Fiscal Year:	FY 2015	**Task Last Updated:**	FY 07/25/2014
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:			
Joint Agency Name:	**TechPort:**		No
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:			
Project Type:	Ground	**Solicitation / Funding Source:**	2012 Space Biology NNH12ZTT001N
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No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
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Flight Program:			
Flight Assignment:			
Key Personnel Changes/Previous PI:	July 2014 report: No changes.		
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 suspension 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 (1day 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.		

Task Progress:	Three batches of studies have been performed for 7 days, 1 and 12 months time points. LDR gamma-irradiation using 57Co plates (0.04Gy at 0.01cGy/h) was delivered to the whole-body of mature 6 month old adult C57BL/6 mice (n=4-6/group) to simulate the radiation component. Anti-orthostatic tail suspension was used to model the unloading, fluid shift, and physiological stress aspects of the microgravity component. Mice were hindlimb suspended and/or irradiated for 21 days. To examine the induction of apoptosis-associated protein profiles in the brain after combined exposure to simulated microgravity and low-dose radiation, brain tissues were isolated for characterization of apoptosis-associated proteins 7 days after the completion of irradiation and unloading. Brain cell lysates from each group were incubated overnight with RayBio Human Apoptosis Antibody Array Membranes. Biotinylated antibodies were used to detect bound proteins and signals were visualized by chemiluminescence. The results were subjected to two-way analysis of variance (ANOVA). BAD, FAS, and FASL proteins were significantly activated ($p<0.05$) at the 7-day time point in the unloaded group compared to controls. There were statistical trends for differences between groups noted in BAX, BCL-2, and HSP27 ($P<0.1$), where hindlimb unloaded mice had a greater than 2-3 fold higher expression level compared with radiation alone or unloaded + radiation groups. These proteins play important roles in induction of apoptosis. There was also a trend for increase in the level of anti-apoptotic protein CD40L in the unloaded + radiation group compared to other groups. This study demonstrates that hindlimb unloading may induce early apoptosis in mouse brain and radiation may exacerbate simulated microgravity-induced damage in brain structure and tissue function. Combined exposure to simulated microgravity and low-dose radiation-induced apoptosis will be examined for longer time points in our future studies. To study the impact of combined exposure on hematological parameters, blood was collected in K2-EDTA coated syringes via cardiac puncture and analysed via the scil Vet ABC Hematology Analyzer. Although there was no significant main effects of either parameter on the count of any major immune subset, there were trends for suspension x interactions for WBC, lymphocyte, and granulocyte counts ($P<0.1$). In all cases, this was due to decreases noted in the suspension alone or radiation alone groups that was not present in the combined treatment group. While there were no effects of LD/LDR radiation on the proportions of each population, there was a suspension-induced proportional shift away from lymphocytes ($P<0.05$) toward granulocytes ($P<0.1$). While there were overall suspension-induced decreases in erythrocyte count and hemoglobin levels ($P<0.05$), these decrease primarily occurred in mice that also received LD/LDR radiation, resulting in significant suspension x radiation interactions ($P<0.05$). A similar interaction was noted in hematocrit ($P<0.05$). There were also suspension-induced increases in both mean corpuscular volume and RBC distribution width ($P<0.05$), with a decreases in mean corpuscular hemoglobin concentration ($P<0.1$). There was only a trend for a radiation-induced increase in mean corpuscular hemoglobin ($P<0.1$). There was no impact of either stressor on platelet count or volume. To study the behavioral response to combined exposure of simulated microgravity and radiation, the mice went through a series of behavioral tests to assess anxiety-related behaviors (elevated zero maze), sensorimotor coordination and balance (rotarod), exploratory behavior and activity levels (open field), and spatial learning / memory (water maze). Preliminary findings show that the all groups performed similarly in the elevated zero maze. Mice exposed to radiation only fell off the rotarod more quickly ($p < 0.05$) compared with control, unloading only, or unloading+irradiation mice (which did not differ). However, all treatment groups trended toward less activity than controls during open field testing and demonstrated spatial learning and/or memory deficits in the water maze. Thus, 3 weeks of Co-57 irradiation, hindlimb unloading, or a combination of both led to sensorimotor coordination and balance deficits, hypoactivity, and spatial learning / memory deficits. Overall, our study demonstrates that hindlimb unloading may induce early changes in mouse brain structure and function and radiation may exacerbate simulated microgravity-induced damage. Our data indicate that environmental factors have differentials responses in immunocytes, erythrocytes, and platelets. These results suggest that multiple factors present in the long-term deep-space environment may have detrimental effects on an astronaut's motor and cognitive abilities. Tissues from this study have been shared with collaborators at various groups and institutes for evaluation and analysis.
Bibliography Type:	Description: (Last Updated: 10/12/2024)
Abstracts for Journals and Proceedings	Mao YX, Pecaut MJ, Campbell-Beachler M, Gifford P, Gridley DS. "Detection of Early-stage Apoptosis in Mouse Brain after Combined Exposure to Simulated Microgravity and Radiation." 30th Annual Meeting of the American Society for Gravitational and Space Research, Pasadena, CA, October 22-26, 2014. Program and abstracts. 30th Annual Meeting of the American Society for Gravitational and Space Research, Pasadena, CA, October 22-26, 2014. In press as of September 2014. , Sep-2014
Abstracts for Journals and Proceedings	Sanders K, Bellone JA, Montanari R, Gifford P, Hartman RE, Mao XW. "Behavioral response to combined exposure of simulated microgravity and radiation." Neuroscience 2014, Washington, DC, November 15-19, 2014. Neuroscience 2014, Washington, DC, November 15-19, 2014. Program#/Poster#: 360.07/SS66. Available at http://www.abstractsonline.com/Plan/ViewAbstract.aspx?mID=3527&sKey=ae2f7f6d-6371-4fdd-aa1f-4348a1849a1a&cKey=afca8d63-5ed1-420b-869e-588f4e26a99e&mKey=54c85d94-6d69-4b09-afaa-502c0e680ca7 ; accessed 11/25/14. , Nov-2014
Abstracts for Journals and Proceedings	Pecaut MJ, Gridley DS, Campbell-Beachler M, Gifford P, Mao XW. "Impact of combined exposure to anti-orthostatic tail suspension and low-dose/low-dose-rate radiation on hematological parameters." 30th Annual Meeting of the American Society for Gravitational and Space Research, Pasadena, CA, October 22-26, 2014. Program and abstracts. 30th Annual Meeting of the American Society for Gravitational and Space Research, Pasadena, CA, October 22-26, 2014. In press as of September 2014. , Sep-2014