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^{28}Silicon Radiation-Induced Enhancement of Synaptic Plasticity in the Hippocampus of Naïve and Cognitively Tested Mice

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The space radiation environment consists of multiple species of high-energy charge particles (HZE), including ^{56}Fe and ^{28}Si nuclei, that may impact neuronal cells, but their damaging effects on the central nervous system (CNS) have been poorly defined. Hippocampus-dependent memory functions have been shown to be highly sensitive to ^{56}Fe HZE particles, which poses a significant risk to the cognitive performance of astronauts during space missions. While low doses of ^{56}Fe radiation do not induce cell death of mature neurons, they affect synaptic plasticity in the CA1 region, the principal neuronal output of the hippocampal formation involved in memory formation. The effects of ^{28}Si on the CNS have not been defined. Compared to behaviorally naïve mice, cognitive testing might affect synaptic plasticity and the effects of ^{28}Si radiation on synaptic plasticity might be modulated by prior cognitive testing. Therefore, in the current study, we quantified the effects of whole-body ^{28}Si radiation (600 MeV/n, 0.25 and 1 Gy) on hippocampus-dependent contextual freezing and synaptic plasticity in the CA1 region of animals not exposed (behaviorally naïve mice) and animals exposed to the contextual freezing test (cognitively tested mice). In behaviorally naïve mice exposed to 0.25 and 1 Gy of ^{28}Si radiation, the magnitude of long-term potentiation (LTP) was enhanced. However, in mice irradiated with 0.25 Gy contextual fear conditioning was enhanced and was associated with a further enhancement of the LTP magnitude. Such increase in synaptic plasticity was not seen in cognitively tested mice irradiated with 1 Gy. Thus, low dose ^{28}Si radiation has effects on synaptic plasticity in the CA1 region of the hippocampus and these effects are modulated by cognitive testing in a contextual fear-conditioning test.

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INTRODUCTION

The space radiation environment contains high-energy charged particles (HZE) such as ^{56}Fe and ^{28}Si nuclei accelerated by magnetic fields to very high velocities. There is emerging evidence at low dose and fluences of these particles that their traversal through neuronal tissue may impact neuronal functions without killing the neurons. HZE particles with the highest relative dose contribution include iron, carbon and silicon.

Currently ongoing ground-based studies indicate that within the brain structures, the hippocampal neurons (both excitatory and inhibitory) might be particularly susceptible to space radiation. Contextual fear conditioning is frequently used to assess hippocampus-dependent memory (*1*), including the acquisition of new memories. Advantages of the fear-conditioning test include the relative ease with which rodents can be trained in fear conditioning paradigms, the use of translational fear conditioning tests in humans (*2*), and the extensive knowledge we already have about the neural circuitry that mediates this form of hippocampus-dependent learning and memory task. Hippocampal long-term potentiation (LTP) has been implicated in the acquisition of conditioned fear (*3*). In addition to the hippocampus (*4*), lesion studies have implicated the amygdala in the formation of new associations between stimuli (*5*) as well as in the expression of the fear response (*6*) and there is support for interdependence of these brain regions in fear conditioning (*7*). In testing fear conditioning in humans, aversive stimuli that are being used are a mild shock to the fingers of the dominant hand, wrist or ankle, aversive noises or small blasts of air directed at the participant's throat or eye (*2*). The stressful conditions that astronauts encounter during space missions include environmental psychological ones, such as interactions with crew members for prolonged periods of time in a relatively confined space and living conditions that require maintenance and repair of equipment for recycling of air and

water, as well as physical stressors, such as moving around in a relatively heavy space suit. Therefore, it is important to include controlled environmental emotional stressors in experimental paradigms used to assess effects of radiation on the ability to learn and acquire new memories during space missions.

It has been shown that ^{56}Fe radiation impacts hippocampus-dependent cognition (*8–12*), including contextual fear conditioning (*8*, *9*), and in formation of hippocampal plasticity and long-term potentiation in the CA1 region of the hippocampus (*13*, *15*),[2] the principal output neurons of hippocampal formation (*15–19*). The hippocampal region was also shown to be important for the effects of photon radiation on hippocampal function. Pellmar *et al.* (*19*) showed for the first time that photon radiation affects neuronal excitability in the CA1 region of the hippocampus. We previously reported that ^{56}Fe radiation (600 MeV/n, head-only exposure) impacts synaptic efficacy and reduced synaptic plasticity in CA1 neurons[2] but we did not assess the behavioral and cognitive correlates.

Cognitive testing affects synaptic plasticity as assessed by *in vitro* hippocampal slice physiological experiments (*20*). In contrast, *in vivo* saturation of LTP (*21*) disrupts subsequent hippocampus-dependent cognitive performance and conversely, cognitive testing itself also affects hippocampal plasticity. Cognitive testing of irradiated animals may modulate the radiation-induced changes in synaptic plasticity. Therefore, to distinguish these effects it is important to include both behaviorally naïve and cognitively tested mice in assessments of risks associated with radiation exposure in the space environment.

Little is known about effects of ^{28}Si on hippocampus-dependent cognitive performance and synaptic plasticity. Therefore, in the current study, we tested the hypotheses that whole-body exposure to ^{28}Si radiation affects hippocampus-dependent contextual fear conditioning and synaptic plasticity in the CA1 region and that radiation effects on synaptic plasticity are modulated by cognitive testing. Behaviorally naïve and cognitively tested mice were either sham irradiated or ^{28}Si irradiated and then utilized for this study. Assessments of synaptic plasticity and cognitive function were performed 3 months after irradiation or sham irradiation, a time sufficient for showing photon- or ^{56}Fe-radiation-induced changes in synaptic plasticity (*13*, *14*) and changes in cognition (*22*, *23*).

MATERIALS AND METHODS

Animals and Study Design

This study was part of a program project involving multiple universities, animal irradiations were performed at Brookhaven National Laboratory (BNL), and throughout the project, associated logistics such as shipping were standardized to minimize environmental variations and to facilitate intercomparisons of measurements performed by the various teams. For these studies, BNL scheduling and multi-user operations, limitations of postirradiation processing rates (one animal per day for electrophysiology) and animal availability were all factored into the study design.

C57BL/6J approximately 9-week-old male mice purchased from Jackson Laboratories (Bar Harbor, ME) were used for this study and were allowed 2 weeks to acclimate to the housing facility at BNL. Mice born on January 11th were irradiated on March 28th (at 76 days old) or April 1st (at 80 days old) and those born on March 29th were irradiated on June 7th (at 80 days old) as parts of BNL experimental campaigns NSRL 11A and NSRL 11B. For electrophysiological measurements of behaviorally naïve animals (Group 1), mice were shipped to Loma Linda University (LLU) and singly housed until use. For behavioral testing (Group 2), 36 animals were shipped directly to Oregon Health and Science University (OHSU) one day after irradiation and group housed with four mice per cage until 2 days before cognitive testing when they were switched permanently to single housed status. Three animals died from unknown causes but 33 were behaviorally tested beginning 91 days postirradiation. Eleven cognitively tested animals (Group 2A) were shipped to LLU for electrophysiological analysis and comparison to behaviorally naïve mice.

After seven days of acclimation at BNL, the mice were randomly assigned to three treatment cohorts that received sham irradiation ($n = 24$) or whole-body exposure of 0.25 Gy ($n = 20$) or 1 Gy ($n = 21$) of Si (600 MeV/n). For exposure, mice were loaded into $8 \times 3 \times 3$ cm plastic boxes with air holes and placed in a foam fixture in the beam line of the NASA Space Radiation Laboratory (NSRL). Mice were exposed to 600 MeV/n $^{28}Si^{14+}$ ions in a rectangular beam of approximately 20×20 cm with a useful central area of 18×18 cm over which intensity varied by less than 5%. The focused beam of high-energy Si particles was generated by the Booster accelerator at BNL and transferred to the experimental beam line at the NSRL facility. Dose calibration was performed with three parallel plate ion chambers that were positioned upstream of the target and a NIST traceable Far West thimble chamber. The values of the thimble chamber were then compared with the upstream ion chambers so that the desired dose could be delivered to the samples based on upstream ion chamber measurements. The NSRL dosimetry system reported the LET for the particles to be 43.6 keV/μm.

Cohorts of mice were exposed to nominal doses of 0, 25 and 100 cGy. Sham-irradiated (0 cGy) animals were loaded into the restraint boxes for 5 min and then returned to their cages. Actual doses received were as follows: behaviorally tested animals (Group 2) received 0 cGy ($n = 24$), 25.04 ± 0.05 cGy ($n = 20$) and 100.15 ± 0.03 cGy ($n = 21$) with dose reported as mean and standard deviation; the subset of behaviorally tested mice subsequently shipped to LLU (Group 2A) received 0 cGy ($n = 4$), 25.02 ± <0.01 cGy [$n = 3$ (but slice preparations from one animal were unsuitable, giving a final $n = 2$)] and 100.18 ± 0.02 cGy ($n = 4$); behaviorally naïve animals used for electrophysiology (Group 1) received 0 cGy ($n = 12$), 25.16 ± 0.09 cGy ($n = 10$) and 101.02 ± 2.02 cGy ($n = 12$). Mice were randomly assigned to dose groups and investigators were blinded to the ^{28}Si radiation dose levels until after completion of measurements. Poisson distribution calculations indicate that for neurons of nuclear area 60 μm^2 typical of CA1 pyramidal cells, 25 cGy results in an average of 2.1 particle traversals per nucleus and 12% of cells will be untraversed (''unhit'') while 100 cGy results in an average of 8.6 traversals per cell nucleus and less than 1 in 5,000 cells will be unhit.

All protocols were reviewed and approved by the Institutional Animal Care and Use Committees (IACUC) of LLU, OHSU and BNL and were in compliance with all Federal regulations.

[2] Vlkolinsky R. Spigelman I. Nelson G. A. Krucker T. Obenaus A, Long-Term Effects of ^{56}Fe Radiation-Induced Impairment of Synaptic Plasticity: The Effect of Lipopolysaccharide in the Mouse Hippocampus, 18th Annual NASA Space Radiation Investigators' Workshop, July 13–15, 2007 Rohnert Park, CA pp. 18.

Cognitive Testing

Cognitive testing was performed 3 months after irradiation or sham irradiation. Animals were singly housed two days before the onset of cognitive testing, which was performed using Kinder Scientific hardware (Poway, CA) and Noldus Ethovision™ video tracking software (Wageningen, The Netherlands), as previously described (*25*). Mice received a foot shock (0.75 mA, 2 s) starting at 178 s during a 195 s training trial. Trained mice displayed fear by ceasing all movement except for respiration, a posture referred to as ''freezing.'' Training took place in a light and sound attenuated chamber (termed the ''conditioning chamber'') that was equipped with a video camera to record freezing behavior for later analysis. Testing for memory occurred 24 h after training and took place in the same environment as the training. Each mouse was again placed in the fear-conditioning chamber for 195 s to assess contextual fear conditioning. No foot shock was delivered during the memory trial. All mice were behaviorally naïve prior to cognitive testing. Freezing levels were hand scored (every 5 s) as previously described (*8*).

Hippocampal Slice Preparation

Postirradiation electrophysiology measurements were conducted over multiple days for logistics reasons, as discussed above. They were pooled from cohorts of mice exposed on three separate days so that the time variation at the nominal 90 day postirradiation point could be minimized but the full range of time points was from 79–117 days postirradiation. For behaviorally naïve animals the measurements occurred at 101 ± 13 days postirradiation for 0 cGy sham-exposed mice, 100 ± 14 days for 25 cGy mice and 94 ± 10 days for 100 cGy mice. For behaviorally tested animals the measurements occurred at 125 ± 2.4 days for 0 cGy, 129 ± 0.7 days for 25 cGy and 135 ± 1.3 days for 100 cGy mice. Thus, the electrophysiological assessments for behaviorally conditioned animals occurred on average 24, 29 and 41 days after those for naïve animals exposed to 0, 25 and 100 cGy.

Hippocampal slices were prepared from the rostromedial part of the right hippocampus only, according to a protocol described previously (*15*). Briefly, the animals were deeply anesthetized with 3.5% isoflurane and decapitated. The brains were rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in m*M*): NaCl, 130; KCl, 3.5; KH_2PO_4, 1.25; $MgSO_4$, 1.5; $CaCl_2$, 2.0; $NaHCO_3$ 24 and glucose 10; with a pH of 7.4 and saturated with carbogen (95% O_2, 5% CO_2). The hippocampus was dissected free and transverse slices (350 μm thick) were cut using a McIlwain tissue chopper. Slices were collected into the incubation chamber (Harvard Apparatus, Holliston, MA) for at least 60 min at 33.0 ± 0.5°C.

In Vitro Electrophysiology

The MED64 multielectrode array (Panasonic, Japan) was used in conjunction with a 16 × 4 channel divider (MED-B02; AlfaMED, Japan), thus synaptic activity in 4 hippocampal slices could be measured simultaneously with 16 active electrodes per slice. Slices were gently transferred (one at the time) using a custom-made plastic dropper on the polyamine-coated multi-electrode MED64 chips (type P210A; AlfaMED, Japan). The slices were carefully positioned under visual guidance (low-magnification inverted microscope; Motic, Speed Fair Co. LTD., Hong Kong, Japan) above the active quadrant (16 × 4 electrode area) that usually covered 60–80% of the dendritic region (*stratum pyramidale*) of the CA1. Slices were covered with 0.7 × 0.7 cm synthetic mesh fabrics to prevent floating, then weighted down by a gold-plated metal U-shaped anchor and visually inspected for proper electrode location. The incubation media was then removed momentarily to enhance slice adherence on the chip and immediately replenished by 0.8 ml fresh prewarmed and oxygenated ACSF. A microphotograph of each slice was used for documentation and later off-line analyses. Chips containing slices were transferred into MED C-03 electrode connector/chip holder. A perfusion cap (MED64, AlphaMED, Japan) was attached to each chip that allowed perfusions of prewarmed (inline-heater connected to TC-344 heat controller, Warner Instruments, Hamden, CT), oxygenated ACSF (2 ml/min) and continuous humidified carbogen delivery (75 ml/min) to the slice microenvironment. Chips containing slices were maintained at 33.0 ± 0.2°C and continuously monitored by two independent thermistor TC-344 heat controllers.

Each of the 16 electrodes could be used for stimulation or for recordings. Stimulation sites were carefully chosen to evoke a well-defined orthodromic excitatory postsynaptic response in the dendritic area of the CA1 neuronal field. The medial perforant pathways were stimulated by delivering biphasic square current pulses of 0.2 ms duration at 0.05 Hz. Compound field excitatory postsynaptic potentials (EPSP) were recorded (Fig. 1).

Evaluation of Synaptic Responses

The field EPSP reflects the magnitude of postsynaptic dendritic depolarization in close vicinity around the recording electrode (*25*). The field EPSP peak-to-peak amplitude, maximal negative amplitude (minimal amplitudes) and initial linear field EPSP slope were measured and statistically evaluated. While all of these measurements reflect the magnitude of synaptic depolarization, they may slightly differ as they are further affected by temporal profile of the excitatory and inhibitory synaptic transients. Peak-to-peak analysis was used as the most robust measurement of field EPSP magnitude, while changes in field EPSP minimal amplitudes (data not shown) and initial linear slopes (data not shown) mostly followed the trends observed in the peak-to-peak analyses. Responses were evaluated online and offline by Mobius™ Win7 installer version 0.3.9 built-in algorithms (WitWerx Inc., AlphaMed, Japan). Measurements were exported to Microsoft Excel for further data processing.

Synaptic Excitability

Tissue excitability was assessed in each individual slice with an input-output (I-O) test, using incrementally increasing stimulus intensities (SIs) ranging from 5–150 μA. Incremental SI recruited an increasing number of presynaptic terminals that resulted in a nearly linear increase of the dendritic field EPSP at this stimulation range. In most cases, the maximal SI used in our experiments was below the threshold for induction of action potentials. Synchronous neuronal

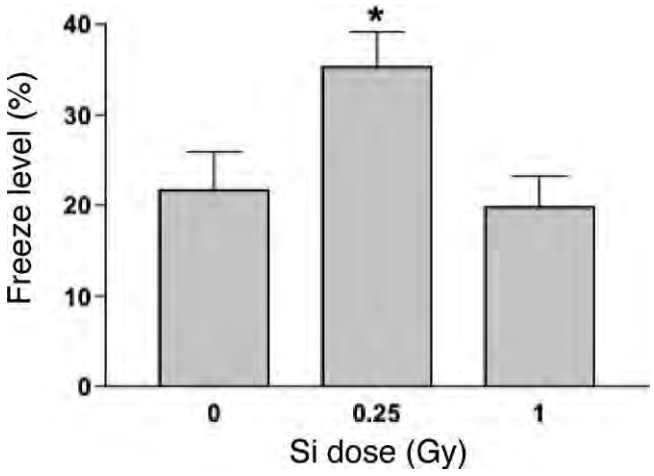

FIG. 1. Percent freezing levels of sham-irradiated mice and mice irradiated with 0.25 Gy or 1 Gy. Mice irradiated with 0.25 Gy froze more than sham-irradiated mice. *$P < 0.05$ vs. sham-irradiated mice. $n = 10$–12 mice/dose. Error bars represent the standard error of the mean.

firing of action potentials was sometimes present in the form of population spikes observed as a subtle distortion superimposed on field EPSP (*14*).

Synaptic Plasticity

Synaptic plasticity was evaluated by induction of long-term potentiation of the field EPSP. SI evoking 30% of the maximal field EPSP ($SI_{30\%}$) was used to record baseline responses for 20 min. LTP was then induced by high-frequency stimulation (HFS: 2 trains of 100 pulses at 100 Hz separated by 20 s). One minute later, the normal stimulation was resumed (at $SI_{30\%}$) and recordings continued for the next 60–80 min. LTP of the field EPSP was expressed as a percentage change relative to the field EPSP in the baseline period.

Statistical Analyses

All data are shown as mean ± SEM. All the statistical analyses were performed using SPSS™ (Chicago, IL) and GraphPad Prism™ (San Diego, CA) software. All figures were generated using GraphPad Prism software. For analyses of the cognitive data, ANOVA was used with radiation dose as the between factor, followed up by Dunnett's post-hoc tests or *t* tests when appropriate. For electrophysiological analyses, ANOVAs were used with radiation dose (i.e., each group of mice represented a distinct radiation exposure condition) and testing condition (i.e., behaviorally naïve or cognitively tested) as the between factors, followed up by Dunnett's post-hoc tests or *t* tests when appropriate. To determine whether the time interval between irradiation and electrophysiological measurements in individual mice affected the LTP magnitude, two-tailed Pearson correlations were used. In cases where there was more than one slice of the same mouse, the average was calculated for that animal. We considered $P < 0.05$ as statistically significant.

RESULTS

Cognitive Data

As expected, the low doses of radiation were well tolerated by the animals and there were no adverse effects observed during the postirradiation follow-up and testing periods.

However, there was an interesting radiation effect on hippocampus-dependent contextual fear conditioning observed (Fig. 1, $F = 4.165$, $P = 0.0253$). Mice irradiated with ^{28}Si at a dose of 0.25 Gy froze more than sham-irradiated mice ($P < 0.05$). In contrast, mice irradiated with ^{28}Si at a dose of 1 Gy showed freezing levels similar to those seen in sham-irradiated mice.

Electrophysiological Data

As a control for the electrophysiology studies, we first determined if variability in postirradiation electrophysiology measurements conducted over multiple days affected the synaptic plasticity-related outcome measures. We found that the time interval between irradiation and LTP magnitude in individual behaviorally naïve mice at any of the three radiation doses did not correlate (0 Gy: $r = 0.1825$, $P = 0.5702$; 0.25 Gy: $r = -0.3216$, $P = 0.3988$; 1 Gy: $r = 0.036$, $P = 0.9270$). Similar results were seen in cognitively tested mice ($P > 0.3$). These data indicated that even though the postirradiation electrophysiology measurements were conducted over multiple days it did not affect the synaptic plasticity-related outcome measurements.

Long-term potentiation was utilized to measure synaptic plasticity in CA1 neurons (see Fig 2A for Mobius™ acquisition software screenshots). Voltage traces from slices of a behaviorally naïve sham-irradiated mouse (control) and a behaviorally naïve irradiated mouse are shown. LTP in CA1 neurons was measured in both naïve (Fig. 2B) and cognitively tested (Fig. 2C) animals. Cognitive testing alone significantly increased the magnitude of LTP in sham-irradiated controls (Fig. 2B and C, $P = 0.0015$, two-way ANOVA) but post-hoc analysis (Bonferroni) did not reveal significant differences between behaviorally naïve and cognitively tested mice at any time point.

In behaviorally naïve animals, the radiation exposure in both dose groups (0.25 and 1 Gy) significantly enhanced LTP compared to the level observed in sham-irradiated mice ($P = 0.0015$, two-way ANOVA) and there was a significant interaction between radiation dose and the time point of LTP ($P < 0.001$). However, in cognitively tested mice an additional radiation-induced LTP enhancement was observed at 0.25 Gy, but not at 1 Gy (Fig. 2C). This enhancement was statistically significant when compared to either behaviorally naïve or cognitively tested sham-irradiated mice immediately after the HFS (from the beginning of the LTP time course, Tukey's post-hoc test). There were effects of radiation ($F = 5.981$, $P = 0.006$), cognitive testing ($F = 6.998$, $P = 0.013$) and radiation × cognitive testing interaction ($F = 5.348$, $P = 0.010$) on the LTP magnitude of the EPSP (Fig. 2), indicating that radiation affected synaptic plasticity differently in behaviorally naïve and cognitively tested mice. Behaviorally naïve mice irradiated with 0.25 Gy or 1 Gy of ^{28}Si showed a higher LTP magnitude than sham-irradiated mice but this effect was not dose dependent. In contrast, cognitive testing enhanced the LTP magnitude in sham-irradiated mice as well as those irradiated at a dose of 0.25 Gy. A dose of 1 Gy failed to affect LTP above that observed in naïve sham-irradiated mice. Thus, the radiation effects on LTP in cognitively tested mice resembled the pattern of radiation-induced changes in contextual freezing.

DISCUSSION

The results of the current study show novel neuronal and synaptic changes in the hippocampus of mice exposed to ^{28}Si radiation, with different functional outcomes in behaviorally naïve and cognitively tested animals. In behaviorally naïve mice, ^{28}Si radiation at 0.25 and 1 Gy enhanced the magnitude of long-term potentiation in the CA1 region. In cognitively tested mice irradiated with 0.25 Gy, contextual fear conditioning was enhanced and this was associated with a further enhancement of the LTP magnitude as compared to that seen in mice not exposed to contextual fear conditioning. Thus, within the dose,

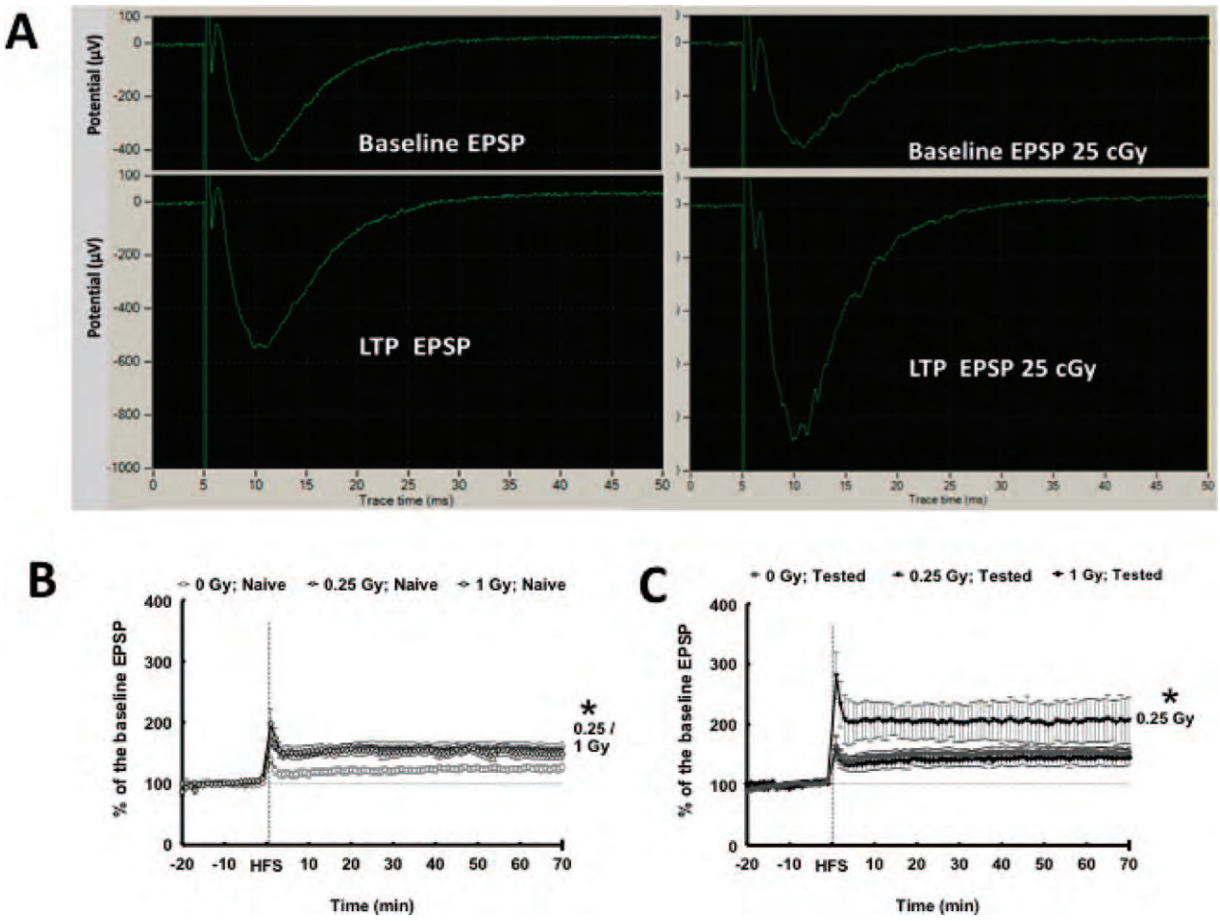

FIG. 2. Effects of radiation and cognitive testing on the long-term potentiation (LTP) magnitude in the CA1 region of the hippocampus. Panel A: Mobius acquisition software screen shots of slices from a behaviorally naïve sham-irradiated mouse and a behaviorally naïve mouse irradiated with a 0.25 Gy dose. Panel B: The time course of LTP of field excitatory postsynaptic potentials (EPSP) in the *stratum radiatum* of the CA1 neurons in ^{28}Si-irradiated naïve mice. LTP was expressed as percentage of the baseline field EPSP (peak-to-peak measurement, horizontal dotted line). When compared to controls, the LTP was significantly increased in 0.25 and 1 Gy dose groups at both time intervals. In slices from the rostral (dorsal) hippocampus we used the multielectrode array (MED64) to measure LTP of the field EPSP. Panel C: The time course of LTP of field EPSP in the stratum radiatum of the CA1 neurons in ^{28}Si-irradiated cognitively tested mice. In cognitively tested mice, additional radiation-induced LTP enhancement was observed at 0.25 Gy, but not at 1 Gy. There were effects of radiation ($F = 5.981$, $P = 0.006$), cognitive testing ($F = 6.998$, $P = 0.013$), and a radiation $\times$ cognitive testing interaction ($F = 5.348$, $P = 0.010$) on the LTP magnitude. A dose of 0.25 Gy or 1 Gy ^{28}Si radiation increased the LTP magnitude compared to sham-irradiated mice. Cognitive testing enhanced the LTP magnitude in sham-irradiated mice and in 25 Gy irradiated mice, but not in 1 Gy irradiated mice. The enhancement of the LTP magnitude after cognitive testing was dose dependent. Behaviorally naïve mice: $n = 31$ mice; sham-irradiated mice: $n = 12$; 0.25 Gy irradiated mice: $n = 10$; 1 Gy irradiated mice: $n = 9$. Cognitive tested mice: $n = 11$; sham-irradiated mice: $n = 4$; 0.25 Gy irradiated mice: $n = 3$; 1 Gy irradiated mice: $n = 4$. *$P < 0.05$ vs. sham-irradiated mice. $n =$ 10–12 mice/dose group. Error bars represent the standard error of the mean.

energy and other experimental conditions used in the current study, ^{28}Si radiation enhanced synaptic plasticity in the CA1 region of the hippocampus and these effects were modulated by cognitive testing. Since astronauts experience psychological and physical stressors during space missions, the fear-conditioning paradigm involving associative learning in the context of aversive stimuli seems an appropriate cognitive test. However, while these data support enhanced hippocampal function, it remains unclear whether an enhanced fear memory would be beneficial or detrimental to the performance and well being of astronauts during space missions.

It is unclear whether our observed enhancement is limited to contextual fear conditioning. It has been shown that long-term environmental enrichment also affects the plasticity in the CA1 region (*26*). In that study, environmental enrichment enhanced spatial memory and the enhancement was associated with, reduced long-term depression in the *stratum radiatum*, reduced depotentiation in the *stratum oriens* and altered paired-pulse inhibition of population spikes evoked in the *stratum oriens* (*26*).

However, it is possible that enhanced memory consolidation of contextual fear 24 h after training might be associated with enhanced extinction of the memory after repeated exposure to the environment in the absence of the aversive stimulus present during training or perhaps have a genetic component. In human apoE targeted replacement mice, apoE3 and apoE4 mice showed more memory

consolidation 24 h after training than apoE2 mice and showed extinction over subsequent days while apoE2 mice did not show extinction (*27*).

While 0.25 Gy ^{28}Si radiation enhanced contextual freezing, indicating a beneficial effect, the direction of the effect of a particular dose of ^{28}Si radiation on cognitive performance might depend on the cognitive test used and as stated above genetic factors may be involved. For example, cognitive-enhancing effects of whole-body ^{56}Fe irradiation on water maze performance were seen in apoE3 mice, whereas reduced cognitive performance after irradiation was seen in apoE2 and apoE4 mice (*10*). These data highlight the importance of including genetic models and mouse strains on different genetic backgrounds in studies aimed at assessing cognitive changes after exposure to space radiation.

Given the role of the CA1 region as an output structure of the hippocampus (*28*, *29*), these functional ''enhancement'' effects might also be due to radiation damage to other brain structures and subsequent hippocampal compensation. For example, radiation damage to the amygdala, which has direct connections with the hippocampus and plays a critical role in fear-related memories (*30*, *31*), may lead to hippocampal overactivity. Future studies are needed to determine the effects of ^{28}Si radiation on synaptic plasticity in the CA3 region and dentate gyrus, as well as the amygdala.

In the cohort of cognitively tested mice irradiated with 0.25 Gy, contextual fear conditioning was enhanced and this was associated with enhancement of the LTP magnitude in the CA1 region. This enhancement was not observed in cognitively tested mice irradiated with 1 Gy. These radiation effects suggest enhanced memory acquisition and enhanced memory consolidation after ^{28}Si irradiation with 0.25 Gy and perhaps is not surprising that CA1 neurons play a role in both (*32*). The molecular mechanism underlying these cognitive and electrophysiological enhancements might involve neurotrophic factors and immediate early genes. For example, the LTP-related immediate early genes such as brain-derived neurotrophic factor (BDNF) and *zif268* that are essential for gene transcription and protein synthesis after induction of LTP are induced 30 min after fear conditioning learning (*33*).

It is also possible that in hippocampal CA1 neurons, the effects of ^{28}Si radiation on synaptic plasticity might reflect improved efficacy of excitatory (glutamate-mediated) synaptic transmission and/or impaired GABA-ergic inhibition. ^{28}Si radiation enhanced the magnitude of LTP in the CA1 region of behaviorally naïve mice at doses of 0.25 and 1 Gy. However, in conditioned (cognitively tested) mice the LTP was only further enhanced at 0.25 Gy and not after the 1 Gy dose. Given that: (1) cognitive testing itself induces plasticity (increased LTP in trained controls); (2) ^{28}Si radiation increased LTP; and (3) the window of plastic changes has its limits, we speculate that a dose of 1 Gy (but not 0.25 Gy) narrowed the window of plastic changes (reduced the ceiling for LTP expression) in cognitively tested mice, resulting in an inverted U-shaped radiation dose-response relationship, whereas in behaviorally naïve mice the LTP in CA1 was increased in both dose groups. Thus, the ceiling effect might not have been reached in behaviorally naïve mice.

The data of this study highlights the complexity of assessing how low dose radiations affect hippocampal function. The data showing effects at a lower dose that are not observed at a higher dose challenges the prevailing theory that the relationship between dose and effect is linear, known as the ''linear no-threshold hypothesis.'' One reason this inverted U-shaped dose-response curve might be observed at relatively higher doses is that a protective compensatory mechanisms might be recruited at higher radiation doses and that does not occur at lower doses.

In summary, after ^{28}Si irradiation, there is a dose-dependent enhancement of synaptic plasticity in the CA1 region of the hippocampus of cognitively tested mice. These data suggest that even a relatively low dose of ^{28}Si can induce cognitive changes and synaptic plasticity-related changes that involve the hippocampus. Future studies are needed to determine the molecular mechanisms responsible for these effects.

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