

Fiscal Year:	FY 2008	Task Last Updated:	FY 06/17/2008
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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		
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:	2004 Radiation Biology NNH04ZUU005N
Start Date:	09/01/2005	End Date:	08/31/2009
No. of Post Docs:	0	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 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)		
Grant/Contract No.:	NNJ05HE63G		
Performance Goal No.:			
Performance Goal Text:			

<p>Task Description:</p>	<p>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 α-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.</p>
<p>Rationale for HRP Directed Research:</p>	
<p>Research Impact/Earth Benefits:</p>	<p>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.</p>
	<p>During this grant year we finished the behavioral analysis and assessment of the number of doublecortin-positive immature neurons following behavioral testing of human apoE2, apoE3, and apoE4 transgenic female mice irradiated with ⁵⁶Fe at a dose of 3 Gy at two months of age and behaviorally tested 3 months later. This experiment is most pertinent to Aim 1B. The data show that ⁵⁶Fe radiation-induced cognitive injury is apoE isoform-dependent. The mice were behaviorally analyzed in a battery of tests over a 5-week period. 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), then for sensorimotor function on the rotarod (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).</p> <p>There were no effects of irradiation on anxiety-like behaviors on any of these tests. Consistent with our previous ¹³⁷Cesium study, an effect of genotype was observed in the plus maze; apoE4 mice spent less time than apoE3 mice in the open arms ($p < 0.01$) and moved less than apoE2 ($p < 0.05$) and apoE3 ($p < 0.01$) mice in the plus maze, indicating increased anxiety and reduced exploratory behavior in apoE4 mice.</p> <p>Irradiation impaired hippocampal-dependent novel location recognition of apoE2 mice. Neither sham-irradiated nor irradiated apoE4 mice spent more time exploring the monkey in its novel location. However unlike sham-irradiated mice, irradiated apoE4 mice showed a trend to explore the monkey more in its novel location ($p = 0.14$). Impairments in novel location recognition were not due to deficits in object recognition, as all mice showed a preference for a novel object in the hippocampal-independent novel object recognition task.</p> <p>The water maze was used to assess spatial learning and memory. Cumulative distance which measures how far from the platform the mice searched was used to measure performance and performance of irradiated mice was compared to the performance of their sham-irradiated genotype-matched mice. Irradiation did not impair swim speed or motor function. Although not significant, irradiation appeared to impair the initial performance of apoE2 ($p = 0.15$) but enhance that of apoE4 ($p = 0.11$) mice in the hidden platform sessions. In the first probe trial, irradiation impaired spatial memory retention of apoE2 ($p < 0.05$) mice and strikingly enhanced that of apoE3 and apoE4 ($p < 0.05$) mice. There was no effect of irradiation with further training.</p> <p>These effects were not associated with a potential differential ability of apoE isoforms to protect against effects of irradiation on neurogenesis. The number of doublecortin-positive immature neurons in the dentate gyrus was dramatically and similarly reduced following ⁵⁶Fe irradiation in all three genotypes ($p < 0.001$). Thus, there is no simple relationship between neurogenesis and cognitive function and other mechanisms contribute to the differential cognitive outcomes in the context of a particular apoE isoform.</p> <p>The ⁵⁶Fe data are opposite to what was observed following irradiation with ¹³⁷Cesium. Following ¹³⁷Cesium irradiation, apoE2 mice were protected while apoE4 mice were most susceptible to cognitive injury. These paradoxical effects may be related to the differential effects of these two sources of irradiation on neurogenesis. Therefore, we also assessed the effects of ¹³⁷Cs irradiation on doublecortin-positive cells in the dentate gyrus of the hippocampus from behaviorally tested mice. Similar reductions in doublecortin-positive cells were observed in all genotypes, indicating that the differential effects of the two types of radiation on hippocampal function were not due to potential differences on inhibition of neurogenesis. It is possible that a different dose rather than a different source of irradiation may</p>

Task Progress:	<p>account for differences in cognitive function following irradiation.</p> <p>As it is possible that the effects of ⁵⁶Fe irradiation on brain function of apoE4 mice are age-dependent, we irradiated 6-month-old human apoE mice with ⁵⁶Fe at a dose of 3 Gy and tested them 3 later. In the first water maze probe trial (following the first day of hidden platform training), irradiation impaired spatial memory retention of only apoE2 mice. While apoE4 sham-irradiated male mice did not show spatial memory retention in the first probe trial, irradiated apoE4 male mice did. These data show that ⁵⁶Fe irradiation at a dose of 3 Gy worsens cognitive function in apoE2 mice but enhances cognitive function in apoE4 mice and that these effects are seen when the mice are irradiated at 2 or at 6 months of age.</p> <p>The ability of ⁵⁶Fe irradiation to potentially impair or enhance cognitive function is not limited to human apoE transgenic mice. To determine whether doses lower than 3 Gy affect hippocampal function 3 months following irradiation, female and male C57Bl/6J wild-type mice were irradiated with ⁵⁶Fe at a dose of 0, 1, 2, or 3 Gy at 2 months of age and cognitively tested 3 months later. This study was also undertaken to assess which lower doses should be used in future studies with apoE transgenic mice. There were no effects of irradiation on behavioral performance in the open field. Consistent with previous studies, the female mice were less active in the open field ($F(1,49) = 9.06$, $p = 0.04$). In the elevated zero maze, there was a sex x dose interaction ($F(3,49) = 0.028$, $p < 0.05$). While irradiation reduced measures of anxiety in the female mice, it increased measures of anxiety in the male mice.</p> <p>To determine whether a more challenging water maze test version involving reversal learning (a novel hidden platform location) is able to detect effects of irradiation on cognitive performance in male mice, C57BL/6J mice were irradiated with ⁵⁶Fe at 0, 1, 2, or 3 Gy ($n = 8$ mice/dose) and cognitively tested 3 months later. While the sham-irradiated mice showed spatial memory retention and searched more time in the target quadrant than any other quadrant, the mice irradiated at 1, 2, or 3 Gy did not. These data show this more challenging water maze paradigm involving 2 instead of 1 hidden platform location is sensitive to effects of irradiation in male mice.</p> <p>Next we determine the effects of irradiation on hippocampus-dependent contextual and hippocampus-independent cued fear conditioning. In the fear conditioning task, mice learn to associate a neutral stimulus (audio tone, CS) with a foot shock (US) and thereby come to fear the previously neutral CS. Trained mice display this conditioned fear by ceasing all movement except for respiration in an attitude called 'freezing.' After a 2 min period, during which the mouse is allowed to explore the experimental chamber, a tone is delivered followed in 30 sec by a foot shock (0.35 or 0.9 mA over 2 seconds). This was repeated 4.5 min later. One day later the mice were placed in the same context (environment) and, 1 hour later, in a new context (novel environment; different floor, walls, and smell) containing only the tone stimulus (cued). In a first experiment, we compared the shock intensity of 0.35 and 0.9 mA on fear conditioning on naïve (not irradiated) Wt male mice. A third group of animals was exposed to the same paradigm but no shock was delivered. Both 0.35 and 0.9 mA increased hippocampal-dependent contextual freezing but the freezing was more pronounced following a shock intensity of 0.9 mA. In contrast, both intensities elicited a comparable hippocampal-independent cued fear conditioning. Based on these data, the 0.35 mA intensity was used to assess fear conditioning in 2-month-old female and male C57BL/6J mice irradiated with ⁵⁶Fe at 0, 1 or 3 Gy ($n = 8$ mice/dose) and cognitively tested 3 mo later. The female mice irradiated at 1 or 3 Gy showed impairments in contextual fear conditioning as compared to sham-irradiated mice. Following irradiation at a dose of 2 Gy, there was a trend towards an effect but that did not reach significance. In contrast, in male mice, irradiation at a dose of 1 or 2 Gy enhanced contextual fear conditioning. Following irradiation at a dose of 3 Gy, there was no enhancement in contextual fear conditioning. In contrast to contextual fear conditioning, no effects of irradiation were seen in cued fear conditioning in female or male mice. Thus, hippocampus-dependent contextual fear conditioning, but not hippocampus-independent cued fear conditioning, is sensitive to the effects of 1 Gy of ⁵⁶Fe ions in a sex-dependent fashion. While female mice perform worse following irradiation, male mice perform better. These data indicate that hippocampus-dependent cognitive impairments occur after relatively low doses of irradiation. Based on these data, mice were irradiated at doses below 1 Gy and they will be cognitively tested in the next grant year.</p>
Bibliography Type:	Description: (Last Updated: 10/30/2023)
Articles in Peer-reviewed Journals	Villasana L, Poage C, van Meer P, Raber J. "Passive avoidance learning and memory of ⁵⁶ Fe sham-irradiated and irradiated human apoE transgenic mice." <i>Radiation Biology Radioecology</i> , 2008 Jan;48:191-4. , Jan-2008
Articles in Peer-reviewed Journals	Acevedo SF, Tittle S, Merry DE, Raber J. "Transgenic expression of androgen receptors improves spatial memory retention of sham-irradiated and ¹³⁷ Cesium irradiated female mice." <i>Radiation Research</i> , provisionally accepted, June 2008. , Jun-2008