Fiscal Year:	FY 2017	**Task Last Updated:**	FY 07/18/2016
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		
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Space Biology Element:	(1) Animal Biology: Vertebrate		
Space Biology Cross-Element Discipline:	(1) Neurobiology		
Space Biology Special Category:	(1) Translational (Countermeasure) Potential		
PI Email:	xmao@llu.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	909-558-8373
Organization Name:	Loma Linda University		
PI Address 1:	Radiation Medicine		
PI Address 2:	11175 Campus Street, Rm A1010, Chan Shun Pavilion		
PI Web Page:			
City:	Loma Linda	**State:**	CA
Zip Code:	92350-0001	**Congressional District:**	31
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2012 Space Biology NNH12ZTT001N
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No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA ARC
Contact Monitor:	Sato, Kevin	**Contact Phone:**	650-604-1104
Contact Email:	kevin.y.sato@nasa.gov		
Flight Program:			
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Key Personnel Changes/Previous PI:	July 2016 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 risks 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 (0.5 Gy at 0.01 cGy/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 tail-unloaded 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.
	To determine whether NADPH oxidase-derived oxidative stress can account for unloading and radiation-induced deleterious effects on endothelial damage and neurovascular remodeling in a Nox2 knock-out (KO) mice model. Wild-type (Nox2 (+/+)) C57BL/6 mice or Nox2 (-/-) (B6. 129S6-CYBBM) mice placed into one of the following groups (n=5-6/group): age-matched control, hindlimb unloading (HLU), low-dose/low-dose-rate radiation (LDR), or HLU+LDR simultaneously for 21 days, and were then sacrificed at day 7 or 1 month. Anti-orthostatic tail suspension was used to model the unloading, fluid shift, and physiological stress aspects of microgravity. The LDR was delivered using 57Co plates (0.04 Gy at 0.01 cGy/h) to the whole body in order to simulate the radiation experienced while in microgravity. Brains were isolated for characterization of various oxidative stress markers and vascular topology. Levels of 4-hydroxynonenal (4-HNE) protein, a specific marker for lipid peroxidation, were measured. Expression of aquaporin-4 (AQ4), a water channel protein expressed in astrocyte endfeet, was quantified. Long-term behavioral effects were also evaluated following chronic exposure of radiation + unloading. Thirty days after simulated space flight, KO mice showed decreased apoptosis ($p<0.05$) in the brain cortex compared to WT counterparts. The HLU-dependent increase in apoptosis in WT mice was not observed in KO mice. Level of 4-HNE protein was significantly elevated in the hippocampus after hindlimb unloading + radiation compared to controls in the WT mice ($p<0.05$), However, there was no significant differences between treated groups and control in the Nox2-KO mice at 7-day and 1-month time points. In contrast to findings in WT animals, superoxide dismutase level and expression of APQ4 was similar among all KO groups. For behavioral tests, KO mice that received irradiation spent significantly less time in the dark portion of the elevated zero maze than KO controls, suggesting abnormal exploratory/risk-taking behaviors ($p<0.05$). In summary, for most of the parameters, the oxidative response to HLU and LDR was suppressed in Nox2(-/-) mice at both time points. This suggests that NOX2-containing NADPH oxidase may contribute to space flight environment–induced oxidative stress. To study the effect of simulated microgravity on metabolic change, brains were isolated at 1, 4, or 9 months after HLS exposure for analysis. Comparative untargeted metabolomics/ lipidomics profiling of the brain tissue was performed by ultra-performance liquid chromatography coupled with electro-spray quadrupole time of flight mass spectrometry (UPLC-ESI-QTOF-MS) to study specific changes that may be indicative of the adaptations in central nervous system (CNS) to space flight condition. Data was pre-processed using the XCMS software while the database search was performed using the Human Metabolome Database (HMDB), Madison Metabolomics Consortium Database (MMCD), LIPID MAPS and Metlin for putative metabolite identification. Although after a month following microgravity exposure, brains did not show significant differences in their metabolomes, we could detect them after longer periods of exposition. After 4 months, some metabolites like PI (18:3/0:0) and tetrahydroaldosterone-3-glucuronide went up ($p<0.05$) and some others such as PE (20:4/16:1), cardiolipin, PS (18:3/18:1), PE (20:3/14:0), PA (22:6/19:1), 11-peroxy-5Z,8Z,12E,14Z-eicosatetraenoate, and linoleamide were significantly ($p<0.05$) less abundant compared to the

Task Progress:	controls. After 9 months following microgravity exposure, some lipids including TG (14:1/18:4/18:4), TG (14:0/14:1/18:3), MG (18:0/0:0/0:0), PG (19:1/20:0), PE (22:4/P-18:0), PI (18:0/18:0), PS (18:3/22:0), and PS (22:4/22:1) went up ($p<0.05$) and some others, such as but-2-enoic acid, ganglioside GT2, LysoPE (20:3/0:0), galactoceramides and ceramides, diglycerides and phosphocholines, were significantly ($p<0.05$) downregulated in mice exposed to microgravity. In summary, our results show significant alterations in lipid profiles particularly glycerophospholipids class. For example, the levels of phospatidyl-ethanolamine and phosphatidyl-serine diminished significantly after four months following HLS in brain tissue which could be a result of increased lipid peroxidation, lipid degradation, or impaired lipid biogenesis in liver or export to the brain. These changes are likely to impact structural and chemical integrity of brain cells leading to structural and functional impairment. Characterization of longitudinal systemic changes in plasma of these mice is ongoing. To compare changes in leukocyte parameters in wild-type (WT) and Nox2(-/-) knockout (KO) mice 30 and 120 days, Six-month-old female Nox2(+/+) and Nox2(-/-) C57BL/6 mice (n=4-6/group) were exposed to whole-body low-dose/low-dose-rate (LDR) gamma-irradiation using 57Co plates (0.04 Gy at 0.01 cGy/h) and/or hindlimb unloaded (HLU) for 21 days. Mice were sacrificed with 100% CO2 at 30 or 120 days after the simulated space flight period and blood was collected via cardiac puncture. An automated ABC Vet Hematology Analyzer was used to obtain white blood cell (WBC), lymphocyte (LYM), monocyte (MON), and granulocyte (GRA) counts and percentages. Thirty days after simulated space flight, KO mice showed increased GRA counts ($P<0.005$) and decreased LYM ($P<0.001$) and MON ($P<0.05$) counts compared to WT controls. This resulted in a shift away from %LYM ($P<0.001$) and %MON ($P<0.001$) towards %GRA ($P<0.001$). Similarly, hindlimb unloading caused increases in WBC ($P<0.05$) and GRA counts ($P<0.05$), leading to proportional shifts away from %LYM ($P=0.005$) and %MON ($P<0.05$) towards %GRA ($P<0.005$). Interestingly, the HLU-dependent increase in GRA was augmented in the KO mice, resulting in a KO x HLU interaction ($P<0.05$). Furthermore, unloading increased %GRA and decreased %MON, but only in the KO mice, leading to significant KO x HLU interactions ($Ps<0.05$). Although %LYM were generally lower in KO mice, the LDR-dependent decrease in this parameter noted in WT was reversed in KO mice. This led to a significant KO x LDR interaction ($P<0.05$). At 120 days after simulated-space flight, there were several significant main effects of KO due to increases in WBC ($P<0.001$), MON ($P<0.001$), and GRA ($P<0.001$) counts. Because the KO-dependent increases in GRA counts was much higher than in other populations, this led to a shift away from %LYM ($P<0.001$) and %MON ($P<0.001$) towards %GRA ($P<0.001$). In contrast to the response at 30 days, the only significant main effect of HLU at 120 days was a decrease in %GRA ($P<0.05$). However, the HLU-dependent increase on MON counts noted in WT mice was amplified in KO mice. The HLU-dependent decrease in LYM counts was reversed in KO mice. This led to significant KO x HLU interactions ($Ps<0.05$) in both of these parameters. Finally, the LDR-dependent increase in %MON noted in WT mice was not observed in KO mice, leading to a significant KO x LDR interaction ($P<0.05$). In conclusion, low-dose radiation had very little impact on leukocyte populations at either time point. Furthermore, many of the changes noted (particularly the changes in percentages) were dominated by large KO-dependent increases in granulocyte counts. In general, the response to hindlimb unloading was limited to day 30. However, the response to HLU was modified in the Nox2(-/-) mice at both time points. This suggests that the effects of microgravity on immune populations may be regulated, in part, by Nox2.
Bibliography Type:	Description: (Last Updated: 10/12/2024)
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