Fiscal Year:	FY 2007	**Task Last Updated:**	FY 06/28/2007
PI Name:	Raber, Jacob Ph.D.		
Project Title:	Neurogenesis and cognition in human apoE transgenic mice following 56Fe radiation		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Radiation health		
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Human Research Program Risks:	(1) **BMed**:Risk of Adverse Cognitive or Behavioral Conditions and Psychiatric Disorders		
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Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
PI Email:	raberj@ohsu.edu	**Fax:**	FY
PI Organization Type:	UNIVERSITY	**Phone:**	503-494-1524
Organization Name:	Oregon Health & Science University		
PI Address 1:	Behavioral Neuroscience and Neurology		
PI Address 2:	L470, 3181 SW Sam Jackson Park Road		
PI Web Page:			
City:	Portland	**State:**	OR
Zip Code:	97239-3011	**Congressional District:**	1
Comments:			
Project Type:	Ground	**Solicitation / Funding Source:**	2004 Radiation Biology NNH04ZUU005N
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No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	0
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
Contact Monitor:		**Contact Phone:**	
Contact Email:			
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Flight Assignment:	NOTE: End date changed back to 8/31/2009 per S. Krenek/JSC (8/07) End date changed to 9/30/2009 per JSC update info (10/06)		
Key Personnel Changes/Previous PI:	0		
COI Name (Institution):	Fike, John (UCSF)		
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Task Description:	Of the major human isoforms of apolipoprotein E (E), E4 is associated with age-related cognitive decline and increased risk to develop Alzheimer's disease (AD). The space radiation environment contains 56Fe. 56Fe radiation exposure causes cognitive injury and might predispose E4 subjects to cognitive injury and an earlier onset or more severe extent of AD. The pathogenesis of this injury may involve loss or injury to neural precursor cells in the dentate subgranular zone (SGZ) of the hippocampus, which is involved in complex learning requiring the ability to learn about multiple relationships among stimuli. Disturbances in hippocampal functioning reduce spatial learning and memory. The granule cells of the dentate gyrus are involved in spatial memory and their radiation-induced depletion implicated in cognitive deficits. Our data show reduced numbers of proliferating cells and immature neurons and spatial learning and memory impairments in wild type mice x-irradiated at 2 months and behaviorally tested 3 months later. Treatments that damage neuronal precursor cells or their progeny might reduce neurogenesis and impair hippocampus-dependent cognitive functions in an E isoform-dependent fashion. Our data indicate that neuronal expression of E3 protects immature neurons against radiation injury and that E3 is more potent than E4 in supporting neuronal proliferation. After irradiation, SGZ precursor cells undergo rapid apoptotic cell death, which might involve oxidative stress. Oxidative stress might also play a critical role in later reductions in hippocampal neurogenesis. E isoforms differ in their ability to protect against neurotoxicity and apoptosis and oxidative stress. Isoform-specific effects of E on 56Fe radiation-induced oxidative stress, apoptosis, and loss of proliferating SGZ cells rapidly after 56Fe-irradiation or oxidative stress later after irradiation might contribute to their effects on cognitive injury later in life. We hypothesize that human E isoforms are associated with different levels of 56Fe-induced loss of neural precursor cells and hippocampus-dependent cognitive injury, and that these effects can be ameliorated by antioxidants. The Specific Aims are: 1A. Determine if E isoform is associated with radiation-induced apoptosis of neural precursor cells in the dentate SGZ; 1B. Determine the role of E isoform in the development of radiation-induced cognitive deficits and whether the severity of these deficits are associated with apoptosis of neural precursor cells in the dentate SGZ. Mice will be tested 3 months following radiation; 1C. Determine how E isoform affects neurogenesis following 56Fe- irradiation, and determine if this effect is related to the severity of radiation-induced cognitive deficits; 2A. Determine if the presence of a specific E isoform is associated with markers of oxidative stress following radiation injury; and 2B. Determine if the antioxidant a-lipoic acid enhances cognitive function and reduces radiation-induced cognitive impairments and whether this ability is E isoform-dependent. Mice will be irradiated with 56Fe ions (0 to 3 Gy). We will assess cognitive impairments using behavioral tests that require hippocampal functioning and quantify of radiation-induced changes using immunohistochemistry.
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	Our research impacts life on earth as it determines whether genetic risk factors for age-related cognitive decline are also risk factors for radiation-induced cognitive impairments. In case the anti-oxidant dietary supplement is able to antagonize these impairments, the impact would also constitute of a potential intervention against these challenges.
Task Progress:	We started to test the hypothesis that 56Fe radiation-induced cognitive injury is sex- and apoE isoform-dependent by sham-irradiating or irradiating two-month-old male and female mice expressing human apoE2, apoE3 or apoE4 under the control of the mouse apoE promoter at 3 Gy and behaviorally testing them in a battery of tests at least three months later. This dose was selected because it was shown to cause a profound reduction in proliferating cells and immature neurons without inducing obvious tissue destruction. This experiment is most pertinent to Aim 1B of our grant proposal: "1B. Determine the role of apoE isoform in the development of radiation-induced cognitive deficits and whether the severity of these deficits are associated with apoptosis of neural precursor cells in the dentate SGZ." We also started to irradiate mice at BNL to look at short term-effects on neurogenesis in the dentate gyrus of the hippocampus. The brains of these mice are being processed for immunohistochemistry. Two-month-old mice (n = 106) bred in our mouse colony at OHSU were sent to BNL for brain only 56Fe irradation (3 Gy) or sham irradiation in June 2006. Each mouse was anesthetized using mouse cocktail. The mice designated for irradiation were placed in positional cradles to stabilize the head position during irradiation. The cradles were placed in a box designed for the beam line. The whole-head of each mouse was irradiated with a 600 MeV/amu iron particles. A few days afterwards, the mice were shipped to OHSU for cognitive testing and tissue analyses. As we can maximally test 25 mice simultaneously in the object recognition and water maze tests, groups of 25 mice each were behaviorally analyzed in a battery of tests over a 5-week period. The researcher testing the mice was blinded to the genotype and treatment of the mice. To minimize the effect of stress on behavioral performance, the mice were tested first for levels of anxiety and exploratory behavior in the open field, elevated zero maze, elevated plus maze, and light dark tests (week 1), than for sensorimotor function on the rotorod (week 2), next for novel location and novel object recognition (week 3), for spatial learning and memory in the water maze (week 4), and last for emotional learning and memory in the passive avoidance test (week 5). The testing of the last cohort of 25 mice started in January, 2007. The researcher testing the mice was blinded to the genotype and treatment of the mice. Exploratory behavior and measures of anxiety were first tested in the open field. There were no effects of apoE on activity or measures of anxiety in the open field. To assess activity and measures of anxiety, the elevated zero maze, elevated plus maze, and light- dark tests were also used. In the zero maze, there was an effect of genotype on activity ($F = 6{,}514$, $p = 0.002$). ApoE4 mice were less active than apoE2 ($p = 0.003$) and apoE3 ($p = 0.011$) mice. Consistent with the elevated zero maze, there was a similar effect of genotype on activity in the light-dark test ($F = 3.947$, $p = 0.023$). ApoE4 mice were less active than apoE2 ($p = 0.047$) and apoE3 ($p = 0.038$) mice. These differential effects of apoE on measures of activity in the elevated zero maze and light-dark tests are similar to the effects found in sham- and Cesium-irradiated 5-month-old apoE2, apoE3, and apoE4 transgenic mice irradiated at 10 Gy at OHSU at 2 months of age. In contrast to the open field, elevated zero maze, and light-dark test, there were effects of genotypes and treatment on performance in elevated plus maze. There were effects of genotype on ratio time spent in the open arms of the elevated plus maze (time spent in open arms/(time spent in open + closed arms) ($F = 8.12$, $p = 0.001$). ApoE4 mice spent less time in the open arms than apoE3 ($p = 0.005$) mice and apoE2 spent less time in the open arms than apoE3 mice ($p = 0.002$). Similarly, there was an effect of genotype on distance moved in the open arms ($F = 11.24$, $p < 0.001$). ApoE4 mice moved less in the open arms than apoE3 mice ($p = 0.001$) and apoE2 mice moved in the open arms than apoE3 mice ($p = 0.002$). For pokes into the open arms, there was an effect of sex ($F = 4.584$, $p = 0.035$) and a sex x treatment interaction ($F = 4.7$, $p = 0.033$). In female mice, irradiated mice poked more into the open arms than sham-irradiated

mice ($p = 0.027$). Such effects were not found for pokes into the closed arms. There was only an effect of sex on pokes into the closed arms ($F = 19.658$, $p < 0.001$). For extending over the edges of the open arms, there was a genotype x sex x treatment interaction ($F = 4.3$, $p = 0.016$). Finally, there were effects of genotype ($F = 12.278$, $p < 0.001$) and treatment ($F = 6.67$, $p = 0.001$) on entries into the intersection. ApoE4 mice entered the intersection less than apoE2 ($p = 0.002$) or apoE3 ($p < 0.001$) mice. In addition, irradiated mice entered the intersection more than sham-irradiated mice ($F = 4.793$, $p = 0.031$). As the intersection is neither closed nor open and relates to measures of risk assessment, these data indicate that 56Fe irradiation increases risk assessment in human apoE transgenic mice. Consistent with interpretation, irradiated female mice poked more into the open arms than sham-irradiated female mice. This indicates that the effects of irradiation on risk assessment is more profound in females than males.

To assess hippocampus-dependent cognitive function, the mice were subsequently tested for novel location and novel object recognition, spatial learning and memory in the water maze, and for emotional learning and memory in the passive avoidance test. Sham-irradiated apoE2 and apoE3 female and male mice showed novel location recognition but irradiated apoE2 and apoE3 female and male mice did not. In contrast, while sham-irradiated apoE4 female and male mice failed to show novel location recognition, irradiated apoE4 female and male mice did. In contrast to novel location recognition, there were no effects of irradiation on novel object recognition. During the visible and hidden session of the water maze, there was an effect of genotype on time to reach the platform (latency) (apoE4 was different from apoE2 and apoE3). During the hidden session of the water maze, there was also a genotype x treatment interaction. Similar to the novel location recognition data, irradiation increased the latency of apoE2 mice but reduced the latency of apoE4 mice. Finally, irradiation impaired spatial memory retention in the probe trial in apoE2 female mice but improved spatial memory retention in apoE4 female and male mice. These data demonstrate that the effects of 56Fe irradiation on hippocampus-dependent cognitive function in mice are critically modulated by apoE isoform. The enhanced cognitive performance of apoE4 mice following 56Fe irradiation is exactly the opposite from the results we obtained in Cesium irradiated mice. Following Cesium irradiation, male mice were not affected and apoE4 female mice were particularly affected. These results indicate that the source of irradiation is critically modulating the direction of the effects of irradiation on brain function. Currently we are processing the brains of the behaviorally tested mice for immunohistochemistry. In particular, we plan to assess neurogenesis in these mice as proposed. As it is possible that these effects of 56Fe irradiation on brain function of apoE4 mice are age-dependent we irradiated older human apoE mice at BNL in May of 2007 and will start testing them at least 3 months following irradiation. This will show whether 56Fe irradiation worsens cognitive function in older apoE4 mice and that the excited, but unexpected, results are only detected in mice irradiated at 2 months of age.

Bibliography Type:	Description: (Last Updated: 10/30/2023)
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Articles in Peer-reviewed Journals	Villasana L, Acevedo SF, Poage C, Raber J. "Sex- and ApoE Isoform-dependent effects of radiation on cognitive function." Radiat Res. 2006 Dec;166(6):883-91. PMID: 17149978 , Dec-2006
Awards	Villasana L, Acevedo S, Poage C, Raber J. "Student Research Forum OHSU 2006, Oregon, U.S.A. Sex- and ApoE Isoform-dependent effects of radiation on cognitive function. Awarded Best Poster. " Sep-2006