Fiscal Year:	FY 2019	**Task Last Updated:**	FY 09/10/2018
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		
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
Start Date:	10/01/2013	**End Date:**	09/04/2019
No. of Post Docs:	0	**No. of PhD Degrees:**	0
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
Contact Monitor:	Sato, Kevin	**Contact Phone:**	650-604-1104
Contact Email:	kevin.y.sato@nasa.gov		
Flight Program:			
Flight Assignment:	NOTE: Extended to 9/4/2019 per F. Hernandez/ARC (Ed., 9/10/18) NOTE change in grant number and end date per NSSC information and F. Hernandez/ARC (Ed., 1/18/2018) NOTE: End date changed to 8/31/2018 per F. Hernandez/ARC (Ed., 6/27/17) NOTE: End date changed to 9/30/2017 per NSSC (Ed., 7/4/16)		
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)		
Grant/Contract No.:	80NSSC17K0693 ; NNX13AL97G		
Performance Goal No.:			
Performance Goal Text:			

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 have 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.
Task Progress:	FINAL REPORTING SEPTEMBER 2019 All the experiments and data analysis have been completed as planned. So far, we have finished four batches of wild-type (WT) animal studies for specific aim 1, at 7 days, 1, 3, and 9 month time points and Nox2 knockout mice study as proposed in specific aims 1 and 2. We have examined protein markers associated with the induction of oxidative stress and apoptosis in the brain using immunostaining, metabolic response using metabolic biochemical assays, hematological parameters, and behavioral responses after combined exposure to simulated microgravity and low-dose radiation. Our data provide the first evidence that prolonged exposure to simulated microgravity and LDR radiation is associated with increased oxidative stress biomarkers which may increase the likelihood of brain injury and reduced antioxidant defense. NOX2-containing nicotinamide adenosine dinucleotide phosphate (NADPH oxidase) may contribute to spaceflight environment-induced oxidative stress. These results suggest that microgravity may lead to changes in exploratory/risk-taking behaviors in the absence of other sensorimotor or cognitive deficits and that combined microgravity and a chronic, low dose of gamma radiation may lead to blood-brain barrier dysfunction. These results have been published in four peer reviewed journals: Radiation Research, Nature npj Microgravity, PLOS One. We have received supplement funding in October, 2017 to use"omics"-based molecular phenotyping approach for identification and characterization of genomic signatures in eye and brain associated with low-dose radiation and simulated microgravity at 1-and 4- month time points. Omics analyses were performed for full genome transcriptomics including RNA-Seq HT Ribo-depletion library preps and RNA spike-control. DNA methylation of reduced representation bisulfite sequencing (RRBS). The dataset has been submitted to NASA GeneLab site, through NASA IT system. One manuscript titled: Mice Exposed to Combined Chronic Low-Dose Irradiation and Modeled Microgravity Develop Long-Term Neurological Sequelae has been published in International Journal of Medical Sciences (IJMS) to evaluate simulated radiation and microgravity induced changes in the brain related to cellular structure, oxidative stress, immune response, and metabolic function. ANNUAL REPORTING SEPTEMBER 2018: The purpose of this study was to determine whether nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-derived stress can account for unloading- and radiation-induced endothelial damage and neurovascular remodeling in a mouse model. Wild-type (WT, Nox2 (+/+)) C57BL/6 mice or Nox2 (-/-) (B6. 129S6-CYBBM) knockout (KO) mice were placed into one of the following groups: age-matched control, hindlimb unloading (HLU), low-dose/low-dose-rate radiation (LDR), or HLU+LDR simultaneously for 21 days, and were then sacrificed 1 month later. 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 (0.04Gy at 0.01cGy/h) to the whole body in order to simulate the radiation experienced while in space. Brains were isolated for characterization of various oxidative stress markers and vascular topology.

	We received supplement funding in October, 2017 to use “omics”-based molecular phenotyping approach for identification and characterization of genomic signatures in eye and brain associated with low-dose radiation and simulated microgravity at 1- and 4– month time points. Omics analyses performed were • RNAseq for full genome transcriptomics including RNA-Seq HT Ribo-depletion library preps and RNA spike-control. • DNA methylation of reduced representation bisulfite sequencing (RRBS). The assay has completed and dataset are in the process of submitting to NASA GeneLab site, through NASA IT system. Many canonical pathways were significantly activated/inhibited in the brain after HLU, LDgammaR, or the combination of both. Most robust changes are observed in LDgammaR + HLU group compared to control. At 1 months, the combination group induced significant activations of EIF2 phosphorylation signaling pathway in the brain, responsible for chronic neurodegeneration. Cellular immune response pathways including leukocyte extravasation signaling, as well as pathways responsible for cell growth, cell repair, and metabolic stress were also significantly altered compared to controls. The mRNA expression for many genes regulating oxidative stress (e.g., GNRH1, UCN3), extracellular matrix remodeling (e.g., Cldn3, FBLN1), endothelial cell biology (e.g., AQP1), and cognitive function (e.g., OXT, AVP) were significantly changed ($p<0.05$) after HLU+ LDgammaR compared to control. Gene analysis of retina tissues also showed that many key pathways responsible for photoreceptor function, oxidative stress, and metabolic function were significantly different between LDgammaR + HLU and control ($p<0.05$).
Bibliography Type:	Description: (Last Updated: 10/12/2024)
Abstracts for Journals and Proceedings	Mao XW, Pecaut MJ, Hartman R, Nishiyama N, Bellone J, Boerma M, Nelson G. "Effects of simulated microgravity and/or low-dose radiation on neurovascular remodeling in the mouse brain and eye." Presented at 42nd Committee on Space Research (COSPAR) 2018 42nd Scientific Assembly, Pasadena, CA, July 14-22, 2018. 2nd Committee on Space Research (COSPAR) 2018 42nd Scientific Assembly, Pasadena, CA, July 14-22, 2018. , Jul-2018
Abstracts for Journals and Proceedings	Mao XW, Nelson G, Jones T, Campbell-Beacher M, Stanbouly S, Nishiyama N, Rodriguez D, Ortloff L, Sun S, Sridharan V, Boerma M, Hauer-Jensen M. "Cardiac and vascular effects of space radiation - CSRR acute risk studies." 2018 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 22-25, 2018. 2018 NASA Human Research Program Investigators’ Workshop, Galveston, TX, January 22-25, 2018. , Jan-2018
Abstracts for Journals and Proceedings	Mao XW. "Effects of Low-dose Radiation on Neurovascular Remodeling." Presented at the The Conference on Normal Tissue Radiation Effects and Countermeasure (CONTREC), Morrilton, AR, May 14-17, 2018. The Conference on Normal Tissue Radiation Effects and Countermeasure (CONTREC), Morrilton, AR, May 14-17, 2018. , May-2018
Abstracts for Journals and Proceedings	Mao XW. "Spaceflight and radiation induced microvessel and structural damage in ocular tissue." International Space Station Microgravity Space Medicine Meeting, American Institute of Aeronautics &Astronautics (AIAA), Manhattan Beach, CA, March 20, 2018. International Space Station Microgravity Space Medicine Meeting, American Institute of Aeronautics &Astronautics (AIAA), Manhattan Beach, CA, March 20, 2018. , Mar-2018
Abstracts for Journals and Proceedings	Mao XW, Boerma M, Nelson G, Shiba D, Shirakawa M, Takahash S, Delp M. "Impact of space flight or simulated microgravity combined with space radiation exposure on retinal oxidative damage." Presented at International Society for Gravitational Physiology (ISGP), Nagoya, Japan, May 26-June 1, 2019. Abstracts. International Society for Gravitational Physiology (ISGP), Nagoya, Japan, May 26-June 1, 2019. , May-2019
Articles in Peer-reviewed Journals	Mao XW, Nishiyama N, Campbell-Beachler M, Gifford P, Haynes KE, Gridley DS, Pecaut M. "Role of NADPH oxidase as a mediator of oxidative damage in low-dose radiated and hindlimb-unloaded mice." Radiation Research. 2017 Oct;188(4):392-9. Epub 2017 Aug 1. https://doi.org/10.1667/RR14754.1 ; PubMed PMID: 28763287 , Oct-2017
Articles in Peer-reviewed Journals	Overbey EG, Paul AM, da Silveira WA, Tahimic CGT, Reinsch SS, Szewczyk N, Stanbouly S, Wang C, Galazka JM, Mao XW. "Mice exposed/ to combined chronic low-dose irradiation and modeled microgravity develop long-term neurological sequelae." Int J Mol Sci. 2019 Aug 22;20(17):E4094. https://doi.org/10.3390/ijms20174094 ; PubMed PMID: 31443374 , Aug-2019