Fiscal Year:	FY 2015	**Task Last Updated:**	FY 04/07/2015
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Project Title:	Functional decline in mice with Alzheimer's-type neurodegeneration is accelerated by charge-particle radiation		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
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:**	2010 Space Radiobiology NNJ10ZSA001N
Start Date:	02/01/2011	**End Date:**	01/31/2015
No. of Post Docs:	1	**No. of PhD Degrees:**	0
No. of PhD Candidates:	1	**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 JSC
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Flight Program:			
Flight Assignment:	NOTE: End date is now 1/31/2015 per NSSC information (Ed., 11/5/13)		
Key Personnel Changes/Previous PI:	Jerome Badaut, PhD terminated participation in our project as of July, 2013. Richard E Hartman, PhD ; Gregory Nelson, PhD ; Attila Szucs, PhD - subcontractor		
COI Name (Institution):	Nelson, Gregory Ph.D. (Loma Linda University) Hartman, Richard Ph.D. (Loma Linda University)		
Grant/Contract No.:	NNX11AE41G		
Performance Goal No.:			
Performance Goal Text:			

Task Description:	Exposure of an astronaut's central nervous system (CNS) to solar particle events (SPE) and galactic cosmic rays (GCR) may accelerate neurodegenerative changes and impact neuronal network activity, leading to cognitive deficits. There are similarities between radiation-induced CNS effects and pathological processes found in the Alzheimer's disease (AD). Common functional and structural findings include profound deficits in neuronal communication (synaptic transmission), cognitive impairments, neuro-inflammatory changes, and reduced neurogenesis. These similarities lead us to hypothesize that subjects with a genetic propensity to develop AD-pathology may be excessively vulnerable to ionizing radiation. We previously showed in transgenic (TG) APP23 mice, a murine model of AD, that irradiation with 600 MeV/n iron particles accelerated the onset of electrophysiological changes in the hippocampus, a brain structure crucially involved in the formation of short-term memory. In this project we use young adult APP/PSEN1E9 (APP/PSEN1) double transgenic (TG) mice and expose them to low doses of 150 MeV proton (irradiations performed at LLU proton treatment facility), 250 MeV/n silicon and 600 MeV/n iron particle radiation to compare and quantify their detrimental effects on hippocampal functions and the onset of AD-like pathology. The APP/PSEN1 TG mice typically exhibit early-onset of age-related behavioral abnormalities and deficits in synaptic transmission. We hypothesized that exposure to even low radiation doses will accelerate the onset of age-related neurodegenerative processes, while in wild-type (WT) animals such damage may stay undetectable. Comparison of proton, silicon, and iron radiation on selected neurophysiological end points in APP/PSEN1 TG mice will provide valuable information whether exposure to space radiation may exacerbate neurodegenerative processes. The functional end points (e.g., electrophysiological and behavioral changes) will be directly correlated with the expression of immuno-histochemical markers of neurodegeneration, including amyloid plaque load, synaptic proteins, and expression of neuro-inflammatory cytokines. If such correlation is found, it may indicate causative relationship between decrements in hippocampal functions and structural changes, which will help to elucidate the pathological mechanisms of radiation-induced neuronal injury and estimate the risks of low dose HZE radiation exposure to the CNS.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The central nervous system (CNS) has been typically described as radiation-resistant tissue. However, there is now sufficient body of evidence, mostly from experiments in rodents, showing that low doses of charged particle radiation (< 1 Gy) may affect some basic neuronal processes, such as synaptic excitability, neuronal firing and propensity for epileptiform activity. In addition, it has been posited by us and other investigators that in subjects prone to develop neurodegenerative diseases such as Alzheimer Disease (AD), an exposure to charged-particle radiation may accelerate the onset and/or alter the progression of AD, increase amyloid plaque load, and promote neuro-inflammatory changes within their brain. While such effects have been observed with high-LET particles (e.g., 1GEv/n Iron nuclei), this hypothesis has not been fully tested with other low- and high-LET particles and protons that represent major components in space radiation spectra. Studying the impact of protons and high-LET radiation on neurodegenerative processes in mammalian CNS is a critical step for critical assessment of the space radiation CNS-risks for astronauts, and e.g. for further development of modern cranial radiotherapies using charged particle radiation. The time-dependent changes in the CNS in patients undergoing cranial irradiations have been well documented, and they range from mild memory deficits to severe delayed demyelination and neurodegeneration. Whether low doses of charged particle radiation may accelerate the onset or affect the severity of AD-related pathology is not known. In the current project we used a murine double transgenic (TG) model of AD (the APP/PSEN1 TG mice commercially available from Jackson Laboratories) that we exposed to low- and high-LET charged-particle radiation and tested whether radiation affects the time course and severity of neurodegenerative processes in these TG mice. The functional changes were also compared to limited cohort of wild type (WT) mice to rule out qualitative differences in response to irradiation. The combination of behavioral, electrophysiological, and histological techniques helped us to gather unique data on radiation effects from 150 MeV proton-, 250 MeV/n silicon-, and 600 MeV/n iron-irradiated APP/PSEN1 TG mice. We identified multitude of functional changes in WT and TG brain and cortex of these mice, such as changes in synaptic excitability in the hippocampal CA1 neurons, and altered short term synaptic plasticity. Interestingly, in WT mice we observed radiation-induced suppression of epileptiform activity, a finding that may be clinically relevant in reducing epileptic seizures. In TG mice irradiated with protons and HZE, we observed altered presynaptic proteins involved in neurotransmitter release and worsened neurodegenerative changes. The acquired data will improve our understanding of pathophysiological processes in irradiated and AD-affected CNS tissue and will help to assess CNS-radiation risks for future manned, deep-space missions, such as the mission to Mars.
	Aim 1 & Aim 3 Activities. In accord with our statement of work, we completed all irradiations, behavioral testing, and in vitro electrophysiological experiments with protons and HZE (silicon 250 MeV/n & iron 600 MeV/n nuclei) particles. Proton irradiated APP/PSEN1 transgenic (TG) and wild-type (WT) mice were behaviorally tested pre- and 3 and 6 months post-irradiation followed by electrophysiological testing either at 6 or 9 months post-irradiation, as planned. HZE-irradiated mice (TG only) were behaviorally tested 3 and 6 months post-irradiation, followed by electrophysiological testing at 6-7 months post-irradiation. In total, from 2010-2013 we irradiated 78 TG and 16 WT mice with protons at Loma Linda University, Proton Treatment Facility. Electrophysiological testing was successfully performed in 82 proton-irradiated mice. We irradiated 120 TG with HZE particles at NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratories. Seven TG mice died spontaneously, thus electrophysiological testing was successfully performed on 113 animals. We used conventional extracellular recordings to monitor evoked synaptic responses and spontaneous epileptiform activity. The analyses of behavioral and electrophysiological data from all irradiated animals, including statistical evaluations, have been completed. We report that at radiation doses ranging from 0.1-1 Gy, whole–body exposures to protons, silicon or iron nuclei did not affect survival of TG mice. Behavioral analyses performed in cohorts of mice irradiated with protons (specific Aim 1) using water maze (WM), Barnes maze (BM), and zero maze (ZM) confirmed previously described, Alzheimer disease (AD) genotype-related deficits in spatial memory that were likely associated with hippocampal dysfunction. Genotype-related behavioral decrements were evident already at 3 months post-irradiation. For example, in control (non-irradiated) APP/PSEN1 TG mice we observed an increased swim distance to the target area in WM, an increased average distance moved in BM, and a reduction of time spent in the dark side of the ZM, when compared to the control (0 Gy) WT mice. Such findings indicate AD-related decrements in spatial memory (WM and BM data), but paradoxically a reduced anxiety-like behavior (ZM data) in TG mice. We observed relatively subtle, proton- and HZE-radiation-induced behavioral effects. Significant radiation-induced effects were only observed in WT mice after exposure to 0.5 Gy protons detected in WM at 6 months post-irradiation. In these mice only (n=16), we observed significantly increased swim distance during reversal learning phase of the WM test, indicating reduced cognitive flexibility. While proton radiation (tested at 0.5 Gy only) affected the performance of

Task Progress:	WT mice, exposures to protons and HZE-radiation (tested at 0.1-1 Gy) did not affect the performance of APP/PSEN1 TG mice. Such result may indicate that low doses of proton and HZE radiation may not necessarily worsen the functional outcomes in mice prone to AD-like pathology, or alternatively such pathology may trump any observable behavioral radiation-induced effects. Interestingly, the TG mice irradiated with 250 MeV/n silicon particles exhibited reduced performance in WM at 3 months only; the decrement was reaching statistical significance at 0.1 Gy only and appeared to be transient as it could not be detected at 6 months post-irradiation. No significant differences were observed for either HZE species in BM and ZM. In TG mice irradiated with 600 MeV/n iron particles we surprisingly observed a trend for improved performance in WM (reduced cumulative distance to the target platform), at 6 months post-irradiation at the dose of 1 Gy. Electrophysiological data indicate that proton radiation at doses from 0.1 to 1 Gy may impact synaptic excitability and short-term synaptic plasticity mediated by presynaptic glutamate release, but it likely does not affect long-term potentiation (LTP; reported previously to be altered in HZE-irradiated mice), the widely used cellular correlate of memory formation in the hippocampus. We observed that proton radiation-induced changes in synaptic excitability are qualitatively different in TG when compared to changes observed in WT mice. In accord with our behavioral findings, the WT mice exhibit different sensitivity to radiation and, for example at 0.5 Gy, we observed increased postsynaptic excitability in CA1 neurons, whereas the TG mice exhibited opposite responses at the same radiation dose. The stimulation paradigms testing the effect of proton radiation on presynaptic glutamate release (paired-pulse facilitation; PPF) revealed that the TG mice (but not the WT mice) at 9 exhibited reduced PPF indicating increased glutamate release. In addition, only in the WT mice irradiated with 0.5 Gy we observed reduced incidence of epileptiform activity (tested in the CA3-CA1 hippocampal network) promoted by activation of NMDA receptors. Interestingly, in TG mice, a radiation exposure to protons or HZE particles had no effect on these spontaneous oscillations. Aim 2 Activities. We completed immunohistological evaluations of ß-amyloid deposits in the brain samples (the cortex and the hippocampus) of APP/PSEN1 TG mice irradiated with protons and HZE particles using 6E10 monoclonal antibody (total amyloid). We confirmed an amyloid depositions in all brains of APP/PSEN1 TG mice at 6 and 9 months post-irradiation time point. At 9 months, we detected proton radiation-induced significant increases of total amyloid deposition at 1Gy in the dorsal cortex, but not the hippocampus. However, we did not identify similar increases in plaque deposition in TG mice irradiated with silicon or iron nuclei. Neuro-inflammation and neurodegenerative changes in TG (and WT) brains (cortex only) exposed to radiation have been assessed by analyses of five cytokines/chemokines (IL-1b, IL-6, TNFa, MCP-1, and IL-10) in homogenates of TG and WT mouse cortices. These signaling molecules have been previously reported to be elevated in irradiated brains and/or have been shown to affect synaptic plasticity in the hippocampus, thus their elevation may be associated with functional decrements observed in these animals. In a cohort of proton-irradiated mice we observed differences in the expression of chemokine IL-10 (CXCL10) between TG and WT mice at 9 months, but the effect was not dependent on the radiation exposure. The other chemokines were not affected by either genotype or radiation, indicating that at 9 months radiation effects on the CNS are not associated with elevated levels of pro-inflammatory cytokines. This also indicated that the electrophysiological and behavioral decrements reported above are not due to elevated levels of cytokines within the CNS, as previously suggested by us and other investigators. We finished analyses of synaptic marker synaptophysin in cortices of WT and TG mice irradiated with protons and HZE radiation by Western blotting (WB). In WT mice at 9 months post-irradiation with 0.5 Gy protons, we observed significantly increased synaptophysin expression when compared to control WT mice. Interestingly, in all TG mice groups (control 0.5 and 1 Gy only) the synaptophysin levels were comparable to those found in irradiated WT mice reflecting presumably an AD-related pathology (a genotype effect). However, the proton-irradiation in TG mice had no further enhancing effects on this presynaptic marker. Thus, we suggest that proton radiation does not affect an elevated expression of synaptophysin in subjects prone to AD-pathology. WB analyses of synaptophysin expression performed in the Si- and Fe-irradiated APP/PSEN1 TG mice at 6-7 mo post-irradiation indicated significant radiation-induced changes. While the Si-irradiated mice exhibited trends of increased expression at 0.1 and 0.5 Gy and reduction to control levels at 1 Gy, the Fe-irradiated mice exhibited significant reduction at 1 Gy. Several manuscripts are in preparation for submission to peer-reviewed journals.
Bibliography Type:	Description: (Last Updated: 04/24/2019)
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Articles in Peer-reviewed Journals	Bellone JA, Rudobeck E, Hartman RE, Szücs A, Vlkolinský R. "A single low dose of proton radiation induces long-term behavioral and electrophysiological changes in mice." Radiat Res. 2015 Aug;184(2):193-202. https://doi.org/10.1667/RR13903.1 ; PubMed PMID: 26207690 , Aug-2015
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