HIGH-ENERGY (HZE) RADIATION EXPOSURE CAUSES DELAYED AXONAL DEGENERATION AND ASTROGLIOSIS IN THE CENTRAL NERVOUS SYSTEM OF RATS

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Introduction
Astronauts assigned to long-duration beyond-earth-orbit space missions will be exposed to numerous forms of radiation that differ from radiation humans encounter on Earth. Currently, radiation concerns for astronauts involve radiation particles such as protons and electrons located in the Van Allen Belt which are entrapped by the Earth’s intrinsic magnetic field. Once outside the Van Allen Belt, astronauts will be exposed to Galactic Cosmic Rays (GCR) and Solar Energetic Particle Events (SPEs) in the form of high-charge high-energy (HZE) radiation particles [Todd, 2003; Hagen, et al., 1989]. An interesting and unique feature of HZE particles is that they are able to distribute their energy in linear fashion, creating single tracks and discrete microlesions [Todd, 1989]. It is theorized that this distinct attribute of HZE radiation will result in the direct killing of non-dividing cells, such as neurons [Hagen, et al., 1989]. However, the effects of HZE on the CNS are not well understood. It has been estimated that up to 13 to 50% of the cells in the CNS will be traversed by at least one HZE particle during a 2 to 3 year mission to Mars. [Curtis, et al., 1998].

Radiation and Inflammation
Astrocytes and microglia are important cellular mediators of CNS inflammation. The activation of astrocytes (reactive astrocitosis or gliosis) is a central response to CNS injury. The characteristics of astrocitosis are astrocytic proliferation, hypertrophy of astrocytic cellular processes and upregulation of glial fibrillary acidic protein (GFAP) expression [Hwang, et al., 2006]. Astroctosis prevents neuroregeneration and neurite outgrowth and form what is termed a glial scar, the permanent sequelae of CNS injury [Chiang, et al., 1993]. It is our hypothesis that HZE exposure will result in reactive changes within the CNS and that these changes will manifest as gliosis with subsequent neuronal loss. It is possible that this neuroinflammatory process may have an important role in the development of cognitive dysfunction in future astronauts.

Materials and Methods
Sprague-Dawley rats were delivered directly to Brookhaven National Laboratory (BNL) from the vendor (Harlan, Oregon, WI). On the day of irradiation rats were transported to the NASA Space Radiation Laboratory (NSRL) beamline at BNL. Each animal was anesthetized with Isoflurane in air (4% induction, 1.5% maintenance) and placed in custom designed stereotaxic cradles to stabilize the head position and facilitate insertion of the animals into the beamline. Controls were treated identically without being inserted into the beamline. Within 24-48 hours after irradiation animals were sent to Loma Linda University (LLU) via courier. All protocols were approved by the Animal Health and Safety Committees of LLU and BNL and are in compliance with Federal regulations.

Animals received whole-body irradiation with a 600 MeV/nucleon 56Fe beam in a single fraction of 0 or 4 Gy. Rats were euthanized at 1, 6, and 12 months post-exposure and brain tissue for histological analysis was stored in cryopreservative at −80°C. Four experimental animals and four controls (n=24) were used at each time point.

Free-floating sections were treated with antibodies directed against GFAP in order characterize the astrocytic response. GFAP-stained sections were scanned and processed with NIH software Image-J in order to quantify the amount of gliosis. Densitometry calculations were performed to determine the percent area staining for GFAP within the cerebral cortex. These percentages were compared to controls and results given as percent of control. In addition, entire brain sections stained with Gallyas silver were examined by light microscopy and the number of degenerating axons counted within the subcortical white matter adjacent to the regions stained with GFAP [Nadler, et al., 1983].

Statistical analyses were carried out by using Student’s t-test with Bonferroni corrections. A P value ≤ 0.05 was considered significant.

Results and Discussion
GFAP immunohistochemistry demonstrated astrogliosis in the form of hypertrophy of astroglial processes in

Gravitational and Space Biology Bulletin 20(2) June 2007 89
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of radiation exposure on astronaut health will be paramount to the development of successful strategies for future long-duration, deep space missions. Our observations suggest that exposure to HZE causes a delayed and sustained injurious process within the CNS of rats. It has been demonstrated elsewhere that HZE exposure results in decreased startle response, taste aversion learning, spatial learning and memory in mouse models. A study by Rabin et al. reported that deficits in these cognitive functions were present one year following irradiation, suggesting that neurological impairment may continue well past the exposure event [Rabin, et al., 2004]. Though the mechanism behind these changes is yet unknown, it can be postulated that they are the result of a neuroinflammatory process, similar to that seen with low-LET, high dose ionizing radiation. Given that patients who have undergone radiation therapy for brain tumors display similar reactive lesions, often in conjunction with cognitive deficits, the HZE-induced CNS damage might have serious implications on the ability of humans to maintain a prolonged presence in interplanetary space. Additional research is needed to fully understand the mechanism of HZE-induced CNS damage so that effective countermeasures can be developed. (Supported by NASA Grant #NNJ04HD80G to AO)

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Figure 1. Amount of gliosis following $^{56}$Fe exposure. Mo = months. Asterisk indicates values significantly different from control (p < 0.05).

When compared to controls, irradiated rats showed evidence of axonal degeneration in white matter tracts. The axonal degeneration was more pronounced at 12 months compared to 1-month post-exposure (1 Mo: 120; 12 Mo: 882 degenerating axons), suggesting a delayed injury mechanism. A p-value <0.05 was calculated at each time point (Figure 2). No statistical difference was observed between the control groups (p>0.5).

These findings demonstrate that exposure to HZE is a potent stimulator of CNS injury and suggest that both gliosis and axonal degeneration occur through a delayed and progressive mechanism.

The risk of radiation induced CNS injury is a major concern of the National Aeronautics and Space Administration (NASA). Understanding the implications of radiation exposure on astronaut health will be paramount to the development of successful strategies for future long-duration, deep space missions. Our observations suggest that exposure to HZE causes a delayed and sustained injurious process within the CNS of rats. It has been demonstrated elsewhere that HZE exposure results in decreased startle response, taste aversion learning, spatial learning and memory in mouse models. A study by Rabin et al. reported that deficits in these cognitive functions were present one year following irradiation, suggesting that neurological impairment may continue well past the exposure event [Rabin, et al., 2004]. Though the mechanism behind these changes is yet unknown, it can be postulated that they are the result of a neuroinflammatory process, similar to that seen with low-LET, high dose ionizing radiation. Given that patients who have undergone radiation therapy for brain tumors display similar reactive lesions, often in conjunction with cognitive deficits, the HZE-induced CNS damage might have serious implications on the ability of humans to maintain a prolonged presence in interplanetary space. Additional research is needed to fully understand the mechanism of HZE-induced CNS damage so that effective countermeasures can be developed. (Supported by NASA Grant #NNJ04HD80G to AO)

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