Fiscal Year:	FY 2022	**Task Last Updated:**	FY 06/13/2022
PI Name:	Nelson, Gregory A. Ph.D.		
Project Title:	VNSCOR: Responses of the Nervous System to Chronic, Low Dose Charged Particle Irradiation		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **HFBP**:Human Factors & Behavioral Performance (IRP Rev H)		
Human Research Program Risks:	(1) **BMed**:Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders (2) **Immune**:Risk of In Mission Impacts, Adverse Health Events or Long-Term Health Impacts due to Altered Immune Response (3) **Sensorimotor**:Risk of Altered Sensorimotor/Vestibular Function Impacting Critical Mission Tasks		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	92350-1700	**Congressional District:**	31
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2016-2017 HERO NNJ16ZSA001N-SRHHC. Appendix E: Space Radiobiology and Human Health Countermeasures Topics
Start Date:	04/15/2018	**End Date:**	08/31/2025
No. of Post Docs:	2	**No. of PhD Degrees:**	
No. of PhD Candidates:	2	**No. of Master' Degrees:**	
No. of Master's Candidates:		**No. of Bachelor's Degrees:**	1
No. of Bachelor's Candidates:		**Monitoring Center:**	NASA JSC
Contact Monitor:	Whitmire, Alexandra	**Contact Phone:**	
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Flight Program:			
Flight Assignment:	NOTE: End date changed to 08/31/2025 per L. Juliette/JSC (Ed., 5/7/22)		
Key Personnel Changes/Previous PI:	June 2022 report, per the PI: Andrew Wroe left Loma Linda University in 2021 for a clinical medical physics position in Miami, Florida and is no longer with this project (Ed, 7/22/22).		
COI Name (Institution):	Hartman, Richard Ph.D. (Loma Linda University) Mao, Xiao Wen M.D. (Loma Linda University) Rosi, Susanna Ph.D. (University of California San Francisco)		
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Task Description:	This project is a combined experimental campaign with "Mechanisms of Radiation-Induced Neurobehavioral Deficits" (PI: C. Davis) to quantify responses for an interrelated set of central nervous system (CNS) outcome measures in mice to acute and protracted exposures to protons, simulated galactic cosmic rays and gamma rays. An initial definition phase review resulted in modifications to the original experimental plan to take advantage of new irradiation capabilities and to coordinate approaches with the Davis project by incorporating both projects into a virtual NSCOR or VNSCOR program project. While the administrative start date for the project is April 15, 2018, funds were received for experimental work in February 2019. The post definition phase task descriptions are provided below. Evidence has accumulated from animal studies that the central nervous system (CNS) undergoes deleterious changes after exposure to charged particle radiation such as protons and high atomic number atomic nuclei that are found in space as galactic cosmic rays and solar particle events. Observed changes include inflammation, oxidative stress, loss of neuron (dendrite) branches and connections (synapses), altered signaling molecules, altered electrical properties, loss of blood vessels, and impaired behavioral performance. If humans respond to charged particles in the same way as animals, then it is possible that deleterious changes may be sufficient to cause cognitive and other behavioral impairments that could compromise spaceflight missions and astronaut health. The current evidence is based primarily on short exposures to single radiation types. However, space radiation is a complex mixture of these particles and exposures accumulate gradually over the course of missions. It is well established in radiation biology that reduction of the dose rate can have a profound effect on the outcome. Therefore, to better simulate the space environment, we propose to expose adult mice to either protons or mixtures of charged particles using the NASA/ Brookhaven National Laboratory (BNL)-developed 33-ion galactic cosmic ray simulation protocol (GCRsim). Then we will deliver the exposures over 4 weeks in 24 short exposures (fractions) compatible with particle accelerator operations. These results would be compared to results from acute exposures to establish the Dose Rate Effectiveness Factors (DREFs) which are needed for risk estimation for astronaut health. We predict that the high numbers (fluence) of protons will result in multiple traversals of cells within short times that may elicit interacting biological responses, whereas the lower fluence of higher charged ions will result in rare independent events. DREFs > 1 are predicted for protons and DREFs ~1 are predicted for high Z particles. We will also compare the "chronic" or fractionated exposures of charged particle mixtures to gamma rays to determine whether they have equivalent dose effects or are more effective. The relative biological effectiveness factor (RBEs) will be derived. These RBEs are utilized in predicting densely ionizing radiation effects in humans for whom only gamma ray and X-ray data are available with the assumption that the ratios obtained in animal models are realistic surrogates for humans. For this project, mice will be irradiated with a broad energy spectrum of protons in acute and protracted (12 fractions over 4 weeks) exposures at a dose of 0.5 Gy and sham controls; acute and protracted (24 fractions over 4 weeks) exposures to 0.25 and 0.5 Gy of charged particles (33 ion GCR simulation, (GCRsim)); and acute and protracted (24 fractions over 4 weeks) exposures to 0.75 and 2.0 Gy of 137-Cs gamma rays. All proposed work will use wild type mice and will be performed under Institutional Animal Care and Use Committee (IACUC) approved protocols in AAALAC-certified facilities at Loma Linda University (LLU), the University of California (UCSF), and Brookhaven National Laboratory (BNL). For all three specific aims the species is Mus musculus, strain C57Bl/6J. Ages are 5 - 6 months at acquisition and the beginning of irradiation procedures. We will test both male and female animals as their responses are not identical and the astronaut population is of mixed sex. Scheduled sacrifices are at 30-45 days, 90-110 days and 9 months post-irradiation. For each of the exposure regimens we will conduct a battery of behavior tests, explore task-driven neuronal pathway activation patterns using c-fos imaging, quantify changes in selected gene expression patterns, and quantify selected biomarkers and the structure of the tissue using state of the art biochemical, histochemical, and microscopy methods. This will allow us to identify the underlying physiological changes most sensitive to dose rate and radiation quality and how they combine to produce behaviors that are adaptive or maladaptive. The Covid-19 pandemic disrupted the 2020 BNL experimental campaign resulting in a 1-year delay in implementing GCR exposures. Therefore, during this period, exploratory studies of chronic mild stress were initiated to model multiple spaceflight stressors (e.g. altered gravity, isolation and confinement, sleep disruption). This will be followed up with experiments using proton exposures with chronic stress to test interactions of multiple stressors with radiation. BNL operations were restored allowing us to expose male animals to GCRsim as planned in the 2021 campaign and most recently females in the 2022 campaign. GCRsim data on males has now been analyzed and behavioral analysis for females will begin in late June 2022. Together the data generated by the project will enhance NASA's ability to translate animal assessments of CNS (central nervous system) structure and function to humans, and to update risk estimates based on single radiation species, high dose rate irradiation protocols, to higher fidelity space-like exposures of charged particle mixtures delivered at dose rates approaching those observed in space.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The primary research impact to NASA is in estimation of potential adverse cognitive and behavioral effects of exposures to space radiation on long (e.g., 3-year Mars missions) deep space missions where exposures are of a chronic nature and are comprised of complex mixtures of charged particles dominated by protons. Such adverse effects could affect in-mission performance as well as post mission health of crew members. The experimental plan uses radiation fields and exposure conditions scaled to the life span of the experimental animal model. On Earth, the principal benefits will be for estimation of health risks to humans from charged particles in the environment (e.g., Radon alpha particles) and potential side effects of particle-based radiotherapy (e.g., proton and carbon beams) which requires pre-clinical animal studies. The current task will provide insight into adverse effects on normal brain tissue from proton exposures similar to those expected for normal tissues outside tumor treatment volumes in head & neck and brain tumor treatment scenarios. Low dose rate exposures to protons as well as 33-ion GCRsim radiation fields with substantial proton and helium components will inform risk estimates for the general population due to Radon exposures and for first responders to radiological accidents (e.g., Fukushima, Chernobyl).

Task Progress:	Protons To date, two proton irradiation campaigns have been conducted and have provided biological replicates for measurements. Subsequent experiments examine simulated galactic cosmic radiation (GCR) spectra for which protons are the main component. A battery of 15 behavioral tests assesses cognitive, affective, and sensorimotor performance in both male and female mice at 1-2, 3-5, & 9 months post irradiation/IR (referred to as “1 month”, “3 month” and “9 month” time points); 9-month measurements were not originally planned, but resulted from Covid-19 related restrictions on animal care facility access and have been continued. Briefly, in cases where 50 cGy proton exposure resulted in altered behavioral parameters, the percent change from sham values ranged up to 74% and the magnitude of change from fractionated exposures often exceeded that for acute exposures. This included: Y-maze, elevated plus maze, light-dark box, open field exploration, novel object and novel place recognition, sociability and social recognition, modified balance beam, tail suspension, and forced swim tests. Fractionated exposures were notably more effective for step-through passive avoidance. Other behaviors were not significantly affected by irradiation. To assess neuronal network function, expression of the immediate early gene c-fos driven by fear memory (passive avoidance) was measured in selected brain regions of male mice at 3 months post IR. Passive avoidance testing elicited strong gene expression in multiple brain regions, compared to home cage controls. Prior radiation exposure resulted in altered c-fos expression reflecting changes in behaviorally driven network activity. To determine which molecular markers and signaling pathways underlie outcomes of the treatment conditions, we used NanoString® technology to profile mRNA expression at 3 months post irradiation of male mice in orbitofrontal cortex, hippocampus, and cerebellum. A set of 760 genes from the nCounter® Mouse Neuropathology panel for 23 fundamental pathways were examined and were characterized according to annotations for: structural integrity, metabolism, neuroinflammation, neuron-glia interaction, plasticity and aging, and neurotransmission. Expression patterns varied with radiation dose rate and brain region. In the hippocampus, pathway activation was generally similar for fractionated vs acute expression, while in the frontal cortex, fractionated exposure differed substantially from either sham or acute exposures. Chronic Mild Stress During the Covid-19 driven delay in the use of Brookhaven National Laboratory (BNL) irradiation facilities, we conducted experiments using the chronic mild stress (CMS) model to simulate combined stressors experienced during spaceflight. This well-vetted model delivered mild stress from disruption of cage environment, lighting, social interactions, predator cues, etc., over a 4-week period as a surrogate for the multisensory set of non-radiation spaceflight stressors. The CMS exposures were conducted from 1/4/2021 to 2/1/2021 and tests were performed at 1-2 or 4-5 weeks after the exposure. We measured selected behavioral outcome measures, as described above, along with stress hormone, corticosterone, and a suite of cytokine. Several anxiety-related outcome measures showed strong increases at 1 and 4 weeks post CMS including light-dark, open field, and elevated plus maze tests while cognitive measures were less responsive. Corticosterone levels were elevated as expected. In the future, the CMS regimen will be combined with a 0.5 Gy proton exposure to characterize interactions of the combined stresses. GCRsim 236 male mice were exposed to 50 cGy GCRsim (sham, acute, and fractionated regimens) and 2 Gy fractionated gamma rays during the NASA Space Radiation Laboratory (NSRL) 2021 campaign in April/May 2021 by special arrangement between NASA and BNL. Behavioral batteries described above for protons were conducted at 1, 3, and 9 month time points with final data acquisition in March 2022. The 3 month c-fos expression histological samples have been archived and are undergoing counting. Brain tissue samples from 7 regions have been frozen for biochemical analysis and hemibrains fixed for histology. 176 female and 60 male mice were exposed to GCRsim and gamma rays from 4/11/22 to 5/6/22 during the NSRL22A campaign and shipped back to Loma Linda University where they are under quarantine. It is expected that behavioral testing will begin the week of June 20, pending health certification. GCRsim exposure elicited a number of behavioral changes in male mice at all three time points, which also allowed us to follow the time course for certain outcome measures. Preliminary results indicate that GCRsim exposure elicited changes in many outcome measures, and gamma rays were also effective, which will permit estimation of relative biological effectiveness (RBE) values. GCRsim exposures did not significantly affect distance or time-in-location measures in the open field, while gamma rays increased locomotion and reduced freezing. Light-dark box tests revealed increased locomotion and reduced anxiety (increased time in light zone and transitions), which was time course dependent. Balance beam revealed elevated locomotion and reduced anxiety. Tail suspension depression-like behavior (learned helplessness) showed enhanced “depression” at 1 month which resolved after 3 months. Working memory (Y maze spontaneous alternation) showed enhancement at late times with fractionated GCRsim and gamma rays. Fear memory (passive avoidance) was insensitive to GCRsim. DREF Estimates Characterization of dose rate effects by a simple parameter like the Dose Rate Effectiveness Factor (DREF) is problematic for central nervous system (CNS) outcome measures. Behavioral outcome measures reflect complex interactions of motivation, sensory and motor function, emotional status, etc., and outcome parameter values reflect the balance between conflicting behavioral drives such as anxiety and curiosity. Thus, deviations from the control values can be positive or negative – reflecting an altered, but stable new state not necessarily interpretable as detrimental – and the raw measures come in a variety of units such as distance, time, force, etc., making them hard to pool. We have turned to standardized effect size measures such as Cohen’s d, Hedge’s g and z-scores to transform the data to a single scale (units of standard deviation from control means) which has enabled us to develop distributions of DREF values for proton and GCRsim exposures (primarily from males). Preliminary DREFs for all pooled behavioral measures were 1.64 +/- 0.55 for protons and 1.4 +/- 0.42 for GCRsim, similar to the estimate of 2 used by radiation risk advisory bodies such as the International Commission on Radiological Protection (ICRP). Reporting To date, presentations of experimental findings have been made to the 2019, 2020, and 2021 annual meetings of the Radiation Research Society and to the 2020, 2021, and 2022 NASA Human Research Program (HRP) Investigators’ Workshops. Publications on proton-irradiations conducted in 2019 - 2020 are in preparation, and one paper describing oxidative stress and vascular changes in the optic nerve head has recently been published.
Bibliography Type:	Description: (Last Updated: 03/19/2025)

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