Fiscal Year:	FY 2018	**Task Last Updated:**	FY 03/22/2019
PI Name:	Lemere, Cynthia Ph.D.		
Project Title:	Impact of Space Radiation on Cognition, Synapses and Biomarkers in Aging and Alzheimer's Disease		
Division Name:	Human Research		
Program/Discipline:			
Program/Discipline--Element/Subdiscipline:			
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SR**:Space Radiation		
Human Research Program Risks:	(1) **BMed**:Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2013 Space Radiobiology NNJ13ZSA001N
Start Date:	06/01/2014	**End Date:**	05/31/2018
No. of Post Docs:	1	**No. of PhD Degrees:**	1
No. of PhD Candidates:	0	**No. of Master' Degrees:**	0
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	2
No. of Bachelor's Candidates:	1	**Monitoring Center:**	NASA JSC
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Flight Program:			
Flight Assignment:	NOTE: change in period of performance to 6/1/2014-5/31/2018 (from 4/29/14-4/28/18) per PI and NSSC information (Ed., 3/17/2015)		
Key Personnel Changes/Previous PI:	None		
COI Name (Institution):	O'Banion, Kerry M.D., Ph.D. (University of Rochester)		
Grant/Contract No.:	NNX14AI07G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	The goal of our work is to identify early and late effects of space radiation on the connections between nerve cells in the brain (i.e., synapses), inflammation, and cognition so that one can assess the central nervous system (CNS) risk to future astronauts involved in long-duration lunar missions and/or a mission to Mars. These early changes, along with changes in brain inflammation that may relay signals between cells in the brain and blood flow, may help define those individuals at risk for developing long-term learning and memory problems. Our studies utilize normal, wildtype (WT) mice and genetic mouse models of Alzheimer's disease. Female and male 4 month-old mice were irradiated once with varying doses of heavy ions (iron) and examined for 2 (early) or 8 (late) months later. A similar study paradigm was used to look at the late effects of protons in female and male mice. Chronic dosing with iron was compared with a single dose for long-term effects as well. A subset of mice underwent positron emission tomography (PET) imaging for brain inflammation and blood flow, and behavioral testing. Alzheimer's amyloid plaques, inflammation and synapses were examined in the brain and inflammatory markers quantified in blood.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	To date, our studies suggest that female mice are somewhat resistant to iron irradiation while male mice appear to be more vulnerable, especially to the long-term central nervous system effects. One caveat is that survival was reduced in female Alzheimer's mice exposed to the higher single dose of iron irradiation; however, those female Alzheimer's mice that survived until the end of the study showed fewer radiation effects on cognition, amyloid pathology, and inflammation compared to their male irradiated counterparts. Iron-irradiated male but not female Alzheimer's mice showed short- and long-term cognitive deficits and long-term increases in brain amyloid and neuroinflammation. Similar to iron irradiation, a single exposure to proton irradiation caused sex- and dose-specific effects that varied according to the mouse model (wildtype or Alzheimer's). For example, motor coordination was worsened by low-dose (but not high dose) proton irradiation in wildtype mice but not Alzheimer's mice, and improved by iron irradiation in Alzheimer's mice. Both proton and iron irradiation induced spatial memory impairment in male but female Alzheimer's mice. And, while iron irradiation increased brain amyloid, proton irradiation decreased brain amyloid in male Alzheimer's mice. Lastly, a comparison of single dose vs. 6 smaller, fractionated doses of the equivalent amount of total iron irradiation in male Alzheimer's mice, using a new genetic knockin mouse model, revealed that fractionated dosing induced fewer detrimental effects than a single larger dose. For example, spatial memory deficits were observed following a single but not fractionated dose of iron irradiation. Neither single nor fractionated iron dosing altered brain amyloid levels in this new mouse model. Importantly, we found no effect of low level dosing of iron or proton radiation for many outcome measures, including general health. These findings will help inform risk estimates for astronauts exposed to space radiation on long-term, deep space missions. In addition, these studies emphasize the need for further research into sex differences in normal and disease conditions. This information may help guide research into developing specific countermeasures for male and female astronauts. In addition, it may help determine factors underlying learning and memory, and potentially lead to new therapies and/or gender-specific treatments for Alzheimer's disease.
	The goal of our work is to identify early and late effects of space radiation on the connections between nerve cells in the brain (i.e., synapses), inflammation and cognition so that one can assess the Central Nervous System (CNS) risk to future astronauts involved in long-duration lunar missions and/or a mission to Mars. These early synaptic changes, along with changes in brain inflammation that may relay signals between cells in the brain and blood flow, may help define those individuals at risk for developing long-term learning and memory problems. Our studies utilize normal wildtype mice and two genetic mouse models of Alzheimer's disease that develop some of the same lesions in the brain and cognitive changes seen in people with Alzheimer's disease. In our first series of experiments, female and male 4 month-old wildtype and Alzheimer's mice (genetic model 1) were irradiated once at Brookhaven National Laboratory (BNL) with varying doses of 56Fe (iron) ions or protons and examined 2 or 8 months later. Our second set of experiments, 12 month-old male Alzheimer's knock-in (genetic model 2) and wildtype mice were exposed to either a single dose or six fractionated doses of smaller amounts of radioactivity over a two-week period. Investigation 1: Early effects of single-dose iron irradiation We began our project in July 2014. In our first study (Aim 1a), we investigated the early effects of single-dose 56Fe irradiation in four-month-old female and male wildtype (non-genetically manipulated) and Alzheimer's transgenic (genetically manipulated) mice. These animals were shipped to BNL in April 2015 and exposed to a single dose of 0, 10 (low dose), or 50 (high dose) cGy 56Fe ions with energies of 1000 MeV/n. Mouse neurobehavioral tests were conducted 1-2 months post-irradiation to examine general health, locomotion, anxiety, depression, strength, motor learning and fatigue, and cognition. Our findings suggested that a single exposure of 56Fe radiation produced early changes in mouse behaviors that vary by sex, genotype, and dose. For example, low dose irradiated female Alzheimer's mice and high dose irradiated male Alzheimer's mice were more active than their non-irradiated counterparts. Unlike other mice in the study, 56Fe-irradiated female Alzheimer's mice had reduced neuroinflammation (by PET scan), reduced amyloid-ß burden, increased levels of a post-synaptic marker in brain, and reduced grip strength but higher motor learning compared to non-irradiated female Alzheimer's mice, suggesting that radiation had some beneficial short-term effects in female Alzheimer's mice but not in males. Instead, high dose iron irradiation led to slight memory impairment in male Alzheimer's mice but not in female Alzheimer's mice nor in any of the wildtype mice. During Year 3 of our grant, we re-analyzed our 18F-GE180 PET scan images for inflammation in the short-term effects study described above to distinguish uptake of the tracer versus stable binding within the brain. We determined that the early radiation-induced reduction in brain inflammation observed in 6 month-old female mice was mainly due to changes in uptake but not stable binding of the tracer in brain, indicating that in the short-term, there were no radiation effects on brain inflammation. We hypothesized that the reduced peak uptake in female irradiated mice might be due to differences in cerebral blood flow or blood brain barrier permeability after radiation. However, by PET imaging, we found no differences in cerebral blood flow between pre- and post-irradiation scans in any groups, indicating that a single dose of iron radiation had no early effects on mouse cerebral blood flow in male or female mice. We performed quantitative staining for inflammatory markers on mouse brain sections and found that iron irradiated 6-month-old female Alzheimer's mice, which had reduced amyloid plaques, also had reduced gliosis (inflammatory cells) compared to non-irradiated females, while no changes were observed in Aß or gliosis in irradiated male Alzheimer's mice.

Task Progress:

During Year 4 of our grant, the early effects of 56Fe (iron) irradiation on body weight pre- and post-irradiation and post-irradiation survival rate of the mice were analyzed. We found that 56Fe irradiation did not affect the body weight of the mice in the short term. Interestingly, although the survival rate differences between mice treated with different doses of 56Fe irradiation were not statistically significant in the short term, we found in the late effects investigation that female Alzheimer's mice were more susceptible to death after a high dose (50 cGy) of 56Fe irradiation than were male Alzheimer's mice.

Investigation 2: Late effects of single-dose iron irradiation

To investigate late effects of 56Fe irradiation, 4-month-old male and female Alzheimer's and wildtype mice were shipped to Brookhaven National Laboratory and received a single dose of 0, 10, or 50 cGy iron radiation in October 2015. Immediately prior to shipping, a subset of mice underwent pre-irradiation PET scanning for neuroinflammation and cerebrovascular blood flow. After irradiation, the mice were shipped back to Boston and were aged until ~12 months of age (May 2016). Neurobehavioral testing, post-irradiation follow-up PET scans, as well as histological and biochemical brain analyses were performed.

Iron irradiation at 4 months of age had no long-term effects on basic health, motor and sensory function, grip strength, fatigue resistance, sensorimotor reactions, or anxiety in Alzheimer's or wildtype mice at ~12 months of age. Long-term radiation effects were observed mostly in male mice. Irradiated male Alzheimer's mice were less active but more coordinated, and had worse short-term spatial memory. Irradiated male wildtype mice had improved motor learning but significantly worse cognition than non-irradiated male wildtype mice. Radiation had no long-term effects on cognition in female mice, including both Alzheimer's and wildtype mice, suggesting that the long-term cognitive effects of iron irradiation are sex-specific. Interestingly, although female Alzheimer's mice have more Aß in brain than males, radiation at 4 months of age increased Aß levels and plaques in 12 month-old male Alzheimer's mice but had no effect on Aß levels and plaques in females. Biochemical detection of synaptic markers showed no radiation effect in male or female Alzheimer's or wildtype mice.

PET scan imaging and staining of mouse brain sections were performed to assess the long-term effects of a single dose of iron irradiation on inflammation in the brain. Neuroinflammation (detected by PET scan) was elevated in 12 month-old non-irradiated male and female Alzheimer's mice compared to wildtype control mice due to the presence of amyloid plaques. However, a single dose of iron irradiation further increased neuroinflammation in male but not in female Alzheimer's mice. As stated above, radiation also increased Aß levels and plaques only in male Alzheimer's mice. Staining of brain sections revealed that 50 cGy iron irradiation increased immune cell activation (i.e., gliosis) in both male and female Alzheimer's mice. Radiation had no effect on cerebral blood flow in any group as assessed by pre- vs. post-irradiation PET scans using a radio-labeled oxygenated water tracer.

During Year 4 of our grant, the late effects of 56Fe (iron) irradiation on mouse body weight and survival rate were also analyzed. Radiation did not affect body weight. Female Alzheimer's mice exposed to high dose iron were more prone to dying within the 8 month follow-up period compared to female Alzheimer's non-irradiated mice. Survival was not affected in the other groups. We also investigated late iron radiation effects on peripheral inflammatory cytokines in the blood, cerebral vascular integrity (microhemorrhages) and brain-region-specific synaptic density, though we found very few late effects of 56Fe radiation in these areas.

Taken together, our results suggest that a single exposure of 56Fe radiation produced long-term changes in mouse behaviors that vary by sex, genotype (wildtype vs. Alzheimer's mice), and dose. Late radiation effects were more predominant in male mice compared with female mice. In particular, iron radiation reduced motor activity, improved motor coordination, impaired short-term spatial memory, increased Aß burden and brain inflammation (GE180 PET scan), and upregulated microglial activation in male Alzheimer's mice compared with non-irradiated male control mice. Radiation had long-term effects on locomotor activity in female mice, but had no effect on memory, brain inflammation (PET) or Aß burden. Iron radiation reduced survival in female Alzheimer's mice (only) and had minimal or no overall long-term effects on vascular integrity, peripheral inflammatory cytokines or synaptic density across all mice.

Investigation 3: Effects of single-dose proton irradiation

In order to compare proton irradiation effects with those late effects we have observed for iron irradiation, female and male wildtype and Alzheimer's mice were shipped to BNL in October 2016 for a single dose of proton irradiation (0, 50, or 200 cGy). The mice were shipped back to Boston shortly thereafter and aged for another 8 months. A battery of 12 behavioral tests was performed in May 2017 when the mice were almost 1 year of age. Following behavioral testing, all mice were sacrificed in June 2017, and histological and biochemical analyses of brain and plasma samples were performed during Year 4 of our grant.

Mice that received a single dose of proton radiation at 4 months of age showed no late effects on general health, depression, or sensorimotor reactivity. However, proton radiation produced significant late effects on locomotor activity, motor coordination, motor learning, and fatigue resistance, which differed by sex, genotype, and dose. Proton radiation produced anti-anxiety like effects in Alzheimer's mice and affected cognition in male Alzheimer's mice only, including impaired spatial novelty memory and improved fear learning (but not fear memory). High dose (200 cGy) proton irradiation lowered Aß plaques in the hippocampi of only male Alzheimer's mice, accompanied by reduced inflammation (gliosis). In addition, proton irradiation lowered levels of the chemokine KC-GRO. No late effects of proton radiation were found on cerebral vascular integrity (microhemorrhages) or synaptic density.

Investigation 4: Effects of fractionated 56Fe exposure

Next, we compared the long-term effects of single vs. fractionated, chronic dosing of iron irradiation in a new Alzheimer's knock-in mouse model and wildtype mice. We bred and aged male mice to almost one year. Before irradiation, a subset of these mice underwent pre-irradiation MRI scans for brain structure and PET imaging for brain inflammation. In early April 2017, we shipped the entire cohort of 12 month-old male Alzheimer's knock-in and wildtype mice to BNL for iron irradiation. Alzheimer's knock-in and wildtype mice were irradiated once with 50 cGy iron (single dose) or with an equivalent dose divided into 6 radiation sessions over 2 weeks (fractionated dose). Control non-irradiated mice were shipped and handled in the same way as the irradiated mice. Upon return to BWH (Brigham & Women's Hospital), we discovered an ongoing error in our breeding of these mice and that the cohort of Alzheimer's knock-in mice assumed to be homozygous Alzheimer's knock-in and wildtype mice were in fact a mixture of homozygous Alzheimer's knock-in, heterozygous Alzheimer's knock-in, and pure wildtype mice. Follow-up imaging

was not repeated due to the low numbers (3 or fewer) of homozygous Alzheimer's knock-in mice in each group.

During Year 4 of our grant, behavioral tests were performed as planned in September/October 2017 on all 21 homozygous Alzheimer's knock-in mice (8, 7, 6 for non-irradiation, single, or fractionated dose, respectively) and 24 wildtype mice (8 mice/group). Upon completion, all mice were sacrificed and their brains were analyzed. In addition to comparing single vs. fractionated dosing, this study also provides new information regarding the effects of radiation exposure during middle age as 12 months of age in mice corresponds to approximately 40-45 years of age in humans. We completed the analyses of these mice by July 31, 2018.

A comparison between single vs. fractionated 56Fe irradiation showed different neurobehavioral effects on the mice. Interestingly, fractionated radiation was not detrimental (except for increased depression-like behavior) and was actually slightly beneficial in regards to increasing locomotion, reducing anxiety in wildtype mice, and improving cognitive learning in Alzheimer's knock-in mice. Single dose irradiation caused an increase in locomotion accompanied by worsened motor coordination and reduced muscle strength and fatigue in wildtype mice and impaired spatial novelty memory in Alzheimer's knock-in mice. Surprisingly, fractionated radiation significantly lowered the number of microhemorrhages, implying a protective effect on cerebral vascular integrity. In addition, single and/or fractionated radiation reduced plasma levels of IL-6, KC/GRO, and IL-10 in WT mice but did not affect these levels in the Alzheimer's knock-in mice. No differences in late effects between single and fractionated radiation were found in Aß pathologies, gliosis, or synapses.

To date, our studies suggest that female mice are somewhat resistant to iron irradiation while male mice appear to be more vulnerable, especially to the long-term central nervous system effects. One caveat is that survival was reduced in female Alzheimer's mice exposed to the higher single dose of iron irradiation; however, those female Alzheimer's mice that survived until the end of the study showed fewer radiation effects on cognition, amyloid pathology, and inflammation compared to their male irradiated counterparts. Iron-irradiated male but not female Alzheimer's mice showed short- and long-term cognitive deficits and long-term increases in brain amyloid and neuroinflammation. Similar to iron irradiation, a single exposure to proton irradiation caused sex- and dose-specific effects that varied according to the mouse model (wildtype or Alzheimer's). For example, motor coordination was worsened by low-dose (but not high dose) proton irradiation in wildtype mice but not Alzheimer's mice, and improved by iron irradiation in Alzheimer's mice. Both proton and iron irradiation induced spatial memory impairment in male but female Alzheimer's mice. And, while iron irradiation increased brain amyloid, proton irradiation decreased brain amyloid in male Alzheimer's mice. Lastly, a comparison of single dose vs. 6 smaller, fractionated doses of the equivalent amount of total iron irradiation in male Alzheimer's mice, using a new genetic knockin mouse model, revealed that fractionated dosing induced fewer detrimental effects than a single larger dose. For example, spatial memory deficits were observed following a single but not fractionated dose of iron irradiation. Neither single nor fractionated iron dosing altered brain amyloid levels in this new mouse model.

These findings will help inform risk estimates for astronauts exposed to space radiation on long-term, deep space missions. In addition, these studies emphasize the need for further research into sex differences in normal and disease conditions. This information may help guide research into developing specific countermeasures for male and female astronauts.

Several manuscripts are in preparation for submission to peer-reviewed journals.

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