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Zip Code:	97239-3011	**Congressional District:**	1
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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. As we show that the anti-oxidant dietary supplement alpha-lipoic acid is able to antagonize these impairments, the impact constitutes a potential intervention against these challenges.
	In the brain, apoE has been implicated in development, regeneration, neurite outgrowth, and neuroprotection. ApoE plays an important role in tissue repair following brain injury. Our data show that apoE isoform critically modulates the pattern of cognitive changes in the water maze in female mice three months following 56Fe irradiation but in a pattern opposite to that found three months following 137Cs irradiation. ApoE4 female mice are more susceptible than apoE2 female mice to develop cognitive injury 3 months following 137Cs irradiation, similar to the enhanced risk in apoE4 than apoE2 carriers to develop age-related cognitive injury and Alzheimer's disease (AD). However, 3 months following 56Fe irradiation, apoE2 female mice show radiation-induced cognitive injury while apoE4 female mice show enhanced cognitive performance. This enhanced cognitive performance 3 months following 56Fe irradiation is highly reproducible and seen in apoE4 female mice irradiated at 2 or 6 months of age and cognitive tested 3 months later. This enhanced cognitive performance is not restricted to apoE4 female mice and also seen in apoE3 female mice. In addition, we demonstrated that there is no simple relationship between reduced neurogenesis following irradiation and cognitive function. In contrast to the apoE isoform-dependent effects of 56Fe irradiation on cognitive function, the number of doublecortin-positive immature neurons in the dentate gyrus is 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. These novel and exciting data have radically changed our understanding of the effects of space irradiation on cognitive function. In the last year of the grant, we performed work pertinent to Aim 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; 1C: Determine how apoE isoform affects neurogenesis following 56Fe- irradiation, and determine if this effect is related to the severity of radiation-induced cognitive deficits; and 2B: Determine if the antioxidant a-lipoic acid enhances cognitive function and reduces radiation-induced cognitive impairments and whether this ability is apoE isoform- dependent. Specifically, we started to test the hypothesis that differences in the levels of reactive oxygen species (ROS) prior and following irradiation contribute to these paradoxical effects. We generated data consistent with such a novel mechanism. As it turned out, these data are not only pertinent to our understanding of the effects of space irradiation on cognitive function in human apoE targeted replacement mice but also to our understanding of the effects of 56Fe irradiation in wild-type mice and the effects of 137Cs irradiation on cognitive performance in wild-type mice. The hypothesized mechanism is based on the dual role of ROS in learning and memory and the increase in ROS levels following irradiation. While ROS is critical for normal synaptic function and learning and memory, chronic highly elevated ROS levels are pathological. Important in the context of different apoE isoforms, apoE2 is more effective as antioxidant than apoE3 or apoE4. The higher ROS levels in apoE3 and apoE4 than apoE2 female mice prior to irradiation might provide a preconditioning challenge to the apoE3 and apoE4 mice and therefore enhance cognitive function in apoE3 and apoE4, but not apoE2, mice. This might not be limited to human apoE mice and 56Fe irradiation. When we behaviorally tested wild-type mice and mice deficient in the extracellular form of superoxide dismutase (EC-SOD) which show elevated levels of oxidative stress following 137Cs irradiation or sham irradiation, irradiation impaired cognitive function in wild-type while it enhanced hippocampus-dependent cognitive function in distinct cognitive tasks. Although the levels of oxidative stress were higher prior to irradiation in the mice deficient in EC-SOD than wild-type mice, irradiation increased oxidative stress levels only in wild-type mice. In EC-SOD deficient mice, the

Task Progress:	levels of oxidative stress did not increase following irradiation. We hypothesize that like the EC-SOD deficient mice, apoE4 female mice have higher levels of oxidative stress prior to irradiation and, as a result of altered oxidative stress levels prior and following 56Fe irradiation, show enhanced cognitive performance 3 months following irradiation. We also hypothesize that this enhanced cognitive performance is time-dependent and not seen following prolonged increased levels of ROS in middle aged mice, who show enhanced ROS levels as part of the aging process. Indeed, apoE3 female mice irradiated with 56Fe at 2 months of age and cognitive tested 10 months later did show impairments in spatial memory retention in the water maze. To determine the role of ROS in cognitive performance following 56Fe irradiation, 9 month-old apoE2 and apoE4 female mice were irradiated with 56Fe or sham-irradiated and either fed a regular diet or a diet containing the anti-oxidant a-lipoic acid (ALA) and cognitively tested 3 months later. At 12 months of age, there was no longer a genotype difference in spatial memory retention in the water maze in the different experimental groups. Therefore, the two genotypes were combined for analysis. Strikingly, while sham-irradiated mice that received ALA-containing food showed worse spatial memory retention than sham-irradiated mice that received the regular diet, the opposite pattern was seen in irradiated mice. Irradiated mice that received the ALA-containing food showed better spatial memory retention than irradiated mice that received the regular diet. To determine whether this effect was restricted to human apoE female mice, we repeated this experiment with wild-type female mice. Consistent with the data in human apoE female mice and the EC-SOD data described above, irradiated wild-type female mice that received the ALA-containing diet outperformed irradiated wild-type mice that received the regular diet but this effect was not seen in sham-irradiated mice. Consistent with this mechanism, we find the same effect in wild-type male mice sham-irradiated or irradiated with 137Cs and treated with a combination of ramipril and statin or a superoxidase mimetic (Euk compound). The sham-irradiated mice show worse cognitive performance with these treatments than sham-irradiated mice that receive vehicle, while a trend towards a therapeutic effect is seen in irradiated mice. Based on these data and as a future perspective, we are planning experiments to assess whether the effects of ALA on cognitive performance in irradiated mice are associated with lower ROS levels. Similarly, it will be important to show that in young apoE4 female mice the cognitive enhancing effects are associated with enhanced ROS levels. In addition to assessments of oxidative stress using dihydroethidium and western blot analyses for 3-nitrotyrosine, we are planning to develop an in vitro slice model to study the nicotinamide adenosine dinucleotide phosphate (NADPH) oxidase complex. NADPH might be important for the role of ROS on cognitive performance following irradiation. It is expressed in neurons, both in soma and the synaps. In addition, irradiation of rat brain endothelial cells in vitro leads to increases in ROS production and expression of p22 phox and p4 phox proteins, and NADPH oxidase can produce brief bursts of superoxide upon stimulation. Western blot analyses can be used to assess total protein levels and the slice system allows assessment of the activity of the complex. So this would allow assessing the effects of space irradiation on the complex and to determine if these effects are critically modulated by apoE isoform. Finally, in the final year of the grant we showed that the dendritic marker microtubule-associated protein 2 (MAP-2) is very sensitive to effects of 56Fe irradiation in an apoE isoform-dependent fashion. To determine whether MAP-2 levels change in an apoE isoform-dependent fashion following 56Fe irradiation, brains of the apoE female mice irradiated at 2 months of age as described above were processed for MAP-2 immunohistochemisry. Group differences were analyzed using a multivariate analysis with genotype and treatment as between subject factors. There was a genotype x treatment interaction ($P < 0.05$, Roy's Largest Root Multivariate Analysis). Irradiation reduced MAP-2 levels in apoE3 and apoE4 ($p < 0.001$), but not apoE2, mice. Expression of MAP-2 is increased in the hippocampus and cortex of aged female and male C56BL/6J wild-type mice. Similarly, we find increased hippocampal MAP-2 levels in the aged female Rhesus monkeys. We hypothesize that this increase might constitute a compensatory change and that therefore the reduced MAP-2 levels in irradiated apoE3 and apoE4 female mice might contribute to pathological aging and reduced MAP-2 levels as seen in late stages of AD. Next we determined whether the reduced MAP-2 levels in irradiated mice are associated with increased levels of the presynaptic marker synaptophysin (SYN). This turns out not to be the case. In aged, but not middle-aged Rhesus monkeys, the levels of SYN are increased. These data indicate that MAP-2 is a particularly suitable and sensitive marker to quantify effects of space irradiation on brain function. Based on the magnitude of the effect, MAP-2 seems also a good candidate to help developing a mathematical framework for risk estimation of age-related changes in brain function following irradiation and as a function of apoE isoform.

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