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Opinion/Position paper

Late effects of ^{1}H irradiation on hippocampal physiology

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ABSTRACT

NASA's Missions to Mars and beyond will expose flight crews to potentially dangerous levels of charged-particle radiation. Of all charged nuclei, ^{1}H is the most abundant charged particle in both the galactic cosmic ray (GCR) and solar particle event (SPE) spectra. There are currently no functional spacecraft shielding materials that are able to mitigate the charged-particle radiation encountered in space. Recent studies have demonstrated cognitive injuries due to high-dose ^{1}H exposures in rodents. Our study investigated the effects of ^{1}H irradiation on neuronal morphology in the hippocampus of adult male mice. 6-month-old mice received whole-body exposure to ^{1}H at 0.5 and 1 Gy (150 MeV/n; 0.35–0.55 Gy/min) at NASA's Space Radiation Laboratory in Upton, NY. At 9-months post-irradiation, we tested each animal's open-field exploratory performance. After sacrifice, we dissected the brains along the midsagittal plane, and then either fixed or dissected further and snap-froze them. Our data showed that exposure to 0.5 Gy or 1 Gy ^{1}H significantly increased animals' anxiety behavior in open-field testing. Our micromorphometric analyses revealed significant decreases in mushroom spine density and dendrite morphology in the Dentate Gyrus, Cornu Ammonis 3 and 1 of the hippocampus, and lowered expression of synaptic markers. Our data suggest ^{1}H radiation significantly increased exploration anxiety and modulated the dendritic spine and dendrite morphology of hippocampal neurons at a dose of 0.5 or 1 Gy.

1. Introduction

NASA's efforts to continue manned space exploration over the next two decades includes a mission to Mars in the late 2030 s, preceded by several Deep Space Gateway missions where humans will be subjected to the deep-space radiation environment without protection from Earth's magnetosphere for extended periods of time. Projected round-trip missions to Mars will range from 560 to 1,100 days depending on launch windows and final mission design (Bret and Drake, 2014). Data from *Curiosity* on Mars and aboard its transit vehicle, the *Mars Science Laboratory* have provided an approximate total dose equivalent for an 850 day mission near solar maximum of approximately 1.01 Sv (Hassler et al., 2014; Zeitlin et al., 2013).

The biological challenges presented by a manned mission to Mars are unique as humans have never been exposed to microgravity or the complex field of the high-energy charged particles comprising the deep-space radiation environment for the duration of multiple years before. Charged particle radiation in deep space originates either from galactic cosmic rays (GCR), which provide a constant exposure, or solar particle events (SPE), which modulate according to the 12-year solar cycle (National Council on Radiation Protection and Measurements 2006). High-energy protons (^{1}H) are the most abundant charged particle in the deep-space environment, constituting approximately 85% of GCR, and the majority of ejected coronal plasma during SPE (National Council on Radiation Protection and Measurements 2006; Nelson, 2016). In addition, SPE are extraordinarily dangerous in that discriminate events are currently unpredictable, and are capable of delivering a high dosage in a short period of time (Bret and Drake, 2014). ^{1}H alone deliver approximately 50%–60% of the constant total organ dose due to GCR exposure in deep space (Nelson et al., 2016).

NASA has adopted a permissible exposure limit for its personnel such that a lifetime risk of death due to work exposure cannot exceed 3% on a 95% confidence interval. This limitation places the lifetime risk of death of astronauts due to radiation on one mission to Mars at

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approximately three times the current limit. However, risk estimates due to deep-space radiation are currently based on radiation-induced carcinogenesis, damage to blood forming organs, skin and lens, most of which would likely manifest several years after return (Nelson et al., 2016). Recent evidence shows that charged particle radiation induces deficits on organ systems such as the circulatory, immune and central nervous systems (CNS). The possible acute CNS risks to in-flight radiation exposure include motor, cognitive and social deficits, which provide serious concerns for in-flight complications, potentially compromising a mission. In addition, there is also concern for the long-term welfare of astronauts, whose CNS may undergo premature aging, and amyloid-β buildup upon return (National Council on Radiation Protection and Measurements 2006; Nelson et al., 2016; National Council on Radiation Protection and Measurements 2000; Cherry et al., 2012).

NASA's risk-factor modeling for radiation-induced detriments to the CNS correspond to the hippocampus, which is vital to the processes of memory consolidation, retrieval, and executive function (Nelson et al., 2016; Cameron and Glover, 2015). The stochastic nature of charged particle interactions with normal tissues presents a challenge in predicting biological outcomes due to irradiation. However, recent *in silico* work involving the interactions of cross-sections via Monte-Carlo simulations with neurons suggests dendritic spine and neurogenesis sensitivity to charged-particle radiation (Alp et al., 2015; Belov et al., 2016; Cacao and Cucinotta, 2016). Indeed, various in vivo studies are beginning to categorize the resulting changes in micromorphologies of hippocampal neurons in response to charged particles of different dosages, energies and atomic number. Recent publications reveal changes in dendritic complexity, length, branching, volume, and spine subtypes (Parihar et al., 2016; Parihar et al., 2015; Allen et al., 2015). In addition, there is evidence of radiation-induced insults to neurogenesis in the granule cell layer of the dentate gyrus (DG) (Rola et al., 2008; Rola et al., 2005; Sweet et al., 2014). Furthermore, high-energy charged particles appear to alter the striatal dopaminergic system associated with some motor and learning processes (Joseph et al., 1992; Denisova et al., 2002).

The processes of memory formation and retrieval are multi-faceted and complex. In this respect, no morphological or molecular experiment can yet single-handedly define cognitive deficits in irradiated animals despite similarities in neurological diseases and radiation treatment. Yet, some studies examining morphological and molecular changes to hippocampal and prefrontal cortex neurons as a result of high-energy ^{1}H irradiation ultimately show behavioral deficits in cognitive task paradigms. Rats exposed to 3 and 4 Gy (250 MeV/n) showed defecits in open field exploratory behavior as well as reduced habituation to acoustic startle habituation and rotarod performance shortly after exposure (Pecaut et al., 2002). Mice exposed to doses of 0.5 or 2 Gy (150 MeV/n) showed deficits in the NOR and Object-in-Place tests at 1-month after radiation and mice irradiated at 0.5 Gy (150 MeV/n) displayed reversal learning deficits in the Morris Water Maze (MWM) paradigm (Parihar et al., 2015; Bellone et al., 2015). NOR deficits have been observed even at the low dose of 0.1 Gy (150 MeV/n) three months postexposure in mice (Raber et al., 2016). However, not all studies examining behavioral changes due to ^{1}H show deficits in cognitive behavior. Rats exposed to 1.5, 3 and 4 Gy (250 MeV/n) showed no changes in conditioned taste aversion, or spatial learning via the MWM (Shukitt-Hale et al., 2004). Mice treated with 2 Gy (150 MeV/n) showed no signs of impaired spatial learning or memory in the MWM task, and a study examining changes in a bar press task in rats irradiated at 4 Gy (250 MeV/n) reports no changes due to treatment (Dulcich and Hartman, 2013; Rabin et al., 2002). This study reports behavioral, morphological and molecular changes to granular and pyramidal neurons of the hippocampus as a result of exposure to ^{1}H at 0.5 or 1 Gy (150 MeV/n) at the novel time point of 9 months post exposure.

2. Materials and methods

2.1. Animals and irradiation

The Institutional Animal Care and Use Committees of the University of Arkansas for Medical Sciences (UAMS) and Brookhaven National Laboratory (BNL) have approved all procedures outlined in this publication. Male C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME) and were housed 5 per cage. Throughout the duration of the study, mice received standard rodent chow that was low in soy (2020X, Harlan Laboratories), water ad libitum, and were housed on a 12:12 h light:dark cycle. At 6 months of age, mice were transported to BNL by overnight airlift. At BNL, mice were again administered the 2020X diet, water ad libitum, and were housed on a 12:12 h light:dark cycle. After a one week acclimation, mice were exposed to whole-body irradiation at the NASA Space Radiation Laboratory (NSRL) at BNL. For this purpose, mice were individually placed in well-ventilated clear Lucite cubes, and then placed within the NSRL beam line, 5 mice at the time. Mice received a single dose of ^{1}H (150 MeV, 0.5 Gy, 0.35–0.55 Gy/min), and immediately after exposure, mice were placed back in their cage. Radiation dosimetry was performed by the NSRL physics team. Sham-irradiated mice were also transported to NSRL and placed in clear Lucite cubes, but were not exposed to ^{1}H. Two days after irradiation or sham treatment, mice were returned to UAMS by overnight airlift. Upon return, mice were administered 2020X chow, containing 150 ppm fenbendazole, for 8 weeks, as a routine UAMS quarantine procedure.

2.2. Behavioral testing

Behavioral testing was performed 9 months after irradiation. Animals underwent behavioral testing for the open field paradigm, a two day procedure in which animals freely explore an empty arena for ten minutes each day. Animals are placed in the center of the arena and measures of locomotor activity and animal location are taken for the duration of the session. The arena is a cube consisting of an aluminum floor, opaque leucite walls and an open ceiling. The arena was wiped clean with 20% EtOH solution after each trial. Each session was recorded on a charge-coupled device video camera, located above the maze for automatic behavioral analysis with EthoVision software version 11 (Noldus Information Technology).

2.3. RNA extraction and quantitative reverse transcription polymerase chain reaction (qRT-PCR)

9 months after irradiation, mice were anesthetized by isoflurane inhalation, and hippocampi were dissected from each treatment group ($n = 10$), immediately frozen in liquid nitrogen, and subsequently stored at −80 °C. Total RNA was extracted from hippocampal tissue with the AllPrep DNA/RNA extraction kit (QIAGEN, Valencia, CA), according to the manufacturer's protocol. RNA quality and quantity was assessed on a Nanodrop 2000 instrument (Thermo Scientific, Waltham, MA). cDNA was synthesized with random primers and a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA), according to the manufacturer's protocol (Life Technologies, Grand Island, NY). The levels of gene transcripts were determined by qRT-PCR with TaqMan Gene Expression Assays (Life Technologies, and Integrated DNA Technologies, Coralville, IA), according to the manufacturer's protocol. In all cases, GAPDH was used as an internal reference gene, and fold changes were calculated with the 2-ddCt method. Measurements were taken in duplicates.

2.4. Golgi staining

To establish the impact of ^{1}H on mature neuronal morphology, brains from mice exposed to ^{1}H were Golgi stained and analyzed for