Title
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Radiation-Induced Alterations in Synaptic Neurotransmission of Dentate Granule Cells Depend on the Dose and Species of Charged Particles


Introduction

Exposure to protons and high-energy charged particle (HZE) radiation during long duration space travel represents a significant health risk for astronauts. Naturally occurring sources of space radiation include trapped radiation belts, galactic cosmic radiation (GCR), and solar particle events (SPE) that are composed of protons and nuclei of heavier elements such as iron and silicon. The traversal of such particles through biological matter is known to trigger intense ionization localized along their path, which is associated with generation of free radicals and clustered damage to macromolecules. Such radiation exposure is associated with oxidative stress to multiple cell types in various organs and tissues (1–3) including neurons in the hippocampus. Therefore, chronic exposure to GCR and SPE could have a detrimental impact on the well being and specifically on the neurophysiological status of astronauts during long-term space exploration (4). Exposure to such radiation can potentially induce a wide range of neurological consequences, and in animals it has been repeatedly shown to induce cognitive impairment (1, 5, 6). Although the mechanisms underlying this radiation-induced cognitive impairment are not clearly understood, previous studies suggest that the pathogenesis likely depends on multiple factors and may involve chronic oxidative stress (7), depletion of neuronal precursor cells of the hippocampus (2, 8, 9), neuroinflammation (10, 11), altered expression of the plasticity-related immediate early gene Arc (6, 12, 13) and impaired neurogenesis (10, 14). Indeed, it has been shown that exposure to iron radiation impairs the proliferation of hippocampal neural precursor cells in mice, and that the reductions in the generation of new neurons correlates in time with cognitive impairments seen in these mice (10).

The hippocampus plays an important role in normal brain function, including learning and memory. It receives major input from the entorhinal cortex through the perforant pathway to the dentate gyrus (DG) granule cells (DGCs). DGCs project through the mossy fiber pathway to the CA3 region pyramidal cells (15), which in turn project to the CA1 pyramidal cells through the Schaffer collaterals (16). The DG has attracted numerous studies because it is a site of active neurogenesis in the adult mammalian brain, which is...
thought to contribute to the formation of new memories (17–19) and has been shown to be highly vulnerable to HZE-particle exposure (20). Moreover, pharmacological studies have demonstrated the importance of GABAergic and glutamatergic synaptic transmission of DGCs in maintaining cognitive health (21–26).

While it is well understood that radiation exposure induces impairments in hippocampal dependent cognitive performance (1, 5, 6, 13), little is known about the molecular mechanisms underlying these changes and whether they are associated with radiation-induced alterations in synaptic properties of DGCs. Due to different patterns of energy deposition and ionization track structures, irradiation with proton, silicon or iron may each have very different effects on cells and the overall neurological outcomes. Previous electrophysiological studies in the CA1 region of the hippocampus revealed that relatively high-dose (1–4 Gy) iron irradiation increases evoked synaptic potentials and decreases long-term plasticity (LTP) in mice (27). The purpose of this study was to determine the effects from exposure to low-dose proton, silicon and iron radiation on synaptic excitability of DGCs and to examine possible synaptic and/or cellular mechanisms that may cause these effects. We hypothesized that after examining radiation-induced changes in the synaptic excitability of DGCs with extracellular multielectrode array recordings, the mechanisms of such modifications could be further investigated at a single neuron level by recordings of pharmacologically isolated excitatory glutamatergic and inhibitory GABAergic synaptic currents. We tested this hypothesis 3 months postirradiation in hippocampal slices prepared from mice that were exposed to low-dose proton, silicon or iron radiation at ~3 months of age.

**MATERIALS AND METHODS**

**Animals and Irradiation**

The experiments described here were part of a program project involving five universities for which animal irradiations at Loma Linda University (LLU) and the Brookhaven National Laboratory (BNL) and associated logistics such as shipping were standardized to minimize environmental variations across the project, thus facilitating intercomparisons of measurements carried out by various expert teams. BNL scheduling and multi-user operations, limitations of postirradiation processing rates (one animal per day for electrophysiology), animal availability and other complex legislation-related factors were all factored into the study design. All animals were obtained from Jackson Laboratories, Bar Harbor, ME as approximately 9-week-old C57BL/6J males and housed at BNL for 1–2 weeks. Mice were irradiated at ages of 10–11 weeks old as part of BNL experimental campaign NSRL 11A (March/April, 2011) using silicon ions and campaign NSRL 11C (October/November, 2011) using iron ions. For postirradiation analyses, mice were shipped to LLU within 1 week after irradiation and then individually housed until later use. A subset of these animals was used for extracellular field recordings at LLU. The other subset was shipped to UCLA after approximately 75 days and used for patch clamp analysis within ~2 weeks of delivery to UCLA.

Animals were shipped directly from the vendor to LLU, where the mice were proton irradiated on a clinical beam line at the James M. Slater Proton Therapy and Research Center in March, July and December 2010. The animals used in the heavy-ion irradiation were also individually housed until later use. A subset of these animals was used for extracellular field recordings at LLU. The other subset was shipped to UCLA after approximately 75 days and used for patch clamp analysis ~2 weeks later. For all animals, electrophysiological recordings had to be conducted on several sequential days around the target date because of logistical constraints.

For radiation exposure, mice were loaded into 8 × 3 × 3 cm plastic boxes with air holes and placed in a foam fixture in the beam line of the NASA Space Radiation Laboratory (NSRL). They were exposed to 600.5 MeV/nucleon 72Si+ ions or 594.7 MeV/nucleon 56Fe+ ions in a rectangular beam of approximately 20 × 20 cm with a useful central area of 18 × 18 cm over which intensity varied by <5%. There was no modulation to the beam aside from beam line components and exposures therefore occurred in the plateau regions of the Bragg curve. The focused beam of HZE 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 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 silicon particles to be 43.6 keV/μm and the LET for the Fe particles to be 174.1 keV/μm.

The mice at LLU were loaded into the same restraint boxes and irradiated in the Bragg plateau region with a 20 × 20 cm circular beam of 150 MeV/nucleon protons (tuned energy) with a 1 cm plastic modulation and ≤3% variation in intensity across the central 18 cm. The beam energy at the target is reduced to ~142 MeV/nucleon with a resulting LET of ~0.57 keV/μm. The dosimetry was provided by the clinical control system calibrated to NIST-traceable ion chambers. Cohorts of mice were exposed to nominal doses of 0, 10, 50 and 100 cGy protons and 0, 25 and 100 cGy for Si and Fe. Sham-irradiated animals (0 cGy) were also loaded into the restraint boxes for 5 min and then returned to their cages. Actual doses received and post-irradiated tissue harvest times are summarized in Table 1.

**Slice Preparation**

Mice were euthanized at 90 ± 10 days after irradiation. Mice were anesthetized with 3.5% isoflurane (Phoenix, St. Joseph, MO) and decapitated. The brain was rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing: 125 mM NaCl, 2.5 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 26 mM NaHCO3 and 10 mM glucose, pH of 7.4 and saturated with 95% O2/5% CO2. Coronal brain slices (400 μm) containing the dorsal hippocampus were obtained with a vibrating slicer (Leica VT 1200S; Nussloch, Germany) from a block of tissue. Slices were allowed to recover for at least 1 h at room temperature.

**Electrophysiology**

Field excitatory postsynaptic potentials (fEPSPs) were recorded using a MED64 multi-electrode array (Panasonic, Japan). The MED64 multi-electrode was used in conjunction with a 16 × 4 channel divider (MED-B02; AlfaMED, Japan), thus evoked synaptic activity in 4 hippocampal slices could be measured simultaneously with 16 active electrodes per slice. Slices were gently transferred (one at a time) using a custom made plastic dropper on the 1% 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, China) above the active quadrant (16 × 4 electrode area) that usually
covered 80–90% of the dendritic region (stratum moleculare) in the enclosed blade of the dentate gyrus neuronal fields. Slices were covered with 0.7 × 0.7 cm synthetic mesh fabrics to prevent floating, then weighed down by gold-plated metal U-shaped anchor and visually inspected for proper electrode location. Incubation media was then removed momentarily to enhance slice adhesion on the chip surface and immediately replenished with 0.8 ml fresh prewarmed and oxygenated ACSF. A microphotograph of each slice was used for documentation and later offline analyses. Chips containing slices were transferred into MED C-03 electrode connector/chip holder. Perfusion caps (MED64, AlphaMED, Japan) were attached to each chip that allowed perfusions of prewarmed (in-line heater connected to TC-344 heat controller, Warner Instruments LLC, Hamden, CT), oxygenated ACSF (2 ml/min) and continuous humidified carbogen delivery (75 ml/min) to the slice microenvironment. Chunks containing slices were maintained at 33.0 ± 0.2°C and continuously monitored by two independent thermistors TC-344 heat controllers. Each of the 16 electrodes (20 μm electrode size, 100 μm inter-polar distance) could be used either for stimulation or for recordings. After careful morphological inspection of each slice, stimulation sites were carefully chosen in the anticipated path of axons of the medial perforant pathway (mPP). Thus, the stimulation sites were typically selected in the dentritic layer (stratum moleculare) of the granular cell located within one third of the enclosed blade of the DG, proximal to the stratum granulosum. Such placement of the stimulation electrode typically evoked a well defined negative fEPSP recorded by the proximal recordings electrodes. While excitatory responses were detected by most of the electrodes, for quantitation we utilized responses originating from electrodes closest to the stimulation electrode (either 100 or 140 μm). Thus, the recording site was usually located between the stimulation electrode and the dentate granule cell layer (∼50 μm from stratum granulosum). The mPP fibers were stimulated by delivering biphasic square current pulses of 0.2 ms duration at 0.05 Hz and compound fEPSPs were recorded in the DG. To ensure selective stimulation of the mPP, paired-pulse protocols were used (20–200 ms inter-pulse interval) to confirm paired-pulse inhibition of the second fEPSP in most recordings (data not shown).

Detection and Analysis of fEPSPs

The evoked fEPSP reflects the magnitude of postsynaptic dendritic depolarization in close vicinity around the recording electrode (28). The fEPSP peak-to-peak amplitude, maximal negative amplitude (minimal amplitudes) and initial linear fEPSP slope were measured and statistically evaluated. While all of these measurements reflect the magnitude of synaptic depolarization, they may slightly differ since 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 fEPSP magnitude, while changes in fEPSP 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 by Mobius TM Window Version 7.0.3.9 (WitWrex Inc., Osaka, Japan) online/offline built-in algorithms. Measurements were exported to Excel for further data processing. Synaptic excitability was assessed in each individual slice with an input-output (I-O) test, using incrementally increasing stimulus intensities (5–150 μA). Incremental stimulation intensity (SI) recruiting an increasing number of presynaptic terminals that resulted in a nearly linear increase of the dendritic fEPSP at this stimulation range. In most cases, the maximal stimulation intensity used in our experiments was below the threshold for induction of action potentials. Thus, synchronous neuronal firing of action potentials typically observed as population spikes was rarely present. If present, population spikes were observed at their threshold as a subtle distortion superimposed on fEPSP that did not confound the measurement of the fEPSP size (27).
Detection and Analysis of mIPSCs and mEPSCs

Data were acquired using pCLAMP™ 10 software (Molecular Devices), and analyzed using Clampfit application software (Molecular Devices) and the Mini Analysis Program (Synaptosoft Inc., Decatur, GA). The recordings were low-pass filtered offline (Clampfit application software) at 2 kHz. The mIPSCs and mEPSCs were detected (Mini Analysis Program, Synaptosoft, Inc., Fort Lee, NJ) with threshold criteria of: amplitude, 5 pA and area, 20 fC. The mean frequency of mIPSCs and mEPSCs was determined for each cell as the number of events per second during a 100–300 s recording period. For kinetic analysis, only single event mIPSCs or mEPSCs with a stable baseline, sharp rising phase and exponential decay were chosen during visual inspection of the recording trace. Double and multiple peaks were excluded. The mIPSC and mEPSC kinetics were obtained from analysis of the averaged single events aligned with half rise-time in each cell (100–300 events per cell). Decay time constants (τ1 and τ2) were obtained by fitting a double or single exponential to the falling phase of the averaged mIPSCs or mEPSCs, respectively. The synaptic charge transfer representing the magnitude of ion flow through GABAARs or AMPARs was measured as the area under the best-fit curve of the average mIPSC or mEPSC, respectively.

Western Blot Analysis

Isolated hippocampus tissues were homogenized in ice-cold homogenate buffer containing: 124 mM NaCl, 3.2 mM KCl, 1.06 mM NaH2PO4, 2.5 mM CaCl2, 1.3 mM MgCl2, 26 mM NaHCO3, and 10 mM glucose, bubbled with 95% O2/5% CO2 to adjust pH at 7.4. Protease inhibitors (1:20; P8340, Sigma-Aldrich LLC, St. Louis, MO) were included in the buffer to minimize proteolysis. The lysate was centrifuged for 15 min (14,000 g, 4°C) and supernatants were collected for Western blot analysis. Protein concentrations were determined with BCA Protein Assay Kit (Pierce Collected for Western blot analysis. Protein concentrations were determined with BCA Protein Assay Kit (Pierce) according to the manufacturer’s instructions. Equal quantities of proteins (20 μg/lane) were electrophoretically separated on a 10% SDS-PAGE (Bio-Rad, Hercules, CA). Proteins were transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad). After saturation in Tris-buffered saline (TBS) with 0.05% Tween™ 20 containing 5% nonfat dry milk (Bio-Rad), the PVDF membranes were probed with GABAARα receptor (GABAAR) α2 or with anti-GABAAR β3 (clone N87/25, UC Davis/NIH NeuroMab Facility, Davis, CA), or with anti-GABAAR γ2 at 1:1,000, or with rabbit polyclonal β-actin (AB8227, Abcam®, Cambridge, MA) at 1:2,000 and were incubated overnight at 4°C. W. Sieghart and colleagues (Medical University of Vienna, Vienna, Austria) kindly provided the s2 and γ2 GABAAR subunit antibodies. After washing in TBS with 0.05% Tween 20, PVDF membranes were incubated with the corresponding peroxidase-conjugated secondary antibody for 1 h at room temperature. Gel bands were revealed by chemiluminescence (ECL, Pierce) and pictures were acquired with a camera system of luminescent image analyzer (LAS-3000, Fujifilm, Tokyo, Japan). Optical density analysis of the signals was performed using ImageJ64 software (ImageJ 1.43u, NIH). The integral of the entire optical density profile was calculated as the final value. β-actin was used as a loading control in all experiments.

Results

Proton Radiation Increases Synaptic Excitability of DGCs

To determine the effect of low-dose proton irradiation on synaptic excitability at perforant pathway to DGCs, we performed an input-output (I-O) test and evaluated the size of field excitatory postsynaptic potentials (fEPSPs) (Fig. 1A). As expected, incremental stimulation intensity significantly increased the fEPSP peak-to-peak amplitude at all 4 experimental groups [F(29,696) = 114.4, ***P < 0.001, two-way RM ANOVA]). Importantly, we observed an interaction between stimulation intensity and proton-radiation doses [F(87,696) = 1.9, ***P < 0.001, two-way RM ANOVA]. Post hoc analysis showed that 1 Gy proton irradiation significantly increases fEPSP peak-to-peak amplitude at the highest stimulation intensities (SI) (SI: 130 nA, 0 Gy; 937 ± 236 μV, n = 5 vs. 1 Gy; 1,792 ± 258 μV, n = 7, *P < 0.05; SI: 150 nA, 0 Gy; 1,000 ± 261 μV, n = 5 vs. 1 Gy; 1,916 ± 272 μV, n = 7; *P < 0.05, Bonferroni post hoc test). However, such effect was not statistically significant at the 0.1 and 0.5 Gy doses. These data suggest that the excitability of the DGCs is increased after a 1 Gy dose of proton radiation.

Proton Radiation Decreases GABAergic Synaptic Transmission in DGCs

The increased synaptic excitability of DGCs observed in proton-irradiated mice could be due to a decrease in inhibitory GABAergic synaptic transmission. To determine the effect of low-dose proton radiation on inhibitory synaptic transmission in DGCs, mIPSCs were recorded from DGCs of 0, 0.1, 0.5 and 1 Gy proton-irradiated mice. Proton irradiation produced significant dose-dependent decreases in the amplitude of mIPSCs (0 vs. 1 Gy, *P <
Proton radiation - GABAergic mIPSCs and GABA_A units

A

0 Gy 0.1 Gy 0.5 Gy 1 Gy

0.1 pA 20 pA 2 s

B

0.1 pA 20 pA 2 s

C

mIPSC amplitude (pA)

0 0.2 0.6 0.8 1.0

Proton dose (Gy)

D

mIPSC charge transfer (pC)

0 0.2 0.6 0.8 1.0

Proton dose (Gy)

E

0 Gy 0.1 Gy 0.5 Gy 1 Gy

α2 β3 γ2

β-actin

F

GABA_A α2 subunit

G

GABA_A β3 subunit

H

GABA_A γ2 subunit

FIG. 2. Exposure of proton radiation decreases GABAergic synaptic transmission in the DG without affecting expression of GABA_AR α2, β3 and γ2 subunits. Panel A: Representative current traces of miniature IPSCs recorded from DGCs of 0, 0.1, 0.5 and 1 Gy proton-irradiated mice. Panel B: Average mIPSC traces from representative DGCs of 0, 0.1, 0.5 and 1 Gy proton-irradiated mice. Panel C: Summary graphs showing the dose-dependent decrease in mIPSC amplitude in proton-irradiated mice (*P < 0.05, n = neurons/mice: 0 Gy, n = 6/4; 0.1 Gy, n = 7/3; 0.5 Gy, n = 7/4; 1 Gy, n = 13/2), while the frequency of mIPSCs was not affected. Panel D: Summary graphs showing that mIPSC rise time and decay time (τ1 and τ2 constant) are not affected by proton radiation, whereas mIPSC area under the curve is significantly decreased in 1 Gy proton-irradiated mice (*P < 0.05). Panel E: Representative Western blots of GABA_AR subunits α2, β3 and γ2 from the DG of 0, 0.1, 0.5 and 1 Gy proton-irradiated mice. β-actin was used as a loading control. Panels F–H: Summary graphs showing the lack of significant effect of proton radiation on the expression of GABA_AR α2 (panel F), GABA_AR β3 (panel G) and γ2 (panel H) subunits. Results are presented as a ratio with β-actin (n = 3 mice per subunit per radiation dose).

0.05, one-way ANOVA with Bonferroni posthoc test) but no changes in the frequency of mIPSCs (P > 0.05, one-way ANOVA) (Fig. 2A–C; Table 2). We also observed a significant decrease in mIPSC charge transfer in 1 Gy proton-irradiated mice (0 vs. 1 Gy, *P < 0.05, Kruskal-Wallis one-way ANOVA on ranks with Bonferroni post hoc test), but no changes in mIPSC rise time (P > 0.05, one-way ANOVA) and decay time (τ1, P > 0.05, one-way ANOVA; τ2, P > 0.05, Kruskal-Wallis one-way ANOVA on ranks) (Fig. 2D and Table 2). These results suggested that proton radiation reduces GABAergic synaptic transmission through postsynaptic mechanisms affecting GABA_AR functional properties and/or expression. This decrease in inhibitory GABAergic synaptic transmission could represent the synaptic mechanism underlying the increased synaptic excitability of DGCs observed in extracellular recordings of fEPSPs from proton-irradiated mice.

Proton Radiation Does Not Modify the Expression of GABA_AR α2, β3 and γ2 Subunits

The channel kinetics and pharmacological sensitivity of GABA_ARs are largely dictated by their subunit composition and phosphorylation/dephosphorylation states (29, 30). Therefore, we examined if the proton radiation-induced decreases in postsynaptic GABA_AR function could be mediated by alterations in the relative expression of individual GABA_AR subunits. We focused on α2, β3 and γ2 subunits, because these are common subunits at DGC inhibitory synapses (31, 32). Immunoblotting analysis revealed no significant changes in the expression of GABA_AR α2, β3 or γ2 subunits in irradiated mice compared
to nonirradiated mice (GABA<sub>R</sub> α2/β-actin: 0 Gy: 100 ± 12%, n = 3 mice; 0.1 Gy: 127 ± 13%, n = 3 mice; 0.5 Gy: 122 ± 23%, n = 3 mice; 1 Gy: 98 ± 16%, n = 3 mice; P > 0.05, one-way ANOVA; GABA<sub>R</sub> β3/β-actin: 0 Gy: 100 ± 28%, n = 3 mice; 0.1 Gy: 103 ± 45%, n = 3 mice; 0.5 Gy: 139 ± 39%, n = 3 mice; 1 Gy: 123 ± 44%, n = 3 mice; P > 0.05, one-way ANOVA; GABA<sub>R</sub> γ2/β-actin: 0 Gy: 100 ± 18%, n = 3 mice; 0.1 Gy: 92 ± 19%, n = 3 mice; 0.5 Gy: 99 ± 6%, n = 3 mice; 1 Gy: 96 ± 10%, n = 3 mice; P > 0.05, one-way ANOVA) (Fig. 2E–H). These data suggested that proton radiation does not affect the expression of GABA<sub>R</sub> subunits in the DG. Thus, one of the effects of proton radiation on GABAergic synaptic transmission may be due to modifications of phosphorylation/dephosphorylation states of postsynaptic GABA<sub>R</sub> function rather than changes in subunit expression.

### Silicon Radiation Does Not Affect Synaptic Excitability in DGCs

Recordings obtained from DGCs of silicon-irradiated mice were used to assess the effect of silicon radiation on DG synaptic transmission. The I-O tests of synaptic excitability in DGCs were not modified in mice irradiated with silicon compared to controls. As expected, increased stimulation intensity significantly increased fEPSP magnitude [F(2,1237) = 141.2, ***P < 0.001, two-way RM ANOVA]. However, silicon radiation did not significantly affect the I-O curves at any of the stimulation intensities [F(2,1237) = 0.11, P > 0.05, two-way RM ANOVA; stimulation intensity: 150 nA, fEPSP peak-to-peak amplitude: 0 Gy: 1272 ± 149 µV, n = 17; 0.25 Gy: 1304 ± 163 µV, n = 12; 1 Gy: 1187 ± 176 µV, n=15] (Fig. 3A).

### Silicon Radiation Does Not Affect GABAergic or Glutamatergic Synaptic Transmission in DGCs

Recordings of inhibitory GABAergic and excitatory glutamatergic synaptic transmission from DGCs of mice irradiated with 0.25 and 1 Gy of silicon were conducted under pharmacological isolation of mIPSCs (Fig. 4) or mEPSCs (Fig. 5). Silicon radiation had no effect on mIPSC amplitude (P > 0.05, one-way ANOVA) or frequency (P > 0.05, Kruskal-Wallis one-way ANOVA on ranks) in the DG (Fig. 4C and Table 2). No effect was observed on mIPSC rise time (P > 0.05, Kruskal-Wallis one-way ANOVA on ranks), decay time (t<sub>1</sub>, P > 0.05, Kruskal-Wallis one-way ANOVA on ranks; t<sub>2</sub>, P > 0.05, Kruskal-Wallis one-way ANOVA on ranks) and area under the curve (P > 0.05, one-way ANOVA) (Fig. 4D and Table 2).

Silicon radiation also had no effect on mEPSC amplitude (P > 0.05, Kruskal-Wallis one-way ANOVA on ranks) and frequency (P > 0.05, Kruskal-Wallis one-way ANOVA on ranks) (Fig. 5C and Table 3), nor on mEPSC rise time (P >

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**TABLE 2**

<table>
<thead>
<tr>
<th>Patch clamp radiation (no. of DGCs recorded)</th>
<th>mIPSC</th>
<th>Amplitude (pA)</th>
<th>Frequency (Hz)</th>
<th>Rise time (ms)</th>
<th>Decay t&lt;sub&gt;1&lt;/sub&gt; (ms)</th>
<th>Decay t&lt;sub&gt;2&lt;/sub&gt; (ms)</th>
<th>Area (IC)</th>
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<tr>
<td><strong>Proton</strong></td>
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<tr>
<td>0 Gy (6)</td>
<td>28.9 ± 3.1</td>
<td>1.8 ± 0.5</td>
<td>0.6 ± 0.03</td>
<td>4.5 ± 0.7</td>
<td>58.6 ± 45.8</td>
<td>255 ± 53</td>
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<td>0.1 Gy (7)</td>
<td>22.8 ± 3.7</td>
<td>1.2 ± 0.2</td>
<td>0.6 ± 0.05</td>
<td>4.4 ± 0.6</td>
<td>44.4 ± 19.8</td>
<td>180 ± 13</td>
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<td>0.5 Gy (7)</td>
<td>19.8 ± 2.1</td>
<td>1.8 ± 0.3</td>
<td>0.6 ± 0.04</td>
<td>5.0 ± 0.5</td>
<td>124.2 ± 82</td>
<td>181 ± 22</td>
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<tr>
<td>1 Gy (13)</td>
<td>15.4 ± 1.4</td>
<td>1.0 ± 0.1</td>
<td>0.7 ± 0.02</td>
<td>5.1 ± 0.4</td>
<td>29.5 ± 9.0</td>
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<td>0 Gy (16)</td>
<td>20.9 ± 1.9</td>
<td>3.2 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>4.0 ± 0.3</td>
<td>13.3 ± 2.1</td>
<td>310 ± 22</td>
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<tr>
<td>0.25 Gy (19)</td>
<td>24.4 ± 1.5</td>
<td>3.3 ± 0.2</td>
<td>0.8 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>11.9 ± 0.8</td>
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<tr>
<td>1 Gy (19)</td>
<td>21.4 ± 1.6</td>
<td>3.2 ± 0.4</td>
<td>1.0 ± 0.2</td>
<td>4.2 ± 0.4</td>
<td>12.7 ± 2.1</td>
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<tr>
<td><strong>Iron</strong></td>
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<tr>
<td>0 Gy (15)</td>
<td>24.8 ± 1.7</td>
<td>1.9 ± 0.2</td>
<td>0.6 ± 0.02</td>
<td>4.8 ± 0.3</td>
<td>14.4 ± 1.8</td>
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<tr>
<td>0.25 Gy (13)</td>
<td>29.4 ± 2.8</td>
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<td>1 Gy (14)</td>
<td>30.5 ± 2.8</td>
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<td>0.6 ± 0.03</td>
<td>4.3 ± 0.4</td>
<td>16.5 ± 3.3</td>
<td>319 ± 33</td>
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</table>

*Note.* 0 Gy vs. 1 Gy.

*P < 0.05, one-way ANOVA.

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**FIG. 3.** Exposure to silicon radiation does not modify synaptic excitability in the DG. Summary of input-output relationship showing that exposure to silicon radiation does not modify the synaptic excitability of DGCs (n = slices/mice: 0 Gy, n = 17/12; 0.25 Gy, n = 13/11; 1 Gy, n = 16/11).
FIG. 4. Exposure to silicon radiation does not affect GABAergic synaptic transmission in the DG. Panel A: Representative current traces of mIPSCs recorded from DGCs of 0, 0.1, 0.5 and 1 Gy silicon-irradiated mice. Panel B: Average mIPSC traces of representative granule cells recorded from 0, 0.25 and 1 Gy silicon-irradiated mice. Panel C: Summary graphs showing that exposure to silicon radiation does not affect mIPSC amplitude and frequency in the DGCs (n = neurons/mice: 0 Gy, n = 16/3; 0.25 Gy, n = 19/5; 1 Gy, n = 19/5). Panel D: Summary graphs showing that mIPSC rise time, decay time (τ1 and τ2 constant) and area under the curve are not affected by exposure to silicon radiation.

FIG. 5. Exposure to silicon radiation does not affect glutamatergic synaptic transmission in the DG. Panel A: Representative current traces of mEPSCs recorded from DGCs of 0, 0.25 and 1 Gy silicon-irradiated mice. Panel B: Average mEPSC traces of representative granule cells recorded from mice irradiated with 0, 0.25 and 1 Gy of silicon. Panel C: Summary graphs showing that silicon radiation does not affect mEPSC amplitude and frequency in the DGCs (n = neurons/mice: 0 Gy, n = 7/3; 0.25 Gy, n = 8/3; 1 Gy, n = 12/3). Panel D: Summary graphs showing that mEPSC rise time, decay time and area under the curve are not affected by silicon radiation.
0.05, Kruskal-Wallis one-way ANOVA on ranks), decay time (P > 0.05, Kruskal-Wallis one-way ANOVA on ranks) or area under the curve (P > 0.05, one-way ANOVA) (Fig. 5D and Table 3). Together, these data on GABAergic and glutamatergic synaptic transmission of DGCs are consistent with the lack of effect of silicon radiation on synaptic excitability and plasticity observed in extracellular recordings.

Iron Radiation Does Not Affect Synaptic Excitability in DGCs

Recordings obtained from DGCs of iron-irradiated mice were used to assess the effect of iron particle radiation on synaptic transmission in the DG. As expected, the input-output curve showed that incremental stimulation intensity significantly increases fEPSP peak-to-peak amplitude [F(29,1247) = 140.1, ***P < 0.001, two-way RM ANOVA] (Fig. 6). Iron radiation did not significantly affect the input-output outcomes [F(2,1247) = 0.56, P > 0.05, two-way RM ANOVA; stimulation intensity: 150 nA, fEPSP peak-to-peak amplitude: 0 Gy: 1,570 ± 200 μV, n = 17; 0.25 Gy: 1,643 ± 259 μV, n = 15; 1 Gy: 1,303 ± 185 μV, n = 14; P > 0.05] (Fig. 6). These results suggest that iron radiation does not affect the synaptic excitability of DGCs.

Iron Radiation Does Not Affect GABAergic, but Modifies Glutamatergic Synaptic Transmission in DGCs

To identify the effects of iron radiation on inhibitory and excitatory synaptic transmission, mIPSCs or mEPSCs were recorded from DGCs of 0, 0.25 and 1 Gy irradiated mice (Fig. 7A). Iron radiation produced no significant changes in mIPSC parameters (Fig. 7C, D). We observed a small trend to increased mIPSC amplitude (P > 0.05, one-way ANOVA), but no discernible trends for mIPSC frequency (P > 0.05, one-way ANOVA) (Fig. 7C and Table 2), rise time (P = 0.05, one-way ANOVA), decay time (P = 0.05, one-way ANOVA) and area (P > 0.05, one-way ANOVA) were not significantly altered (Fig. 8D). These results suggest that iron radiation may potentiate glutamatergic synaptic transmission in DGCs through presynaptic increases in the vesicular release probability of glutamate.

DISCUSSION

The assessment of potential health risks associated with exposure to proton and HZE particle radiation is important for future long duration space missions. Here we demon-
FIG. 7. Exposure to iron radiation does not affect GABAergic synaptic transmission in the DG. Panel A: Representative current traces of mIPSCs recorded from DGCs of 0, 0.25 and 1 Gy iron-irradiated mice. Panel B: Average mIPSC traces of representative DGCs recorded from 0, 0.25 and 1 Gy iron-irradiated mice. Panel C: Summary graphs showing that iron radiation does not affect mIPSC amplitude and frequency in the DGCs (n = neurons/mice: 0 Gy, n = 15/4; 0.25 Gy, n = 13/4; 1 Gy, n = 14/4. Panel D: Summary graphs showing that mIPSC rise time, decay time (t1 and t2 constant) and area under the curve are not affected by iron radiation.

FIG. 8. Exposure to iron radiation modifies glutamatergic synaptic transmission in the DG. Panel A: Representative current traces of mEPSCs recorded from DGCs of 0, 0.25 and 1 Gy iron-irradiated mice. Panel B: Average mEPSC traces of representative granule cells recorded from 0, 0.25 and 1 Gy iron-irradiated mice. Panel C: Summary graphs showing the dose-dependent increase in mEPSC frequency in mice exposed to iron radiation (*P < 0.05), while mEPSC amplitude is unchanged (n = neurons/mice: 0 Gy, n = 10/4; 0.25 Gy, n = 8/4; 1 Gy, n = 12/4. Panel D: Summary graphs showing that mEPSC rise time, decay time and area under the curve are not affected by iron radiation.
strate that proton and HZE particle radiation have distinct effects on synaptic excitability and inhibitory synaptic transmission of DGCs in the dorsal hippocampus. We also show that iron radiation induces changes in excitatory synaptic transmission in the same cells. However, exposure to silicon radiation did not modify synaptic excitability or excitatory and inhibitory neurotransmission of DGCs. This study is the first to describe the effects of silicon radiation in the DG neurons. Learning and memory in the mammalian brain have prominent electrophysiological manifestations in the DG of the hippocampus. Synapses in the DGCs exhibit LTP (33), a specific form of synaptic plasticity is believed to contribute to long-term memory formation (24, 33–35). Synaptic excitability plays a critical role in the expression of LTP in the hippocampus. It has been shown that the reduction in LTP of CA1 neurons was attributed to radiation-induced increases in excitability, placing synapses in a potentiated state such that high-frequency stimulation in vitro fails to produce further increases in fEPSP (27). Thus, radiation-induced cognitive impairments could be associated with electrophysiological alterations in synaptic excitability of DGCs. In this study, we observed that 1 Gy proton irradiation increased synaptic excitability of DGCs. These neuroadaptive changes induced by proton radiation could lead to a dysregulation in the processing and integration of information within the hippocampus, which could play a crucial role in space radiation-induced impairment of hippocampal-dependent cognitive functions.

A fine balance between the excitatory glutamatergic and inhibitory GABAergic transmission in the DG is critical for proper operation of neuronal networks and cognitive health (21–26). It has been shown that the GABAergic-mediated synaptic inhibition plays a critical role in the modulation of synaptic plasticity, such as LTP, in the DG compared with the CA1 region (25, 36). In our extracellular recordings, the fEPSP responses to electrical stimulation are a sum of activated postsynaptic excitatory glutamatergic receptors and inhibitory GABAergic conductances. Thus, decreases in GABAergic inhibition allow for increased synaptic excitability and promote LTP (25), whereas enhanced GABAergic inhibition can reduce synaptic excitability and completely prevent the induction of LTP (25). In this study, we observed that exposure to proton radiation alters GABAergic synaptic transmission of the DGCs. The decreases in mIPSC amplitude and charge transfer observed in the DGCs of proton-irradiated mice suggest that proton radiation decreases the function of synaptic GABA<sub>R</sub>s. The channel kinetics and pharmacological sensitivity of GABA<sub>R</sub>s are largely dictated by their subunit composition and phosphorylation/dephosphorylation states (29, 30, 37). For example, we have previously demonstrated that altered expression and cellular localization of individual GABA<sub>R</sub> subunits greatly affects postsynaptic GABA<sub>R</sub> function of DGCs (38). It has also been shown that decreased synaptic clustering and expression of postsynaptic GABA<sub>R</sub>s produced decreases in mIPSC amplitude (39). However, in this current study we found that the expression of GABA<sub>R</sub> subunits α<sub>2</sub>, β<sub>3</sub> and γ<sub>2</sub>, common to DGC inhibitory synapses (31, 32), was not modified in proton-irradiated mice suggesting that the decreased synaptic GABA<sub>R</sub> function was likely due to alterations in their phosphorylation state or decreased synaptic clustering. This decrease in GABAergic synaptic inhibition may represent a cellular mechanism underlying proton radiation-induced increases in synaptic excitability of DGCs.

The effects of low-dose silicon radiation on cognitive functions and hippocampal synaptic neurotransmission are still unclear. Recent studies have shown that low doses of silicon radiation increase synaptic plasticity in the CA1 region of the hippocampus in naïve mice, and even further in cognitively tested mice (14, 40). In this current study, we found that exposure to silicon radiation has no effects on synaptic excitability. GABAergic or glutamatergic synaptic transmission of DGCs. These data suggest that the effects of low-dose silicon radiation on hippocampal neurotransmission and consequently on cognitive function may be selective for specific regions of the hippocampal formation.

It has been shown that relatively high doses of iron radiation (1–4 Gy) increase synaptic excitability and reduce LTP in CA1 pyramidal neurons of the hippocampus (27). Compared to DGCs, CA1 neurons are generally more excitable, have a weaker tonic inhibitory GABAergic drive and in slice recordings exhibit more depolarized membrane potentials. In this study, we showed that exposure to iron radiation does not significantly alter the synaptic excitability of DGCs. These results suggest that low-dose iron radiation can have different effects on synaptic properties in different regions of the hippocampus.

A previous study has demonstrated that low doses (0.6 Gy) of iron radiation induced a persistent reduction in glutamatergic readily releasable pool in hippocampal presynaptic terminals, suggesting that exposure to iron radiation may decrease the glutamate release probability in the hippocampus (41). In contrast, we found that exposure to iron radiation produces a dose-dependent increase in frequency of mEPSCs in DG granule cells suggestive of increased presynaptic glutamate release. This increase in mEPSC frequency suggests that the effects of iron radiation in the DG may be mediated by presynaptic increases in the probability of glutamate release (42, 43). However, this increase in presynaptic glutamate release probability was not paralleled by increases in evoked synaptic excitability in the DG granule cells in our extracellular I-O recordings (Fig. 6), nor in modifications in GABAergic mEPSCs (Fig. 7), nor in paired-pulse stimulation responses (see Materials and Methods; data not shown) that indirectly test for changes in presynaptic glutamate release. Therefore, since extracellular recordings demonstrated that fEPSPs were preferentially evoked by stimulation of the mPP axons, it is possible that the increased frequency of mEPSCs in intracellular measurements originated from synapses of other input pathways such as the commissural fibers.
Moreover, all mEPSC recordings were performed during blockade of GABA$_A$ and GABA$_B$ receptor blockers, as opposed to fEPSP recordings where modulatory effects of GABAergic interneuron activation may have precluded detection of changes in fEPSPs evoked by intense presynaptic stimulation. Thus, our study suggests that modification of the glutamatergic synaptic transmission, rather than alteration of GABAergic inhibition and synaptic excitability in the DG as shown with proton irradiation, may cause the impairment of hippocampal-dependent cognitive functions induced by exposure to iron radiation. Recent studies have shown that low-dose iron radiation induces a persistent deficit in hippocampal-dependent cognitive functions, including spatial learning (5) and contextual freezing, which was correlated to the number of neurons expressing the protein Arc, a plasticity-related protein essential for memory consolidation (44), in the enclosed (bottom) and free (upper) blade of the DG (6). Our current extracellular and whole cell patch-clamp recordings have not addressed possible differences in the electrophysiological properties and radiation responses of Arc-positive neurons, which may be a fruitful area for future investigations.

The molecular mechanisms by which particle radiation affects synaptic neurotransmission and cognitive performance are still unclear. The data presented in this study suggest that radiation-induced alterations in DG neurotransmission may be quite complex and involve mechanisms that need further investigation. Damage induced by ionizing radiation to living cells is directly related to the track structure of ionizing radiation in matter (45, 46). Insults from ionizing radiation take the form of highly structured tracks of ionized and excited molecules leading to the production of free radicals and reactive oxygen species (ROS) (2, 3, 47, 48) that are highly damaging to DNA, lipids and proteins in living cells (49). Poisson distribution calculations indicate that for neurons of m2 typical of hippocampal neurons (50), a 0.25 Gy dose of silicon radiation results in an average of 2.1 particle traversals per nucleus with 12% of cells untraversed “unhit” while a 1 Gy dose results in an average of 8.6 traversals per cell nucleus and with less than 0.02% of the cells remaining unhit. For iron radiation, a 0.25 Gy dose results in an average of 0.54 particle traversals per nucleus and 58% of cells remaining unhit, while a 1 Gy dose results in an average of 2.2 traversals per cell nucleus and 12% of cells being unhit. For protons, all doses ≥0.1 Gy result in at least 66 traversals per cell and essentially no unhit cells. Thus, it is likely that the differential effects of proton, silicon and iron radiations on DG synaptic neurotransmission could be related to the ability of their different track structures to effectively generate oxidative stress within the cells. Several studies have shown that ROS can regulate signaling pathways (51), modulate synaptic transmission (52) and plasticity in the hippocampus (51, 53). Therefore, the differential effects of ionized radiation on cellular ROS production could be responsible for the differential effects of proton, silicon and iron on synaptic transmission of DGCs. Overexpression of the mitochondrial-targeted human catalase (MCAT) transgene in mice, has been shown to decrease the overall production of free radicals, significantly reduced mitochondrial ROS and improved hippocampal neurogenesis after low-dose exposure to proton radiation (54). Examination of differences in synaptic neurotransmission and radiation responses of DGCs from MCAT mice would be useful to determine if their resistance to the harmful effects of low-dose radiation also extends to protection from radiation-induced changes in DG synaptic neurotransmission.

In conclusion, this study demonstrates that space radiation induces long-lasting neuroadaptive changes in the synaptic neurotransmission of the hippocampal DG network, which could represent the cellular and molecular mechanisms responsible for space radiation-induced hippocampal-dependent cognitive dysfunctions. Nevertheless, the relationship between radiation-induced cognitive impairment and synaptic neurotransmission is complex and needs to be carefully assessed within the context of a set of radiation exposures to better understand the mechanisms involved, and to develop innovative strategies to effectively protect astronauts against the detrimental effects of space radiation during long-term space exploration.

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REFERENCES


