

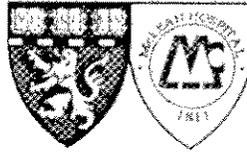
Tab 7



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25 January 2009

Dr. Francis A. Cucinotta
NASA Johnson Space Center
Mail Code SN3
Houston TX 77058

Dear Dr. Cucinotta,

Enclosed please find a revised Statement of Work for our proposal 09-Rad09Step2-0060. We have revised this document in response to peer review and to incorporate points raised in discussion with NASA.

Please do not hesitate to call or write me should you require further information.

Very sincerely yours,

Jack Bergman, Ph.D.
Harvard Medical School / McLean Hospital





Project Summary (submitted with Step 1 application)

NRA-NNJ09ZSA001N identifies an urgent need to determine the long-term effects of exposure to high-energy radiation (low fluence galactic cosmic rays and solar particle events) on the central nervous system (CNS). This information gap significantly hinders our ability to realistically estimate radiation risk associated with human space exploration and, consequently, impedes the development of future human deep space missions. To address these knowledge gaps, we propose to evaluate the long-term effects of space radiation on *in vivo* central nervous system (CNS) function in non-human primates. Our goals are to: 1) develop a range of procedures to assess the long-term effects of two different types (protons and ^{56}Fe) of space radiation on CNS function in monkeys, and 2) identify neurobehavioral and neuropharmacological markers in monkeys that will be useful predictors of long-term CNS radiation risk associated with human space exploration. Our results will, for the first time, systematically monitor and characterize changes in several neurobehavioral assays that are targeted towards specific brain systems in nonhuman primates over a period of three to four years post-radiation exposure. These assays include monitoring changes in overt behavior, motivation and reward processes, cognition, and learning. Our main strategy is to apply relevant receptor-directed behavioral pharmacology to investigate long-term CNS changes associated with exposure to space radiation. To determine meaningful cause-effect relationships between the radiation doses and the observed neurobehavioral and neuropharmacological results, we will also develop computational dosimetry for nonhuman primates. This will permit accurate dose assessments in specific regions of the brain, and in other organs and whole body. Our proposed work in nonhuman primates will yield critical new information towards estimating long-term space radiation risks to the CNS, thereby accelerating NASA's Space Radiation Program in an efficient and easily translational manner.



Revised Statement of Work for Proposal 09-Rad09Step2-0060

Research Statement

A. Integrated Research Plan Risks and Gaps

This proposal responds to NRA-NNJ09ZSA001N regarding the critical need for ground-based radiobiological studies to identify long-term effects of space radiation on the central nervous system (CNS) and other non-cancer related degenerative risks. This application address the 2nd and 3rd categories of space radiation risk and gaps outlined in the Human Research Program Integrated Research Plan (HRPIRP): *1.2 Risk of Acute or Late CNS Effects from Space Radiation; specific gaps: CNS-2 and CNS-6 (NRA, pg 33, 37). 1.3 Risk of Degenerative Tissue or other Health Effects from Space Radiation; specific gaps: Degen 1 – 3 (NRA, pg 33, 38).*

B. Statement of Problem

The long-term risk of neurobiological and non-cancer related degenerative damage to humans induced by space radiation during deep space travel is the most poorly explored health risk in ground-based studies of space radiobiology. Future deep space missions, such as long-term lunar and Mars missions, require travel beyond the Earth's protective magnetic field. Such continuous exposure leads to different types of space radiation hazards such as galactic cosmic rays (GCRs) and solar particle events (SPEs), which consist of particles of high energy and charge (HZE) and protons. Exposure to these radiations may pose a significant health risk for astronauts and, notably, low doses of GCRs or proton exposure caused by SPEs may be the primary sources of space radiation hazards on long duration missions. Recent studies suggest that the CNS, like the gastrointestinal tract and the lens of the eye, may be a radiosensitive organ.¹ Thus, exposure to these types of space radiation, in addition to jeopardizing mission success, may cause considerable and potentially long-term damage to the astronaut's normal CNS functioning and overall health. Although astronauts will be exposed to low doses of HZE particles (e.g. ⁵⁶Fe) and protons during deep space travel, studies to date have primarily focused on the short-term CNS effects of high doses of HZEs and protons.²⁻²⁴ Surprisingly little information is available on the long-term CNS and degenerative consequences of different types of space radiation with exposures that may be more representative of a space radiation environment. This information gap has significantly hindered NASA's ability to realistically estimate radiation risk associated with human space exploration and, consequently, impeded the development of future human deep space missions. The studies proposed in this application will address this information gap by developing a set of *in vivo* procedures in nonhuman primates that will examine the long-term effects of two different dose levels each for ⁵⁶Fe, ²⁸Si, and protons on CNS function and obtain comparative information regarding effects of these treatments on protein aggregation in the lens of the eye.

C. Overall Objectives, Specific Aims and Hypotheses

1. Program Objective. The overall objective of our program is to obtain data from studies in nonhuman primates that help predict long-term deleterious effects of space radiation on CNS function and the lens of the eye in humans. We propose to undertake this effort with neurobehavioral pharmacological studies in monkeys to evaluate the long-term (up to 4 yrs) effects of exposure to two doses of ⁵⁶Fe ion (0.1 or 0.5 Gy), ²⁸Si (0.1 or 0.5 Gy), and protons (0.5 or 1.0 Gy).

2. Specific Aims. Specifically, we aim to:

a. Evaluate effects of space radiation on overt behavior. The dose-related effects of dopaminergic drugs on targeted overt behavior—i.e., visual scanning, eye-blinking, self-scratching—have been used to study CNS dopamine (DA) receptor-mediated mechanisms (D_1 , D_2) in monkeys.²⁵⁻²⁷ We will compare pre- and post-radiation exposure effects of receptor-selective drugs on overt behavior to determine whether DA receptor-mediated function in monkeys is altered by exposure to ^{56}Fe and ^{28}Si particles and protons.^{2,14,16-18}

b. Assess effects of space radiation on motivational processes. Progressive-ratio (PR) procedures, used to measure the amount of behavior a subject will emit for food reinforcement, provide an established operant index of reinforcement value. In turn, this information can be used to study the role of different CNS systems in motivational processes. We plan to assess the effects of drugs that selectively increase or decrease different types of receptor activity implicated in brain reward systems [e.g. DA, cannabinoid CB_1 , glutamate (GLU)]⁴⁴ to determine how exposure to ^{56}Fe and ^{28}Si particles and protons alters their effects on PR behavior.

c. Examine space radiation effects on cognitive processes. Behavioral procedures such as the 'Stop Signal Response (SSR) Task' to measure impulsivity and performance monitoring, and the 'Stimulus Discrimination /Reversal (SD/R) Task' to measure cognitive functions, have been used to study aspects of impulsivity and cognitive function in both nonhuman primates and humans.⁴⁵⁻⁴⁸ We will use these highly translational procedures in squirrel monkeys to determine how ^{56}Fe and ^{28}Si particles and protons may alter the effects of drugs that target different brain systems implicated in cognitive processes (i.e. cholinergic; DA, cannabinoid CB_1).

d. Effects of space radiation on lens protein aggregation. The lens of the eye, among the most radiosensitive tissues in the body, is subject to dose-dependent changes in molecular structure following biologically relevant ionizing radiation. These radiation-induced lenticular changes can be non-invasively assessed *in vivo* by quasi-elastic light scattering (QLS) analysis. In collaborative studies with ██████████ at Boston University, a purpose-adapted non-invasive laser eye scanner will be used to obtain longitudinal QLS information with which to quantitate pre-cataractous changes in the lens of the eye as a function of radiation dose, type, and time following exposure. The QLS technology proposed for these studies is safe, simple, and highly sensitive. The platform that will be used has been extensively validated and has been deployed in a wide-range of studies involving laboratory animals, nonhuman primates, and human subjects. In particular, we will perform *in vivo* molecular biodosimetry (MBD) analysis of the lens in squirrel monkeys to determine how exposure to ^{56}Fe and ^{28}Si particles and protons alters lens protein aggregation and whether these changes correlate with neurobehavioral function. The proposed studies in non-human primates are complementary to mouse studies aimed at assessing the effects of low-dose cosmic (NASA) or X-ray (DOE) irradiation.

3. **Hypotheses.** Although the absence of comparable studies in other species does not permit confident prediction, we anticipate that:

a. studies of overt behavior will reveal long-term alterations in both D_1 and D_2 receptor mechanisms, consistent with a role for DA systems in motivation and cognition.

b. exposure to ^{56}Fe and ^{28}Si particles and protons will alter the pharmacology of motivational and cognitive processes in nonhuman primates and, in particular, the effects of DA-related drugs on PR behavior and in SSR and SD/R tasks.

c. exposure to ^{56}Fe and ^{28}Si particles and protons will potentiate dose- and time-dependent protein aggregation within the nonhuman primate lens that will correlate with changes observed in behavioral tasks.

D. Relevance, Significance and Feasibility of Proposed Studies

Our overall research strategy is pharmacologically mechanistic. We plan to compare the effects of CNS receptor-selective drugs before and after exposure to evaluate long-term neurobehavioral effects of ^{56}Fe and ^{28}Si particles and proton. This approach is based on observations that exposure to high doses of GCR and SPE leads to: 1) short-term detrimental effects in some rodent neurobehavioral assays;²⁻¹⁵ 2) *in vitro* changes in brain DA striatal tissue;^{2,14,16-18} 3) long-term CNS changes that are comparable to those observed in normal human aging.^{2,13-22} 4) acceleration of the aging process and an increased risk of early onset of neurodegenerative disease^{2,5,13-22} and 5) molecular and cellular damage in CNS.²³⁻²⁴ These findings come mainly from *in vitro* preparations or neurobehavioral assays in rodents, and provide a strong foundation for further assessing the immediate and long-term CNS effects of space radiation. Cognizant of anatomical and functional variations across species that limit extrapolation of laboratory data from rodents to nonhuman primates or humans,³⁵ our approach will employ well-established procedures in nonhuman primates to study the effects of ^{56}Fe , ^{28}Si , and proton radiation on brain systems that may be involved in motivational and cognitive processes, and whether these CNS changes can be associated with molecular pathological changes in the lens.

1. Relevance of Proposed Studies. There presently is little understanding of potential long-term neurobehavioral consequences of exposure to space radiation. In this regard, NASA's Space Radiation Program-HRPIRP has identified studies of immediate and late (i.e., long-term) effects of space radiation on CNS functions as a major research focus (NRA, pg 11);³⁴ The relevance of our proposed research is that we will directly address this need with ground-based studies in monkeys to examine the long-term effects of HZE and proton radiation in neurobehavioral assays of motivational and cognitive processes. In addition, our proposed experiments will investigate the utility of non-invasive assessment of molecular pathology in the lens of the eye as a stable quantitative marker of exposure to biologically relevant radiation. This information will help advance NASA's ability to predict the long-term risk of space radiation for astronauts.

2. Significance of Proposed Studies. To our knowledge, the neurobehavioral effects of exposure to space radiation have not been previously examined in nonhuman primates. Overall, a highly significant aspect of our proposal lies in our use of nonhuman primates Several features of nonhuman primate research facilitate the translation of experimental results to humans with reasonable predictive validity.³⁵⁻³⁸ For example: 1) nonhuman primates and humans are similar in their genetic, physiological, pharmacokinetic, and neurobiological characteristics; 2) within-subject designs similar to those used in human laboratory studies permit meaningful conclusions or inferences to be based on the evaluation of all treatment effects in individuals as well as in groups; and 3) nonhuman primates are reliable subjects in long-term behavioral and pharmacological studies.³⁸⁻⁴⁰ Considerations such as these suggest that nonhuman primates are especially well-suited for ground-based research to study long-term neurobehavioral effects of space radiation.

Previous reports suggest that HZE particle radiation may have deleterious effects on DA function. Thus, we have chosen to first evaluate the long-term consequences of exposure to ^{56}Fe , ^{28}Si , and proton radiation on CNS DA-related systems. Studies of overt behavior outlined in Specific Aim 1 (see section G.2.a, G.6.a, and H.1) will enable us to determine whether exposure alters the effects of DA receptor subtype-selective drugs or of drugs that activate DA receptors indirectly. The significance of these studies is that they will advance NASA's Space Radiation

Program in a manner that is logically based on previous findings of DA-related abnormalities and that is highly translational.

Under **Specific Aims 2 and 3** we will employ operant procedures that are widely acknowledged to measure aspects of motivation and cognition. These procedures also have been used to study the effects of receptor-selective drugs that act through CNS systems implicated in these neurobiological processes. Our proposed research will use this pharmacological approach to analyze the long-term effects of exposure to radiation on motivation and cognition by evaluating changes in baseline performance over time, and by comparing pre- and post-exposure effects of receptor-selective drugs. The significance of these studies is that they will provide data in nonhuman primates that will lead to more accurate prediction of long-term effects of space radiation on motivational and cognitive processes.

Finally, in **Specific Aim 4** we will test a recently-developed innovative laser-based diagnostic procedure to non-invasively measure molecular pathology (e.g., radiation-induced protein denaturation, aggregation, crosslinking) within the nonhuman primate lens as a function of radiation dose, type, and post-exposure time. The significance of this work is that it will permit meaningful quantitative analyses of cause-effect relationships between radiation dose and effects in neurobehavioral studies in monkeys.

Taken together, the results of these studies will provide previously unavailable information on the long-term CNS effects of ^{56}Fe and ^{28}Si particles and proton radiation in nonhuman primates. Regardless of study outcomes, such quantitative and qualitative neurobehavioral and neuropharmacological data, as well as, measurement of lens protein aggregation will be uniquely valuable to two stated goals of NASA's Space Radiation Program: a) significant progress toward estimating space radiation risks to humans and b) development of a strong and clear foundation for NASA to successfully design future human deep space missions.

3. *Feasibility of Proposed Studies.* Given that similar ground-based studies in space radiobiology have not been previously conducted in nonhuman primates, it is important to recognize the feasibility of our planned research. Our research group includes investigators with as much as 30 years of experience in conducting complex behavioral pharmacology studies in nonhuman primates [REDACTED] radiation biology expertise in cellular and molecular CNS effects of space radiation,²³⁻²⁴ [REDACTED] and sophisticated capabilities for non-invasively analyzing the relationship between protein aggregation in the lens and CNS function during normal and abnormal aging processes [REDACTED]

With regard to our proposed neurobehavioral studies, we have substantial experience in studying drug effects on overt behavior in squirrel monkeys (**Specific Aim 1**), and we have demonstrated that different types of DA receptor-related drugs exhibit unique and characteristic behavioral profiles (see section G.6.a).^{25,27,42-44} Our laboratory also has extensive experience in training nonhuman primates in complex behavioral tasks that are used to measure both motivational and cognitive processes (**Specific Aims 2 and 3**). For example, we currently are conducting studies in squirrel monkeys using PR performance as an index of motivation and we have recently trained monkeys to leverpress or withhold leverpressing depending on the color of visual stimulus lights as a measure of impulsivity.⁴⁹ Except for the use of a touch screen in place of stimulus lights and levers, this task is nearly identical to the SSR task that we will use in our proposed research.⁴⁵ Finally, SD/R learning is routinely used in the context of drug discrimination or choice research in our laboratory. Based on our extensive experience in training squirrel monkeys to perform these and other complex behavioral tasks, we do not

anticipate methodological problems in achieving the goals of this application. Regarding **Specific Aim 4**, our project team has substantial experience in using QLS instrumentation to investigate the molecular architecture of the lens in rats, mice, nonhuman primates, and humans.

Finally, to further ensure that the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory (BNL, NY) can support our planned studies in nonhuman primates, we recently visited [REDACTED] (a consultant on our proposal) and [REDACTED] [REDACTED] BNL Animal Facility Manager). Following careful inspection of the NSRL and the animal care facility at BNL, and as well, discussion with [REDACTED] we are highly confident that: 1) the irradiation of squirrel monkeys at NSRL will be conducted without difficulty; and 2) our subjects will be properly housed and maintained at BNL during the irradiation protocol.

E. Future Direction of Planned Nonhuman Primate Studies.

Our proposed research in nonhuman primates is designed as an initial set of studies to assess the *in vivo* CNS effects of exposure to relatively low doses of ^{56}Fe and ^{28}Si particles and protons. We understand that in these initial studies, monkeys will be exposed to particle radiation that does not fully mimic the chronic low dose situation in a mixed radiation field. Based on guidance from NASA, we expect future research to include studies of long-term CNS risks associated with other radiation types, including a combination of ^{56}Fe and proton irradiation. In addition to space radiation, other external stressors also may have detrimental effects on normal CNS function during long-lasting deep space missions. These include microgravity, altered light-dark cycle, elevated CO_2 levels in ambient air, confinement, and high physical and mental workload.⁴⁶ Consequently, future studies in this program also may be designed to evaluate the short- and long-term impact of a combination of ^{56}Fe and/or proton irradiation and external stress factors (e.g., microgravity) on normative behavior in nonhuman primates. Finally, previous work in rodents suggests that we will observe DA-related behavioral effects of exposure in our initial studies. If earlier findings are confirmed in our studies, we are prepared to measure ^{56}Fe , ^{28}Si , and proton radiation-induced changes in DA neurochemistry in monkeys, using *in vivo* microdialysis procedures that are ongoing in our laboratory.³⁹

Although identifying specific studies is premature at this time, we recognize that the major goals of this program will include, first, research into the relationship between molecular and cellular changes in the primate brain and in the lens of the eye in relationship to neurobehavioral and pharmacological effects of exposure to HZE particles and protons radiation and, second, the development of effective pharmacological and/or dietary countermeasures to lessen the risks of HZE and proton radiation and/or other external stressors to CNS function. Data from our planned studies will provide a strong first step in these directions.