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# $^{56}$Fe particle exposure results in a long-lasting increase in a cellular index of genomic instability and transiently suppresses adult hippocampal neurogenesis *in vivo*

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ABSTRACT

The high-LET HZE particles from galactic cosmic radiation pose tremendous health risks to astronauts, as they may incur sub-threshold brain injury or maladaptations that may lead to cognitive impairment. The health effects of HZE particles are difficult to predict and unfeasible to prevent. This underscores the importance of estimating radiation risks to the central nervous system as a whole as well as to specific brain regions like the hippocampus, which is central to learning and memory. Given that neurogenesis in the hippocampus has been linked to learning and memory, we investigated the response and recovery of neurogenesis and neural stem cells in the adult mouse hippocampal dentate gyrus after HZE particle exposure using two nestin transgenic reporter mouse lines to label and track radial glia stem cells (Nestin-GFP and Nestin-CreER$^{T2}$/R26R:YFP mice, respectively). Mice were subjected to $^{56}$Fe particle exposure (0 or 1 Gy, at either 300 or 1000 MeV/n) and brains were harvested at early (24 h), intermediate (7 d), and/or long time points (2–3 mo) post-irradiation. $^{56}$Fe particle exposure resulted in a robust increase in 53BP1+ foci at both the intermediate and long time points post-irradiation, suggesting long-term genomic instability in the brain. However, $^{56}$Fe particle exposure only produced a transient decrease in immature neuron number at the intermediate time point, with no significant decrease at the long time point post-irradiation. $^{56}$Fe particle exposure similarly produced a transient decrease in dividing progenitors, with fewer progenitors labeled at the early time point but equal number labeled at the intermediate time point, suggesting a recovery of neurogenesis. Notably, $^{56}$Fe particle exposure did not change the total number of nestin-expressing neural stem cells. These results highlight that despite the persistence of an index of genomic instability, $^{56}$Fe particle-induced deficits in adult hippocampal neurogenesis may be transient. These data support the regenerative capacity of the adult SGZ after HZE particle exposure and encourage additional inquiry into the relationship between radial glia stem cells and cognitive function after HZE particle exposure.



## 1. Introduction

Astronauts traveling to Mars during space flight missions will be exposed to damaging doses (~0.60 Sv/year) of galactic cosmic radiation (GCR) (Zeitlin et al., 2013). About half of this total annual dose is comprised of high linear energy transfer (LET) particles, including various high-energy, high-charge nuclei (HZE) that can damage cells and tissues due to their inherent properties. The damaging nature of these particles poses a serious risk to astronauts and therefore to mission success (Zeitlin et al., 2013). In particular, a major risk for mission success is the long-term effect of GCR on the adult brain (Cucinotta et al., 2009). Certain brain regions – like the hippocampus – continually give rise to new neurons throughout life (Kempermann, 2003), and the dividing cells in the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) appear particularly vulnerable to the damaging effects

*Abbreviations:* LET, linear energy transfer; HZE, high-energy, high charge nuclei; GCR, galactic cosmic radiation; GFP, green fluorescent protein; YFP, yellow fluorescent protein; SGZ, subgranular zone; DG, dentate gyrus; BrdU, 5-bromo-2′-deoxyuridine; DCX, doublecortin; NeuN, neuronal nuclei; IR, irradiation; BNL, Brookhaven National Laboratories.

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of GCR (Rola et al., 2004, 2005, 2008; Casadesus et al., 2005; Limoli et al., 2007; Encinas et al., 2008; Manda et al., 2008; Rosi et al., 2012; Rivera et al., 2013). It is notable that when GCR is mimicked using ground-based HZE particle radiation, deficits in DNA damage responses *in vitro*, DG SGZ adult neurogenesis *in vivo*, and cognition have been found (Manda et al., 2008; Rosi et al., 2012; Asaithamby and Chen, 2011; Britten et al., 2012; Cherry et al., 2012). However, studies have yet to examine the DNA damage response within the DG and compare various energies on SGZ adult neurogenesis. Therefore, it is important for the field of space research to assess the cellular response and array of energies that astronauts may receive during long-term space flight missions.

Currently, the literature supports a persistent deficit in hippocampal neurogenesis after HZE particle exposure. Many studies label exogenous (5-bromo-2′-deoxyuridine, BrdU) or endogenous (Ki67; doublecortin, DCX; and neuronal nuclei, NeuN) markers of adult neurogenesis to show HZE exposure-induced deficits in neurogenesis (Rola et al., 2004, 2005, 2008; Casadesus et al., 2005; Manda et al., 2008; Rivera et al., 2013). In addition, studies have also used transgenic reporter mouse lines to quantify, label, and track adult-generated hippocampal neurons and their putative source, nestin-expressing radial glial-like Type-1 cells (Encinas et al., 2008; Rivera et al., 2013). Assessment of these different "stages" of neurogenesis are important given the distinct capabilities of cells in each stage. For example, Type-1 stem cells rarely divide, but are multipotent, giving rise to both neurons and astrocytes; progenitors divide frequently, but are unipotent, giving rise to only neurons (DeCarolis et al., 2013; Encinas et al., 2011; Lagace et al., 2007). Therefore, the resistance or susceptibility of Type-1 stem cell number to HZE particle irradiation (IR) may determine whether repopulation of hippocampal neurons is possible in the long-term, even if there is a decrease in hippocampal neurons (e.g. BrdU/NeuN immunoreactive [+] neurons) or even rapidly-dividing progenitors (e.g. Ki67+ cells). Two recent studies examined the influence of HZE particles on Type-1 stem cells (Encinas et al., 2008; Rivera et al., 2013). While both studies showed radiation-induced deficits in neurogenesis, the one study that looked several months post-irradiation (post-IR) showed no change in the number of nestin-expressing radial glia Type-1 cells (Rivera et al., 2013). Given this persistence of radial glia Type-1 cells at a long time point post-IR, is it possible for adult neurogenesis to recover from $^{56}$Fe particle exposure? This question of recovery has important implications for risk assessment for spaceflight missions but also is important in understanding the dynamic life-long process of adult neurogenesis.

The main goal of this study was to employ nestin transgenic reporter mouse lines to clarify to what extent neurogenesis deficits recover after $^{56}$Fe particle exposure to various mission-relevant doses and LET. In addition, a secondary goal of this study was to examine the influence of energy of $^{56}$Fe particle on neurogenesis and genomic instability. We exposed mice from two different nestin transgenic lines (one constitutive and one inducible reporter) to $^{56}$Fe particles, and quantified the changes in neurogenesis at early (24 hours [h]), intermediate (7 days [d]), and long (2–3 months [mo]) time points post-IR. The constitutive Nestin-GFP mice allow quantification of adult neural stem cells (e.g. radial glia Type-1 cells, Encinas et al., 2008; Rivera et al., 2013; Yamaguchi et al., 2000, which are GFP+ with a radial morphology). The NestinCreER$^{T2}$/R26R:YFP mice allow inducible labeling of neural stem cells and their progeny (Lagace et al., 2007). We combine the use of these nestin transgenic lines with 2 more classic approaches to assess neurogenesis: 1) administration of a mitotic marker, BrdU, and quantification of cells at time points post-injection to assess proliferating, differentiating, and surviving cells; 2) quantification of immature neurons that express the endogenous protein DCX. Based on prior studies showing long-term deficits in adult hippocampal neurogenesis (Manda et al., 2008; Rivera et al., 2013; Britten et al., 2012; Cherry et al., 2012) and behavior (Britten et al., 2012; Cherry et al., 2012; Shukitt-Hale et al., 2000; Villasana et al., 2010) after HZE particle exposure, we hypothesized there would be no recovery of neurogenesis after $^{56}$Fe particle exposure. In keeping with our recent work (Rivera et al., 2013), HZE particle exposure did not decrease the number of radial glia Type-1 cells. Consistent with prior work showing long-term deficits in neurogenesis (Rola et al., 2004; Manda et al., 2008; Rivera et al., 2013) and *in vitro* work (Asaithamby and Chen, 2011), HZE particle exposure induced long-term cellular evidence of genomic instability, as indicated by increase in 53BP1 puncta (Groesser et al., 2011). Surprisingly, HZE particle exposure resulted in only a transient decrease in the number of adult-generated hippocampal neurons. These data support the regenerative capacity of the adult SGZ after HZE particle exposure and encourage additional inquiry into the relationship between radial glia Type-1 cells and cognitive function after HZE particle exposure.

## 2. Methods

### 2.1. Animals

Animal procedures and husbandry were in accordance with the National Institutes of Health and Guide for the Care and Use of Laboratory Animals and were approved by the UT Southwestern Medical Center (UTSW) Animal Care and Use Committee. Mice were group housed (n = 2–5/cage) with *ad libitum* access to food and water on a 12 h light-dark cycle. Homozygous Nestin-GFP mice (Yamaguchi et al., 2000) were generated and maintained on a C56BL/6J background. Homozygous Nestin-CreER$^{T2}$ transgenic mice were also generated and maintained on a C57BL/6J background and were crossed to homozygous R26R:YFP knock-in mice to produce Nestin-CreER$^{T2}$/R26R:YFP ("Nestin-Cre/YFP") bitransgenic mice. Genotyping and characterization of both mouse lines have been described elsewhere (Rivera et al., 2013; Lagace et al., 2007; Yamaguchi et al., 2000; Battiste et al., 2007; Ables et al., 2010). At 5–6 weeks of age, Nestin-Cre/YFP mice were administered tamoxifen (TAM, 180 mg/kg/day, i.p.) for 5 d to induce recombination in nestin-expressing cells and their progeny (DeCarolis et al., 2013; Lagace et al., 2007). For all studies, mice from both sexes were used since pilot studies showed no sex-dependent differences (Lagace et al., 2007).

### 2.2. Particle irradiation

Mice acclimated to Brookhaven National Laboratories (BNL) 2–5 d before irradiation. Mice received whole-body irradiation with 1 Gy $^{56}$Fe at 1000 MeV/n (LET: 148 keV/μm, Figs. 1–2) or 300 MeV/n (LET: 240 keV/μm, Figs. 1, 3–4) with a dose rate of 1 Gy/min at BNL NASA Space Radiation Laboratory (NSRL) as previously described (Rivera et al., 2013). We elected to use 1 Gy because this dose permits each cell in the hippocampus to have 1–3 hits per cell (Encinas et al., 2008) and has been widely used for rodent studies (Rola et al. 2004, 2008; Limoli et al., 2007; Encinas et al., 2008; Rivera et al., 2013; Cherry et al., 2012; Shukitt-Hale et al., 2003). We chose these energies because 1000 MeV/n is the highest energy available at BNL [NSRL Technical Note TN10-001] and has been commonly used for *in vivo* studies (Rola et al., 2004; Limoli et al., 2007; Encinas et al., 2008; Britten et al., 2012; Cherry et al., 2012; Shukitt-Hale et al., 2000, 2003); 300 MeV/n has also been used for *in vivo* studies (Rola et al., 2005; Rivera et al., 2013). An additional reason to use these parameters is that we wanted to compare our findings in whole body irradiation non-anesthetized mice to prior work where cranially-directed 1000 MeV/n and 1 Gy decreased Nestin-GFP+ stem cell number