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PI Name:	Goldstein, Lee M.D., Ph.D.		
Project Title:	Effects of Space Radiation on Hippocampal-Dependent Learning and Neuropathology in Wild-Type and Alzheimer's Disease Transgenic Mice		
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Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
Joint Agency Name:		**TechPort:**	No
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Human Research Program Risks:	(1) **BMed**:Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders		
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COI Name (Institution):	Blakely, Eleanor (Lawrence Berkeley National Laboratory) Moncaster, Juliet (Boston University) Stanton, Patric (New York Medical College)		
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Task Description:	The hippocampus and dentate gyrus are critically important brain regions required for long-term memory formation. Damage to these critical brain regions contributes to memory deficits in patients with Alzheimer's disease. The hippocampus and dentate gyrus are also notable as sites where brain stem cells differentiate into new neurons throughout life, a process called neurogenesis. Exposure to space radiation can result in impairments in learning and long-term reduction in hippocampal neurogenesis. It is unknown how radiation causes these impairments and whether and by what mechanism(s) radiation exposure might predispose individuals to develop Alzheimer's disease. This proposal will utilize a well-characterized and widely used Alzheimer's disease transgenic mouse model (Tg2576) to address the following research objectives: (1) examine the long-term impact of space radiation (SR) on hippocampal-dependent spatial learning and memory, (2) evaluate the potential of SR to accelerate Alzheimer's disease pathogenesis and neuropathology, (3) evaluate a novel non-invasive laser-based eye scanner to detect and monitor molecular changes in the lens of the eye induced by radiation exposure and Alzheimer's disease pathology (Goldstein, et al., Lancet, 2003). A complementary companion study will utilize the same cohort of animal subjects to: (1) evaluate electrical communication between neurons, and changes in function and fine structure of neurons, including dendritic spines where synaptic contacts enable neuronal communication, (2) determine whether SR, in reducing neurogenesis, also alters the functionality of newly-born neurons, and (3) assess whether SR differentially affects electrical or physical function of neurons, and/or accelerates the Alzheimer's disease process. Our proposed studies directly address key objectives of the NASA Human Space Flight Program, including determination of potential space-related SR dependencies related to late CNS risks such as early-onset dementia or Alzheimer's disease, assessment of SR effects on molecular, cellular and tissue environment changes in hippocampus indicative of increased risk of dementia or Alzheimer's disease, and evaluation of biological models of Alzheimer's disease or other forms of dementia that occur in humans. The existing knowledge gap is immense and presents a major obstacle to rational assessment of short- and long-term risk to the central nervous system posed by SR exposure expected during extended human space travel. Our experiments will examine, for the first time, the mechanisms by which SR impairs synaptic function in normal brain, assess whether SR does, in fact, enhance long-term risk of Alzheimer's disease, and provide an experimental system to identify and evaluate new radiation countermeasures. The proposed interdisciplinary research program will provide an integrated scientific foundation to assess and reduce SR-induced risk to the brain, thus enabling a safe path forward for extended human space exploration.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	This project investigates Alzheimer's Disease (AD) vulnerability in the setting of exposure to low-dose particle space radiation. The goal of extended human space flight is predicated on establishing a solid scientific foundation for rational assessment of Central Nervous System (CNS) risk from exposure to space radiation. The same argument applies to development of prophylactic countermeasures. Exposure to particle radiations during long-duration space travel may induce subtle but deleterious late effects in neuronal function and propensity for neurodegenerative diseases, including AD. While acute CNS damage is a hallmark injury following exposure to high-dose radiation, investigation of late effects following exposure to low-dose particle space radiation may predispose the brain to development of slowly progressive age-dependent neurodegenerative disease. Moreover, if an AD diathesis is induced by space radiation exposure, the need for effective countermeasures will be of paramount importance for the human space flight program. In this project, we are using the well-characterized Tg2576 AD transgenic mouse model (Hsiao et al., 1996) to determine whether and to what extent exposure to low doses of 28Si and 56Fe particle radiation influence AD risk, latency, progression, and penetrance.
	A. Project Hypotheses & Specific Aims Project Hypotheses We hypothesize that exposure to low-dose particle space radiation will negatively and synergistically impact: (i) hippocampal-dependent learning and memory, and (ii) Alzheimer's disease (AD)-linked pathology in the brain and lens. We anticipate that these effects will be dose- and time-dependent. Furthermore, we hypothesize that cerebral microvasculature disruption and reactive neuroinflammation are critical radiation-induced pathogenic mechanisms by which hippocampal neurocognitive dysfunction and age-dependent AD pathology are synergistically accelerated. Understanding of these relationships is essential for rational assessment of CNS risk and efficient development of prophylactic countermeasures for extended human space travel. Specific Aim 1 Identify and characterize the effects of low-dose particle space radiation exposure on hippocampal-dependent spatial learning and memory using the Morris water navigation task (or alternative hippocampal-dependent spatial reference paradigm) in Tg2576 AD transgenic mice compared to age-matched wild-type controls. Specific Aim 2 Identify and characterize the effects of low-dose particle space radiation exposure on AD biochemistry and histopathology in Tg2576 AD transgenic mice compared to age-matched wild-type littermate controls. Specific Aim 3 Identify, characterize, and track the effect of low-dose particle space radiation on AD progression using a novel noninvasive laser-based eye scanner to quantitatively assess AD-linked Aß pathology in the lens. B. YR1 PROGRESS TO DATE (Sep 1, 2011 through Aug 31, 2012) Gain of Experience at the Brookhaven National Laboratory (BNL) NSRL facility September 2011 – June 2012: All Boston University and BNL IACUC protocols were submitted and approved for this project. Dr Goldstein's team conducted all necessary training online and on-site to be eligible to conduct experiments safely at BNL NSRL. Submission of beamline time request was submitted by Dr Goldstein and approved for 2 sessions.

Task Progress:

First session was conducted in June 2012 (Summer 2012) and the second in November 2012 (Fall 2012).

April 2012: Prior to the start of this project, Dr Lee Goldstein and his team had never worked at the BNL NSRL facility. Dr Goldstein has a longstanding collaboration with Dr Eleanor Blakely, Lawrence Berkeley National Laboratory, who is Co-I on this project. Her role is to advise Dr Goldstein regarding radiobiology experimental design and execution based on her prior experience in the field and at the BNL NSRL facility. In early April 2012, Dr Goldstein and his research technician Mark Wojnarowicz observed Dr Eleanor Blakely and her team conduct experiments at the NSRL facility.

First Experiments Conducted at BNL NSRL

June 2012: Dr Goldstein, Dr Juliet Moncaster, Mark Wojnarowicz (senior research technician), and Andrew Fisher (BU bioengineering doctoral candidate in the Goldstein laboratory) conducted their first experiments on the BNL NSRL beamline. The experiment involved whole-body irradiation vs sham irradiation of 2-month-old male C57BL/6 mice using beamline 56Fe (600 MeV/u, 181 keV/µm). Experiment objectives: (i) establish detailed workflow strategy and standard operating protocols (SOPs) for experiments conducted at NSRL, and (ii) evaluate background effects of 56Fe exposure on cerebral microvasculature and neuroinflammation assessed by ultrastructural (EM) neuropathological analysis. In consultation with Eleanor Blakely, Ph.D., Lawrence Berkeley National Laboratory (Dr. Blakely serves as Radiobiology Collaborator on this project), 2 particle radiation doses were chosen: 0 and 100 cGy. Each group was comprised of n=12 mice and will be sacrificed at 4, 10, and 20 months-of-age. Animal numbers were calculated as follows: 1 irradiation types (Fe) x 2 doses (0, 100 cGY) x 3 timepoints (4, 10, 20 mos) = 6 groups x 12 per group = 72 mice total. This pilot study will not only establish team familiarity with NSRL procedures and protocols, but also provide important information for our subsequent studies.

Experimental Changes: In our original proposal, we identified the Morris water maze (MWM) (Morris, 1984; Goldstein, 2003) as our behavioral assay of hippocampal-dependent spatial learning and memory. While the MWM is widely used for this purpose, this behavioral assay was designed specifically for rats, a species well adapted for navigating water environments. The MWM works poorly in certain mouse strains that are not water tolerant and innately assume a motionless floating posture when placed in a water environment. This behavioral response defeats the assay and undermines experimental utility. To overcome this obstacle in our studies, we now plan to deploy the Barnes maze (Barnes CA, J Comp Physiol Psychol, 1979), a well-characterized hippocampal-dependent, spatial learning and memory assay that does not utilize a water environment for behavioral testing. The Barnes maze provides a robust and reliable test of hippocampal-dependent spatial learning and memory in many different inbred mouse strains (Fox, 1999; Nguyen, 2000; Holmes, 2002; Koopmans, 2003; O'Leary, 2011; O'Leary, 2012) and Alzheimer's disease (AD) transgenic mouse models (Pompl, 1999; Brown, 2007; Reiserer, 2007; O'Leary, 2009). Importantly, the Barnes maze also provides secondary measures of gross locomotor function, exploratory behavior, higher-order working memory, and thigmotaxis (a sensitive measure of murine anxiety), that are relevant to our research objectives and NASA program priorities. We recently deployed the Barnes maze to assess hippocampal-dependent spatial learning and memory deficits in C57BL/6 mice exposed to a single experimental blast ((Goldstein, 2012), Science Translational Medicine). We will use the same behavioral testing paradigm in our NASA studies.

Barnes Maze Test of Hippocampal-Dependent Spatial Learning and Memory Retrieval: Neurobehavioral assessment will be performed using a combination open-field test and Barnes maze (Med-Associates, Inc., St. Albans, VT, USA). Open-field testing enables assessment of baseline locomotor functioning (average velocity), exploratory activity (total distance), and thigmotaxis (number of central zone entries). The test is performed by placing each test mouse in the middle of a 42.5 cm x 42.5 cm open arena and monitoring movement for 10 min using a 3D infrared diode motion detector system (Any-Maze, Stoelting Co., Inc., Wood Dale, IL). The Barnes maze utilizes the same experimental system to quantitatively assess hippocampal-dependent spatial learning and memory retrieval. Barnes maze evaluation is conducted using a 20-box apparatus illuminated with 900 lux surface light intensity. Mice are familiarized with the test apparatus by placement on the platform and gentle guidance to the escape box. Training sessions are conducted across four training trials per day for four days. The order of testing of individual subjects is the same throughout daily sessions, but randomized across the four test days for a total of 16 trials. To initiate testing, a single mouse is placed in the start box in the middle of the maze and released. Test subjects are evaluated while locating a single escape box placed at a constant position. Spatial learning is assisted by visual cues that remain constant during and across test sessions. Movement is tracked and recorded electronically as in the open-field test. Latency to find the escape box, trajectory velocity to the escape box, and total trajectory distance is assessed and recorded daily. Memory retrieval is evaluated by replacing the escape box with a blank box 24 hours after the last training session. Memory retrieval is electronically assessed by recording the number of nose pokes into the blank box as a percentage of total nose pokes.

We will test the influence of exposure to low-dose 56Fe particle radiation on cognitive impairment using the Barnes Maze in the male C57/bl mice we irradiated in June 2012. Behavioral results will be correlated with ex vivo analysis of brains via immunohistopathology, electron microscopic ultrastructural pathology focusing on microvasculopathy, and assessment of neuroinflammation using a variety of specific markers and techniques, including TSPO imaging of activated microglia and astrocytes (Chen, 2008).

C. YR2 EXPERIMENTAL PLAN

Fall 2012 Campaign: We will irradiate 4-month-old mice Tg2576 and wild-type controls using Beamline 56Fe (600 MeV/u, 181 keV/µm) at Brookhaven National Laboratory (BNL). These studies will be conducted in consultation with NASA and Eleanor Blakely, Ph.D., Senior Staff Biophysicist, Lawrence Berkeley National Laboratory, who serves as Radiobiology Collaborator on this project. We have chosen 4 particle radiation doses: 0, 10, 50, and 100 cGy. Each group will be comprise of n=12 mice and will be sacrificed at 10 months-of-age (+6 months s/p irradiation), and 18 months-of-age (+14 months s/p irradiation). Behavior in these mice will be assessed before irradiation, at the midpoint between irradiation, and again just prior to sacrifice and tissue harvest. Animal numbers are calculated as follows: 2 groups (Wt, Tg) x 2 genders (F, M) x 1 irradiation types (Fe) x 4 doses (0,10, 50, 100 cGY) x 2 timepoints (10mos, 18mos) = 32 groups x 12 per group = 384 mice total.

Spring 2013 Campaign: We plan to irradiate Tg2576 and wild-type controls mice with Beamline 28Si (300 MeV/u, 70 keV/µm) at BNL using the same doses (0,10, 50, 100 cGY) and age timepoints. Animal numbers are calculated as follows: 2 groups (Wt, Tg) x 2 genders (F, M) x 1 irradiation types (Si) x 4 doses (0,10, 50, 100 cGY) x 2 timepoints (10mos, 18mos) = 32 groups x 12 per group = 384 mice total.

All mice will be behaviorally assessed on a hippocampal-dependent spatial learning and memory task using the Barnes Maze before irradiation and evaluated using a battery of neurobehavioral, neuropathological, and biomarker endpoints

	at selected post-irradiation intervals endpoints as a function of: (i) particle radiation exposure (Z, energy, dose), (ii) genotype (Tg, Wt), (iii) post-exposure interval, (iv) age and gender. See our recent publication (non-NASA support) for additional details regarding immunohistochemical, ultrastructural, and neurobehavioral index metrics (Goldstein, 2012, Science Transl Med; http://dx.doi.org/).
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