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Neurogenic Effects of Low-Dose Whole-Body HZE (Fe) Ion and Gamma Irradiation

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Abstract

Understanding the dose-toxicity profile of radiation is critical when evaluating potential health risks associated with natural and man-made sources in our environment. The purpose of this study was to evaluate the effects of low-dose whole-body high-energy charged (HZE) iron (Fe) ions and low-energy gamma exposure on proliferation and differentiation of adult-born neurons within the dentate gyrus of the hippocampus, cells deemed to play a critical role in memory regulation. To determine the dose-response characteristics of the brain to whole-body Fe-ion vs. gamma-radiation exposure, C57BL/6J mice were irradiated with 1 GeV/n Fe ions or a static ¹³⁷Cs source (0.662 MeV) at doses ranging from 0 to 300 cGy. The neurogenesis was analyzed at 48 h and one month postirradiation. These experiments revealed that whole-body exposure to either Fe ions or gamma radiation leads to: 1. An acute decrease in cell division within the dentate gyrus of the hippocampus, detected at doses as low as 30 and 100 cGy for Fe ions and gamma radiation, respectively; and 2. A reduction in newly differentiated neurons (DCX immunoreactivity) at one month postirradiation, with significant decreases detected at doses as low as 100 cGy for both Fe ions and gamma rays. The data presented here contribute to our understanding of brain responses to whole-body Fe ions and gamma rays and may help inform health-risk evaluations related to systemic exposure during a medical or radiologic/nuclear event or as a result of prolonged space travel.

INTRODUCTION

Humans are exposed to low-dose or low-fluence ionizing radiation from natural and man-made sources in our environment, including cosmic and terrestrial radiation, medical

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procedures and occupational exposure (1). For decades, there has been extensive research on the health effects of such exposures, using standard epidemiological and toxicological approaches, which endeavors to establish exposure limits that will protect the public and relevant worker populations from adverse radiation effects, such as radiation-associated carcinogenesis. In general, the research approach has been to characterize responses of both populations and individuals to high-dose radiation and then, as appropriate, combine those data with quality factors, relative biological effectiveness factors and dose-rate modifiers derived from experimental models (2). However, despite this body of work, the health risks from low-dose radiation, such as the uncontrolled release from the Fukushima nuclear power plant (3) or long-duration space missions (4, 5), remain poorly determined. Of particular concern to the space industry are the effects of low-dose, high-energy cosmic radiation on the central nervous system (CNS). It has been suggested that these may include altered cognitive and emotional function, as well as detriments in short-term memory, reduced motor function and behavioral changes, outcomes that could affect mission astronaut performance and health; potential later effects include an increased risk of neurological disorders, such as Alzheimer's disease or other dementias (6).

Importantly, when determining radiation risk, especially at low doses, there is a need to understand which cells and functions are vulnerable to radiation injury. For example, in humans, the generation of new neurons (neurogenesis), occurs within the hippocampus throughout adulthood (7, 8). Although it is difficult to assess its contribution in humans, evidence in rodent studies suggests that adult hippocampal neurogenesis contributes to both cognitive function and emotional regulation (9–13). For example, selective suppression of adult neurogenesis impairs performance of cognitive tasks (14–22), abrogates the behavioral effects of antidepressant medications (23, 24) and impairs response to stress (25–28). Given the proliferative aspects of this critical function, it is not surprising that there is a significant body of evidence linking radiation exposure of the CNS to cognitive and emotional dysfunction in humans and mice (13, 26, 27, 29–35). Therefore, since radiation exposure of the hippocampus specifically may contribute to these cognitive deficits (29, 31), we have focused on the effects of radiation on hippocampal neurogenic end points. Furthermore, while the ability of localized irradiation to inhibit neurogenesis is evident (30, 36–41), the existence or significance of a dose-response relationship of neurogenesis in the context of whole-body irradiation at low doses is less clear and was a primary focus of this study.

MATERIALS AND METHODS

Animals

Male C57BL/6J mice (N = 260) were purchased from Jackson Laboratory (Bar Harbor, ME) and shipped to Brookhaven National Laboratory (BNL; Upton, NY) to acclimate for one week before entering the study. Animals were irradiated at 8–10 weeks of age and either sacrificed at 48 h or shipped to the University of Rochester (Rochester, NY), where they were housed until euthanized at one month postirradiation. In general, mice were housed five per cage and kept on a 12:12 h light-dark schedule in a temperature-controlled environment ($23 \pm 3^\circ\text{C}$) with access to food and water *ad libitum*. Mice were routinely monitored for health issues and had no observable problems at the time of euthanasia. All

studies were approved by the Institutional Animal Care and Use Committees of the University of Rochester and BNL.

Irradiations

Animals received Fe-ions or gamma whole-body irradiation at the NASA Space Radiation Laboratory and the Gamma Radiation Source Facility, respectively, at BNL. Due to logistical necessity, mice were exposed to Fe ions and gamma rays in two different runs, but variables including housing and handling conditions were minimized as much as possible.

The Fe-ion irradiation setup and dosing have been described elsewhere (42). Briefly, awake mice were loaded into ventilated 50 ml polystyrene conical tubes and irradiated, 10 at a time, using a foam tube holder positioned perpendicular to the center of a 20×20 cm beam of ^{56}Fe ions accelerated to 1 GeV/n. For a 1 GeV/n Fe beam, the field is uniform $\pm 2\%$. Doses were delivered at 1, 3, 10, 30 or 100 cGy, with dose rates ranging from 1 to 100 cGy/min. Control mice were similarly placed in tubes and sham irradiated. A total of 120 mice were used in this experiment with a group size of 20 for each dose.

For gamma-ray exposures, awake mice in their home cages were placed at various distances from a static ^{137}Cs source. The Biology Department at BNL maintains and calibrates the dose rate of this source. Doses of 1, 3, 10, 30, 100 or 300 cGy were delivered at dose rates ranging from 2 to 27 cGy/min. A control group of mice was placed in the source room but not irradiated. A total of 140 mice were used in this experiment with 20 mice per group for each dose.

Bromodeoxyuridine Injections

Animals destined for histological examination at the 48 h and one month postirradiation time points were injected intraperitoneally with 75 mg/kg bromodeoxyuridine (BrdU, Sigma-Aldrich® LLC, St. Louis, MO). Animals received four injections at 2 h intervals, starting at 2 h postirradiation.

Tissue Collection

Mice were euthanized at 48 h or one month postirradiation for histological analysis (9–10 mice for each dose and time point). At each time point, animals were deeply anesthetized using a mixture of ketamine (50 mg) and xylazine (10 mg) before performing transcardial perfusion via the left ventricle. A perfusate containing 0.15 M phosphate buffer (PB) with 0.5% w/v sodium nitrite and 2 IU/ml heparin was flushed through each animal to dilate blood vessels and prevent clotting. This was followed by perfusion with approximately 50 ml of freshly made, ice-cold 4% paraformaldehyde (00380; Polysciences Inc., Warrington, PA) in 0.15 MPB, pH 7.2. Brains were carefully removed, immersion fixed for 2 h at 4°C, dehydrated overnight in 30% sucrose in 0.15 MPB, snap frozen using dry ice and isopentane and stored at -80°C until sectioned. Brains were sectioned coronally (random initiation) to a thickness of 30 μm on a sliding microtome and free-floating sections stored in cryoprotectant until assayed.

Immunohistochemical Staining

Systematic random sampled sections of the brain from each mouse at each time point and dose were washed in 0.15 MPB, reacted in 3% H₂O₂ and blocked with 3% rabbit serum (Gibco®, Carlsbad, CA) with 0.4% Triton™ X-100 (Sigma-Aldrich). For examination of BrdU incorporation, brain sections were subject to an antigen retrieval process prior to primary antibody incubation. Briefly, sections were mounted on slides and allowed to dry overnight, then incubated in 4 NHC1 for 30 min at room temperature. Trypsin treatment (0.1% in 0.15 MPB for 10 min at 37°C) further improved antigen retrieval. Sections were then incubated with primary antibody: rat anti-BrdU (ab6326; Abcam®, Cambridge, MA) diluted at 1:300. For examination of Doublecortin (DCX) immunoreactivity, staining of free-floating sections was performed using goat anti-DCX antibody (sc-8066; Santa Cruz, Dallas, TX) diluted at 1:1,000. DCX antigen retrieval was performed by incubating 20 min at room temperature in liberate antibody binding (L.A.B.) solution (Polysciences). Antibody staining was visualized using biotinylated secondary antibodies (Vector Laboratories, Burlingame, CA or Jackson ImmunoResearch Laboratories Inc., West Grove, PA), diluted at 1:2,000 or 1:4,000, respectively, Elite® avidin-biotin complex (PK-6100) and a 3,3-diaminobenzidine (DAB, SK-4100) substrate kit (Vector Laboratories). Sections were cover slipped with DPX (13512; Electron Microscopy Sciences, Hatfield, PA).

Immunohistochemical Analysis

All analyses were performed blinded to treatment on sections taken at regular intervals from the brain (every 24th section). For counting BrdU-immunopositive (BrdU⁺) cells, sections were viewed with an Axiophot microscope, and light microscope images of representative sections were captured on an Axioplan Ili microscope (both from Carl Zeiss Microscopy, Jena, Germany). BrdU⁺ cells were counted in the subgranular zone (SGZ)/granule cell layer (GCL) in all brain tissue sections stained for each animal at both time points (4–5 sections per animal). For each radiation type, data were calculated as the percentage of the number of BrdU⁺ cells at 48 h in the sham-irradiated animals.

For counting DCX⁺ cells, sections were viewed on a Zeiss Axioplan Ili microscope and DCX⁺ cells in the SGZ/GCL were counted in the first two sections of brain tissue containing both blades of the dentate gyrus. For area calculations, light microscope images of each hemisphere of the first hippocampal section containing both blades of the dentate sections were captured at 10× magnification. From each image, the area corresponding to the SGZ/GCL was determined using ImageJ Software (National Institutes of Health, Bethesda, MD). Data are displayed as a percentage of the number of DCX⁺ cells per 0.1 mm² in the sham-irradiated sample for each radiation type. Images of representative sections were captured at 10× magnification.

Statistics

The D'Agostino-Pearson omnibus test or, under conditions when the N was small, the Shapiro-Wilk test, was used to confirm the normality of the data. Bartlett's test was used to assess homoscedasticity of the data. ANOVA analysis and Dunnett's post hoc tests were used when required assumptions were met. For situations of unequal variance, Welch's ANOVA and Games-Howell post hoc tests were used. Statistical computation and graph

creation was performed in JMP statistical software, version 5 (SAS Institute, Cary, NC) and GraphPad Prism, version 6 (GraphPad Software Inc., San Diego, CA). Statistical significance was considered at $P = 0.05$.

RESULTS

Acute (48 h) Effects of Whole-Body Fe Ions vs. Gamma Rays on BrdU Labeling

Neurogenesis persists in the adult mouse hippocampus, with new neurons arising from the proliferation of neural stem cells and intermediate progenitor cells that reside in the SGZ in the dentate gyrus (7, 43). To determine whether whole-body exposure to either Fe ions or gamma rays affected neurogenesis, we assessed BrdU labeling at 48 h and one month postirradiation. At 48 h, radiation-induced apoptosis of adult-born dentate gyrus cells is generally complete (44, 45), and at one month, adult-born dentate gyrus cells have achieved a stage of maturation such that they could have a functional influence on the dentate gyrus network (46). Across the brain, the cells that had incorporated BrdU were detected predominantly in neurogenic regions and, within the hippocampus itself, BrdU⁺ cells were detected in the SGZ along both blades of the dentate gyrus and, occasionally, in the hilus and GCL near the SGZ (Fig. 1A–D).

To quantify effects on proliferation, BrdU⁺ cells were counted throughout the dentate gyrus. Figure 1E shows a dose-dependent decline in the number of BrdU⁺ cells in the dentate gyrus at 48 h after high-energy charged (HZE) irradiation. Welch's ANOVA comparing all doses tested revealed a significant effect of radiation dose on the number of BrdU⁺ cells in the hippocampus [$F(5,23) = 123.50$, $P < 0.0001$]; Games-Howell post hoc tests revealed significant effects at doses of 30 cGy and higher (30 cGy: 66 ± 12 ; 100 cGy: 22 ± 5) compared to controls (0 cGy: 100 ± 22). Although a trend towards lower numbers was indicated in the low-dose groups, the reductions were not significant. As a comparison with the effects of whole-body Fe ion irradiation, Fig. 1F shows the quantification of the BrdU⁺ cells in the dentate gyrus of mice 48 h after whole-body gamma irradiation. Again, Welch's ANOVA comparing all doses tested revealed a significant effect of radiation dose on the number of BrdU⁺ cells in the hippocampus [$F(6,22) = 300.36$, $P < 0.0001$]. However, Games-Howell post hoc test revealed a significant effect only at doses of 100 cGy and greater (100 cGy: 45 ± 16 ; 300 cGy: 6 ± 2) compared to controls (0 cGy: 100 ± 13).

Delayed (One Month) Effects of Whole-Body Fe Ions vs. Gamma Rays on BrdU Labeling

The acute study showed a substantial loss of proliferating cells after whole-body irradiation with either gamma rays or Fe ions. However, even after 100 cGy irradiation with either type, 20% of the BrdU⁺ cells appeared to be viable and, indeed, BrdU⁺ cells persisted in the SGZ at one month postirradiation (Fig. 2A–D). To quantify these surviving cells and their progeny, we counted BrdU⁺ cells throughout the dentate and SGZ at one month postirradiation; data are shown for each radiation type as a percentage of the number of BrdU⁺ cells detected with respect to the sham-irradiated animals at the 48 h time point. Figure 2E shows the BrdU⁺ cells in the dentate gyrus of male mice at one month after whole-body Fe-ion irradiation. ANOVA comparing all doses tested revealed a significant effect of Fe-ion radiation on the number of BrdU⁺ cells; this included the 0 Gy cohort,

which displayed significant age-related attrition of adult-born neurons, as reported by Biebl *et al.* (47). Dunnett's post hoc tests revealed a significant decrease in the number of BrdU⁺ cells after 100 cGy (100 cGy: 6 ± 2) compared to controls (0 cGy: 15 ± 3), with no significant effect of Fe-ion radiation at any lower dose. Figure 2F similarly quantifies BrdU⁺ cells in the dentate gyrus of male mice at one month after whole-body gamma irradiation. A Welch's ANOVA comparing all doses tested revealed a significant effect of radiation on the number of BrdU⁺ cells remaining [$F(6, 24) = 97.49$, $P < 0.0001$] with a Games-Howell post hoc test revealing a significant decrease in the number of BrdU⁺ cells remaining at doses of 100 cGy and greater (100 cGy: 10 ± 2 ; 300 cGy: 2 ± 1) compared to controls (0 cGy: 16 ± 3). Again, the low-dose groups did not show a significant effect from whole-body gamma-ray exposure.

In our previously published study, we reported that exposure to proton radiation increased the proliferation of cells undergoing division at the time of exposure (48). The effect was first noted when we examined the ratio of the average number of BrdU⁺ cells at one month to the average number of BrdU⁺ cells at 48 h. In the context of the current experiments, we again examined this ratio after either gamma or Fe-ion irradiation over the range of doses tested, but did not detect an effect of either exposure; ANOVA analysis did not reveal a significant effect at $P > 0.05$ [gamma: $F(6, 55) = 0.08476$, $P = 0.9975$, Fe: $F(5, 51) = 0.2302$, $P = 0.9476$]. Consequently, we did not pursue staining with proliferative markers to more directly test whether or not gamma or Fe radiation alters the subsequent proliferation of cells undergoing division at the time of exposure.

Effects of Fe Ions vs. Gamma Rays on Neuronal Differentiation

New neurons arise from the proliferation and differentiation of progenitor cells that reside in the SGZ in the dentate gyrus (43). While a reduction in generated neurons in the adult brain is often reflective of a decrease in proliferating SGZ cells, the processes of proliferation, neurogenesis and survival can also be differentially regulated (49). To determine whether radiation exposure alters the number of newly differentiated neurons, we examined DCX staining in the dentate gyrus of whole-body Fe-ion- and gamma-irradiated mice. Figure 3A–D shows representative images of DCX staining in sham-irradiated and irradiated mice one month postirradiation. Figure 3E quantifies DCX immunoreactivity in the dentate gyrus of the hippocampus of mice one month after whole-body Fe-ion irradiation and demonstrates that exposure reduced the number of newly differentiated neurons relative to the sham-irradiated controls (Fe ion). ANOVA analysis comparing all doses tested revealed a significant effect of whole-body Fe-ion irradiation on the percentage of DCX⁺ cells remaining [$F(5, 54) = 8.909$, $P < 0.0001$], with Dunnett's post hoc tests revealing a significant decrease in the percentage of DCX⁺ cells remaining at doses of 100 cGy and greater (100 cGy: 63 ± 12) compared to controls (0 cGy: 100 ± 11); there was no significant effect of whole-body Fe-ion irradiation on the percentage of DCX⁺ cells at any lower dose level. Similarly, Fig. 3F quantifies DCX immunoreactivity in the dentate gyrus of the hippocampus of mice one month after whole-body gamma irradiation and also demonstrates that exposure reduces the number of newly differentiated neurons relative to sham-irradiated controls (gamma). ANOVA analysis comparing all doses tested revealed a significant effect of whole-body gamma irradiation on the percentage of DCX⁺ cells remaining [$F(6, 62) =$

7.452, $P < 0.0001$]. Dunnett's post hoc tests revealed a significant decrease in the percentage of DCX⁺ cells at doses of 100 cGy and greater (100 cGy: 81 ± 12 , 300 cGy: 67 ± 12) compared to controls (0 cGy: 100 ± 5), with no significant effect at lower dose levels.

DISCUSSION

Many published studies have shown an effect of ionizing radiation on the survival of adult-born neurons in the dentate gyrus of the hippocampus (30, 36, 39, 41, 44, 50–57). However, remarkably few have examined the dose-response relationship for whole-body irradiation with respect to this proliferating cell population over a low-dose range. We therefore chose to focus our investigation on whole-body HZE (Fe) ions and ¹³⁷Cs gamma-ray exposures, since they represent relevant forms of ionizing radiation encountered in the space environment and after radiologic/nuclear disasters, respectively.

Our analysis of DCX staining in the dentate gyrus of the hippocampus, which provides an indication of the numbers of neuronal precursor cells and immature neurons, showed a statistically significant reduction at one month after whole-body Fe-ion and gamma irradiation at doses 100 cGy, and was mirrored by findings from the BrdU⁺ analysis. These data, therefore, are consistent with previously published studies suggesting that brain irradiation induces a prolonged reduction in neurogenesis (38, 44, 50, 54, 55). It is important to note that a persistent downregulation of DCX immunoreactivity has been shown to correlate with effects on depression-like hippocampal-related behaviors (41). However, not all previous studies using Fe ions have shown evidence of a reduction in neurogenesis after HZE irradiation (41). Furthermore, Son *et al.* demonstrated a persistent effect on DCX immunoreactivity at higher doses (10 Gy) of cranial exposure to ¹³⁷Cs gamma rays, but not at the 100 cGy dose level (51). These differential findings may be partially explained by the differences in exposure volume (cranium vs. whole body). Furthermore, the natural age-dependent reductions in these cell populations may have further confounded the investigators' ability to detect DCX immunoreactivity at later time points, especially beyond one month postirradiation (58, 59). Indeed, we also detected a large attrition in adult-born cells between the 48 h and one month postirradiation time point, as evidenced by the percentage of BrdU⁺ cells remaining at one month compared to 48 h in both cohorts of sham-irradiated mice (0 Gy; Fig. 2E and F). However, there was not an increase in the relative variability (coefficient of variation) of our measurement after 30 cGy Fe-ion irradiation (compared to lower Fe-ion doses), or to gamma irradiation at the same dose, suggesting that the failure to detect a significant effect at one month was not due to a decrease in the total number of BrdU⁺ cells detected.

The shared dose threshold for the observed effect after 100 cGy irradiation using both radiation types at one month was unanticipated, since gamma rays and Fe ions induce biologically distinct damages. The track structure of Fe ions provides a narrow (nanometer) core of energy deposition (ionizing events), with a penumbra of diffuse secondary ionizations (60), resulting in heterogeneous and complex damage (61–63) distributed nonuniformly within a tissue volume. In contrast, the more sparsely ionizing ¹³⁷Cs (and X-ray) exposures result in fewer ionization events, but with a more homogeneous damage distribution (64).

Support for such a differential dose distribution was borne out by our analysis of the acute (48 h) effect of radiation on the incorporation of BrdU by proliferating cells in the SGZ of the dentate gyrus, which demonstrated a clear distinction between the two radiation types. After gamma irradiation, the lowest dose seen with a statistically significant effect (reduction) on BrdU incorporation was 100 cGy at 48 h postirradiation, with little to no changes relative to controls at any of the low-dose levels. This compared to a threshold of 30 cGy after Fe-ion irradiation, with a nonsignificant trend being observed at all low-dose levels (1–10 cGy). This threshold in effect was lower than had been previously reported (39, 41, 55), although many of these studies had not examined exposures specifically in this relatively low-dose range (10–100 cGy). Nonetheless, the extent of the decrease we saw in the fraction of cycling cells (BrdU⁺) after 100 cGy Fe-ion irradiation is in general agreement with that reported by Encinas *et al.* (39), DeCarolis *et al.* (41) and Rivera *et al.* (55), despite methodological differences between these studies and ours with respect to BrdU dosing protocol, time points of assessment and genetic background of mice. Furthermore, our ability to demonstrate a differential between the effects of Fe ions and gamma rays on proliferation at doses as low as 30 cGy may be the result of the specific BrdU dosing protocol used, since multiple injections over the first 8 h may have provided us with a more sensitive detection rate.

The difference in the threshold for detecting an effect of Fe-ion radiation at the acute and more chronic time point begs the question: If the effect demonstrated at 48 h after e.g., 30 cGy irradiation (though the argument could be made at any dose) does not persist at one month, is it relevant? The short answer to this question is unclear, though it has been suggested that dividing cells in the dentate are functionally important independent of their role in producing progeny, and that altering their proliferation may affect behavior both acutely and more chronically (51, 65–70). Further exploration of the influence of proliferating progenitors on the hippocampal circuit may give better insight into this question.

A full understanding of the biological effect of low-dose radiation is hampered by the time and money required to evaluate the dose response and time course of each different radiation type. This highlights the importance of understanding the dose-response profile either prior to designing experiments or when evaluating experiments to determine radiation risks. Interestingly, it has been suggested that LET-dependent trends for changes in neurogenesis would be difficult to demonstrate due to the complexity of the CNS radiation response (6). To inform the evaluation of CNS risk associated with exposure to space radiation, we examined responses in the C57BL/6 mouse after a single Fe-ion whole-body dose. There are obvious experimental limitations when attempting to replicate space radiation on Earth, therefore, there are some caveats in applying our data to considerations of risk for space travel. First, we examined the effect of exposure to only a single type of ion, Fe. Thus, our data are not reflective of physical or biological interactions that may be present with exposure to mixed particle radiation (71). Second, Fe ions encountered in space present with a wide range of energies that intermittently and unpredictably bombard astronauts during interplanetary space flights (72). We examined the effect of exposure to 1 GeV Fe ions, which are near the upper range of the spectrum of energies encountered, whereas the more plentiful low-energy Fe-ion particles deposit relatively more of their energy in tissue, thus

their impact may not be inferable from our studies (73). Third, the fluence rates of the combination of ions and particles that make up galactic cosmic rays correspond to a tissue dose of approximately 0.3–0.6 mGy/day (74). Therefore, extrapolating findings observed after the relatively high-dose rates used at BNL to the potential outcomes that may result from exposure in space must be treated with caution, especially since the biology of radiation-induced damage and the subsequent DNA repair alters profoundly with changes in dose rate.

Low-dose ^{137}Cs exposure represents a relevant form of radiation encountered in medical therapeutic devices, but also may be a constituent of exposures after nuclear power plant accidents or terrorist attacks. To inform the evaluation of risk associated with radioactive ^{137}Cs , we examined responses in the C57BL/6 mouse after a single whole-body exposure to a static source of ^{137}Cs . As suggested, there are caveats in applying the findings of our data to considerations of risk for human exposed to Fe ions. For example, radiation exposure can occur from either external or internal sources, the latter through inhalation or ingestion (75). We used an external source, thus our model will not represent the physiological effects that can occur from internal contamination. Ongoing experiments in our laboratory are underway to examine the effects of internal contamination in isolation and in conjunction with external contamination on neurogenic end points. Secondly, as part of a nuclear incident, individuals would likely receive a highly nonuniform dose, and there could be other confounding variables, such as concomitant exposure to burn, trauma or infection (75). Previous experiments in our laboratory have explored the possible synergism between thermal injury and radiation and demonstrated that thermal injury lowers the threshold for radiation-induced neuroinflammation (76).

There are also some caveats that are relevant to both types of radiation tested. Both environmental enrichment and forced running have been shown to increase adult neurogenesis after irradiation (77–80). Thus, the standard laboratory housing conditions used in our studies may not recapitulate the effects of radiation experienced in a more complex or physically demanding environment. Additionally, radiation exposure in space is chronic, and has the potential to be chronic after a nuclear incident or terrorist act. Examining chronic exposures to low-dose radiation is rarely experimentally feasible since few facilities have either the capabilities or time available. Therefore, out of necessity, we examined responses to a single exposure. Whether a single exposure is more or less detrimental than chronic exposure to the same total dose is unclear, but the two types of exposures likely affect cycling and static processes differently, thus our model may not best represent cases of either varied or chronic exposure.

In summary, our data indicate that whole-body exposure to low-dose HZE (Fe) ions or gamma rays may provide a CNS-related health risk. Our analysis of the dose-response relationship to neurogenic end points indicates there are measurable radiation effects from doses as low as 30 and 100 cGy for Fe-ion and gamma-ray exposures, respectively. While this study does not represent a robust model of human exposure from medical therapeutic devices, nuclear power plant accidents, terrorist attacks or human exploratory space missions, this work does indicate that there are important dose-response relationships for ^{137}Cs and Fe-ion exposure on neurogenic end points, which may inform future

experimental design and aid in the determination of risk associated with low-dose systemic exposure. To inform decisions related to the risk of radiation exposure, it would be of great utility to determine in future experiments whether or not the complexity of the environment, including environmental enrichment and/or stressors, influences the threshold at which we can detect an effect of ionizing radiation.

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FIG. 1.

Acute response of BrdU⁺ cells 48 h after whole-body irradiation at a range of doses (0–300 cGy). Panels A–D: Representative images of BrdU⁺ cells in the dentate gyrus of the hippocampus of irradiated mice 48 h after sham or 100 cGy Fe-ion or gamma irradiation. Scale bar = 100 μ m. Panels E and F: Graphs of BrdU⁺ cells 48 h after Fe-ion or gamma irradiation. The responses are presented as the percentage of the average number of BrdU⁺ cells detected in the corresponding sham-irradiated mice at 48 h postirradiation. Error bars represent SEM. N = 9–10 mice per dose. Data were analyzed with Welch's ANOVA

followed by Games-Howell post hoc tests. *Significant effects are reported at a threshold of $P < 0.05$.

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FIG. 2.

Persistence of BrdU⁺ cells one month after whole-body irradiation at a range of doses (0–300 cGy). Panels A–D: Representative images of BrdU⁺ cells in the dentate gyrus of the hippocampus of irradiated mice one month after sham or 100 cGy Fe-ion or gamma irradiation. Scale bar = 100 μ m. Panels E and F: Graphs of BrdU⁺ cells one month after Fe-ion or gamma irradiation. Responses are presented as the percentage of the average number of BrdU⁺ cells detected in the corresponding sham-irradiated mice at 48 h. Error bars represent SEM. N = 7–10 mice per dose. Data were analyzed with Welch's ANOVA

followed by Games-Howell post hoc tests. *Significant effects are reported at a threshold of $P < 0.05$.

FIG. 3.

DCX⁺ cells in the dentate gyrus of the hippocampus after whole-body irradiation. Panels A–D: Representative images show DCX⁺ cells in the dentate gyrus of the hippocampus in sham and 100 cGy irradiated mice one month after Fe-ion or gamma irradiation. Scale bar = 100 μ m. Panels E and F: Graphs of DCX⁺ cells one month after either Fe ion or gamma irradiation. The responses are presented as the percentage of the average number of DCX⁺ cells/100,000 μ m² detected in the corresponding sham. Error bars represent SEM. N = 9–10

mice per dose. Data were analyzed with ANOVA followed by Dunnett's post hoc tests. Significant effects were reported at a threshold of $*P < 0.05$; $***P < 0.0001$.