Fiscal Year:	FY 2016	**Task Last Updated:**	FY 07/17/2015
PI Name:	Mao, Xiao Wen M.D.		
Project Title:	Role of Oxidative Stress in Mediating the Effects of Combined Exposure to Simulated Microgravity and Radiation on Neurovascular Remodeling in Mouse		
Division Name:	Space Biology		
Program/Discipline:	SPACE BIOLOGY		
Program/Discipline--Element/Subdiscipline:			
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Human Research Program Elements:	None		
Human Research Program Risks:	None		
Space Biology Element:	(1) Animal Biology: Vertebrate		
Space Biology Cross-Element Discipline:	(1) Neurobiology		
Space Biology Special Category:	(1) Translational (Countermeasure) Potential		
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Zip Code:	92350-0001	**Congressional District:**	31
Comments:			
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No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
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Flight Program:			
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COI Name (Institution):	Gridley, Daila Ph.D. (Loma Linda University) Hartman, Richard Ph.D. (Loma Linda University) Pecaut, Michael Ph.D. (Loma Linda University)		
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Task Description:	One of the main concerns for long-term deep manned space missions are health risk associated with altered gravitational environment and prolonged exposure to low-dose radiation above levels normally found on Earth. Microgravity and radiation exposure has been known to produce a number of neurological disturbances and neurodegeneration by space flight condition. However, the pathophysiological process from adaptive response to irreversible oxidative damage in the brain vasculature and the underlying mechanism(s) of these disturbances are less studied and remain unclear. Our proposal seeks to fill in the gap by testing the hypothesis that NADPH oxidase is a critical source of the neurovascular oxidative stress following space flight conditions that mediates vascular remodeling in the brain, thus disrupting communication between endothelial cells and astrocytes and altering production of extracellular matrix (ECM) proteins. It is further proposed that these changes will contribute to increased vascular permeability and blood-brain barrier (BBB) disturbance, thus resulting in neurological deficit. Our specific aims are 1) Define the causal relationships between space flight condition induced NADPH oxidase expression, vascular damage, and BBB function following microgravity and/or low-dose irradiation in mature mice using neuropathology, stereological, and automated image analysis, and neurobehavioral outcomes. 2) Determine if space flight condition-induced oxidative stress is mediated through NADPH oxidase in brain microvasculature. Nox2, (a subunit of NADPH oxidase) gene knockout (Nox2(-/-)) mice, and wild-type (Nox2(+/+)) C57BL/6 mice will be used in this ground-based animal study. Hindlimb unloading (HLU) will be used to model the unloading, fluid shift, and physiological stress aspects of the microgravity component. Low-dose/low-dose-rate (LDR) gamma-irradiation (0.5Gy at 0.01cGy/h) will be delivered to the whole-body of mature adult mice to simulate the radiation component for over 21 days while the animals are tailed-suspended in cages for microgravity simulation. We will evaluate the radiation- and microgravity-induced brain vascular and tissue remodeling at multiple time points (1 day to 12 months post-irradiation). Together, our unique, integrative, and quantitative activities with advanced imaging techniques, stereological analysis, and behavioral tests will provide insight into the molecular mechanisms of space flight condition-induced oxidative damage on brain tissue and vascular remodeling. Understanding how factors and environmental stress impact on vasculature, tissue remodeling, and function will increase our knowledge and focus toward more effective countermeasures during human space flight and planetary exploration. Our study will also lend new insights into the causes and possible treatments of debilitating neurovascular-related disease and neurodegeneration by targeting NADPH oxidase activation.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Oxidative stress in central nervous system (CNS) is a major contributor to brain injury and aging. There are strong indications that the physiological effects of space flight are similar to those seen in some neurodegenerative diseases and aging: multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease. Our study will provide the first detailed description of combined effects of microgravity and LDR radiation on oxidative stress-induced brain tissue and microvessel network remodeling and underlying mechanism(s) of potential interaction of space flight environmental components over a 12-month observation period. Our research will provide important input to elucidate cellular pathways of response and adaptation to stress imposed by environmental conditions in the brain vasculature. Understanding how factors and environmental insults impact on vasculature and tissue remodeling and function will increase our knowledge and help focus the approach toward more effective countermeasures during human space flight and planetary exploration. Our study might also lend new insights into the causes and possible treatments of debilitating neurovascular-related diseases and neurodegeneration.
	Experiments and data analysis are ongoing as planned. So far, we have finished four batches of animal studies for specific aim 1, at 7 days, 1, 3, and 9 month time points. We also started Nox2 knockout mice study as proposed in specific aim 2. Both 7 day and 1 month time points study are ongoing. We examined the gene expression using microarray analysis, protein markers associated with the induction of oxidative stress and apoptosis in the brain, metabolic response using metabolic biochemical assays, hematological parameters and behavioral responses after combined exposure to simulated microgravity and low-dose radiation at day 7 and 9 months. To study the role of oxidative stress in the mouse brain after simulated microgravity and low-dose radiation exposure, the level of 4-HNE protein was significantly elevated in the hippocampus after hindlimb unloading + radiation compared to controls ($p<0.05$). Unloading + radiation group had highest level of NOX2 expression compared to controls ($p<0.05$). There was a significant increase in SOD protein expression in the group that received unloading or combination of unloading + radiation compared to control ($P<0.05$). SOD expression also showed a strong trend for an interaction between unloading and radiation ($p=0.09$). Significantly higher levels of AQ4 production in the mouse hippocampus were also noted after combined exposure or unloading only compared to controls ($p<0.05$). This study suggests that hindlimb unloading and radiation may induce oxidative stress in mouse brain. Nox2-containing nicotinamide adenosine dinucleotide phosphate (NADPH oxidase) may contribute to combined exposure to simulated microgravity and low-dose radiation–induced oxidative stress. The long-term impact of combined exposure to simulated microgravity and low-dose radiation-induced oxidative stress will be examined in additional time points in future studies. A gene network analysis was performed to provide information on biological pathways underlying the impact of combined exposure. Total RNA samples were extracted from brains and processed for gene expression microarray. The activation/inhibition ($\|Z\text{-score}\|=2$, p-value =0.05) of Global Canonical Pathways was analyzed by Ingenuity Pathway Analysis (IPA). At day 7, there was no significant activation/inhibition of any pathways after HLU, LD gamma R or the combination of both. At 9 months, HLU induced significant activations of HMGB1 signaling, a risk factor for memory impairment and chronic neurodegeneration. Cellular immune response pathways including PKC theta signaling, PI3K signaling, as well as the production of nitric oxide and reactive oxygen species (ROS) signaling were also activated. PPAR signaling was significantly inhibited at 9 months after HLU. The combination of HLU and LD gamma R induced significant activations of HMGB1 and Neuregulin signaling only. The mRNA expression of many genes that regulated oxidative stress (e.g., MPO, SERPINA1, NOXa1), extracellular matrix remodeling (e.g., ADAM8, COL9a), endothelial cell biology (e.g., ITGA5, MMP1), and inflammation (e.g., SOS1, TNFRSF1A) were significantly altered ($p<0.05$) after HLU or HLU+ LD gamma R compared to control. Our findings provide candidate genes and biological pathways underlying phenotypes induced by HLU and LD/LDR. These data indicate that space environmental factors may have an impact on pathological and functional consequences associated with late neurodegeneration.

Task Progress:	To study the impact of long-term behavioral effects of chronic exposure of low-dose radiation and simulated microgravity, Mice then underwent a series of behavioral tests at 4 time-points (1 week, 1 month, 4 months, and 8 months post-exposure) to assess anxiety-related behaviors (elevated zero maze), sensorimotor coordination and balance (rotarod), exploratory behavior/activity levels (open field), learned helplessness/depression-like behavior (tail suspension test), and spatial learning/memory (water maze). Mice that received hindlimb unloading (i.e., the unloading and combination groups) were hypoactive in the open field compared to the radiation-only group. Mice that received a combination of unloading and irradiation spent significantly less time in the dark portion of the elevated zero maze than controls, suggesting abnormal exploratory/risk-taking behaviors. No differences were observed on the water maze, rotarod, or tail suspension tests. These results suggest that microgravity and/or a chronic, low dose of gamma radiation may lead to changes in exploratory/risk-taking behaviors, but that neither cause deficits in spatial learning, coordination and balance, nor learned helplessness/depression-like behavior. Such findings have implications for extended manned space missions planned in the near future. We also examined time-dependent changes of hematological parameters after hindlimb unloading and low-Dose/low-dose-rate radiation. Blood was collected via cardiac puncture. An automated ABC Hematology Analyzer (scil Vet, Inc.) was used to obtain white blood cell (WBC), red blood cell (RBC), and platelet (PLT) measurements. There were no significant main effects of day, radiation, or unloading on the count of any major immune subset. However, significant day-dependent decreases were observed in lymphocyte ($P<0.001$) and monocyte ($P<0.001$) percentages, with corresponding increases in granulocytes ($P<0.001$), suggesting an effect of age. In erythrocytes, there were decreases noted in the combined treatment group (LDR+HLU) on day 7 post-exposure, and increases in all non-control groups on day 30 for RBC, hemoglobin (HGB) levels, and hematocrit (HCT). This led to main effects of day in RBC, HGB, and HCT ($P<0.001$) and a Day x HLU interaction on HGB ($P<0.05$). Furthermore, with the exception of day 30, the LDR+HLU group was consistently low in all three parameters at other time points, resulting in significant LDR x HLU interactions ($P<0.05$) in HGB and HCT. Similarly, RBC distribution width (RDW) is increased at the early time points in the unloading groups, resulting in significant main effects of day ($P<0.001$) and HLU ($P<0.001$), as well as a Day x HLU interaction ($P=0.001$). There were no significant effects or interactions involving day, radiation, or unloading for platelet count or volume. The long term impact of the spaceflight environment on immune populations was minimal. The early decreases in the LDR+HLU group noted in RBC, HGB, and HCT, along with increases in RDW, are consistent with reports that spaceflight leads to anemia. Furthermore, the increases noted in erythrocyte parameters on day 30 suggest that both microgravity and radiation may cause a change in hematopoiesis, resulting in overcompensation. Although the effects of simulated spaceflight appear to be mild on these measures, further study is required to determine the functional consequences of these changes.
Bibliography Type:	Description: (Last Updated: 10/12/2024)
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Abstracts for Journals and Proceedings	Mao XW, Pecaut MJ, Campbell-Beachler M, Gifford P, Nishiyama N, Gridley DS. "Role of Oxidative Stress in Combined Effects of Hindlimb Unloading and Low-dose Radiation in Mouse Brain." 15th International Congress of Radiation Research (ICRR), Kyoto, Japan. May 25-29, 2015. 15th International Congress of Radiation Research, Kyoto, Japan. May 25-29, 2015. , May-2015
Abstracts for Journals and Proceedings	Mao YX, Nishiyama N, Pecaut MJ, Campbell-Beachler M, Gifford P, Gridley DS. "Simulated microgravity and low-dose radiation induces oxidative damage in the mouse hippocampus." American Society for Gravitational and Space Research (ASGSR), Alexandria, VA November 11-14 2015. 31st Annual Meeting of the American Society for Gravitational and Space Research, Alexandria, VA, November 11-14, 2015. , Nov-2015