Fiscal Year:	FY 2014	**Task Last Updated:**	FY 12/04/2013
PI Name:	Vlkolinsky, Roman Ph.D.		
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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Zip Code:	92350-1700	**Congressional District:**	41
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 (Loma Linda University) Hartman, Richard Ph.D. (Loma Linda University)		
Grant/Contract No.:	NNX11AE41G		
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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 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/PSEN1?E9 (APP/PSEN1) double transgenic (TG) mice and expose them to low doses of 150 MeV/n 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 immunohistochemical markers of neurodegeneration, including amyloid plaque load, synaptic proteins and expression of neuroinflammatory 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 space radiation exposure to the CNS.		
Rationale for HRP Directed Research:			
Research Impact/Earth Benefits:	While the central nervous system (CNS) has been typically described as radiation-resistant tissue, we have previous electrophysiological and new behavioral evidence showing that even low doses of ionizing radiation may affect basic neuronal processes, such as synaptic transmission, neuronal excitability, and formation and consolidation of spatial memory. Specifically in the hippocampus, a brain structure intimately involved in the formation of memory, the ionizing radiation has been shown to impact synaptic excitability and plasticity. In addition, it cannot be excluded that ionizing radiation, even at very low doses of 0.1-1 Gy, may promote the onset of neurodegenerative disorders that affect the hippocampus, such as Alzheimer's disease (AD). However, this hypothesis has not been fully tested with different low- and high-LET particles. Studying the impact of protons and high-LET radiation on neurodegenerative processes in mammalian CNS is a critical step, not only for the assessment of the space radiation risks for astronauts, but also 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 model of AD that we exposed to low- and high-LET charged-particle radiation to attempt to answer this question. We tested whether radiation affects the time course and severity of neurodegenerative processes in these AD-prone subjects. The combination of behavioral, electrophysiological, and histological data will help us to identify functional decrements and the neurodegenerative changes in the brains of the irradiated mice. The acquired data will improve our understanding of pathophysiological processes in irradiated and AD-affected CNS tissue.		

Task Progress:

We received an approval for one year no-cost extension of our project ending in January 31, 2015.
Dr. Badaut, PhD, has been a co-investigator and lead for the immunohistochemical (IHC) aspect of our project. In July, Dr. Badaut had announced his intention to relocate to France and on his request his participation in our project was terminated as of July, 2013. Dr. Shalini Mehrotra, PhD, a first year postdoctoral fellow, was trained by Dr. J. Badaut. She was working with us since June 2012 and she was mostly responsible for performing the IHC analyses. Dr. Mehrotra had announced terminating her employment with LLU in August, 2013. Thus, the IHC part of our study was paused since Dr. Badaut's laboratory equipment (e.g., fluorescent microscope with Mercator software package) became unavailable. Nonetheless, we intend to complete the IHC part in full in collaboration with Dr. Nelson (co-investigator) and students trained by Dr. Badaut.

Re-allocation of funds allowed us to hire part time Mr. Gordon Harding as of September 2013, a senior research associate with significant experience in Western blotting and other protein quantification techniques. Mr. Harding performed initial experiments with presynaptic marker synaptophysin in mice irradiated with protons.

Technical progress: Summary Results by Aims.

Aim 1 & Aim 3 Activities. In accord with our statement of work (SOW), we completed all irradiations, behavioral testing, and in vitro electrophysiological experiments with protons and HZE (iron 600 MeV/n & silicon 250 MeV/n) 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. Pooling two electrophysiological time points (6 and 9 months) to one in HZE-irradiated animals was inevitable to boost the power of consequent statistical analyses. This change to the original SOW was opted for after observing considerable variability in electrophysiological results along with increased mortality specifically in iron-irradiated TG mice (likely unrelated to irradiation) that reduced the total numbers of experimental subjects per group. The change to the electrophysiological time point was applied for both HZE species to allow for direct comparisons of the radiation effects on relevant endpoints.

In total, from 2010-2013 we irradiated 78 TG and 16 WT mice with protons at Loma Linda University, Proton Treatment Facility. Twelve TG mice died; thus, electrophysiological testing was successfully performed in 82 proton-irradiated mice. We irradiated 120 TG with HZE particles at Brookhaven National Laboratories. Seven TG mice died spontaneously and thus electrophysiological testing was successfully performed in 113 animals. Ninety of these TG animals were electrophysiologically tested in 2013. We used conventional extracellular recordings to monitor both evoked synaptic responses and spontaneous activity. The behavioral and electrophysiological data from all proton- irradiated animals, including statistical evaluation, have been 95% completed. The analyses of electrophysiological and behavioral data from HZE-irradiated mice have been ~65% completed.

Behavioral analyses of proton and HZE-irradiated animals have been completed. Data from the water maze (WM) and the Barnes maze confirmed previously described deficits in spatial memory in control (0Gy) APP/PSEN1 TG mice (increased swim distance to the target area) when compared to the WT mice. We also observed that proton radiation (0.5 Gy) affected the performance of WT mice, but did not affect the performance of APP/PSEN1 TG mice. This may indicate that low radiation may not necessarily worsen the AD-like pathology, or that such pathology trumps any radiation-induced effects. In APP/PSEN1 TG mice irradiated with 600 MeV/n iron particles we surprisingly observed improved performance in WM (reduced cumulative distance to the target platform), the effect became significant at 6 months post-irradiation at the dose of 1 Gy. Interestingly, the TG mice irradiated with 250 MeV/n silicon particles exhibited reduced performance in WM at 3 months; the decrement was statistically significant 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 the Barnes maze or zero maze.

Electrophysiological data show 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), 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 APP/PSEN1 TG and 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 using two (paired) stimulation pulses were used to evaluate the effect of proton radiation on presynaptic glutamate release (paired-pulse facilitation; PPF). In TG mice at 6 months post-irradiation with protons we observed reduced PPF indicating increased glutamate release and this change became more pronounced at 9 months post-irradiation. Changes in PPF were not detected in WT mice. On the other hand, WT mice exhibited sensitivity to proton radiation because at the dose of 0.5 Gy we observed radiation-induced decrements in frequency of sharp wave-ripple complexes, which are implicated in memory consolidation process in the hippocampus. Interestingly, in TG mice, a radiation exposure to protons or HZE particles had no effect on these spontaneous oscillations.

Aim 2 Activities. We partly completed immunohistological evaluations of ß-amyloid deposits in the brain samples (the cortex and the hippocampus) of APP/PSEN1 TG mice irradiated with protons using thioflavin-S staining (fibrillar form of amyloid) and by IHC using 6E10 monoclonal antibody (total amyloid). Both methods confirmed amyloid depositions in the brains of APP/PSEN1 TG mice at 6 and 9 months post irradiation. In the dorsal cortex (but not the hippocampus) at 1 Gy of protons we observed significant increase of total amyloid by 9 months post-irradiation detected by 6E10 antibodies. The IHC on brain samples irradiated with HZE particles was temporarily paused due to departure of Drs. Badaut and Methorta. Nonetheless, the IHC analyses of HZE irradiated samples is planned for the fourth year of the project (the no-cost extension has been approved) by hardware provided by Dr. Nelson (co-investigator) and performed by other team members trained in Dr. Badaut's lab and by student volunteers.

Neuroinflammation and neurodegenerative changes in TG (and WT) brains (cortex only) exposed to radiation have been assessed by determination of five cytokines/chemokines (IL-1 beta, IL-6, TNF alpha, MCP-1, and IL-10). These 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. The Luminex assays have been completed in samples irradiated with protons, the assays with HZE-irradiated brains will be completed by December, 2013. In a cohort of proton-irradiated mice we observed differences in the expression of chemokine IL-10 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 are currently performing the analyses of synaptic markers in WT and TG mice irradiated with protons by Western blotting. The initial analyses in APP/PSEN1 TG mice irradiated with protons indicates that such exposure may increase the expression of synaptic vesicle glycoprotein and presynaptic marker synaptophysin, which may explain the radiation-induced changes in PPF described above. This marker has been previously shown to be affected by exposure to iron radiation, which awaits confirmation in APP/PSEN1 TG mice planned for the next year. Analyses in cortices irradiated with 0.1 and 1 Gy of protons and with HZE particles will be ensuing.

Bibliography Type:	Description: (Last Updated: 04/24/2019)
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Articles in Peer-reviewed Journals	Rudobeck E, Szücs A, Vlkolinsky R. "Effects of Proton Radiation on Evoked and Spontaneous Neuronal Activity in the Hippocampus of APP/PSEN1 Transgenic Mice." Journal of Radiation Research. In press, as of December 2013. To be published January 2014. , Dec-2013