Fiscal Year:	FY 2024	**Task Last Updated:**	FY 02/10/2024
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		
PI Email:	grnelson@llu.edu	**Fax:**	FY 909-558-4035
PI Organization Type:	UNIVERSITY	**Phone:**	909-558-8364
Organization Name:	Loma Linda University		
PI Address 1:	Basic Sciences, Div. Radiation Research 11175 Campus Street		
PI Address 2:	Chan Shun Pavilion, Room A-1024		
PI Web Page:			
City:	Loma Linda	**State:**	CA
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:**	12/31/2025
No. of Post Docs:	2	**No. of PhD Degrees:**	1
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:**	
Contact Email:	alexandra.m.whitmire@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: End date changed to 12/31/2025 per JSC Grants Office (Ed., 1/21/25) NOTE: End date changed to 12/31/2024 per JSC Grants Office (Ed., 4/26/23) NOTE: End date changed to 08/31/2025 per L. Juliette/JSC (Ed., 5/7/22)		
Key Personnel Changes/Previous PI:	Subcontract by Susanna Rosi, Ph.D. at the University of California San Francisco has been completed. Per the PI, the setup of behavioral experiments with Dr. Richard Hartman was completed by end of Year 2; after Year 2, Dr. Hartman left the project (Ed., 5/21/23).		
COI Name (Institution):	Mao, Xiao Wen M.D. (Loma Linda University) Rosi, Susanna Ph.D. (University of California San Francisco)		
Grant/Contract No.:	80NSSC18K0785		
Performance Goal No.:			

Performance Goal Text:	
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. The start date for the project is April 15, 2018, and the period of performance has been extended to December 31, 2024 under supplement P00021 to cover additional experimental procedures and data analysis for animals irradiated during the 2023 experimental campaign at Brookhaven National Laboratory. The statement of work has also been adjusted from the original grant submission in coordination with NASA science teams. 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 endeavored 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). The radiation would be delivered in either a single exposure or over 4 weeks in 24 short exposures (fractions) compatible with particle accelerator operations. These results would be compared 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 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) is derived from this comparison. 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 are 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 0.5 Gy of charged particles (33 ion GCR simulation, (GCRsim)); and protracted (24 fractions over 4 weeks) exposures to 2.0 Gy of 137-Cs gamma rays. An additional set of acute exposures to GCRsim was completed in April 25 & 25 2023 to determine the form of the dose-response with acute exposures to 0, 0.15, 0.25, 0.5 and 0.75 Gy and an acute 137-Cs gamma ray exposure. All work uses wild type mice and is 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 aims the species is Mus musculus, strain C57Bl/6J. Ages are 5 - 6 months at acquisition and the beginning of irradiation procedures. We test both male and female animals as their responses are not identical, and the astronaut population is of mixed sex. Scheduled sacrifices and tissue harvests follow behavioral testing. For each of the exposure regimens we 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 allows 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). Findings from this pilot will enable future experiments using proton exposures in combination with chronic stress to test interactions of multiple stressors with radiation. BNL operations were restored allowing us to expose male and female animals to GCRsim in the 2021 and 2022 campaigns. GCRsim behavioral data on males and females has now been analyzed and histological and biochemical analyses are in progress. This includes quantitation of c-fos expression after behavioral stimulation of GCRsim irradiated animals to understand how network activity associated with a behavioral task is modulated by prior radiation exposure. Assessment of glial cell activation using IBA-1 and GFAP markers is used to inform how neuroinflammation correlates with behavioral outcomes. Transcriptomics analysis was performed on GCRsim-irradiated animals to complement prior measurements with proton-irradiated animals. Results indicate that expression patterns (for a series of 770 genes associated with neuropathology) depend on brain region, sex, and type of radiation. A dose response irradiation experiment was completed in April, 2023 during the NSRL23A/B campaign for which behavioral analyses continued into November 2023. Tissue harvests followed the behavioral testing and microglial activation marker histology is in progress. 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 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, hindlimb unloading, 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 770 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 hippocampus, pathway activation was generally similar for fractionated vs acute expression while in frontal cortex fractionated exposure differed substantially from either sham or acute exposures. When the data was analyzed for the top genes based on absolute fold expression value, the 8 highest differentially expressed genes were associated with oxidative stress, protein homeostasis, and inflammation. They included: Arc and Fos which are immediate early genes expressed after synaptic activity and oxidative stress, Cp (Ceruloplasmin) which may have antioxidant activity in astrocytes, regulate monoamine pathways and serve as a copper transporter, Des (Desmin) which is associated with astrocyte activation and is expressed along with GFAP, Nqo1 (NADPH dehydrogenase quinone) which is associated with adaptation to stress and may regulate Poly (ADP-ribose) formation (DNA repair, apoptosis) and proteasome activity on denatured proteins, Pla2g4 (Phospholipase A2) which regulates signaling in neuroinflammation and oxidative stress, Psmb9 (Proteosome subunit 9) which regulates protein degradation, and Shh (Sonic Hedgehog) which is a central nervous system (CNS) morphogen that also regulates autophagy and shows protective activity for neurogenesis and oxidative stress. Similar differentially expressed gene sets were observed for male and female mice exposed to simulated galactic cosmic rays but there were significant sex differences in both hippocampus and frontal cortex.

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 space flight 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. 3-month c-fos expression histological samples have been archived and are undergoing counting. Brain tissue samples from 7 regions were 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 were quarantined for 7 weeks after which behavioral testing began. Behavioral testing was completed in November 2022 and tissues were archived for histology and biochemistry. Fixed samples were processed for c-fos and neuroinflammatory marker immunohistochemistry and imaging and quantification is still in progress. To better understand the shape of the dose-response curve and to improve RBE estimates, a final cohort of 180 female (120) and male (60) mice were irradiated acutely during the NSRL23A/B campaign (April 25 and 26, 2023) with the 33-ion GCRsim field at doses of 0, 15, 25, 50 and 75 cGy complemented by a 2 Gy acute exposure to 137-Cs gamma rays. These were processed for behavioral and histological endpoints as per previous animals exposed to fractionated GCRsim.

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 enabled 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 and hindlimb unloading depression-like behavior (learned helplessness) revealed enhancement of “depression” at 1 month which resolved at 3 and 9 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. For females subjected to the same behavioral battery, there was generally a smaller effect than for males. Open field distance and center time measures were reduced

	in fractionated exposure animals but time immobile was increased. Novel object recognition memory was not significantly affected. Elevated plus maze measures indicated reduced anxiety in males but not females while females were found to move 25% more than males. Y maze working memory and passive avoidance fear memory measures were not significantly altered, nor was depression-like behavior in the hindlimb unloading test. For the dose-response series of acute exposures in females, there were similar outcomes to those described above but the magnitude of changes was subtle. Both linear and non-linear dose responses were observed depending on endpoint measures. We are exploring the use of data transformed to Z-scores and Hedge's g or Cohen's d effect sizes to clarify which outcome measures are the most robust, not just the most significant based on sampling statistics. To reduce the influence of particular experimental set-ups we are combining Z-scores for selected parameters into integrated Z-scores for broadly defined behavioral domains such as emotionality and locomotory behaviors. For example, locomotory behavior is assessed in open field, Y-maze, novel object, and elevated plus maze arenas, each of which has its own geometry, lighting, and external cues. Distance, entry, and speed parameters from these arenas all reflect locomotory behavior so that Z-scores at the individual animal level for the multiple parameters can be combined and normalized to a consensus score to eliminate noise and test bias. Dose Rate Effectiveness Factor (DREF) and RBE values calculated from these consensus scores are expected to be superior to those from individual test parameters. Histological samples from NSRL23A/B mice are being photographed for quantitation of IBA-1 and GFAP glial activation markers reflecting neuroinflammation. mRNA samples from NSRL22 and 21 mice were prepared for transcriptomics analysis using the NanoString® technology reported for protons. The data have been reported back from the vendor and are undergoing analysis for pathway scores and differential expression. A first look suggests that gene expression patterns are dependent on sex, brain region, and radiation type. Despite the fact that protons numerically, and as a dose fraction, dominate the GCRsim field, the GCRsim expression patterns differ significantly from those observed for protons. DREF Estimates Characterization of dose rate effects by a simple parameter like DREF is problematic for 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 metric (units of standard deviation from control means) which has enabled us to develop distributions of DREF values for proton and GCRsim exposures. Preliminary DREFs for all pooled behavioral measures were approximately 1.65 for both protons and GCRsim, similar to the estimate of 2 used by radiation risk advisory bodies such as the International Commission on Radiological Protection (ICRP). Male and female values were similar. RBE estimates were also derived from effect size measures with linear interpolation of dose responses and yielded values of approximately 3.9. These values were limited to behavioral data sets which were normally distributed and for which effect sizes (Hedge's g) exceeded 0.2. Effect size and integrated Z-score approaches will be used for DREF and RBE estimates and the dose-response curve from the NSRL23A/B experiments will be used to correct for curvature in the GCRsim data which currently assumes linearity.

Bibliography Type:	Description: (Last Updated: 03/19/2025)
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