despite the increased in LET.

CONCLUSION

In these experiments, we wanted to determine whether low-dose/low-dose-rate proton irradiation was capable of producing measurable changes in behavior. If such changes were found, we were also interested in the time course of the deficit. The results indicated that exposure to low-dose/low-dose-rate proton radiation similar to that of a large SPE appears to produce transient changes in the spontaneous open-field locomotor activity, the habituation of acoustic startle, and the rotorod performance of C57BL/6 mice.

It therefore appears that, as long-duration space flights are being designed, it will be important to consider the impact of exposure to proton radiation from such sources as SPEs. Although these effects are generally transient, there is the potential for devastating consequences if critical operations must be carried out shortly following exposure. In the design of mission countermeasures, it will therefore be important to clarify the extent of the impairment for the various performance tasks, to determine which structures of the central nervous system are particularly vulnerable to proton and other types of radiation, to assess the risks associated with changes in fluence and LET, and the protection available from various types of shielding.

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